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INSTRUCTIONS FOR AUTHORS (2021)

GENERAL

The *Journal of the Serbian Chemical Society* (the *Journal* in further text) is an international journal publishing papers from all fields of chemistry and related disciplines. Twelve issues are published annually. The Editorial Board expects the editors, reviewers, and authors to respect the well-known standard of professional ethics.

Types of Contributions

Original scientific papers	(up to 15 typewritten pages, including Figures, Tables and References) report original research which must not have been previously published.
Short communications	(up to 8 pages) report unpublished preliminary results of sufficient importance to merit rapid publication.
Notes	(up to 5 pages) report unpublished results of short, but complete, original research
Authors' reviews	(up to 40 pages) present an overview of the author's current research with comparison to data of other scientists working in the field
Reviews[a]	(up to 40 pages) present a concise and critical survey of a specific research area. Generally, these are prepared at the invitation of the Editor
Surveys	(about 25 pages) communicate a short review of a specific research area.
Book and Web site reviews	(1 - 2 pages)
Extended abstracts	(about 4 pages) of Lectures given at meetings of the Serbian Chemical Society Divisions
Letters to the Editor	report miscellaneous topics directed directly to the Editor

[a]Generally, Authors' reviews, Reviews and Surveys are prepared at the invitation of the Editor.

Submission of manuscripts

Manuscripts should be submitted using the **OnLine Submission Form**, available on the JSCS Web Site (**http://www.shd-pub.org.rs/index.php/JSCS**). The manuscript must be uploaded as a Word.doc or .rtf file, with tables and figures (including the corresponding captions – above Tables and below Figures), placed within the text to follow the paragraph in which they were mentioned for the first time.

Please note that **Full Names (**First Name, Last Name), **Full Affiliation** and **Country** (from drop down menu) of **ALL OF AUTHORS** (written in accordance with English spelling rules - the first letter capitalized) must be entered in the manuscript Submission Form (Step 3). Manuscript Title, authors' names and affiliations, as well as the Abstract, **WILL APPEAR** in the article listing, as well as in **BIBLIOGRAPHIC DATABASES (WoS, SCOPUS...)**, in the form and in the order entered in the author details

Graphical abstract

Graphical abstract is a one-image file containing the main depiction of the authors work and/or conclusion and must be supplied along with the manuscript. It must enable readers to quickly gain the main message of the paper and to encourage browsing, help readers identify which papers are most relevant to their research interests. Authors must provide an image that clearly represents the research described in the paper. The most relevant figure from the work, which summarizes the content, can also be submitted. The image should be submitted as a separate file in **Online Submission Form - Step 2**.

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No additional text, outline or synopsis should be included. Please do not use white space or any heading within the image.

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Manuscripts must be accompanied by a cover letter (strictly uploaded in **Online Submission Step 2**) in which the type of the submitted manuscript and a warranty as given below are given. The Author(s) has(have) to warranty that the manuscript submitted to the *Journal* for review is original, has been written by the stated author(s) and has not been published elsewhere; is currently not being considered for publication by any other journal and will not be submitted for such a review while under review by the *Journal*; the manuscript contains no libellous or other unlawful statements and does not contain any materials that violate any personal or proprietary rights of any other person or entity. All manuscripts will be acknowledged on receipt (by e-mail).

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*International Committee of Medical Journal Editors ("Uniform Requirements for Manuscripts Submitted to Biomedical Journals"), February 2006

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All contributions will be peer reviewed and only those deemed worthy and suitable will be accepted for publication. The Editor has the final decision. To facilitate the reviewing process, authors are encouraged to suggest up to three persons competent to review their manuscript. Such suggestions will be taken into consideration but not always accepted. If authors would prefer a specific person not be a reviewer, this should be announced. The Cover Letter must be accompanied by these suggestions. Manuscripts requiring revision should be returned according to the requirement of the Editor, within 60 days upon reception of the reviewing comments by e-mail.

The *Journal* maintains its policy and takes the liberty of correcting the English as well as false content of manuscripts **provisionally accepted** for publication in the first stage of reviewing process. In this second stage of manuscript preparation by JSCS Editorial Office, the author(s) may be required to supply some **additional clarifications and corrections**. This procedure will be executed during copyediting actions, with a demand to author(s) to perform corrections of unclear parts before the manuscript would be published OnLine as **finally accepted manuscript (OLF Section of the JSCS website)**. Please note that the manuscript can receive the status of **final rejection** if the author's corrections would not be satisfactory.

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Tables, figures and/or schemes must be embedded in the main text of the manuscript and should follow the paragraph in which they are mentioned for the first time. **Tables** must be prepared with the aid of the **WORD table function**, without vertical lines. The minimum size of the font in the tables should be **10 pt**. Table columns must not be formatted using multiple spaces. Table rows must not be formatted using any returns (enter key; ↵ key) and are **limited to 12 cm width**. Tables should not be incorporated as graphical objects. **Footnotes to Tables** should follow them and are to be indicated consequently (in a single line) in superscript letters and separated by semi-column.

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IUPAC recommendations for the naming of compounds should be followed. SI units, or other permissible units, should be employed. The designation of physical quantities must be in italic throughout the text (including figures, tables and equations), whereas the units and indexes (except for indexes having the meaning of physical quantities) are in upright letters. They should be in Times New Roman font. In graphs and tables, a slash should be used to separate the designation of a physical quantity from the unit

(example: p / kPa, j / mA cm^{-2}, t / °C, T_0 / K, τ / h, ln (j / mA cm^{-2})…). Designations such as: p (kPa), t [min]…, are not acceptable. However, if the full name of a physical quantity is unavoidable, it should be given in upright letters and separated from the unit by a comma (example: Pressure, kPa; Temperature, K; Current density, mA cm^{-2}…). Please do not use the axes of graphs for additional explanations; these should be mentioned in the figure captions and/or the manuscript (example: "pressure at the inlet of the system, kPa" should be avoided). The axis name should follow the direction of the axis (the name of y-axis should be rotated by 90°). Top and right axes should be avoided in diagrams, unless they are absolutely necessary.

Latin words, as well as the names of species, should be in *italic*, as for example: *i.e.*, *e.g.*, *in vivo*, *ibid*, *Calendula officinalis* L., *etc*. The branching of organic compound should also be indicated in *italic*, for example, *n*-butanol, *tert*-butanol, *etc*.

Decimal numbers must have decimal points and not commas in the text (except in the Serbian abstract), tables and axis labels in graphical presentations of results. Thousands are separated, if at all, by a comma and not a point.

Mathematical and chemical equations should be given in separate lines and must be numbered, Arabic numbers, consecutively in parenthesis at the end of the line. All equations should be embedded in the text Complex equations (fractions, integrals, matrix…) should be prepared with the aid of the **Microsoft Equation 3.0** (or higher) or **MathType** (Do not use them to create simple equations and labels). **Using the Insert -> Equation option, integrated in MS Office 2010 and MS Office 2013, as well as insertion of equation objects within paragraph text IS NOT ALLOWED.**

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- SUPPLEMENTARY MATERIAL (optional**)**

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Main text – should have the form:

- **INTRODUCTION,**
- **EXPERIMENTAL (RESULTS AND DISCUSSION),**
- **RESULTS AND DISCUSSION (EXPERIMENTAL),**
- **CONCLUSIONS,**
- **NOMENCLATURE (optional) and**
- ***Acknowledgements*: If any.**
- REFERENCES (Citation of recent papers published in chemistry journals that highlight the significance of work to the general readership is encouraged.)

The sections should be arranged in a sequence generally accepted for publication in the respective fields. They subtitles should be in capital letters, centred and NOT numbered.

- The INTRODUCTION should include the aim of the research and a concise description of background information and related studies directly connected to the paper.
- The EXPERIMENTAL section should give the purity and source of all employed materials, as well as details of the instruments used. The employed methods should be described in sufficient detail to enable experienced persons to repeat them. Standard procedures should be referenced and only modifications described in detail. On no account should results be included in the experimental section.

Chemistry

Detailed information about instruments and general experimental techniques should be given in all necessary details. If special treatment for solvents or chemical purification were applied that must be emphasized.

Example: Melting points were determined on a Boetius PMHK or a Mel-Temp apparatus and were not corrected. Optical rotations were measured on a Rudolph Research Analytical automatic polarimeter, Autopol IV in dichloromethane (DCM) or methanol (MeOH) as solvent. IR spectra were recorded on a Perkin-Elmer spectrophotometer FT-IR 1725X. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively), and on a Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) employing indicated solvents (*vide infra*) using TMS as the internal standard. Chemical shifts are expressed in ppm (δ / ppm) values and coupling constants in Hz (J / Hz). ESI-MS spectra were recorded on Agilent Technologies 6210 Time-Of-Flight LC-MS instrument in positive ion mode with CH_3CN/H_2O 1/1 with 0.2 % HCOOH as the carrying solvent solution. Samples were dissolved in CH_3CN or MeOH (HPLC grade purity). The selected values were as follows: capillary voltage = 4 kV, gas temperature = 350 °C, drying gas flow 12 L min^{-1}, nebulizer pressure = 310 kPa, fragmentator voltage = 70 V.The elemental analysis was performed on the Vario EL III- C,H,N,S/O Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau-Germany). Thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F254 and RP-18 F254 plates. Column chromatography was performed on Lobar LichroPrep Si 60 (40-63 μm), RP-18 (40-63 μm) columns coupled to a Waters RI 401 detector, and on Biotage SP1 system with UV detector and FLASH 12+, FLASH 25+ or FLASH 40+ columns pre packed with KP-SIL [40-63 μm, pore diameter 6 nm (60 Å)], KP-C18-HS (40-63 μm, pore diameter 9 nm (90 Å) or KP-NH [40-63 μm, pore diameter 10 nm (100 Å)] as adsorbent. Compounds were analyzed for purity (HPLC) using a Waters 1525 HPLC dual pump system equipped with an Alltech, Select degasser system, and dual λ 2487 UV-VIS detector. For data processing, Empower software was used (methods A and B). Methods C and D: Agylent Technologies 1260 Liquid Chromatograph equipped with Quat Pump (G1311B), Injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and Detector 1260 DAD VL+ (G1315C). For data processing, LC OpenLab CDS ChemStation software was used. For details, see Supporting Information.

1. Synthesis experiments

Each paragraph describing a synthesis experiment should begin with the name of the product and any structure number assigned to the compound in the Results and Discussions section. Thereafter, the compound should be identified by its structure number. Use of standard abbreviations or unambiguous molecular formulas for reagents and solvents, and of structure numbers rather than chemical names to identify starting materials and intermediates, is encouraged.

When a new or improved synthetic method is described, the yields reported in key experimental examples, and yields used for comparison with existing methods, should represent amounts of isolated and purified products, rather than chromategraphically or spectroscopically determined yields. Reactant quantities should be reported in weight and molar units and for product yields should be reported in weight units; percentage yields should only be reported for materials of demonstrated purity. When chromatography is used for product purification, both the support and solvent should be identified.

2. Microwave experiments

Reports of syntheses conducted in microwave reactors must clearly indicate whether sealed or open reaction vessels were used and must document the manufacturer and model of the reactor, the method of monitoring the reaction mixture temperature, and the temperature-time profile. Reporting a wattage rating or power setting is not an acceptable alternative to providing temperature data. Manuscripts describing work done with domestic (kitchen) microwave ovens will not be accepted except for studies where the unit is used for heating reaction mixtures at atmospheric pressure.

3. Compound characterization

The Journal upholds a high standard for compound characterization to ensure that substances being added to the chemical literature have been correctly identified and can be synthesized in known yield and purity by the reported preparation and isolation methods. For **all new** compounds, evidence adequate to establish both **identity** and **degree of purity** (homogeneity) must be provided.

Identity *- Melting point.* All homogeneous solid products (*e.g.* not mixtures of isomers) should be characterized by melting or decomposition points. The colors and morphologies of the products should also be noted.

Specific rotations. Specific rotations based on the equation $[\alpha]^t; D = (100\ \alpha) / (l\ c)$ should be reported as unitless numbers as in the following example: $[\alpha]^{20}$; D = -25.4 (c 1.93, $CHCl_3$), where c / g mL^{-1} is concentration and l / dm is path length. The units of the specific rotation, (deg mL) / (g dm), are implicit and are not included with the reported value.

Spectra/*Spectral Data.* Important IR adsorptions should be given.

For all new diamagnetic substances, NMR data should be reported (^1H, ^{13}C, and relevant heteronuclei). ^1H NMR chemical shifts should be given with two digits after the decimal point. Include the number of protons represented by the signal, signal multiplicity, and coupling constants as needed (*J* italicized, reported with up to one digit after the decimal). The number of bonds through which the coupling is operative, ^xJ, may be specified by the author if known with a high degree of certainty. ^{13}C NMR signal shifts should be rounded to the nearest 0.01 ppm unless greater precision is needed to distinguish closely spaced signals. Field strength should be noted for each spectrum, not as a comment in the general experimental section. Hydrogen multiplicity (C, CH, CH_2, CH_3) information obtained from routine DEPT spectra should be included. If detailed signal assignments are made, the type of NOESY or COSY methods used to establish atom connectivity and spatial relationships should be identified in the Supporting Information. Copies of spectra should also be included where structure assignments of complex molecules depend heavily on NMR interpretation. Numbering system used for assignments of signals should be given in the Supporting Information with corresponding general structural formula of named derivative.

HPLC/LCMS can be substituted for biochemistry papers where the main focus is not on compound synthesis.

HRMS/*elemental analysis.* To support the molecular formula assignment, HRMS data accurate within 5 ppm, or combustion elemental analysis [carbon and hydrogen (and nitrogen, if present)] data accurate within 0.5 %, should be reported for new compounds. HRMS data should be given in format as is usually given for combustion analysis: calculated mass for given formula following with observed mass: (+)ESI-HRMS *m*/*z*: [molecular formula + H]$^+$ calculated mass, observed mass. Example: (+)ESI-HRMS *m*/*z*: calculated for [$C_{13}H_8BrCl_2N + H^+$] 327.92899, observed 327.92792.

NOTE: in certain cases, a crystal structure may be an acceptable substitute for HRMS/elemental analysis.

Biomacromolecules. The structures of biomacromolecules may be established by providing evidence about sequence and mass. Sequences may be inferred from the experimental order of amino acid, saccharide, or nucleotide coupling, from known sequences of templates in enzyme-mediated syntheses, or through standard sequencing techniques. Typically, a sequence will be accompanied by MS data that establish the molecular weight.

Example: Product was isolated upon column chromatography [dry flash (SiO_2, eluent EA, EA/MeOH gradient 95/5 → 9/1, EA/MeOH/NH_3 gradient 18/0.5/0.5 → 9/1/1, and flash chromatography (Biotage SP1, RP column, eluent MeOH/H_2O gradient 75/25 → 95/5, N-H column, eluent EA/Hex gradient 6/3 → EA). was obtained after flash column chromatography (Biotage SP NH column, eluent hexane/EA 4:6 → 2:6). Yield 968.4 mg (95 %). Colorless foam softens at 96-101 °C. $[\alpha]^{20}$; D = +0,163 (c =2.0×10^{-3} g/mL, CH_2Cl_2). IR (ATR): 3376w, 2949m, 2868w, 2802w, 1731s, 1611w, 1581s, 1528m, 1452m, 1374s, 1331w, 1246s, 1171m, 1063w, 1023m, 965w, 940w, 881w, 850w, 807w, cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.46 (*d*, 1H, J = 5.4, H-2'), 7.89 (*s*, 1H, J = 2.0, H-8'), 7.71 (*d*, 1H, J = 8.9, H-5'), 7.30 (*dd*, 1H, J_1 = 8.8, J_2 = 2.1, H-6'), 6.33 (*d*, 1H, J = 5.4, H-3'), 6.07 (*s*, HN-Boc, exchangeable with D_2O), 5.06 (*s*, 1H, H-12), 4.92-4.88 (*m*, 1H, H-7), 4.42 (*bs*, H-3), 3.45 (*s*,CH_3-N), 3.33 (*bs*, H-9'), 3.05-2.95 (*m*, 2H, H-11'), 2.70-2.43 (*m*, 2H, H-24) and HN, exchangeable with D_2O), 2.07 (*s*, CH_3COO), 2.04 (*s*, CH_3COO), 1.42 (*s*, 9H, $(CH_3)_3$C-N(Boc)), 0.88 (*s*, 3H, CH_3-10), 0.79 (*d*, 3H,J = 6.6, CH_3-20), 0.68 (*s*, 3H, CH_3-13). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 170.34, 170.27, 151.80, 149.92, 148.87, 134.77, 128.36, 125.11, 121.43, 117.29, 99.98, 75.41, 70.82, 50.43, 49.66, 47.60, 47.33, 44.97, 43.30, 41.83, 41.48, 37.65, 36.35, 35.44, 34.89,

34.19, 33.23, 31.24, 28.79, 28.35, 27.25, 26.45, 25.45, 22.74, 22.63, 21.57, 21.31, 17.85, 12.15. (+)ESI-HRMS (*m/z*): calculated for $[C_{45}H_{67}ClN_4O_6 + H]^+$ 795.48219, observed 795.48185. Combustion analysis for $C_{45}H_{67}ClN_4O_6$: Calculated. C 67.94, H 8.49, N 7.04; found C 67.72, H 8.63, N 6.75. HPLC purity: method A: RT 1.994, area 99.12 %; method C: RT 9.936, area 98.20 %.

Purity **-** Evidence for documenting compound purity should include one or more of the following:

a) Well-resolved high field 1D ^{1}H NMR spectrum showing at most only trace peaks not attributable to the assigned structure and a standard 1D proton-decoupled ^{13}C NMR spectrum. Copies of the spectra should be included as figures in the Supporting Information.
b) Quantitative gas chromatographic analytical data for distilled or vacuum-transferred samples, or quantitative HPLC analytical data for materials isolated by column chromatography or separation from a solid support. HPLC analyses should be performed in two diverse systems. The stationary phase, solvents (HPLC), detector type, and percentage of total chromatogram integration should be reported; a copy of the chromatograms may be included as a figure in the Supporting Information.
c) Electrophoretic analytical data obtained under conditions that permit observing impurities present at the 5 % level.

HRMS data may be used to support a molecular formula assignment **but cannot be used as a criterion of purity**.

4. Biological Data

Quantitative biological data are required for all tested compounds. Biological test methods must be referenced or described in sufficient detail to permit the experiments to be repeated by others. Detailed descriptions of biological methods should be placed in the experimental section. Standard compounds or established drugs should be tested in the same system for comparison. Data may be presented as numerical expressions or in graphical form; biological data for extensive series of compounds should be presented in tabular form. Tables consisting primarily of negative data will not usually be accepted; however, for purposes of documentation they may be submitted as supporting information. Active compounds obtained from combinatorial syntheses should be resynthesized and retested to verify that the biology conforms to the initial observation.

Statistical limits (statistical significance) for the biological data are usually required. If statistical limits cannot be provided, the number of determinations and some indication of the variability and reliability of the results should be given. References to statistical methods of calculation should be included. Doses and concentrations should be expressed as molar quantities (*e.g.*, mol/kg, μmol/kg, M, mM). The routes of administration of test compounds and vehicles used should be indicated, and any salt forms used (hydrochlorides, sulfates, *etc.*) should be noted. The physical state of the compound dosed (crystalline, amorphous; solution, suspension) and the formulation for dosing (micronized, jet-milled, nanoparticles) should be indicated. For those compounds found to be inactive, the highest concentration (*in vitro*) or dose level (*in vivo*) tested should be indicated.

- The RESULTS AND DISCUSSION should include concisely presented results and there significance discussed and compared to relevant literature data. The results and discussion may be combined or kept separate.
- The inclusion of a CONCLUSION section, which briefly summarizes the principal conclusions, is recommended.
- NOMENCLATURE is optional but, if the authors wish, a list of employed symbols may be included.
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Authors are encouraged to presentthe information and results non-essential to the understanding of their paper as SUPPLEMENTARY MATERIAL (can be uploaded inStep 4 of Online Submission). This material may include as a rule, but is not limited to, the presentation of analytical and spectral data demonstrating the identity and purity of synthesized compounds, tables containing raw data on which calculations were based, series of figures where one example would remain in the main text, etc. The Editorial Board retain the right to assign such information and results to the Supplementary material when deemed fit. Supplementary material does not appear in printed form but can be downloaded from the web site of the JSCS.

Mathematical and chemical equations should be given in separate lines and must be numbered, Arabic numbers, consecutively in parenthesis at the end of the line. All equations should be embedded in the text Complex equations (fractions, integrals, matrix…) should be prepared with the aid of the Microsoft Equation 3.0 (or higher) or MathType (Do not use them to create simple equations and labels). Using the Insert -> Equation option, integrated in MS Office 2010 and MS Office 2013, as well as insertion of equation objects within paragraph text IS NOT ALLOWED.

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Prior to submission, the crystallographic data included in a manuscript presenting such data should be deposited at the appropriate database. Crystallographic data associated with organic and metal-organic structures should be deposited at the Cambridge Crystallographic Data Centre (CCDC) by e-mail to **deposit@ccdc.cam.ac.uk**

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- JSCS aspires to have a uniform look for all artwork contained in a single article. Hence, it is important to be aware of the style of the journal.
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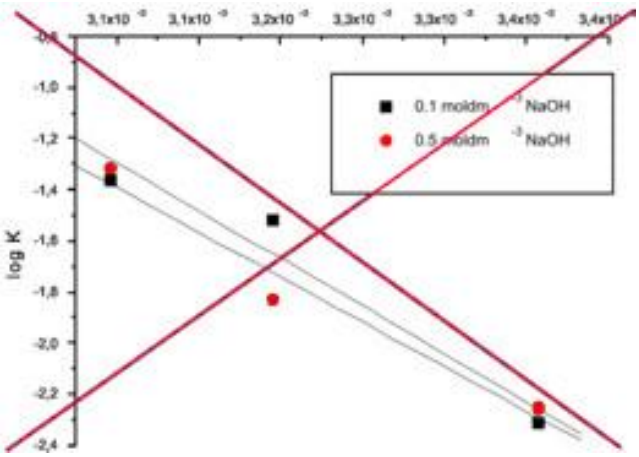

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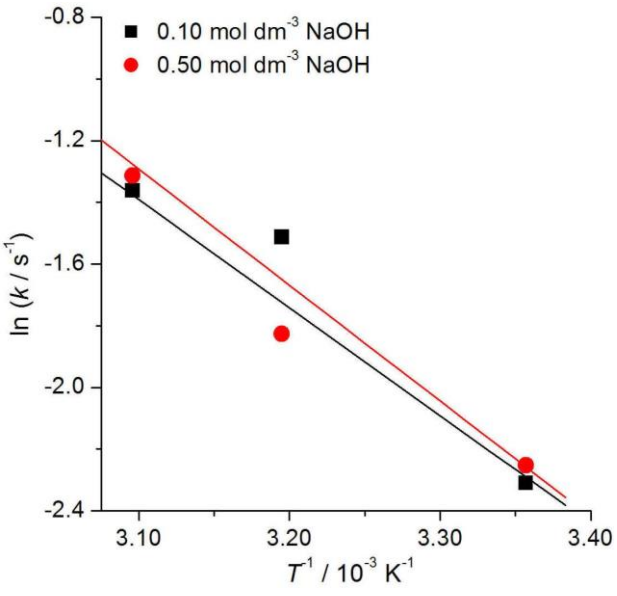

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CONTENTS*

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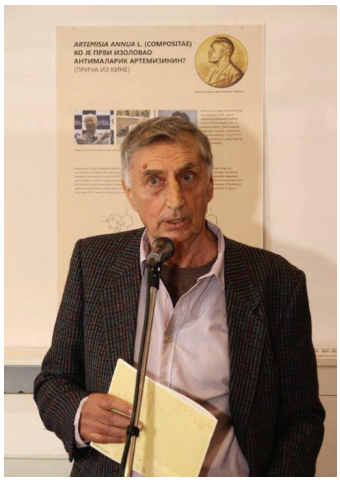
EDITORIAL

This issue of the *Journal of the Serbian Chemical Society* is dedicated to an accomplished scientist and excellent mentor Academician Slobodan Milosavljević, Professor Emeritus at the University of Belgrade – Faculty of Chemistry, on the occasion of his 80th birthday, in honor of his many achievements in chemistry, his contribution to university education, structural instrumental analysis and natural products chemistry at the University of Belgrade.



Slobodan was born in Belgrade on 30 December, 1941. He grew up in Belgrade, where his mother was philologist and the father university professor, and where he finished the high school. He received B.S. (in 1965) from Faculty of Technology and Metallurgy, University of Belgrade, M.S. (in 1970) as well as PhD (in 1974) at Department of Chemistry, Faculty of Science and Mathematics (later Faculty of Chemistry) University of Belgrade.

The two years of postdoctoral studies (1974––1976) included the synthesis of natural products, at The Polytechnic of North London, with Dr. A. P. Johnson.

The first job was in INEP Institute for Application of Nuclear Energy 1965, but Slobodan soon moved to Institute for Chemistry 1966, and finally to Faculty of Chemistry 1970, where stayed till retirement. He became assistant, associate, and full professor at the University of Belgrade in 1970, 1986 and 1992, respectively.

Slobodan's lifelong interest for structural analysis started when he joined prof Jeremić at the Institute for Chemistry in 1966, and helped develop modern Laboratory for instrumental analysis.

The Laboratory, later Center for instrumental analysis (CIA), equipped with IR, UV–Vis, MS and NMR gradually became a core for further teaching and

scientific work in chemistry. Main task and interest was structure determination of organic compounds.

Slobodan dedicated most of his energy creating better conditions for scientific work in chemistry and similar, related sciences, like biology, that use the aforementioned analytical tools. He is still involved in work of this Centre assisting in solving problems concerning structure elucidation of various compounds and commercial products.

As a researcher, Slobodan mostly deals with investigation and interpretation of NMR and mass spectra of syntetised and natural compounds. It presents a permanent scientific interest and have started with M.S. thesis.

The main research topic in last two decades was isolation and identification of natural products, the constituents of wild-growing plant species from Serbia and Montenegro by means of spectroscopic and chromatographic methods (1D and 2D NMR, MS, GLC–MS, IR, UV–Vis, HPLC). Lately he is involved in metabolomics investigations of plants.

Besides intensive scientific work (about 150 original papers and reviews), Slobodan was dedicated to teaching 1979-1986 "*Structural instrumental methods*" at the same faculty. From 1979 teaching in "*Organic chemistry of natural products*" for the third-year undergraduate students of Molecular biology at the Faculty of Biology, Belgrade; from 2000 teaching in "*Structural instrumental methods II*" at the Faculty of Chemistry.

In his very good textbook "*Structural instrumental methods*" (in Serbian), II edition published at the Faculty of Chemistry (2004) Slobodan treats the subjects in more than 500 pages.

Slobodan also served as a visiting professor, teaching Structural instrumental methods at the University of Niš and Kragujevac. He led numerous students in their diploma (B.S.) works, M.S. theses, and Ph.D. dissertations. As a member of committees, he selflessly helped students and degree candidates mentored by his colleagues.

Students, associates and colleagues respect Slobodan as a scholar and admire him as a man for his modesty and fine humor. He has trained many academics, collaborated with others in research, and influenced large number of students over the decades of his academic career. Some of these students contribute to this Special Issue.

Serbian Academy of Sciences and Arts elected Slobodan as a Corresponding Member in 2009 and as a Full Member in 2018.

Serbian Chemical Society gave Slobodan a Medal for his lasting and outstanding contribution to science in 2006. University of Belgrade appointed him Professor Emeritus 2012.

Besides chemistry, Slobodan enjoys fishing, tennis and skiing.

The papers in this issue deal mostly with natural products chemistry. Space limits prevented us from inviting contributions by other, equally deserving authors affiliated with Faculties Chemistry, Biology and Pharmacy. We thank all contributors for their efforts, as well as for financial support.

On behalf of your colleagues, collaborators and students, we dedicate this Special Issue to you, Slobodan, and hope that you will enjoy it for years to come.

Belgrade, December 2021

Guest Editors

Dr. Vele Tešević, Full Professor
University of Belgrade – Faculty of Chemistry

Dr. Ljubodrag Vujisić, Associate Professor
University of Belgrade – Faculty of Chemistry

SELECTED PUBLICATIONS

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My collaboration with Prof. Slobodan Milosavljevic

It is hard for me to say when exactly I met Prof. Milosavljevic (Sloba) for the first time, it was so long ago, further than my memory could reach. But I remember how Prof. Jeremic (then the chair of the Center for Instrumental Analysis) brought us together.

After I returned from two years postdoc at ETH in September of 1981, Prof. Jeremic shown a keen interest in my postdoc experience. I was telling him what I did and about new development in the field: 2D NMR, COSY, NOESY, double--quantum spectroscopy, *etc*. He was really impressed as he understood immediately that this was the future. Then Prof. Jeremic asked Sloba, then his young collaborator to come over and join us in the conversation, and in no time, we realized that we have complementary interests and expertise that could be aligned and that we can collaborate fruitfully. At that time, we did not have proper equipment as the only NMR instrument in the Center was outdated A-60. (A year earlier I saw the same model in London's Science Museum as an exhibit of the lab of 1960s.) Since I was travelling around the globe for my own research, I was taking along Sloba's samples and kept recording the spectra wherever I went.

First few papers went through smoothly but on one sent to Magnetic Resonance in Chemistry we got a mixed review with major complaint that "2D NMR spectrum could be recorded by any average lab". We were flattered by this rejection as we interpreted this that in previous papers we were above the average. Then we wrote back that there is nothing wrong being an average. (In USA there was very famous show about fictitious place called Lake Wobegon where "… all the children are above average.") This paper was eventually published but the lesson learned was that we must raise the papers on higher level. And Sloba indeed did just that. There was nothing more I could contribute, I was just recording and occasionally interpreting the spectra. And we went on and on. Later when I moved to the USA we included other colleagues from the Chemistry department who had interest in NMR.

Many years ago, our collaboration naturally winded down as 2D NMR became quite common and generating useful spectra no longer needed specialized expertise. But our friendship has continued till today. We rarely met in Belgrade outside his lab. But I am grateful for the time we got to spent together in Rochester (Minnesota), Minneapolis, Chicago, Rio de Janeiro…). Even more interesting is that both of us had passion for skiing, tennis, and fishing but never went together to enjoy these activities. I gave up sports long time ago, but I hope there's time to go fishing. Happy anniversary, my friend.

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Slobodan Milosavljević, dear friend, distinguished scientist

It is difficult to describe dedication and devotion of Prof. Slobodan Milosavljević, member of Academy of Sciences and Arts, tireless scientist, postdoc at London University, researcher and promoter, who selflessly transferred knowledge to students and associates of faculty for chemistry as well as biology.

My dear friend Slobodan is specially excelling in the field of education and formation of young people through mutual work and numerous tasks performed on the Faculty of Chemistry in Belgrade. He led students from basic level to the highest degrees, with great knowledge and enthusiasm, so later they felt at home in world of chemistry and biology. Slobodan is still in this noble mission.

I shall remark on his work as much as I can see it from our longstanding collaboration and friendship. We have spent time mostly on the Montenegro mountains and in the botanic garden Dulovina in Kolašin. Academician Slobodan took part, with group of botanist led by dr Nebojsa Menković, in long-term quest to discover medicinal and endemic plants of east Balkans, namely Montenegro and Serbia. The stress was on the complex mountain system Prokletije, and the mountain massif Orjen. Going through unexplored landscape, through rocky paths and inaccessible remote stones they have collected many precious medicinal as well as endemic plants. All mentioned activities were well documented, with photographs and botanical specimens for herbarium. Those investigations were important for botanical garden in Kolašin and later for garden Velemun in Plav. Furthermore, the research was significant and useful for many institutions in Serbia and Montenegro such as: Faculty for Chemistry, Faculty for Biology, Institute Josif Pančić in Belgrade and Biotechnical institute, in Podgorica.

One of the results of the explorations is the book on medicinal plants by Nebojsa Menković *et al*. entitled:”A guide to medicinal plants”.

Slobodan made a great contribution to development and growth of the botanic garden Dulovina, however it was not only scientifically significant, but the garden has become more visible. In other words, papers published in domestic and international magazines made mountains of Montenegro and their botanical gardens more popular and more frequently visited by general public.

Daniel Vincek (1926–2021),
Founder and director of the botanic garden Dulovine

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REVIEW

Phytochemicals from bryophytes: Structures and biological activity

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Abstract: Little attention has been paid to the bryophytes as sources for human diet despite the presence of 23,000 species in the world. Some mosses contain Vitamin B1, tocopherols, prostaglandin-like highly unsaturated fatty acids and phenolic compounds. On the other hand, liverworts contain enantiomeric mono-, sesqui- and diterpenoids similar to those found in vascular plants. Additionally, they possess bibenzyls, bis-bibenzyls and polyketides, many of them showing various bioactivity, such as antimicrobial, antiviral, anti-inflammatory, cytotoxicity against cancer cell lines, muscle relaxing, antioxidant and others. In this paper, the structures of phytochemicals from bryophytes and their biological activities are discussed.

Keywords: bryophytes; terpenoids; bibenzyls; bis-bibenzyls; antimicrobial; antiviral; anti-inflammatory; cytotoxic.

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1. INTRODUCTION

Bryophytes are found everywhere in the world except in the sea, on each continent including The Antarctic. They grow on wet soil or rocks in rivers, lakes and ponds, on the trunks of trees, even on the heads of some lizards. The bryophytes are placed taxonomically between algae and pteridophytes and there are about 23,000 species in the world. They are further divided into three phyla, Bryophyta (mosses 14,000 species), Marchantiophyta (liverworts 6,000 species) and Anthocerotophyta (hornworts 300 species). They are considered to be the oldest terrestrial plants, although no strong scientific evidence for this has appeared in the literature. This hypothesis is mainly based on the resemblance of the present-day liverworts to fossils of the first land plant, the spores of which date back almost 500 million years. Among the bryophytes, almost all liverworts possess beautiful cellular oil bodies, which are peculiar, membrane-bound cell organelles that consist of ethereal terpenoids and aromatic oils suspended in a carbohydrate- or protein-rich matrix, while the other two phyla do not. These oil bodies are very important biological markers for the taxonomy of liverworts.[1–10]

The phytochemistry of bryophytes has been neglected for a long time because they are morphologically very small, difficult to collect in large amounts as pure samples, and their identification is very difficult even under the microscope. Despite of the existence of more than 20,000 species, they are considered to be nutritionally useless to humans because there are many other edible products from nature, such as vegetables, mushrooms, algae and even several ferns and lichens. In fact, no references concerning the use of liverworts as food for humans have been found.

However, some bryological researchers tried to eat some liverworts. *Bazzania pompeana* was prepared as Tempura (Japanese cuisine). Such fried material was edible although the strong mushroomy smell remained in the foods and nothing from sickness occurred (Inoue, private communication). *Marchantia polymorpha* was also fried with flour at 250 °C in a restaurant, and the author (YA), his wife and the chief of the restaurant ate it. The taste was very good like seagrass but the mossy note was retained in the food. Many liverworts produce hot-tasting substances, such capsaicin from paprika and compounds from pepper, that originated from some sesquiterpene and diterpene dialdehydes (see later). Some mosses, for example *Fissidens* and *Rhodobryum* species, elaborate strong sweet taste. These tasty liverworts could be useful as certain spices for foods or as food additives. Some liverworts and mosses produce high amounts of vitamins B_2 and E, and compounds related to them, as discussed later. Thus it is considered that the bryophytes have potentially important food properties.

On the other hand, many moss species have been used as medicinal plants. Most bryophytes used medicinally have been applied as decoctions. Additionally, bryophytes can be crushed and the resulting powder mixed with oil to make an ointment that reputedly heals cuts, burns and external wounds. North American Indians used *Bryum*, *Mnium*, *Philonotis* species and *Polytrichum juniperinum* as medicinal mosses to heal burns, bruises and wounds.[11] *M. polymorpha* has been used as a diuretic in Europe. French liverwort was soaked with a white liquor and the patients drank the resulting mixture of liquor and extracts.[12]

In the literature of the Chinese medicinal spore-forming plants, 24 lichens, 74 sea-algae, 22 mosses, 5 liverworts, 112 fungi and 329 ferns have been listed and their Latin names, morphological characteristics, distribution locations, pharmacological activities and effects, and their prescriptions given in detail.[13] Several mosses have been widely used medicinally in China, to heal burns, bruises, external wounds, snake bites, pulmonary tuberculosis, neurasthenia, fractures, convulsions, scald, uropathy, pneumonia, neurasthenia, *etc*.[7,8,14]

Many species of liverworts show characteristic fragrant odors and an intense pungent, sweet or bitter taste. Generally, bryophytes are not damaged by bacteria and fungi, insect larvae and adults, snails, slugs and other small mammals, which indicated their potential bioactivity. Furthermore, some liverworts cause intense allergenic contact dermatitis and allelopathy. Although liverworts possess such pharmacologically interesting substances, their isolation and structural elucidation were neglected for almost a century. Then interest emerged for the application of bryophytes as foods for human or domestic animals and for the isolation and the structural elucidation of biologically active substances from bryophytes and their bioassay. Since 1972, more than 1,000 species of bryophytes collected in the world, especially Europe, South America, South–Eastern Asia, Japan, Madagascar and New Zealand, were chemically analyzed with respect to their chemistry, pharmacology, and application as sources of medicinal or agricultural drugs and cosmetic products. The biological activities of liverworts are due to terpenoids and aromatic compounds that are significant constituents of oil bodies in each species.[5,6,15–25]

In this paper, bio- and chemical diversity of the liverworts and the chemical structures of their bioactive compounds are surveyed. A few biosynthesis and hemi- and total synthesis of biologically active compounds are also discussed. Physical characteristics, such as odor and taste, and the possibility of use of bryophytes as foods are also discussed.

2. BIODIVERSITY OF BRYOPHYTES

The Marchantiophyta (liverworts) include two subclasses, the Jungermaniidae and Marchantiidae, 6 orders, 49 families, 130 genera and 6,000 species. There are 54 endemic genera in southern hemispheric countries, such as New

Zealand and Argentina.[24] In Asia, including Japan, a relatively large number of endemic genera has been recorded,[25] while South Africa, Madagascar and both North America and Europe are very poor regions of endemic genera.[26] The richness of endemic genera of bryophytes in the southern hemisphere suggests that the bryophytes might have originated from the past Antarctic islands since 350,000,000–400,000,000 years ago and developed to the northern hemisphere with a long range evolutionary process. In South–East Asia and South America, there are rain forests where many liverworts species still found, but many different species, such as the Lejeuneaceae species, are intermingled with them and their purification is time consuming work.

3. CHEMICAL DIVERSITY OF BRYOPHYTES

The extraction of oil bodies with *n*-hexane or diethyl ether, using ultrasonic apparatus, is very easy for stem-leafy liverworts giving a large amount of crude extract. In the case of thalloid liverworts, the specimens are ground mechanically and then extracted with non-polar solvents. At present, several hundred new compounds have been isolated from liverworts and more than 50 new carbon skeletal terpenoids and aromatic compounds have been found in this class. Most of the liverworts possess characteristic odiferous, pungent and bitter tasting compounds many of which show different activities: antimicrobial, antifungal, antiviral, allergenic contact dermatitis, cytotoxic, insecticidal, anti-HIV, superoxide anion radical release, plant growth regulatory, neurotrophic, NO production inhibitory, muscle relaxing, antiobesity, piscicidal and nematocidal activity. The biological activity ascribed to the liverworts is mainly due to lipophilic sesqui- and diterpenoids, phenolic compounds and polyketides, which are constituents of the oil bodies.

The most characteristic chemical phenomenon of liverworts is that most of sesqui- and diterpenoids are enantiomers of those found in higher plants, although there are a few exceptions, such as germacrane- and guaiane-type sesquiterpenoids. It is very noteworthy that different species of the same genera, such as *Frullania tamarisci* and *F. dilatata* (Frullaniaceae), each produces different sesquiterpene lactone enantiomers. Some liverworts, such as *Lepidozia* species (Lepidoziaceae), biosynthesize both enantiomers. Flavonoids, fatty acids and phytosterols are ubiquitous components in bryophytes. However, the presence of nitrogen- or sulfur-containing compounds in bryophytes was very rare. Recently, several compounds with sulfur and nitrogen in the structure **1**–**4** have been isolated from the Mediterranean liverwort *Corsinia coriandrina* (Corsiniaceae, Marchantiales),[27] and also two prenyl indole derivatives (**5**, **6a**) from the *Riccardia* species (Riccardiaceae), Fig. 1.[2] Skatole (**6b**) have been isolated from or detected in *Asterella* or *Mannia* (Aytoniaceae)[28] and the Tahitian *Cyathodium foetidissimum* (Cyathodiaceae)[29]

and benzyl- (**7a** and **b**) and *β*-phenethyl *β*-methylthioacrylates (**7c**) from the Isotachidaceae (Fig. 1).[2]

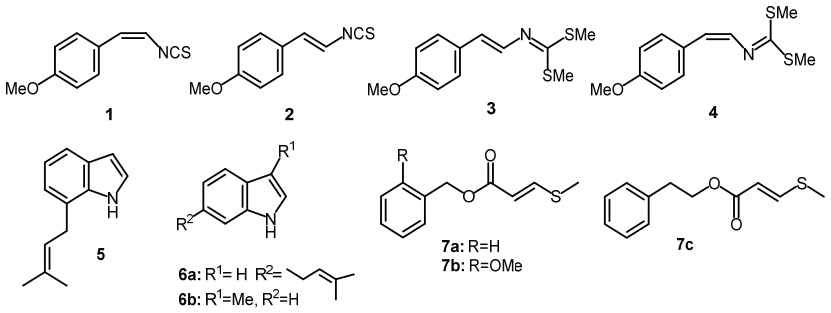


Fig. 1. Compounds isolated from the Corsiniaceae, Riccardiaceae, Aytoniaceae, Cyathodiaceae and Isotachidaceae families.

Highly evolved liverworts belonging to the Marchantiaceae produce phytosterols, such as campe-, stigma- and sitosterol. Almost all liverworts elaborate *α*-tocopherol and the sterol precursor squalene. The characteristic components of the Bryophyta are highly unsaturated fatty acids and alkanones, such as 5,8,11,14,17-eicosapentaenoic acid, 7,10,13,16,19-docosapentaenoic acid and 10,13,16-nonadecatrien-7-yn-2-one and triterpenoids. Neolignans are one of the most important chemical markers of the Anthoceratophyta.[2] The presence of hydrophobic terpenoids is very rare in the Marchantiophyta. A few bitter kaurene glycosides have been found in *Jungermannia* species. Moreover, a number of flavonoid glycosides have been detected both in liverworts and mosses.[1,2,6]

4. BRYOPHYTES AS FUTURE FOODS

The mosses, *Barbella pendula*, *B. enervis*, *Floribundaria nipponica*, *Hypnum plumaeforme* and *Neckeropsis nitidula* contain high amounts of riboflavin, Vitamin B_2. Chickens and puppies fed on a diet including these powered bryophytes gained more weight than did the control animals. The supplement did not cause any sickness or distaste.[30] Since there are more than 14,000 species of mosses, more species possessing high amount of Vitamin B_2 will be discovered.

The liverworts, *M. polymorpha*, *Pellia endiviifolia* and the mosses *Atrichum undulatum* and *Mnium hornum* produce Vitamin E (*α*-tocopherol) (**8**), Vitamin K (**9**), plastoquinone (**10**), plastohydroquinone (**11**) and *α*-tocoquinone (**12**), Fig. 2).[31,32] The last compound was also found in the moss *Racomitrium japonicum* (Fig. 2).[33–35]

Nishiki and coauthors analyzed 700 liverworts chemically and found that almost all of them contained *α*-tocopherol and squalene.[34] Prostaglandin-like highly unsaturated fatty acids have been found in many mosses, such as *Dicranum scoparium*, *D. japonicum* and *Leucobryum* species (**13**–**17**), Fig. 3.[5,6,36,37] These

and other unsaturated fatty acids are viscous liquids and it is thought that they help in protecting herbivorous animals living in very cold places from cold. For example, arachidonic acid has not been found in higher plants.[38] Such unsaturated fatty acids, like those obtained from fish oils, play an important role as antioxidant in the body. Acetylcholine (**18**) and a cytokinin-like compound N_6-(D_2-isopentenyl)adenine (**19**), Fig. 3, have been found in the hybrid of *Funaria hydrometrica*×*Physcomitrium pyriforme*.[39,40]

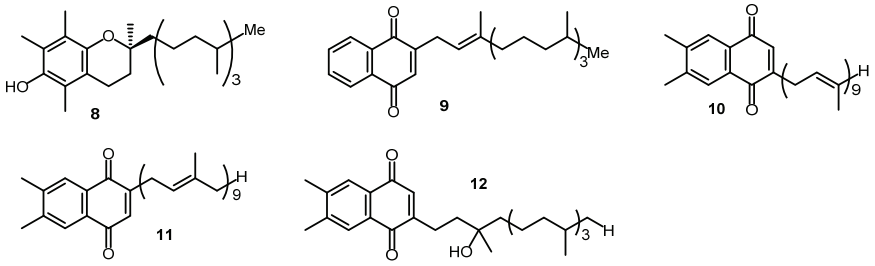


Fig. 2. Lipophilic vitamins and related compounds isolated from *Marchantia polymorpha*, *Pellia endiviifolia*, *Atrichum undulatum* and *Mnium hornum*.

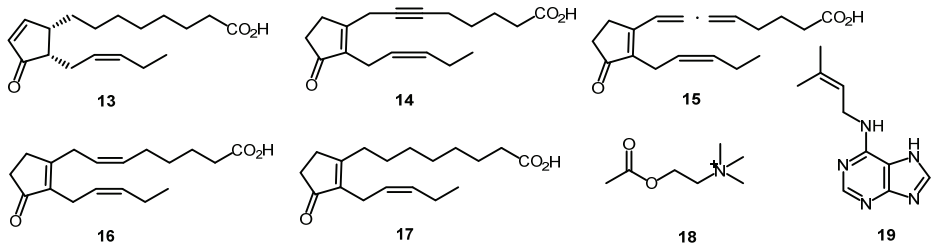


Fig. 3. Prostaglandin-like highly unsaturated fatty acids isolated from *Dicranum* and *Leucobryum* species, and acetylcholine and cytokinin-like compounds from the hybrid of *Funaria hydrometrica* × *Physcomitrium pyriforme*.

Many of the liverworts produce hot-tasting substances that could be used as spices for foods and their food preservation effect because they possess potent antimicrobial and antifungal activity (see later).

5. BIOLOGICALLY ACTIVE COMPOUNDS FROM BRYOPHYTES

The bryophytes could be used as rather medicinal plants as human diet plants at present since they elaborate a number of biologically active secondary metabolites, as shown in this paragraph. The biological characteristics of the secondary metabolites obtained from bryophytes are: 1) characteristic scents, 2) pungency and bitterness, 3) allergenic contact dermatitis, 4) cytotoxic, 5) antimicrobial, antifungal and antiviral, 6) insect antifeedant, mortality, and nematocidal, 7) superoxide anion radical release inhibitory, 8) 5-lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase, DNA polymerase β and α-glucosidase inhibitory, 9) antioxidant, 10) pisci-

cidal, 11) neurotrophic, 12) muscle relaxing and calcium inhibitory, 13) cardiotonic and vasopressin antagonist, 14) liver X-receptor (LXR)α agonist and LXRβ antagonist, 15) cathepsins B and L inhibitory, antithrombin, 16) farnesoid X-receptor (FXR) activation, 17) nitric oxide production inhibitory, 18) plant growth inhibitory, 19) tubulin polymerization inhibitory, 20) sex pheromones. In addition, antiplatelet and brine shrimp lethal activity are included.

5.1. Characteristic scent compounds

All of the liverworts emit a very strong odor when crushed. Lipophilic terpenoids and aromatic compounds found in the oil bodies are responsible for intense sweet-woody, turpentine, sweet-mossy, fungal-like, carrot-like, mushroomy, or seaeed-like scents.[19,31,41–43] Almost all liverworts containing mushroom odor contain 1-octen-3-ol (**20a**) and its acetate (**20b**) which are responsible for the mushroom odor. The unidentified Malaysian liverwort (*Asterella* or *Mannia*) emits 20 % of skatole (**6b**) which is responsible for the unpleasant smell of this liverwort and 80 % of 3,4-dimethoxystyrene (**21**).[28] The stink bug smell of *Cheilolejeunea pallidus* is attributable to (*E*)-dec-2-enal (**22**) and its analogues (**23**-**25**).[44] The characteristic cresol-like smell of *Leptolejeunea elliptica* is due to *p*-ethylanisol (**26**), *p*-ethylphenol (**27**), and *p*-ethylphenylacetate (**28**), Fig. 4.[45]

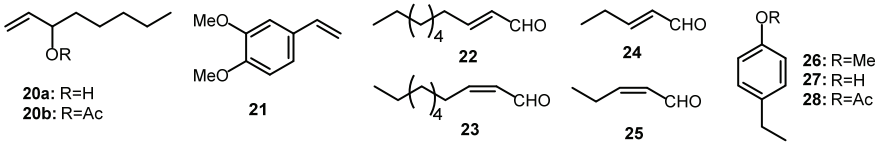


Fig. 4. Some scent compounds from liverworts.

A mixture of (*R*)-dodec-2-en-1,5-olide (**29**) and (*R*)-tetradec-2-en-1,5-olide (**30**) is responsible for the strong milky smell of the liverwort *Cheilolejeunea imbricata* (Fig. 5).[45] *Plagiochila sciophila* elaborates bicyclohumulenone (**31**), which shows an aroma reminiscent of a variety of scents based on a strong woody note, resembling the odor of patchouli, vetiver, cedar wood, iris, moss and carnations.[2] Tamariscol (**32**) from *F. tamarisci* subsp. *tamarisci, F. tamarisci* subsp. *obscura*, *F. nepalensis,* and *F. asagrayana* similarly possesses a remarkable aroma reminiscent of the woody and powdery green notes of mosses, hay, costus, violet leaves and seaweeds (Fig. 5). Both compounds are commercially important. They are used as perfumes and as perfume components of the powdery floral-, oriental bouquet-, fantastic chypre-, fancy violet- and white rose-types in various cosmetics. It is noteworthy that *Frullania* species producing tamariscol only grow in high mountains.[46,47] Total synthesis of (±)-tamariscol (**32**) has been accomplished using commercially available *p*-methoxyacetophenone in 13 steps.[48] A synthetic minitamariscol, 1-hydroxy-1-(2-methyl-1-propenyl)-cyclohexane (**33**) has a sweet mossy aroma similar to that of tamariscol

itself.[47] There are three chemo-types of liverwort, *Conocephalum conicum*. The types 1, 2 and 3 emit (–)-sabinene (**34a**), (+)-bornyl acetate (**34b**), and methyl cinnamate (**34c**) as the major components, respectively, which are responsible for the characteristic odor of each type.[49] An intense carrot-like odor of *Jungermannia obovata* arises from 4-hydroxy-4-methylcyclohex-2-en-1-one (**35**), Fig. 5.[50,51]

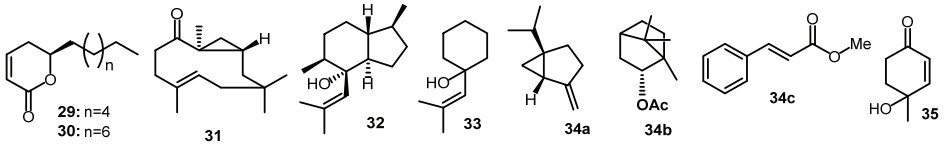


Fig. 5. Scent compounds from *Cheilolejeunea imbricata*, *Plagiochila sciophila*, *Frullania* species, *Conocephalum conicum* and *Jungermannia obovate*.

The strong and distinct mossy odor of *Lophocolea heterophylla* and *L. bidentata* is due to a mixture of (-)-2-methylisoborneol (**36**) and geosmin (**37**) (Fig. 6).[52] The latter compound has also been found in *in vitro* cultured *Symphyogyna brongniartii.*[53] The strong sweet mossy note of *Mannia fragrans* is attributed to grimaldone (**38**).[54] The sweet turpentine-like odor of French *Targionia hypophyll*a is due to a mixture of *cis*- and *trans*-pinocarveyl acetates (**39**, **40**), Fig. 6.[55] The strong sweet-mushroomy scent of the ether extract of *Wiesnerella denudata* is due to (+)-bornyl acetate and a mixture of the monoterpene hydrocarbons, *α*-terpinene, *β*-phellandrene, terpinolene, *α*-pinene, *β*-pinene and camphene.[35] The odor of the steam distillate of *W. denudata* is weaker than that of its ether extract. The steam distillate contains nerol (14%), neryl acetate (27%), and *γ*-terpinene (31 %), but the content of 1-octen-3-ol (7 %, **20a**) and its acetate (**20b**, 2 %), Fig. 4, is lower than that of *C. conicum* belonging to the same genus of *Wiesnerella* (Asakawa, unpublished results). *Gackstroemia decipiens* emits a characteristic scent that is due to the presence of a mixture of (–)-13-hydroxybergamota-2,11-diene (**41**) and the santalene derivatives (**42**–**45**). These compounds were characterized by olfactory effects.[56] Isoafricanol (**46**), Fig. 6, isolated from *Pellia epiphylla* is responsible for the typical odor of its sporophyte.[57]

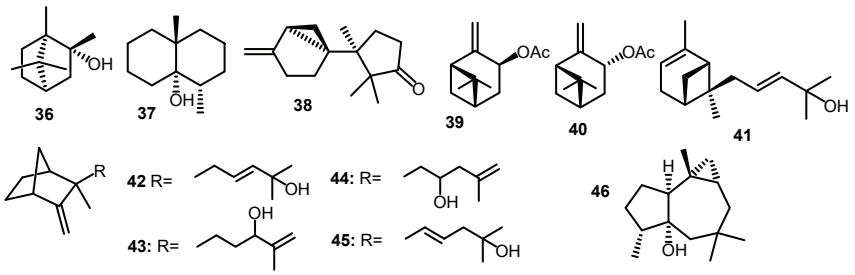


Fig. 6. Scent compounds from *Lophocolea*, *Mania*, *Targionia*, *Wiesnerella*, *Gackstroemia* and *Pellia* species.

5.2. Pungent and bitter tasty compounds

Some genera of liverworts, such as the *Hymenophyton*, *Pellia*, *Porella*, *Trichocoleopsis* and *Wiesnerella* species, elaborate potent pungent constituents,[2,19,58] which exhibit interesting biological activities described in subsequent sections. Most of the North American liverworts contain unpleasant substances, some of which taste like immature green pea seeds or pepper.[59] The *Anastrepta*, *Lophozia*, *Scapania,* and many other stem-leafy liverworts, produce intense bitter principles.

Porella vernicosa complex (*P. arborisvitae*, *P. fauriei*, *P. gracillima*, *P. obtusata* subsp. *macroloba*, *P. roellii* and *P. vernicosa*) contain surprisingly intense pungent substances. *Jamesoniella autumnalis* contains an intense bitter principle the taste of which resembles that of the leaf of lilac and *Swertia japonica* or the root of *Gentiana scabra* var. *orientalis*, but these bitter principles have not yet been isolated. The strong hot taste of the *P. vernicosa* complex is due to (–)--polygodial (**47**),[1,18,41,60] the major component of the Japanese medicinal plant, *Polygonum hydropiper*, Malaysian *P. minus* and Argentinean *P. punctatum* var. *punctatum* (Polygonaceae), Fig. 7.[61] The sacculatane diterpene dialdehyde sacculatal (**48**), two eudesmanolides, diplophyllolide (**50**) and ent-7*α*-hydroxydiplophyllolide (**51**) and germacranolide, tulipinolide (**52**), which possess potent pungency, were isolated from *Pellia endiviifolia*, *Trichocoleopsis sacculata*, *Chiloscyphus polyanthos* and *Wiesnerella denudata*, respectively (Fig. 7). An additional pungent 1-*β*-hydroxysacculatal (**49**) was obtained from *Pellia endiviifolia*, together with several sacculatane-type diterpenoids.[62] The hot taste of *Pallavicinia levieri* and *Riccardia robata* var. *yakushimensis* (belonging to the Metzgeriales) is also due to sacculatal (**48**).[63] Polygodial (**47**) and sacculatal (**48**) have been obtained from cell suspension cultures from *P. vernicosa* Lindb. and polygodial has also been detected in *Pellia neesiana* and *P. endiviifolia*, respectively (Fig. 7).[64,65] When the whole plant of the stem-leafy liverwort, *Plagiochila asplenioides*, *P. fruticosa*, *P. ovalifolia* and *P. yokogurensis* that contain plagiochiline A (**53**) and plagiochiline I (**54**), is chewed a potent pungent taste is slowly felt.

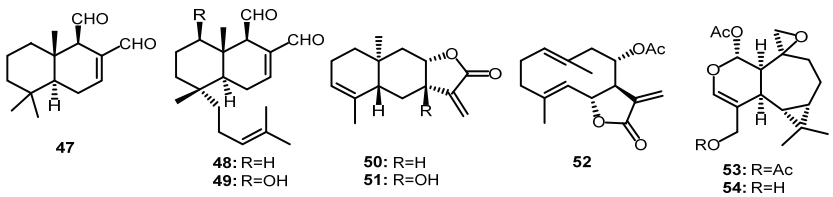


Fig. 7. Pungent and bitter substances from *Porella*, *Pellia*, *Trichocoleopsis*, *Chiloscyphus Wiesnerella* and *Plagiochila* species.

It is suggested that both compounds might be converted into pungent unsaturated dialdehyde by human saliva. In fact, enzymatic treatment of **53** with amyl-

ase in phosphate buffer or with human saliva produces two strong pungent, plagiochilal B (**55**) the partial structure of which is similar to that of the pungent drimane-type sesquiterpene dialdehyde, polygodial (**47**), and furanoplagiochilal (**56**), Fig. 8.[66]

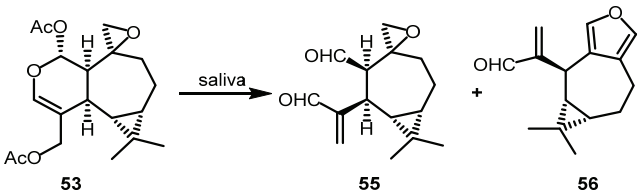


Fig. 8. Formation of pungent di- (**55**) and monoaldehyde (**56**) from plagiochiline A (**53**) by saliva.

The pungent taste of *Porella acutifolia* subsp. *tosana* is due to the presence of hydroperoxysesquiterpene lactones, 1*α*- (**57**), and 1*β*-hydroperoxy-4*α*,5*β*-epoxygermacra-10(14),11(13)-dien-12,18*α*-olides (**58**), Fig. 9.[67] The New Zealand liverwort, *Hymenophyton flabellatum* produces a different pungent tasting substance from the other aforementioned liverworts. 1-(2,4,6-Trimethoxyphenyl)-buta-(2*E*)-en-1-one (**59**) is responsible for the pungency of this liverwort (Fig. 9).[68] Most of the species belonging to the Lophoziaceae produce bitter substances. *Gymnocolea inflata* is persistently bitter and induces vomiting when a few leaves are chewed for several seconds. This surprisingly intense bitterness is due to the clerodane diterpene lactone, gymnocolin A (**60**).[31] *Jungermannia infusca* has an intense bitter taste that is due to the presence of the infuscasides A-E (**61**–**65**), Fig. 9. These were the first reported isolation of glycosides from liverworts.[69]

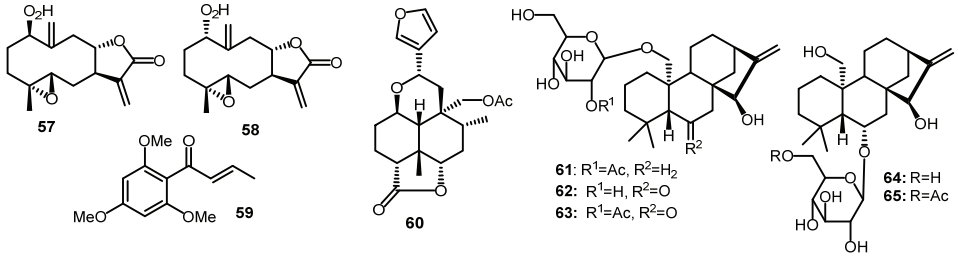


Fig. 9. Pungent and bitter substances from *Porella*, *Hymenophyton*, *Gymnocolea* and *Jungermannia* species.

Anastrepta orcadensis, *Barbilophozia lycopodioides* and *Scapania undulata* are also bitter liverworts from which the highly oxygenated bitter diterpenoids, anastreptin A (**66**), barbilycopodin (**67**)[50,70] and scapanin A (**68**)[71] have been isolated, respectively, Fig. 10.

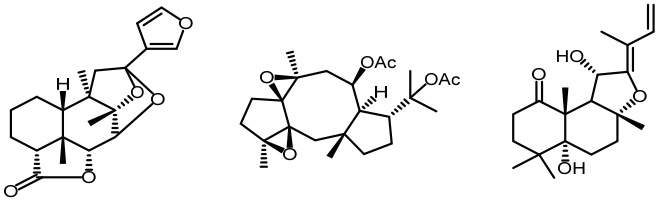


Fig. 10. Bitter substances from *Anastrepta*, *Barbilophozia,* and *Scapania* species.

5.3. Allergenic contact dermatitic compounds

Frullania species are notable as liverworts that cause very intense allergenic contact dermatitis. The allergy-inducing substances are sesquiterpene lactones, (+)-frullanolide (**69**) and (–)-frullanolide (**70**), which have been isolated from *Frullania dilatata* and *F. tamarisci* subsp. *tamarisci*, respectively (Fig. 11).[1] Both dihydrofrullanolides (**71**, **72**) with α-methyl-γ-butyrolactones isolated from the liverworts mentioned above do not cause allergy. *F. asagrayana*, *F. bolanderi*, *F. brasiliensis*, *F. eboracensis*, *F. franciscana*, *F. inflata*, *F. kunzei*, *F. nisquallensis*, *F. riparia* and the other *Frullania* species, which contain sesquiterpenes (**73**–**79**) with α-methylene-γ-butyrolactones, cause strong allergenic contact dermatitis (Fig. 11).[72]

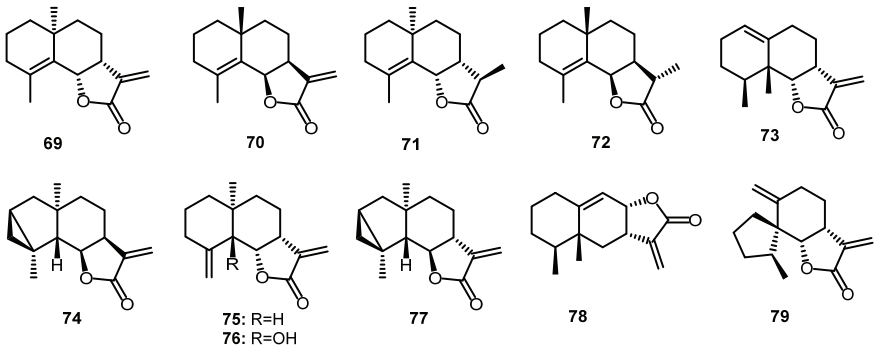
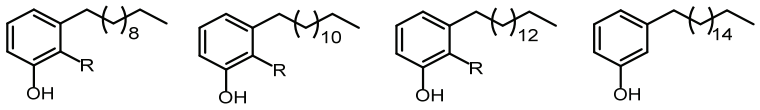


Fig. 11. *Frullania* sesquiterpene lactones.

The allergens of *Schistochila appendiculata* are a mixture of long chain alkylphenols, such as 3-undecyl- (**80**), 3-tridecyl (**81**), 3-pentadecyl (**82**), and 3-heptadecyl phenols (**83**), long chain alkyl salicylic acids, 6-undecyl- (**84**), 6-tridecyl- (**85**), 6-pentadecyl salicylates (**86**) and their potassium salts, potassium 6-undecyl- (**87**), 6-tridecyl- (**88**), and 6-pentadecyl salicylates (**89**) as well as 6-undecyl catechol (**90**), Fig. 12.[72] Such dermatitis is similar to that caused by the long chain alkylphenols of the fruit of *Ginkgo biloba* and Anacardiaceae plants, such as *Toxicodendron vernicifluum* and *Rhus succedranea*.



80: R=H
84: R=CO_2H
87: R=CO_2K
90: R=OH

81: R=H
85: R=CO_2H
88: R=CO_2K

82: R=H
86: R=CO_2H
89: R=CO_2K

83

Fig. 12. *Schistochila appendiculata* allergenic substances.

5.4. Cytotoxic compounds

Germacranolides, and guaianolides isolated from liverworts, exhibit cytotoxic activity against KB nasopharyngeal and P-388 lymphocytic leukemia cells.[2] The crude ether extracts of liverworts *B. pompeana*, *Kurzia makinoana*, *Lophocolea heterophylla*, *Makinoa crispata*, *Marsupella emarginata*, *Pellia endiviifolia*, *Plagiochila fruticosa*, *P. ovalifolia*, *Porella caespitans*, *P. japonica*, *P. perrottetiana*, *P. vernicosa* and *Radula perrottetii* showed cytotoxicity against P-388 cells (IC_{50} value range 4–20 μg mL^{-1}). In contrast, the crude extracts of *Frullania diversitexta*, *F. ericoides*, *F. muscicola*, *F. tamarisci* subsp. *obscura, Lepidozia vitrea*, *Pallavicinia subciliata*, *Plagiochila sciophila*, *Spruceanthus semirepandus* and *Trocholejeunea sandvicensis* were inactive against this same cell line (IC_{50} values > 20 μg mL^{-1} – Asakawa, unpublished results).

Several sesquiterpene lactones, such as eudesmanolides, 2*α*,5*β*-dihydroxybornane-2-cinnamate (**91**) from *C. conicum* and lunularin (**92**), Fig. 13, from *Dumortiera hirsuta*, exhibited cytotoxic activity against human HepG2 cells, with IC_{50} values of 4.5 and 7.4 μg mL^{-1}, respectively.[73] Many *Plagiochila* species contain cytotoxic plagiochiline A (**53**, 0.28 μg mL^{-1}) against KB cell.[31] The ether extract of *Plagiochila ovalifolia* showed inhibitory activity against P 388 murine leukemia cells, and its constituents, plagiochiline A (**53**), plagiochiline A-15-yl octanoate (**94**) and 14-hydroxyplagiochiline A-15-yl (2*E*,4*E*)--dodecadienoate (**95**), Fig. 13, exhibited IC_{50} values of 3.0, 0.05 and 0.05 μg mL^{-1}, respectively.[74] Lunularic acid (**93**) and plagiochiline A-15-yl decanoate (**96**) from *P. ovalifolia*, polygodial (**47**) from *P. vernicosa* complex, as well as sacculatal (**48**), Fig. 7, from *P. endiviifolia* showed cytotoxic activity against a human melanoma cell line (IC_{50} value range 2–4 μg mL^{-1}). Compound **48** was also cytotoxic for Lu1 (IC_{50} 5.7 μg mL^{-1}), KB (3.2), LNCaP (7.6) and ZR-75-1 cells (7.6) (Cordell, Pezzuto, Asakawa, unpublished results). Aponte *et al.*[75] also reported that plagiochilines A (**53**), I (**54**) and M (**96**), Fig. 13, showed cytotoxic activity against a panel of human tumor cell lines, 3T3, H460, DU145, MCF-7, M-14, HT-29, K562 and VERO. Among them, compound **53** exhibited the strongest activity against all the above-mentioned cell lines at a concentration between GI_{50} 1.4–6.8 μM. Compound **53** possessed antileishmania activity against *Leishmania amazonensis* axenic amastigotes at a concentration of IC_{50}

7.1 μM and trypanocidal activity against *Trypanosoma cruzi* trypmastigotes at *MIC* 14.5 μM. Lepidozenolide (**97**), Fig. 13, showed potent cytotoxicity when evaluated in the P-388 murine leukemia cell line (IC_{50} 2.1 μg mL^{-1}).[76] The liverwort *Chandonanthus hirtellus* produces a new sesquiterpene lactone, chandolide (**98**),[77] 13,18,20-tri-*epi*-chandonanthone (**99**)[78] and anadensin (**100**), Fig. 13, that were evaluated for cytotoxic activity against the HL-60 leukemia cell line, and exhibited IC_{50} values of 5.3, 18.1 and 17.0 μg mL^{-1}, respectively.

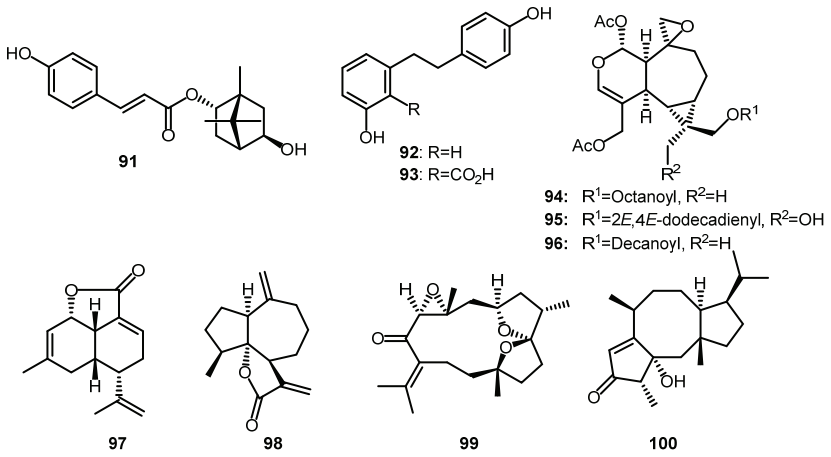


Fig. 13. Cytotoxic compounds from *Conocephalum*, *Dumortiera*, *Plagiochila*, *Pellia*, *Leishmania*, and *Chandonanthus* species.

6*α*-Methoxyfusicoauritone (**101**) isolated from the same liverwort showed some cytotoxicity against KB cells (IC_{50} 11.2 μg mL^{-1}), although compounds **99** and **100** were inactive.[77,79] 13-Hydroxychiloscyphone (**102**) from *Chilosyphus rivularis* was tested against the RS322, RS188N and RS321 yeast strains. It showed IC_{12} values of 75 and 88 μg mL^{-1} for strains RS321 and RS322. These data are characteristic of a selective DNA-damaging agent that does not act as a topoisomerase I or II inhibitor. Compound **102** also showed cytotoxic activity against lung carcinoma A-549 cells (IC_{50} value 2.0 μg mL^{-1}).[80] (–)-*ent*-Arbusculin B (**103**) and (–)-*ent*-costunolide (**104**) from *Hepatostolonophora paucistipula*, showed cytotoxic activity against P388 murine leukemia cells, with IC_{50} values of 1.1 and 0.7 μg mL^{-1} (Fig. 14).[81] Costunolide (**105**) isolated from *Frullania nisquallensis* showed growth inhibitory activity against the A-549 human lung carcinoma cell line with an IC_{50} value of 12 μg mL^{-1} and moderate, but selective, DNA-damaging activity against the RS321N, RS322YK and RS167K mutant yeast strains, with IC_{12} values of 50, 150, and 330 μg mL^{-1}.[82] Naviculyl caffeate (**106**), Fig. 14, from *Bazzania novae-zelandiae*, demonstrated growth inhibitory effects against P-388 murine leukemia cells with a GI_{50} value

of 0.8–1.1 μg mL^{-1}, although naviculol (**107**), Fig. 14, was inactive.[83] Riccardiphenol C (**108**), Fig. 15, from *Riccardia crassa* showed slight cytotoxicity against BSC-1 (African green monkey kidney epithelial) cells at 60 μg disc^{-1}.[84] The ether and methanol extracts of the Tahitian *Mastigophora diclados* showed cytotoxic activity against HL 60 cells at IC_{50} 2.4 and 13.1 μg mL^{-1} and KB cells at 14.6 and 32.5 μg mL^{-1}, respectively.[85]

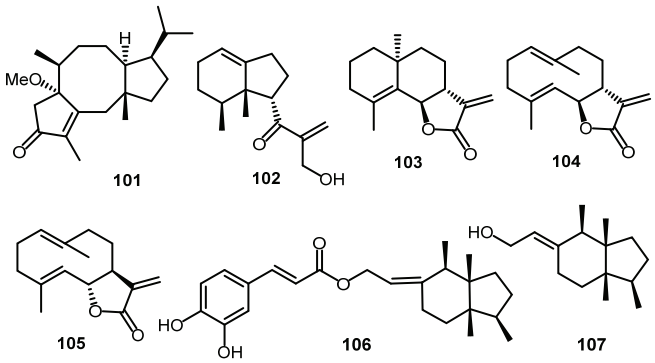


Fig. 14. Compounds from *Chilosyphus*, *Hepatostolonophora*, *Frullania* and *Bazzania* species.

(–)-Diplophyllolide (**43**), *α*-herbertenol (**109**), (–)-herbertene-1,2-diol (**110**), mastigophorene C (**113**) and mastigophorene D (**114**) isolated from both extracts were cytotoxic against HL 60 cells with IC_{50} values of 2.5, 1.4, 12.8, 1.4 and 2.4 μg mL^{-1} (Fig. 15). They also showed cytotoxicity against KB cells (IC_{50} values of 14.2, 3.3, 12.5, 11.8 and 14.8 μg mL^{-1}). 2-Methoxy (**111**) and diacetoxy derivatives (**112**) of (–)-herbertene-1,2-diol (**110**) showed evidence of having less potent cytotoxicity than the parent compound against both HL 60 and KB cells (Fig. 15). However, (–)-diplophyllin (**115**) did not indicate cytotoxicity against either of these cell lines.[85] Glaucescenolide (**116**), Fig. 15, from *Schistochila glaucescens* showed cytotoxic activity against P-388 mouse leukemia cells (IC_{50} 2.3 μg mL^{-1}).[86] *ent*-1*β*-Hydroxykauran-12-one (**117**), isolated from *Paraschistochila pinnatifolia* and 1*α*-hydroxy-*ent*-sandaracopimara-8(14),15-diene (**118**), Fig. 16, from *Trichocolea mollissima* showed IC_{50} values of 15 and >25 μg mL^{-1}, when evaluated against this cell line.[87]

The ethanol-soluble extract of *Lepidolaena taylorii*, which showed cytotoxicity against the P-388 cell line (IC_{50} 1.3 μg mL^{-1}), was purified to give the 8,9--secokaurane diterpenoids, rabdoumbrosanin (**119**), 16,17-dihydrorabdoumbrosanin (**120**), 8,14-epoxyrabdoumbrosanin (**121**) and their related compounds **122**–**125**, and also the *ent*-kaur-16-en-15-ones (**127**–**130**), Fig. 16. In turn, *L. palpebrifolia* also elaborated the 8,9-*seco*kauranes (**119**–**121**). The cytotoxicity of these *ent*-8,9-*seco* and *ent*-kaurenes was tested against mouse P-388 leukemia and several human tumor cell lines, inclusive of six leukemia and a range of

organ-specific cancer cell lines. Compounds **119** and **121** showed the most potent cytotoxic activities (mean IC_{50} values of 0.006 and 0.27 μg mL^{-1}; GI_{50} values of 0.10 and 1.2 μM, respectively). Compound **120** also showed cytotoxicity against P-388 cell at 0.80 μM). Compound **119** (including 10 % of **120**) and **121** showed differential cytotoxicity *in vitro* when tested against five further leukemia cell lines with **119** showing an average IC_{50} value of 0.4 μM; however, cell growth was not inhibited by **121** (IC_{50} > 50 μM). The growth of seven colon cancer cell lines were inhibited also by **119** (mean IC_{50} value, 6 μM).[88,89] Compounds **119** and **121** were tested in an *in vivo* hollow fiber model system, in which neither compound was active at the doses tested (18 and 12 mg kg^{-1} for **108** and 150 and 100 mg kg^{-1} for **110**), Fig. 15. Compound **114** was the most active against several leukemia cell lines (mean GI_{50} 0.3 μM) and least active against various central nervous system cancer cell lines (mean GI_{50} 6 μM).[89] *Ent*-kaurene (**131**) and (**132**) from the New Zealand *Jungermannia* species (Fig. 16) showed weak cytotoxic activity against P-388 at 0.48 and 25 μg mL^{-1}, respectively.[90] Among the isolated compounds, 8,9-secokaurenes (**119**, **121**, **125**), Fig. 16, showed selective toxicity amongst human tumor cell lines at a concentration of 1.2, 2.5 and 1.5 μM, respectively. The mode of action for the cytotoxicity of the *ent*-8,9-*seco*-kaur-16-en-15-one and *ent*-kaur-16-en-15-one series was supported by Michael addition of a thiol to the C-16–C-17 double bond of **119**, but the C-8–C-14 double bond of **120** was relatively unreactive.[88,89] Clavigerins A–D (**133**–**136**) isolated from *Lepidolaena clavigera* (Fig. 16) showed weak cytotoxicity (30 μg disc^{-1}) against BSC cells.[91] A new atisane-2 derivative (**137**) from *Lepidolaena clavigera* exhibited weak inhibitory activity against mouse lymphocytic leukemia cells (P-388) with an IC_{50} value of 16 μg mL^{-1}.[92] *α*-Zeorin (**138**) has been isolated from several liverworts and displayed cytotoxic activity against P-388 cells with an IC_{50} of 1.1 μg mL^{-1}.[93,94] (Fig. 16).

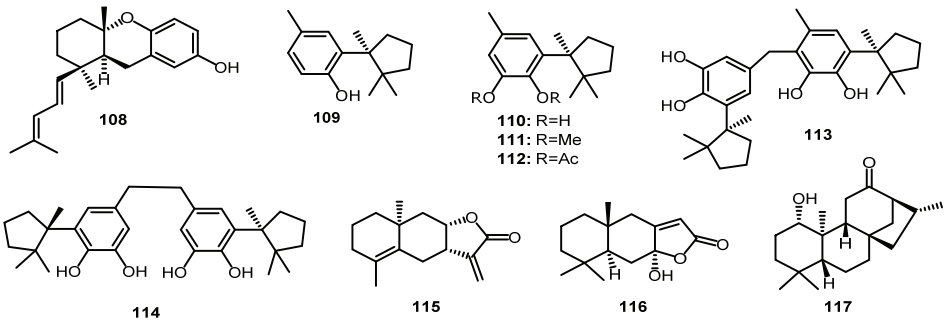


Fig. 15. Cytotoxic compounds from *Riccardia*, *Schistochila* and *Paraschistochila* species.

The crude ether extract of two unidentified Indonesian and Tahitian *Frullania* species exhibited cytotoxic activity against both the HL-60 and KB cell

lines, with at EC_{50} values of 6.7 and 1.6 μg mL^{-1} (HL-60 cells) and 1.6 and 11.2 μg mL^{-1} (KB cells), respectively.[95]

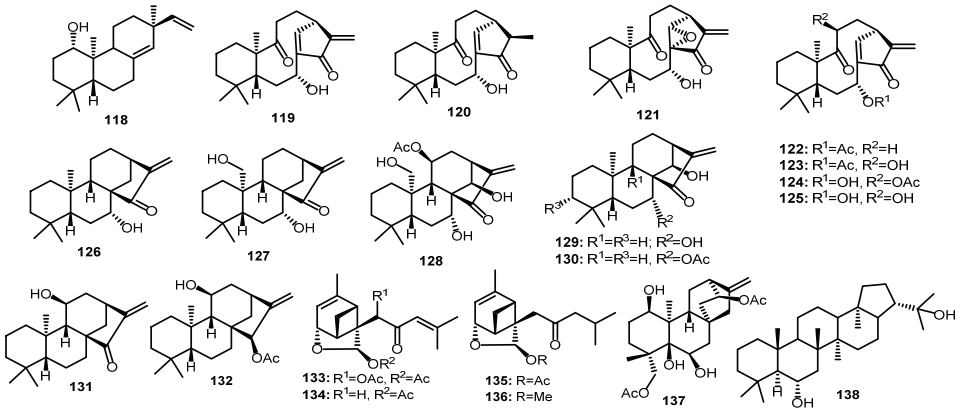


Fig. 16. Cytotoxic compounds from *Lepidolaena* and *Jungermannia* species.

Bioactivity-guided fractionation of the Indonesian sample led to the isolation of (+)-3*α*-(4'-methoxybenzyl)-5,7-dimethoxyphthalide (**139**), (–)-3*α*-(3'-methoxy--4',5'-methylenedioxybenzyl)-5,7-dimethoxyphthalide (**140**), together with 3-methoxy-3',4'-methylenedioxybibenzyl (**141**), 2,3,5-trimethoxy-9,10-dihydrophenanthrene (**142**) and atranorin (**143**), among which **139** possessed the most potent cytotoxic activity against HL-60 and KB cells showing IC_{50} values of 0.92 and 0.96 μM (Fig. 17). The other compounds (**140**–**142**) and the 6'-nitro derivative of **141** indicated much less activity against both cell lines (HL-60 IC_{50} value range, 6.3–96.6 μM; KB IC_{50} value range, 5.5–124.3 μM). From the Tahitian sample, tulipinolide (**52**) and costunolide (**105**) were obtained and the latter germacranolide showed cytotoxic activity against the HL-60 cell line (IC_{50} 4.6 μM).[95] *Porella perrottetiana* produced cytotoxic compounds against both HL-60 and KB cell lines.[95] The same treatment as mentioned above gave 4*α*,5*β*-epoxy-8-*epi*-inunolide (**144**), perrottetianal A (**145**), Fig. 17, and 7-keto-8-carbomethoxy-pinguisenol (**146**), Fig. 18.

The former two compounds exhibited moderate or weak cytotoxicity against HL-60 (IC_{50} 8.5 and 2.7 μM) and KB cells (IC_{50} 52.4 and 46.3 μM).[95] 7*α*-Hydroxy-8-carbomethoxypinguisenol (**147a**) and acutifolone A (**147b**) prepared from **146** by reduction and dehydration (Fig. 18) were evaluated against HL-60 (IC_{50} 83.10 and >177 μM) and KB cells (IC_{50} 2.7 and 46.6 μM). It was suggested that the dienone group plays an important role in the mediation of cytotoxicity on HL-60 cells.[95]

Macrocyclic bis-bibenzyls such as marchantin A (**148a**) and riccardin A (**151b**), Fig. 19, were firstly isolated from liverworts by Asakawa *et al.*[1,3] Up to now, more than 100 macrocyclic and acyclic bis-bibenzyls have been isolated from

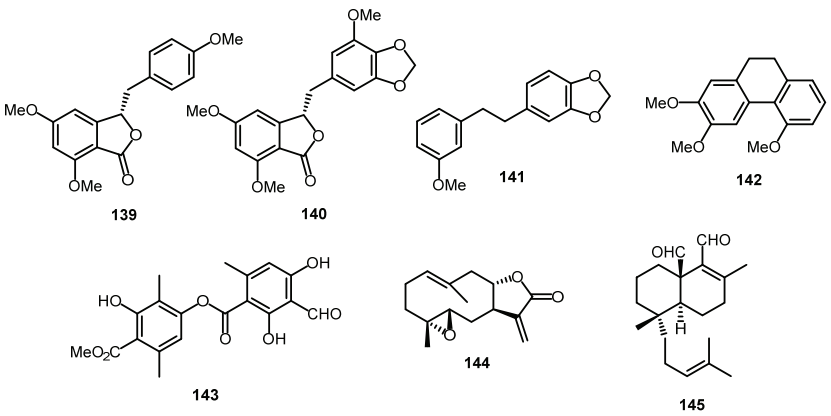


Fig. 17. Cytotoxic compounds from Tahitian and Indonesian *Frullania* species.

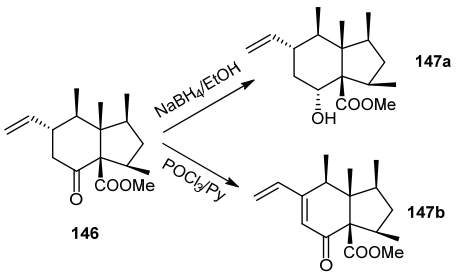
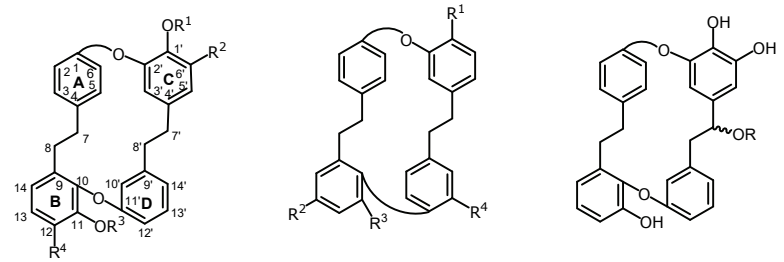


Fig. 18. Preparation of diol (**147a**) and dienone (**147b**) from **146.**



148a: $R^1=R^3=R^4=H$, $R^2=OH$
148b: $R^1=R^3=Me$, $R^2=OMe$, $R^4=H$
148c: $R^1=R^3=Ac$, $R^2=OAc$, $R^4=H$
149a: $R^1=R^3=H$, $R^2=R^4=OH$
149b: $R^1=R^3=Me$, $R^2=R^4=OMe$
150: $R^1=R^2=R^3=R^4=H$

151a: $R^1=R^2=R^4=OH$; $R^3=H$
151b: $R^1=R^4=OH$; $R^3=H$; $R^2=OMe$
151c: $R^1=OMe$; $R^2=R^4=OH$; $R^3=H$
151d: $R^1=OMe$; $R^3=R^4=OH$; $R^2=H$

152: R=H
153a: R=Me
153b: R=Et

Fig. 19. Bis-bibenzyls of the marchantin and riccardin type.

many liverworts and their stereo structures established.[2,15,23,96] The cyclic bis-bibenzyls such as marchantin (*e.g.*, **148**, **149**, **150**) and riccardin series (*e.g.*, **151**), Fig. 19, might be biosynthesized from bibenzyls that correspond chemically to dihydrostilbenes.[97] This assumption was proved by feeding experiments using radioactive and ^{13}C-labelled precursors, such as L-[*U*-^{14}C]-phenylalanine,

[*U*-^{14}C]dihydro-*p*-coumaric acid, [2-^{13}C]acetate and L-[^{13}COOH]phenylalanine.[98,99] Marchantin C (**150**) was biosynthesized by the coupling of two lunularic acid (**93**), followed by cytochrome P-450, named marchantin C hydroxylase to afford marchantin A (**148a**), Fig. 20.

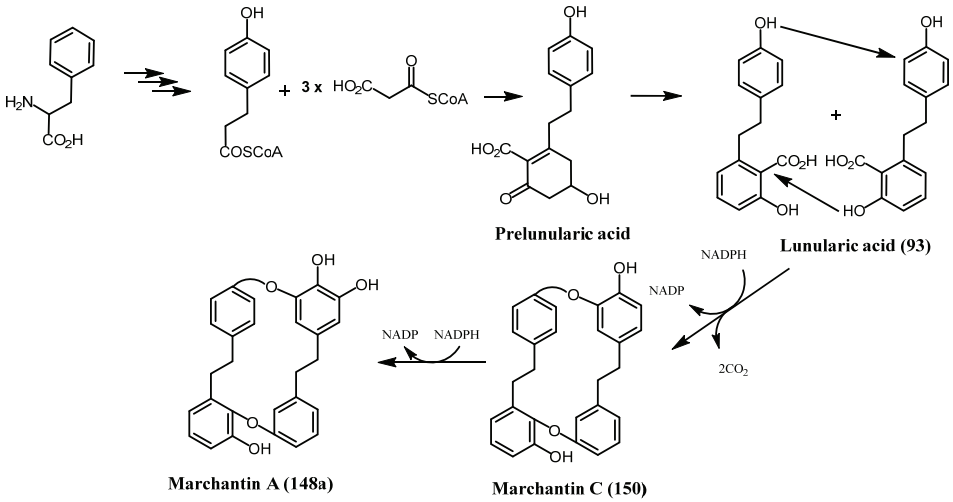


Fig. 20. Biosynthetic pathway of marchantin A.

Macrocyclic bis-bibenzyls produced in liverworts possess various biological activities, such as antimicrobial, antifungal, muscle relaxant, cytotoxicity against KB cells, inhibitory activity against DNA-polymerase *β*, cardiovascular activity, anti-HIV, and antitumor activity.[1,2,32,100] The methanol extract (**105g**) of a Japanese *M. polymorpha* was chromatographed over silica gel and Sephadex LH-20 to give the cyclic bis-bibenzyls, marchantin A (**148a**, 30 g), and its analogues, marchantins B (**148b**), C (**150**), D (**152**), E (**153a**), G (**154**), J (**153b**, Figs. 19 and 21. The yield of marchantin A (**148a**) is dependent upon the particular *Marchantia* species being investigated. Pure **148a** (80–120 g) was isolated from 6.67 kg of dried *M. paleacea* var. *diptera*. This thalloid liverwort elaborates not only the marchantin series, including marchantins A (**148a**), B (**149a**), D (**152**) and E (**153a**, Fig. 19, but also the acyclic bis-bibenzyls, perrottetin F (**155b**) and paleatin B (**156**). Marchantins A (**148a**), B (**149a**), D (**152**), perrottetin F (**155b**) and paleatin B (**156**), Fig. 21, showed cytotoxicity against KB cells (IC_{50} range 3.7––20 μM) and P-388 (*T*/*C* 117).[32]

The Italian liverwort, *Lunularia cruciata* elaborates seven new bis-bibenzyls (**153c**–**i**), along with lunularin (**92**), perrottetins E (**155a**) and F (**155b**), riccardin C (**151a**), F (**151c**), and G (**151d**). Compounds **153e** and **153g** and riccardin G (**151d**) showed cytotoxicity against the A549 lung cancer cell line with IC_{50} values of 5.0, 5.0 and 2.5 μM (Fig. 22).[101]

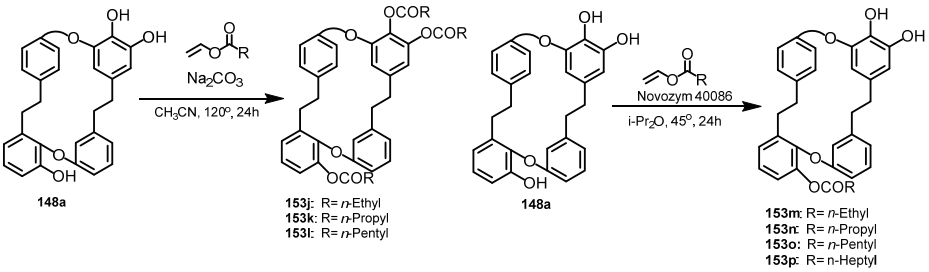


Fig. 21. Marchantin A derivatives obtained by chemical and enzymatic modifications.

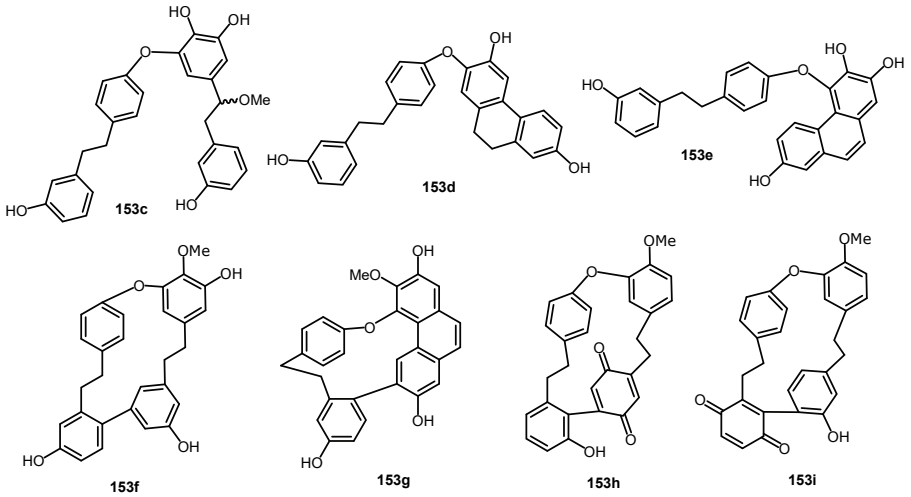


Fig. 22. Seven new bis-bibenzyls from *Lunularia cruciata.*

Marchantin A (**148a**) induced cell growth inhibition in human MCF-7 breast cancer cells at IC_{50} 4.0 μg mL^{-1}. Fluorescence microscopic and a Western blot analysis indicated that compound **148a** induced apoptosis of MCF-7 cells through a caspase-dependent pathway. The phenolic hydroxy groups at C-1′ and C-6′ are responsible for inducing cytotoxic and antioxidant activity.[103] In order to confirm the above hypothesis, seven previously undescribed marchantin A ester derivatives (**153j**–**p**) were synthesized chemically and enzymatically and tested on MRC-5 healthy human lung fibroblast, A549 human lung cancer, and MDA-MB--231 human breast cancer cell lines. All tested compounds were less cytotoxic in comparison to marchantin A (**148a**), but they also exhibited lower cytotoxicity against healthy cells.[102] The above results showed the C-ring plays an important role in cytotoxic activity.

Marchantin C (**150**, Fig. 17, and its dimethyl ether, 7,8-dehydro-marchantin C and its dimethyl ether were synthesized and their possible modulatory effects on P-glycoprotein in VCR-resistant KB/VCR cells were investigated.[104] The

results indicated that **150** was the most potent inhibitor of cell proliferation in both KB and KB/VCR cells among these four synthetic compounds, while the three derivatives of **150** have a little antiproliferative activity. Potent apoptosis in KB/VCR cells was induced by treatment with 16 μM of the dimethyl ether of marchantin C (**150**) and 0.2 μM VCR for 48 h.[104] Marchantin C also showed the induction of apoptosis of human glioma A172 cells at 8–16 μM.[105]

Marchantin C (**150**), neomarchantins A (**157**) and B (**158**), and a mixture of sesquiterpene/bis-bibenzyl dimers, GBB A (**159**) and GBB B (**160**), Fig. 23, from *Schistochila glaucescens* showed growth inhibitory activity against the P-388 cell line, with IC_{50} values of 18, 7.6, 8.5 and 10.3 μg mL^{-1}, respectively.[86] Riccardin D (**161**) from *Monocolea forsteri*[2] and *M. polymorpha*[106,107] indicated antiproliferative activity on human glioma A172 cells and induction of apoptosis at 16 μM. Compound **161** also showed potent effects in reversing P-glycoprotein-mediated multidrug resistance.[106]

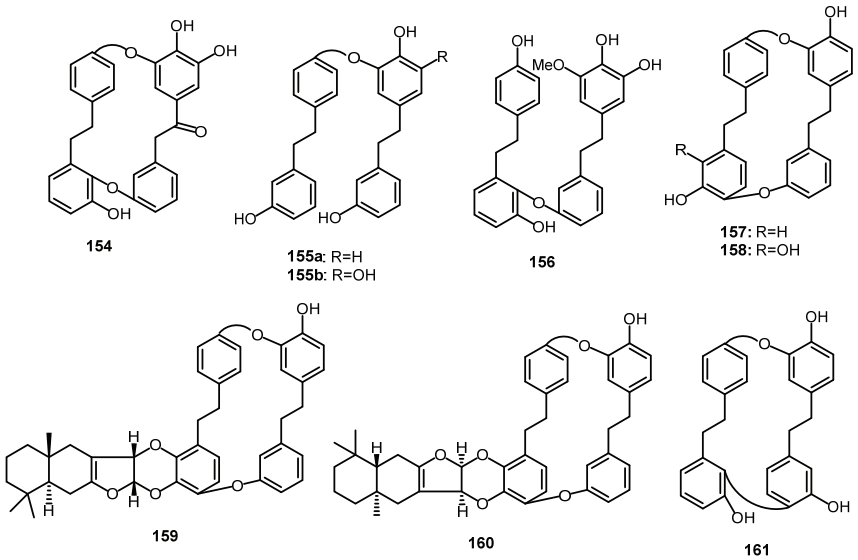


Fig. 23. Bis-bibenzyls from *Schistochila glaucescens* and *Marchantia polymorpha*.

2-Hydroxy-3,4,6-trimethoxyacetophenone (**162**) and 2-hydroxy-4,6-dimethoxyacetophenone (**163**) from *Plagiochila fasciculata* were inactive against the P-388 cell line (IC_{50} values of > 50 μg mL^{-1}).[108] *Trichocolea lanata* and *T. tomentella* produced tomentellin (**164**), Fig. 24, which showed inhibitory activity against African green monkey kidney epithelial (BSC-1) cells at 15 μg mL^{-1}, with no antiviral effects against herpes simplex or polio viruses. Demethoxytomentellin (**165**) from *T. tomentella* showed a similar cell growth inhibitory effect, indicating that both an allylic ether and a conjugated enone substructure are

required for such activity.[109] Methyl-4-[(2*E*)-3,7-dimethyl-2,6-octadienyl]oxy]-3-hydroxybenzoate (**166**), isolated from *T. hatcheri*, showed a lack of cytotoxicity (IC_{50} > 100 μM) against both KB and SK-MEL-3 human melanoma cells, as well as NIT 3T3 fibroblasts (Fig. 24).[110]

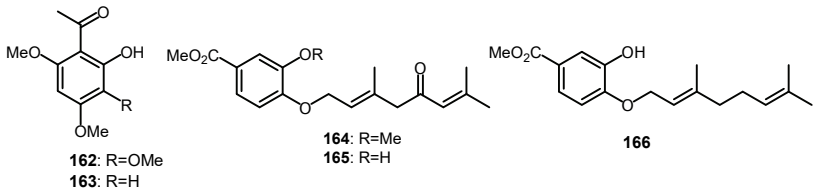


Fig. 24. Some of the isolated compounds from *Plagiochila fasciculata* and *Trichocolea* species.

The *ent*-kauranes and kaurenes **131** and **167**–**169** isolated from *Jungermannia* species inhibited HL-60 cells with IC_{50} values of 0.49, 7.0, 0.59 and 0.28 μM, respectively. Treatment of **131** and **167**–**169** caused proteolysis of ply(ADP--ribose) polymerase, a sign of activation of the apoptotic machinery, whereas the feature of cell death induced by treatment with compounds **167** and **168** was necrosis. Treatment with compound **169** induced apoptosis (see below).[111] The *ent*-kaurene diterpenoids **170**, **171** and **172**–**174** from a *Jungermannia* species showed cytotoxicity for HL-60 cells with IC_{50} values of 1.00, 0.40, 1.21, 1.28, and 0.78 μM, respectively (Fig. 25).[112]

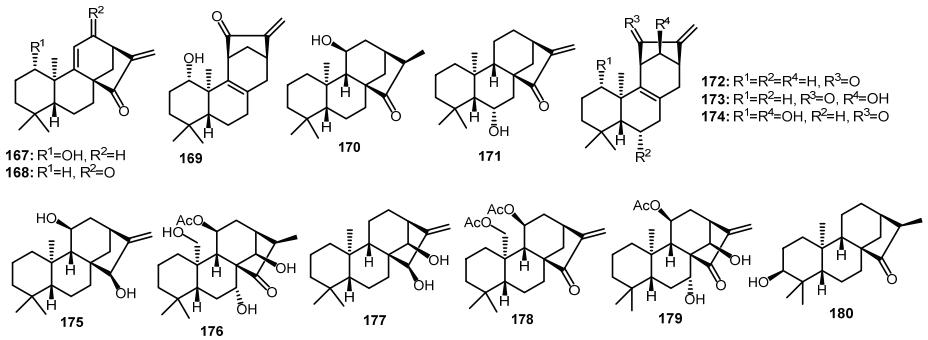


Fig. 25. Cytotoxic kaurenes from *Jungermannia* species.

The *ent*-kaurenes **131**, **175**–**180**, Fig. 25, isolated from the Japanese liverwort *Jungermannia truncata*, were evaluated for cytotoxicity against HL-60 human leukemia cells. Of these, *ent*-11α-hydroxy-16-kauren-15-one (**131**) induced apoptosis (programmed cell death) in this cell line partly through a caspase-8 dependent pathway.[90,113] The presence of an enone group in this class of molecule appears to be essential for the induction of apoptosis and the activation of caspases in human leukemia cell lines.[111,114] *ent*-Kaurenes **131**, **127** and **179** and

ent-9(11),16-kauradien-12,15-dione (**168**) and the rearranged *ent*-kaurene, jungermannenone A (**169**), selectively inhibited the nuclear factor-κB (NF-κB)-dependent gene expression due to treatment with TNF-*α*. Compound **131**, in combination with TNF-*α*, caused a dramatic increase in apoptosis in human leukemia cells accompanied by activation of caspases. Compound **120**, when combined with camptothecin, also caused an increase in apoptosis.[115,116] Jungermannenones A–D (**169**–**174**), Fig. 25, obtained from *Jungermannia* species, induced cytotoxicity against human leukemia HL-60 cells at 50 % inhibitory concentrations of 1.3, 5.5, 7.8 and 2.7 μM, respectively, and DNA fragmentation and nuclear condensation.

Both are biochemical markers of apoptosis induction, and apoptosis was induced through a caspase-independent pathway. Compounds **169** and **174** showed inhibitory activity for NF-κB, which is a transcriptional factor of anti-apoptotic factors. Thus, *ent*-kaurene diterpenoids from liverworts may be promising candidates as antitumor agents.[117,118] Some monoterpenoids, such as bornyl acetate (**181**), Fig. 26, found in liverworts, demonstrated potent apoptosis-inducing activities against the cultured cells of *M. polymorpha.*

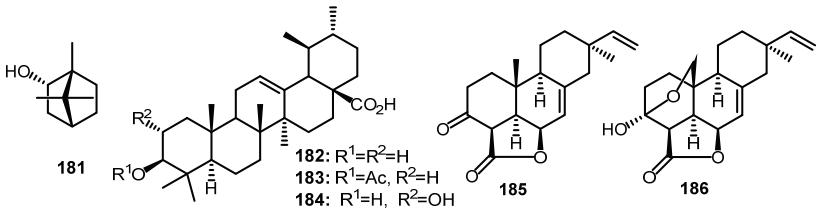


Fig. 26. Cytotoxic compounds from *Ptilidium* and *Hypnum* species.

Apoptosis induced by monoterpenoids occurs *via* the production of active oxygen species such as H_2O_2.[119] The ursane triterpenoids from the liverwort *Ptilidium pulcherrimum*, ursolic acid (**182**), 2*α*,3*β*-dihydroxyurs-12-en-28-oic acid (**183**) and acetoxyursolic acid (**184**), showed inhibition of the growth of PC3 human prostate cancer cells, at concentrations between 10.1±1.00 and 39.7±2.98 μM.[120] Previously, two pimarane diterpenoids momilactones A (**185**) and B (**186**), which were identified as phytoalexins in rice, were isolated from the moss *H. plumaeforme* (Hypnaceae), Fig. 26.[121] Momilactone B (**186**) was shown to have cytotoxicity against human colon cancer HT-29 and SW620 cells at 1 μM.[122]

Pallidisetin A (**187**) and pallidisetin B (**188**), isolated from the moss *Polytrichum pallidiscetum*, showed cytotoxicity against human melanoma (RPMI-7951) and human glioblastoma multiforme (U-251 MG) cells, with ED_{50} values of 1.0 and 1.0 μg mL^{-1} and 2.0 and 2.0 μg mL^{-1}, respectively.[123] Three cytotoxic compounds, 1-*O*-methylohioensin B (**189**), 1-*O*-methyldihydroohioensin B (**190**) and

1,14-di-*O*-methyldihydroohioensin B (**191**), Fig. 27, were also isolated from the moss *P. pallidiscetum.* Compound **189** proved to be cytotoxic for human colon adenocarcinoma (HT-29), human melanoma (RPMI-7951), and human glioblastoma multiforme (U-251 MG) cells, with ED_{50} values of 1.0, 1.0, and 2.0 μg mL^{-1}, respetively. Compound **190** showed inhibitory activity only against U-251 cells (ED_{50} 0.8 μg mL^{-1}) while **191** inhibited the growth of A549 lung carcinoma A549 (ED_{50} 1.0 μg mL^{-1}) and RPMI-7951 melanoma (ED_{50} 1.0 μg mL^{-1}) cell lines.[123] Ohioensin H (**192**) from *P. commune* did not show any cytotoxicity against the five human cancer cell lines in which it was evaluated (IC_{50} in all cases > 5 μg mL^{-1}).[124] Marsupellone (**193**) and acetoxymarsupellone (**194**) from *Marsupella emarginata* showed cytotoxicity (ID_{50} 1.0 μg mL^{-1}) against P388.[16,125] Riccardins A (**151**) and B (**195**) which were the first bis-bibenzyls from the Japanese liverwort, *Riccardia multifida* subsp. *decrescens* inhibited KB cells at a concentration of 10 and 12 μg mL^{-1}, respectively. *Radula perrottetii* contained cytotoxic perrottetin E (**155a**), Fig. 23, against KB cells (12.5 μg mL^{-1}).[31]

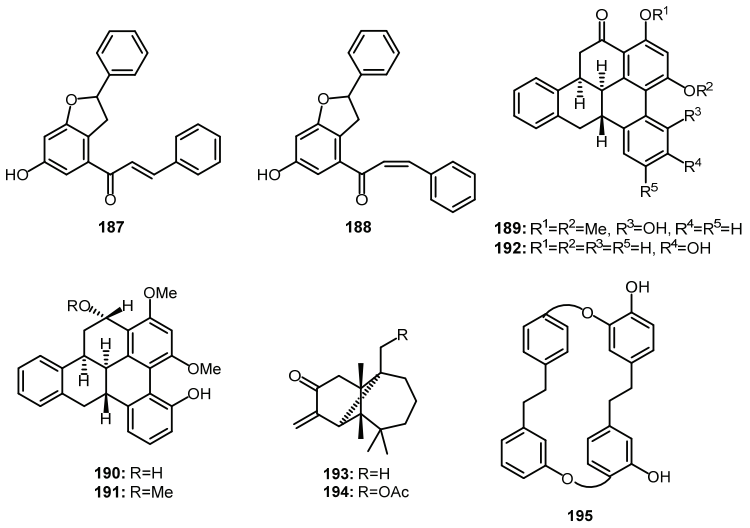


Fig. 27. Cytotoxic compounds from *Polytrichum*, *Marsupella* and *Riccardia* species.

5.5. Antimicrobial, antifungal and antiviral compounds

Several liverworts, *Bazzania* species, *C. conicum*, *Diplophyllum albicans*, *Dumortiera hirsuta*, *M. polymorpha*, *Metzgeria furcata*, *Lunularia cruciata*, *Pellia endiviifolia*, *Plagiochila* species, *P. vernicosa* complex, *P. platyphylla*, and *Radula* species show antimicrobial activity.[31] The essential oil of *Marchesinia mackaii* showed antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Salmonella pullorum*, *Staphylococcus aureus* and *Yersinia enterocolitica*.[126] Sacculatal (**48**) from *P. endiviifolia* showed potent antibacterial activity against

Streptococcus mutans (a causative organism for dental caries), exhibiting a LD_{50} value of 8 μg mL^{-1}. Polygodial (**47**), Fig. 7, was inactive (LD_{50} 100 μg mL^{-1}) in the same bioassay.[72] Lunularin (**92**), Fig. 13, from *Dumortiera hirsuta* also showed antimicrobial activity against *Pseudomonas aeruginosa* at a concentration of 64 μg mL^{-1}.[73] Lepidozenolide (**97b**), Fig. 13, from *Lepidozia fauriana* showed a positive response to methicillin-resistant *S. aureus* at 100 μg mL^{-1}. Riccardiphenol C (**108**) from *Riccardia crassa* showed antibacterial activity against *B. subtilis* at 60 μg disc^{-1} but not against fungi *Candida albicans* or *Trichophyton mentagrophytes*.[84] Glaucescenolide (**116**) from *Schistochila glaucescens*, exhibited antifungal activity against *T. mentagrophytes*.[86] *Ent*-1μ-Hydroxykauran-12-one (**117**) from *Paraschistochila pinnatifolia* demonstrated weak antifungal activity against *C. albicans* (Fig. 15).[87]

Marchantin A (**148a**) from many *Marchantia* species shows antibacterial activity against *Acinetobacter calcoaceticus* (*MIC* 6.25 μg mL^{-1}), *Alcaligenes faecalis* (100 μg mL^{-1}), *Bacillus cereus* (12.5 μg mL^{-1}), *B. megaterium* (25 μg mL^{-1}), *B. subtilis* (25 μg mL^{-1}), *Cryptococcus neoformans* (12.5 μg mL^{-1}), *Enterobacter cloacae, E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella typhimurium* (100 μg mL^{-1}) and *S. aureus* (3.13–25 μg mL^{-1}).[31] They also exhibit antifungal activity against *Alternaria kikuchiana*, *Aspergillus fumigatus* (*MIC* 100 μg mL^{-1}), *A. niger* (25–100 μg mL^{-1}), *C. albicans*, *Microsprorum gypseum*, *Penicillium chrysogenum* (100 μg mL^{-1}), *Piricularia oryzae* (12.5 μg mL^{-1}), *Rhizoctonia slain* (50 μg mL^{-1}), *Saccharomyces cerevisiae, Sporothrix schenckii* (100 μg mL^{-1}), and dermatophytic *T. mentagrophytes* (3.13 μg mL^{-1}) and *T. rubrum* (100 μg mL^{-1}).[31] Marchantins A (**148a**), B (**148b**), D (**141**), perrottetin F (**155b**) and paleatin B (**156**), Figs. 22 and 23, showed anti-HIV-1 activity (IC_{50} range 5.3–23.7 μg mL^{-1}).[7,72] Marchantin C (**150**), neomarchantins A (**157**) and B (**158**), Fig. 23, from *Schistochila glaucescens* showed antimicrobial activity against the Gram-positive bacterium, *B. subtilis*, with *MIC* values of 2, 1.5 and 2 μg mL^{-1}, and were also active against *T. mentagrophytes*, with *MIC* values of 0.5, 1, and 0.5 μg mL^{-1}, respectively.[86]

Riccardin D (**161**), Fig. 23, from *M. polymorpha* showed antifungal activity against the fluconazole-resistant *C. albicans* strains, QL-14, QL-28, SDEY-24R and SDEY-09R with *MIC* values of 16, 32, 16 and 16 μg mL^{-1}, respectively. When riccardin D (**161**) was mixed with fluconazole, the antifungal activities were substantially more potent (*MIC* values in the range 0.313 to 0.375 μg mL^{-1}).[127] The antifungal activity of **161** in *C. albicans* might be attributed to its inhibitory effect on cell wall chitin synthesis.[126] Riccardin D exerts its antifungal activity through mitochondrial dysfunction-induced accumulation of reactive oxygen species in *C. albicans*. Compound **161** also induced apoptosis in *C. albicans* through the activation of a metacaspase.[129,130]

A disc diffusion assay on 2-hydroxy-3,4,6-trimethoxyacetophenone (**162**) and 2-hydroxy-4,6-dimethoxyacetophenone (**163**) from *Plagiochila fasciculata* at 150 μg disc^{-1} showed both to have antifungal activity against *T. mentagrophytes* and *Cladosporium resinae*, but not against *B. subtilis* or the *Gram*-negative bacteria *E. coli* and *P. aeruginosa.* Compound **162** showed inhibitory activities against *E. coli*, *Proteus mirabilis* and *S. aureus* at a concentration of 100 μg mL^{-1}. Compound **162** also showed antifungal activity against *C. albicans.*[106] Tomentellin (**164**) and demethoxytomentellin (**165**) from *Trichocolea tomentella* and *T. mollissima* showed mild antifungal activity against *C. albicans* and *T. mentagrophytes* (Fig. 24).[107] Methyl-4-[(2*E*)-3,7-dimethyl-2,6-octadienyl)oxy]-3-hydroxybenzoate (**166**), from *T. hatcheri*, showed a lack of antimicrobial activity against *Staphylococcus epidermidis* (*MIC* 1000 μg mL^{-1}).[108] Reinvestigation of the antifungal activity of bis-bibenzyls from *M. polymorpha* using a bioautographic method showed that neomarchantin A (**157**), riccardin D (**161**) and 13,13'-*O*-isopropylidene riccardin D (**196**), Fig. 28, possessed antifungal activity against *C. albicans* with respective *MID* (minimum inhibitory dose) values of 0.25, 0.2 and 0.4 μg, respectively, compared to that of 0.01 μg for the positive control miconazole. Moreover, marchantin A (**148a**), marchantin B (**148b**), marchantin E (**153a**) and riccardin H (**197**) showed moderate growth inhibitory activities against the same fungus, with *MID* values of 2.5, 4.0, 2.5 and 4.0 μg, respectively.[131]

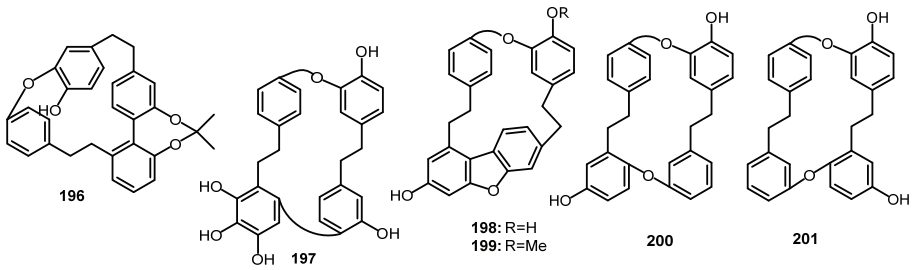


Fig. 28. Bis-bibenzyls from *Asterella angusta* showing activity against *Candida albicans*.

Direct TLC bioautographic detection of the antifungal activity of an ether extract of *Asterella angusta* showed activity against *C. albicans*. Ten bis-bibenzyls, riccardin D (**161**), riccardin B (**195**), perrottetin E (**155a**), asterelin A (**198**), asterelin B (**199**), 11-demethylmarchantin I (**200**), dihydroptychantol (**201**), marchantin H (**202**), marchantin M (**203**) and marchantin P (**204**) (Figs. 23, 27, 28 and 29) were tested against the yeast *C. albicans*. All of the compounds tested showed antifungal activity, exhibiting *MIQ* (minimum inhibitory quantity) values between 0.25–15.0 μg mL^{-1}, and *MIC* values in the range 16–512 μg mL^{-1}.[102,132] The free phenolic hydroxy group seems to play an important role in mediating antifungal activity because bis-bibenzyls possessing a methoxy group displayed decreased

potencies in this regard.[131,133] Six bis-bibenzyls, neomarchantin A (**157**), marchantin H (**202**), riccardin C (**151a**), riccardin F (**151c**), isoriccardin C (**205**) and pakyonol (**206**) isolated from *Plagiochasma intermedium* possessed weak *in vitro* antifungal activity against fluconazole-sensitive and resistant strains of *C. albicans*, with *MIC* valuess ranging from 32 to > 512 μg mL^{-1}. 6',8'-Dichloroisoplagiochin C (**207**), isoplagiochin D (**208**) and 6'-chloroisoplagiochin D (**209**) (Fig. 29) from *Bazzania trilobata*, showed discernible antifungal activity in a microtiter plate test against *P. oryzae* at IC_{50} values of 3.9, 4.0 and 2.6 μg mL^{-1} and *S. tritic* at IC_{50} values of 23.5, 15.9 and 4.5 μg mL^{-1}, respectively. Compounds **207** and **208** also demonstrated inhibitory activity against *B. cinerea* at IC_{50} values of 18.9 and 7.6 μg mL^{-1}. The free hydroxy groups on the benzene rings of bis-bibenzyls play an important role in mediating inhibitory activity against fungi such as *C. cucumerinum* (Fig. 29).[133]

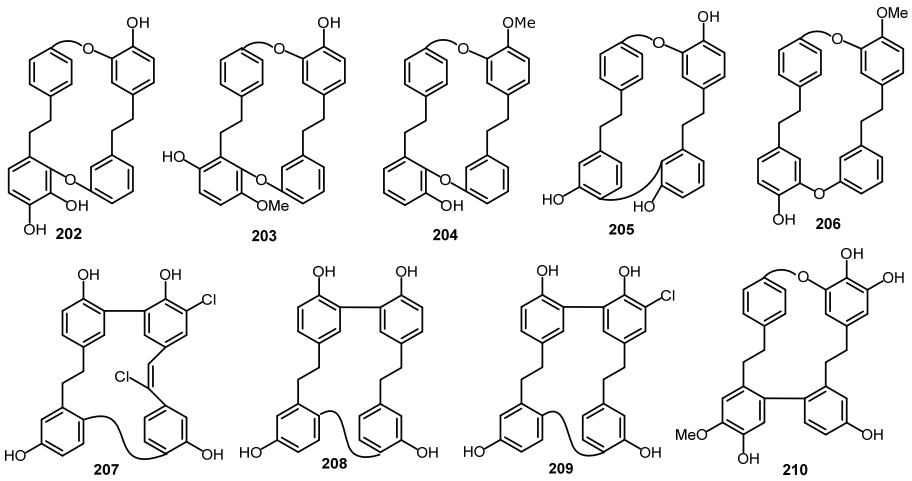


Fig. 29. Antimicrobial compounds from *Asterella*, *Plagiochasma* and *Bazzania* species.

The H1N1 and H5N1 influenza A virus caused pandemics throughout the world in 2009. Influenza A possesses an endonuclease within its RNA polymerase comprised of PA, PB1, and PB2 subunits. In order to obtain potential new anti-influenza compounds, 33 different types of phytochemicals were evaluated using a *in vitro* PA endonuclease inhibition assay.[134] Among them, the bis-bibenzyls, marchantins A (**148a**), B (**148b**) and E (**153a**) and plagiochin A (**210**), Fig. 29, inhibited influenza PA endonuclease activity at a concentration of 10 μM. This was the first evidence that phytochemicals derived from liverworts could inhibit influenza A endonuclease.

The herbertane sesquiterpenoids, *α*-herbertenol (**109**), *β*-herbertenol (**211**), herbertene-1,2-diol (**110**), mastigophorene C (**113**), mastigophoric acid methyl ester (**212**), *α*-formyl herbertenol (**213**) and 1,2-dihydroxyherberten-12-al (**214**),

Fig. 30, isolated from *Mastigophora diclados*, were tested against *S. aureus* strain, using an agar diffusion method. These sesquiterpenoids showed weaker activity than the standard antibiotics chloramphenicol (22 mm) and kanamycin (23 mm). However, of the compounds tested, mastigophorene C (**113**), a dimer of herbertene-1,2-diol (**110**), showed the most potent antibacterial activity (17 mm), while its monomer (**110**) displayed significant activity (13 mm) in this regard.[135]

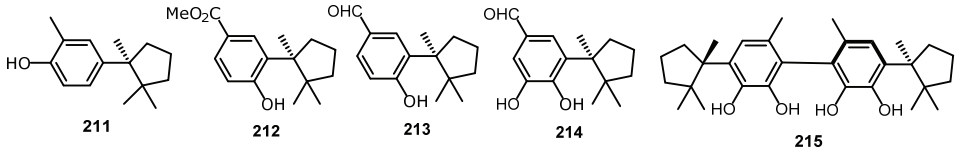


Fig. 30. Antibacterial herbertane sesquiterpenoids from *Mastigophora diclados*.

The crude ether and methanol extracts of the Tahitian *M. diclados* showed antimicrobial activity against *B. subtilis* and *Streptococcus aureus* (*MIC* 16 μg mL^{-1}).[85] Bioactivity-guided fractionation of both extracts gave (–)-*α*-herbertenol (**100**), (–)-herbertene-1,2-diol (**110**), (–)-mastigophorene C (**113**), (–)-mastigophorene D (**114**), diplophyllin (**115**), (–)-diplophyllolide (**43**) and mastigophorene A (**215**) (Fig. 30), among which **110**, **113,** and **114** showed moderate antimicrobial activity against *B. subtilis* at an *MIC* of 2–8 μg mL^{-1}. Only diol (**110**) indicated weak antimicrobial activity against *Klebsiella pneumoniae* at an *MIC* of 100 μg mL^{-1}.[85]

Chlorophyl decomposed compounds, phaeophytin a (**216**), 13^2-hydroxy-(13^2--*S*)-phaeophytin a (**217**), 13^2-hydroxy-(13^2-*R*)-phaeophytin a (**218**), and 13^2--(MeO_2)-(13^2-*R*)-phaeophytin a (=phaeophytin a hydroperoxide) (**219**), Fig. 31, isolated from the methanol-soluble extract of a cell suspension culture of *Plagiochila ovalifolia* showed antimicrobial activity against *E. coli* and *B. subtilis.*[136] *Bazzania trilobata* contained six antifungal active sesquiterpenoids, viridiflorol (**220**), gymnomitrol (**221**), 5-hydoxycalamenene (**222**), 7-hydroxycalamenene (**223**), drimenol (**224**) and drimenal (**225**), Fig. 32.[133] Viridiflorol (**220**) was found to have antifungal activity against *Cladosporium cucumerinum.*[137]

Also showed was the antifungal activity against *Pyricularia oryzae* at an IC_{50} value of 105.2 μg mL^{-1}. Gymnomitrol (**221**) showed strong inhibition against *Phytophthora infestans*, *P. oryzae* and *Septoria tritici* at IC_{50} values of 97.0, 1.7 and 53.0 μg mL^{-1}, respectively.[133] 5-Hydroxycalamenene (**222**) showed inhibitory activity against *Pyricularia oryzae* at an IC_{50} value of 1.7 μg mL^{-1} while 7-hydroxycalamenene (**223**) had potent antifungal activity against *Cladosporium cucumerinum, P. oryzae*, and *Septoria tritici* at IC_{50} values of 97.0, 1.7 and 53.0 μg mL^{-1}, respectively.

Compound **223** was tested for its *in vivo* activity against *Plasmopara viticola* on grape vine leaves and showed inhibitory activity at a concentration of 250

ppm. The infection was reduced from 100 % in the control to 30 % in the treated plants in a greenhouse.[133] 7-Hydroxycalamenene (**223**) from *Tilia europaea* is a phytoalexin. Drimenol (**224**) is less active than the calamenenes described above.[138] It inhibited the growth of *C. cucumerinium* and *S. tritici* at concentrations of 6.6 and 80.1 μg mL^{-1}, respectively.[133] Drimenal (**225**) exhibited moderate growth inhibitory activity against *B. cinerea* and *P. oryzae* at IC_{50} values of 81.8 and 61.6 μg mL^{-1}, respectively, and more potent activity against *S. tritici* and *P. infestans* at IC_{50} values of 17.6 μg mL (Fig. 32).[133]

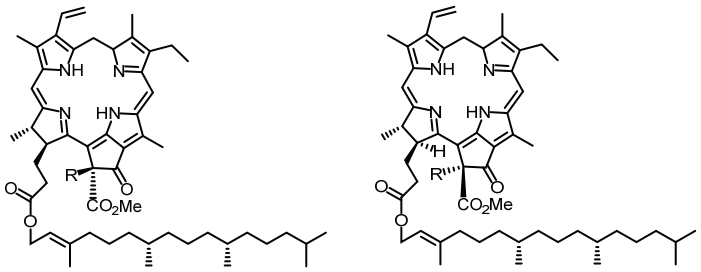


Fig. 31. Antibacterial chlorophyl decomposed compounds from *Plagiochila ovalifolia*.

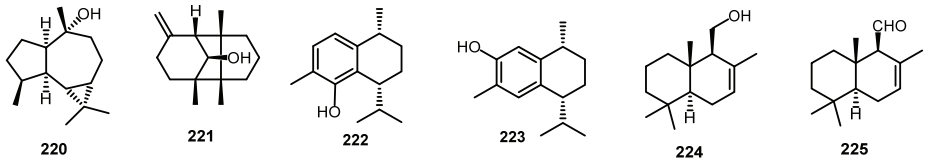


Fig. 32. Antibacterial compounds from *Bazzania trilobata* and *Tilia europaea*.

Dehydrocostus lactone (**226**), acetyltrifloculoside lactone (**227**), and 11α,13-dihydrodehydrocostuslactone (**228**), Fig. 33, from *Targionia lorbeeriana* showed antifungal activity against *Cladosporium cucumerinum* with *MIC* values of 0.5, 10, and 3 μg mL^{-1}, respectively, using a bioautographic TLC method. Dehydrocostus lactone (**226**) exhibited the same activity at 20 μg mL^{-1} against *C. cucumerinum* in an agar dilution assay. Compound **226** also showed larvicidal activity against *Aedes aegypti*, with an LC_{100} value of 12.5 ppm and antifungal activity against *C. albicans* (*MIC* 5 μg mL^{-1}) in a bioautographic TLC method.[139]

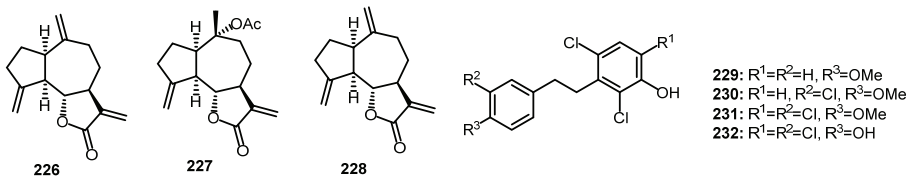


Fig. 33. Antibacterial compounds from *Targionia lorbeeriana*.

Some species of *Riccardia* elaborate high concentrations of the bioactive polychlorinated bibenzyls, 2,6-dichloro-3-hydroxy-4'-methoxybibenzyl (**229**), 2,6,3'-trichloro-3-hydroxy-4'-methoxybibenzyl (**230**), 2,4,6,3'-tetrachloro-3-hydroxybibenzyl (**231**) and 2,4,6,3'-tetrachloro-3,4-dimethoxybibenzyl (**232**), Fig. 33, in order to protect them from pathogens and herbivores. On TLC-bioautography with a *Cladosporium herbarum* culture, compounds **229**, **230** and **232** showed fungicidal activities, as manifested by inhibition zones of 1.2–2.9 cm, which were greater than those obtained with the fungicide, ketoconazole. Compound **231** was inactive against *C. herbarum.*[140]

The dichloromethane extract of *Balantiopsis cancellata* demonstrated strong antifungal activity against *Cladosporium herbarum*, a rot fungus, at 0.01 μg spot^{-1}. Five aromatic esters, isotachin B (**7c**), 2-phenylethyl benzoate (**233**), (*R*)-2-hydroxy-2-phenylethyl benzoate (**234**), 2-phenylethyl (*Z*)-cinnamate (**235**) and its (*E*)-cinnamate (**236**), Figs. 1 and 34, were isolated, among which compound **7c** showed the most potent antifungal activity against *C. herbarum* at 0.006 μg spot^{-1}, on TLC-bioautography.

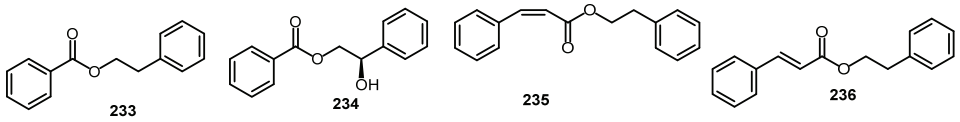
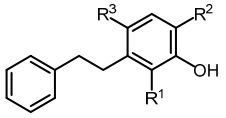


Fig. 34. Antibacterial compounds from *Balantiopsis cancellata*.

This activity is lower than that required with either pure ketoconazole or commercial captan as observed in dilution experiments. The benzoate (**234**) also showed the same activity as mentioned above at 0.05 μg spot^{-1}.[141]

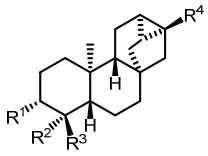
The crude extract of *Riccardia marginata* showed antimicrobial activity against the *Gram*-positive bacterium, *B. subtilis*, and the dermatophytic fungus, *T. mentagrophytes*. The active compounds are the chlorinated bibenzyls, 2,4,6--trichloro-3-hydroxybibenzyl (**237**), 2,4-dichloro-3-hydroxybibenzyl (**238**) and 2-chloro-3-hydroxybibenzyl (**239**), Fig. 35, which showed activity against *B. subtilis*, *T. mentagrophytes*, *C. albicans*, and the plant pathogenic fungus *Cladosporium resinae*, at a concentration of 30 μg disc^{-1}. However, these compounds proved to be inactive against the *Gram*-negative bacteria, *E. coli* and *P. aeruginosa*. Compound **231** showed its most potent activities against *T. mentagrophytes* (12 mm) (zone of inhibition in mm for 5 mm disc) and *C. resinae* (2 mm).[142]



237: $R^1=R^2=R^3=Cl$
238: $R^1=R^2=Cl$, $R^3=H$
239: $R^1=Cl$, $R^2=R^3=H$

Fig. 35. Antibacterial chlorinated bibenzyls from *Riccardia marginata*.

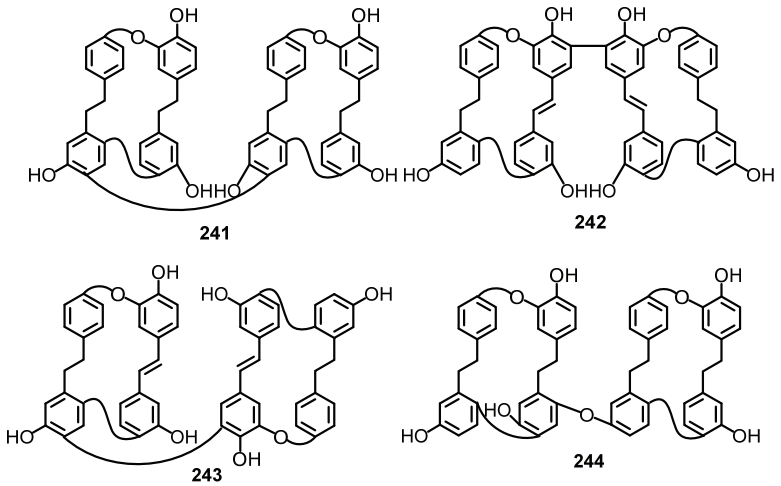
In the critical search for new antituberculosis, compounds from bryophytes lead,[143] 14 trachylobane diterpenoids from the liverwort *Jungermannia exsertifolia* subsp. *cordifolia* were isolated, among which *ent*-trachyloban-17-al (**240a**) showed the most potent activity against the virulent *Mycobacterium tuberculosis* H37Rv strain, with an MIC_{90} value of 24 μg mL^{-1}. *ent*-3*β*-Acetoxy-19-hydroxytrachylobane (**240b**), *ent*-trachylobane-3-one (**240c**), *ent*-3*β*-hydroxytrachylobane (**240d**), and *ent*-3*β*-acetoxytrachylobane (**240e**), Fig. 36, demonstrated moderate inhibitory activities against the same microbe (MIC_{90} values of 59, 50, 61 and 111 μg mL^{-1}, respectively). The remaining trachylobanes (**240f**–**l**) showed *MIC* values of > 128 μg mL^{-1} and were thus considered to be inactive.



240a: R^1=H, R^2= R^3=Me, R^4=CHO
240b: R^1=OAc, R^2=CH_2OH, R^3=R^4=Me
240c: R^1=O, R^2=R^3=R^4=Me
240d: R^1=OH, R^2=R^3=R^4=Me
240e: R^1=OAc, R^2=R^3=R^4=Me
240f: R^1=OAc, R^2=R^4=Me, R^3=CH_2OH
240g: R^1=OH, R^2=R^4=Me, R^3=CH_2OAc
240h: R^1=OAc, R^2=R^4=Me, R^3=CHO
240i: R^1=OH, R^2=CHO, R^3=R^4=Me
240j: R^1=OAc, R^2= R^3=Me, R^4=CH_2OH
240k: R^1=OAc, R^2=CO_2H, R^3=CH_2OAc, R^4=Me
240l: R^1=OH, R^2= R^3=Me, R^4=CO_2H

Fig. 36. Trachylobane diterpenoids from the liverwort *Jungermannia exsertifolia* subsp. *cordifolia*.

Blasia pusilla produces the bis-bibenzyl dimers, pusilatins A-D (**241**–**244**), Fig. 37. Pusilatins B (**242**) and C (**243**) showed weak HIV-RT inhibitory activity.[144]



241 **242** **243** **244**

Fig. 37. Pusilatins from *Blasia pusilla*.

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ИЗВОД

ФИТОХЕМИКАЛИЈЕ ИЗ БРИОФИТА: СТРУКТУРА И БИОЛОШКЕ АКТИВНОСТИ

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На бриофите као изворе за људску исхрану се обраћа мала пажња иако их има преко 23000 врста. Неке маховине садрже витамин Б1, токофероле, простагландинима слична једињења, вишеструко незасићене масне киселине и фенолна једињења. С друге стране, јетрењаче садрже моно-, сескви- и дитерпеноиде енентиомерне онима пронађеним у васкуларним биљкама. Поред њих, оне садрже и бибензиле, бис-бибензиле и поликетиде, од којих многи показују антимикробну, антивирусну, анти-инфламаторну, цитотоксичну на ћелије рака, миорекласантску, антиоксидативну и друге. У овом раду су продискутоване структуре и биолошка активност фитохемикалија из јетрењача.

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SURVEY

Phytochemical study of the genus *Amphoricarpos*

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Abstract: Phytochemistry deals with the study of secondary metabolites produced by plants that synthesize these compounds for many reasons, including their own protection against attack of herbivores and plant diseases. Secondary metabolites are believed to represent plant adaptation to various environmental factors and that they enabled the survival of the species. Secondary metabolites of plants can have curative or toxic effects in humans and animals. Herbal medicine has a long tradition in folk medicine and until the early 20th century, when synthetic organic chemistry began to develop, plants were the main source of medicines. The two basic goals of our phytochemical research are: isolation and identification of new (biologically active) compounds – potential drugs, and chemotaxonomy (chemosystematics). In the following text through one selected example, the genus *Amphoricarpos* Vis., our phytochemical research is shown on both aspects.

Keywords: phytochemistry; amphoricarpolides; chemosystematics; metabolomics.

CONTENTS

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1. INTRODUCTION

Phytochemistry is the study of secondary metabolites produced by plants considering their structural compositions, the biosynthetic pathways, functions, mechanisms of actions in the living systems as well as their medicinal, industrial, and commercial applications. Phytochemistry is an important part of the range of disciplines, such as systematic botany, taxonomy, ethnobotany, conservation biology, plant genetic and metabolomics, evolutionary sciences, and plant pathology. The achievements in phytochemistry in the discovery of bioactive compounds is applied in pharmacy and pharmacognosy, complementary and alternative medicine, ethnomedicine, biochemistry, microbiology, bioinformatics, and computational chemistry. In the biotechnology and process engineering, nutrition and food sciences, as well as in organic chemistry, the expertise in phytochemistry is very important for improvement of processes yielding natural products. In the control of environmental pollution, for the application of bioremediation techniques, such as phytoremediation for the removal of harmful substances, the competence in phytochemistry is essential. Since phytochemistry is closely related to many biosciences, there are different opinions about the place of phytochemistry as a discipline. Some scientists consider it a subfield of botany and chemistry, while others believe that it should be a part of food and medical chemistry due to its wide application in drug discovery. In any case, it is difficult to single out phytochemistry as a discrete discipline.

To study chemical interactions in plants based on the chemical knowledge to successfully isolate components and determine molecular structure by studying their properties, it is necessary to know the basics of plant science, isolation, and identification of molecules from plants. Besides, knowledge and expertise on various analytical techniques for extraction, characterization and quality assessment are prerequisite. In addition, understanding natural products induction, metabolomics profiling (nuclear magnetic resonance (NMR), mass spectrometry (MS), micro-fractionation, natural products database, e-bioprospecting) is required. Expertise in the state-of-art techniques including the various extraction methods, for example, solvent extraction methods, supercritical fluid extraction, microwave-assisted extraction, chromatographic fingerprinting, and marker compound analysis is necessary. Advances in chromatographic techniques (liquid chromatography–MS; liquid chromatography–NMR), gas chromatography–MS, anti-microbial and antioxidant studies will help in a comprehensive analysis of natural product extracts. In the recent years, studies are being conducted in relation to the stress induction of natural products under metabolomics view (plant

metabolomics). The use of metabolomics in conjunction with direct NMR profiling approaches is important for the understanding metabolic reactions in plants.[1]

The aim of this paper is to present the basic goals of our phytochemical research on a single model example – gender *Amphoricarpos* Vis.:

1) isolation and identification of new (biologically active) compounds – potential drugs and

2) chemotaxonomy (chemosystematics).

The classification of the genus *Amphoricarpos*, an endemic species of the western part of the Balkan Peninsula, is somewhat vague. In the examination of European *Amphoricarpos* complex, Blečić and Mayer[2] reported two endemic species: *A. neumayeri* Vis. and *A. autariatus* Blečić *et* Mayer, the latter comprising two subspecies, ssp. *autariatus* and ssp. *bertisceus* Blečić *et* Mayer. The occurrence of *A. neumayeri* is limited to coastal Montenegro mountains over Boka Kotorska Bay, Orjen and Lovćen, whereas *A. autariatus* could be found throughout the wider area. The taxon growing on mountains of Bosnia, Herzegovina and northwest Montenegro was assigned as *A. autariatus* ssp. *autariatus* and the remaining one, mostly inhabiting mountain group Prokletije (situated between Montenegro, Kosovo, and Albania) and the mountains of north Greece was denoted as *A. autariatus* ssp. *bertisceus*. On the other hand, Webb[3] recognized only a single species, *A. neumayeri* Vis., divided in two subspecies, *i.e.*, ssp. *neumayeri* and ssp. *murbeckii* Bošnjak (syn. *Amphoricarpos autariatus* Blečić & E. Mayer).

Since the insight into secondary metabolites could provide additional information about the systematics of this taxon, a phytochemical study of plant species of the genus *Amphoricarpos* from different localities, collected over the years, was undertaken.

2. RESULTS AND DISCUSSION

2.1. Analysis of volatile components

Volatile components of plants of the genus *Amphoricarpos* from various localities[4] were obtained by steam distillation on a Likens–Nickerson continuous distillation–extraction apparatus. The volatiles were concentrated in dichloromethane and analyzed by GC-FID and GC–MS techniques. The components were identified based on the retention indices and comparison with reference spectra (Wiley and NIST databases). The relative percentage of the identified compounds was computed from GC-FID peak area, and the results are presented in the Supplementary material to this paper (Table S-I of the Supplementary material to this paper).

According to the GC-FID and GC–MS analyses, caryophyllene oxide is the main component in almost all samples. Based on the relative distribution of main components, three groups of samples are recognized.

In the sample OR04, *A. neumayeri* Vis. according to Blečić and Mayer[2], the main components are caryophyllene oxide (37.68 %), palmitic acid (9.93 %) and *β*-caryophyllene (6.80 %).

In the second group, caryophyllene oxide (28.53–36.68 %), decanal (12.83 to 13.58 %) and palmitic acid (7.50 to 8.18 %) are dominant. This group consists of samples that according to Blečić and Mayer[2] belong to *A. autariatus* ssp. *autariatus*.

The third group consists of samples belonging to *A. autariatus* ssp. *bertisceus*, in which results are inhomogeneous.

The results of analysis of volatile components are in support of the claims by Blečić and Mayer[2].

2.2. Analysis of nonvolatile components

Determination of sesquiterpene lactones content in plant species of the genus Amphoricarpos. Crude extracts of grounded aerial parts (BEM) of various *Amphoricarpus* species from different localities, collected over several years were analyzed by ^{1}H-NMR (Table I).[4] In all investigated samples the signals from the sesquiterpene lactones were present. Since the overall content of sesquiterpene lactones in the studied species was rather high (≥ 1–2 %, calculated per weight of the dried plant material), ^{1}H-NMR spectroscopy was applied to determine the total content of sesquiterpene lactones in the aerial parts of the examined plant species of the *Amphoricarpos* genus. The analysis was performed by comparison of the integral of the exomethylene H-13 proton of sesquiterpene lactones (δ ~6.2) with that of the two-proton singlet (δ ~7) of 2,6-*bis*(1,1-dimethylethyl)-4-methylphenol (BHT), used as internal standard.

The percentage of the lactones in the dry plant material was calculated as follows:

$$w_{\text{lakt}} = 100 \frac{m_{\text{BHT}} M_{\text{lakt}} 2 I_{\text{lakt}}}{M_{\text{BHT}} I_{\text{BHT}} m_{\text{sb}}} \quad (1)$$

w_{lakt} / %, content of lactone in dry plant; m_{BHT} / mg, the mass of BHT added to the plant material; M_{lakt}, average molar mass of lactone (270 g/mol); 2, the number of protons from which the BHT signal originates; I_{lakt}, the value of the integral of the exomethylene proton lactone, H-13; M_{BHT}, molar mass of BHT (220 g/mol); I_{BHT}, the value of the integral of the aromatic BHT protons, H-3 i H-5; m_{sb} / mg, mass of weighed dry plant material.

Quantitative ^{1}H-NMR analysis showed that plant species of the genus *Amphoricarpos* are characterized by a relatively high content of sesquiterpene lactones. The largest mass fraction of lactones is present in the samples of *A. neumayeri* Vis. – more than 1.50 %, calculated by weight of dry plant material. Samples of *A. autariatus* ssp. *autariatus* contain about 0.50 % of sesquiterpene lactones, and samples of *A. autariatus* ssp. *bertisceus* (according to Blečić and

Mayer) contain between 0.36 and 1.12 % of these compounds, depending on the location and time of collection of plant material.

TABLE I. Content of sesquiterpene lactones in crude extracts of aerial parts (BEM) of various species of the genus *Amphoricarpos*, based on quantitative ^{1}H-NMR analysis; dpm – dried plant material (aerial parts); BEM – petrol ether–diethylether–methanol (1:1:1 volume ratio)

Sample	m_{dpm} / mg	m_{BHT} / mg	I_{lact}	I_{BHT}	$w_{lact\ BEM}$ / %
OR04[a]	1061.4	7.264	51.19	48.81	1.76
OR06[a]	1020.0	4.728	58.98	41.02	1.60
OR07[a]	1030.0	8.274	53.39	46.61	2.26
KT04[b]	1029.4	2.238	49.35	50.65	0.52
KD05[b]	1041.4	2.179	50.05	49.95	0.52
KT05[b]	1013.0	2.179	52.01	47.99	0.57
PR01[c]	1041.8	2.775	48.27	51.73	0.61
ZEL02[c]	1002.7	2.903	51.35	48.65	0.75
VIS04[c]	1013.4	4.718	44.87	55.13	0.93
SINJ04[c]	1027.1	4.104	46.72	53.28	0.86
VIS05[c]	1055.1	7.264	38.18	61.82	1.04
VK05[c]	1002.5	4.100	45.22	54.78	0.83
PLK05[c]	1005.6	2.361	55.61	44.39	0.72
POP05[c]	1001.6	4.903	48.35	51.64	1.12
SINJ05[c]	1035.3	4.540	47.31	52.69	0.97
KOT06[c]	820.0	2.955	29.06	70.94	0.36
GR07[c]	530.0	2.955	54.46	45.54	0.52
VIS07[c]	1030.0	4.728	33.41	66.59	1.06

[a]*A neumayeri* Vis.; [b]*A. autariatus* ssp. *autariatus*; [c]*A. autariatus* ssp. *Bertisceus* (according to Blečić and Mayer[2])

Leaf-surface waxes of plant species of the genus Amphoricarpos. Intact air--dried leaves were sonicated with dichloromethane and extract filtered and evaporated. The solid residue was treated with *n*-hexane to separate non-polar from a more polar fraction.[4]

GC/FID and GC–MS analysis identification of the components of non-polar fraction was done based on the retention indices and comparison with reference spectra (Wiley and NIST databases). The relative percentage of the identified compounds was computed from GC/FID peak area. The results are given in Table II.

In all analyzed samples the non-polar fraction contained *n*-alkanes with chain lengths ranging from 27 to 33 carbons, with over 90 % of odd-number of carbons.

There was no pattern in *n*-alkane distribution, so their analysis did not contribute to the classification of *Amphoricarpos* genus.

During the extraction of surface waxes with dichloromethane and re-extraction with *n*-hexane, it turned out that the fraction insoluble in hexane contained, to a large extent, sesquiterpene lactones.[4] They were separated from the other ingredients by methanol extraction (SL). By measuring the plant material and solid

extracts in each extraction phase, approximate mass fractions of lactone in whole dry leaves were obtained. The results are shown in Table III.

TABLE II. Results of GC/FID and GC/MS analyses of non-polar fraction of leaf-surface waxes

Sample	Content of *n*-alkanes, %							Total content of *n*-alkanes, %
	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	
OR04[a]	12.94	0.77	41.53	1.14	33.35	0.59	2.75	93.07
OR06[a]	1.43	2.77	17.45	1.77	44.18	1.46	26.36	95.42
OR07[a]	1.88	–	19.55	0.89	47.06	1.09	23.80	94.27
KT04[b]	10.54	0.74	35.81	1.22	39.11	0.50	3.31	91.23
KD05[b]	16.01	0.90	38.41	1.45	30.17	0.72	2.96	90.62
KT05[b]	19.63	1.32	42.03	1.76	29.47	–	2.46	96.67
PR01[c]	13.28	0.94	43.66	1.23	31.74	–	1.39	92.24
ZEL02[c]	13.35	0.64	42.73	1.02	33.88	0.59	2.54	94.75
VIS04[c]	15.50	0.90	43.40	1.09	30.96	–	2.07	93.92
SINJ04[c]	20.58	0.91	44.68	0.95	26.69	–	–	93.81
VIS05[c]	9.22	1.18	39.69	1.53	38.08	0.90	3.34	93.94
VK05[c]	12.52	1.02	42.91	1.53	35.81	0.44	3.08	97.31
PLK05[c]	15.82	0.77	46.97	1.17	28.20	–	2.45	95.38
POP05[c]	22.82	1.27	47.12	1.09	21.20	–	–	93.52
SINJ05[c]	12.67	0.99	42.13	1.86	31.69	1.10	2.65	94.10
KOT06[c]	1.95	1.71	17.42	1.76	44.86	1.50	26.31	95.51
GR07[c]	1.24	1.44	22.09	1.52	45.78	1.08	22.98	96.13
VIS07[c]	1.38	1.81	36.76	3.75	32.04	–	20.59	94.95

[a]*A neumayeri* Vis.; [b]*A. autariatus* ssp. *autariatus*; [c]*A. autariatus* ssp. *Bertisceus* (according to Blečić and Mayer[2])

TABLE III. Approximate percentage of sesquiterpene lactones on the leaf surface (LS) of plants of the genus *Amphoricarpos*

Sample	m_{leaves} / g	m_{lact} / mg	$w_{lact\ SL}$ / %
OR04[a]	1.04	18.3	1.76
OR06[a]	1.06	22.5	2.23
OR07[a]	1.01	33.9	3.36
KT04[b]	1.01	13.0	1.29
KD05[b]	1.26	16.2	1.28
KT05[b]	1.08	15.4	1.42
PR01[c]	0.98	10.2	1.04
ZEL02[c]	1.04	17.1	1.64
VIS04[c]	1.00	14.8	1.48
SINJ04[c]	1.05	16.8	1.60
VIS05[c]	1.04	16.7	1.60
VK05[c]	1.04	10.8	1.04
PLK05[c]	1.00	19.3	1.93
POP05[c]	1.02	16.1	1.58
SINJ05[c]	1.02	15.4	1.51
KOT06[c]	1.05	12.6	1.20
GR07[c]	0.52	5.3	1.02
VIS07[c]	1.06	17.6	1.66

[a]*A neumayeri* Vis.; [b]*A. autariatus* ssp. *autariatus*; [c]*A. autariatus* ssp. *Bertisceus* (according to Blečić and Mayer[2])

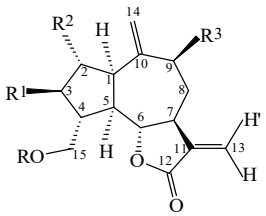
Considering these data and the previous results of ^{1}H-NMR quantifitation, it is shown that the largest mass fraction of lactones is present in the samples of *A. neumayeri* Vis., and the content of sesquiterpene lactones in *A. autariatus* ssp. *bertisceus* (according to Blečić and Mayer[2]) also varies depending on the locality and collection time of plants material.

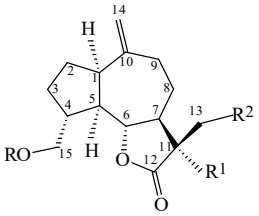
2.3. Isolation and characterization of secondary metabolites

Isolation of secondary metabolites begun with classic methods of extraction of aerial parts[5] followed by dry-flash, column and preparative thin-layer chromatography. In this manner, 23 new sesquiterpene lactones (SL, named amphoricarpolides, Table IV), all of them guaianolides, were isolated.[4,6,7] The structures of isolated SL were elucidated by detailed analysis of IR, NMR, and MS data.

Further 9 SL were isolated from extracts gained as a polar fraction (SL) during leaf-surface waxes investigation.[8,9] Given the great diversity of lactones isolated by classical separation methods, the study was continued by semi-preparative LC analysis. The SL isolated solely in this manner are presented in the Table IV.

TABLE IV. Sesquiterpene lactones (SL) isolated using classical extraction and separation methods, and semi-preparative LC





1–17, 23					18–22			
Lactone	R	R^1	R^2	R^3	Lactone	R	R^1	R^2
1	H	H	H	H	**18**[a]	H	OH	OH
2	H	OH	H	H	**19**	H	OH	OH
3	Ac	OH	H	H	**20**	Ac	OH	OH
4	Ac	OAc	H	H	**21**	Ac	OH	Cl
5[a]	Ac	OAc	H	H	**22**	H	OH	Cl
6	*i*-Val	OAc	OH	H				
7	Ac	OAc	OH	H				
8	Ac	OH	OH	H				
9	*i*-Val	OH	OH	H				
10	H	OAc	OH	H				
11	Ac	OAc	H	OH				
12	Ac	OAc	OAc	H				
13	*i*-Val	OAc	H	OH				
14	Ac	OH	H	OH				
15	*i*-Val	OH	H	OH				
16	Ac	H	H	H				
17[a]	H	H	H	H				
23[a]	Ac	OH	H	H				

TABLE IV. Continued

SL isoladed by semi-preparative LC								
24–31					**32**			
Lactone	R	R^1	R^2	R^3	Lactone	R	R^1	R^2
24[b]	H	H	H	H	**32**	Ac	H	OH
25[b]	Ac	H	H	H				
26[c]	Ac	H	H	H				
27	Ac	OAc	H	α-OH				
28	Sen	OAc	H	OH				
29	*i*-Val	OAc	H	H				
30	H	OH	H	OH				
31	H	OH	OH	H				

[a]10α(14)-Epoxy; [b]11(13)-epoxy; [c]10α(14)-epoxy

2.4. Statistical analysis of data obtained by phytochemical analyses of plant extracts of the genus Amphoricarpos

To gain insight into the possible pattern of synthesis of secondary metabolites in different species of plants of the genus *Amphoricarpos*, plant extracts were subjected to LC-DAD and LC-ESI–MS analyses.[4] The extracts were obtained by standard procedure[5] (BEM), and by dichloromethane extraction, after separation of non-polar fraction of surface waxes (SL). The results obtained by aforementioned analyses, as well as quantitative NMR and gravimetric analyses (Supplementary material, Table S-II), were subjected to statistical analysis.

Principal component analysis (PCA) was performed as *unsupervised* multivariate analysis for easier understanding of the structure of elements and characteristics in extensive tabular data. Discriminant analysis (DA) was employed to differentiate *a priori* defined groups and to assort the elements within the predefined groups. Cluster analysis (CA) was performed with the aim of identification of the relations among populations. Statistical analyses were all carried out with the software Statgraphics Plus (version 5.0; Statistical Graphics Corporation, USA) and Statistica (version 5, StatSoft. Inc., USA).

^{1}H-NMR, gravimetric and LC-DAD analyses, 18 populations. PCA explained 66.66 % of the total variance for the two main axes (axis 1, 40.10 %; axis 2, 26.56 %, Fig. 1).

The greatest influence on the formation of the first axis (axis 1) have the content of sesquiterpene lactones in BEM extracts of plants of the genus *Amphoricarpos* based on quantitative ^{1}H-NMR analysis ($w_{\text{lact BEM}}$), the content of sesquiterpene lactones in the whole dry leaves (SL extracts) based on gravimetric analysis ($w_{\text{lact SL}}$), and lactones **3**–**8** (in uniform proportion), and on the formation of the second axis (axis 2) lactones **1** and **19**. The plot of factor scores showed a tendency to form three groups. The first group consists of populations 1–3 characterized by higher content of lactones **3**, **4**, **7** and **8** (Table S-II, Supple-

mentary material). The second group consists of populations 4, 5 and 7 characterized by high content of lactones **9**, **11** and **13**. The third group is the least homogeneous and consists of other populations.

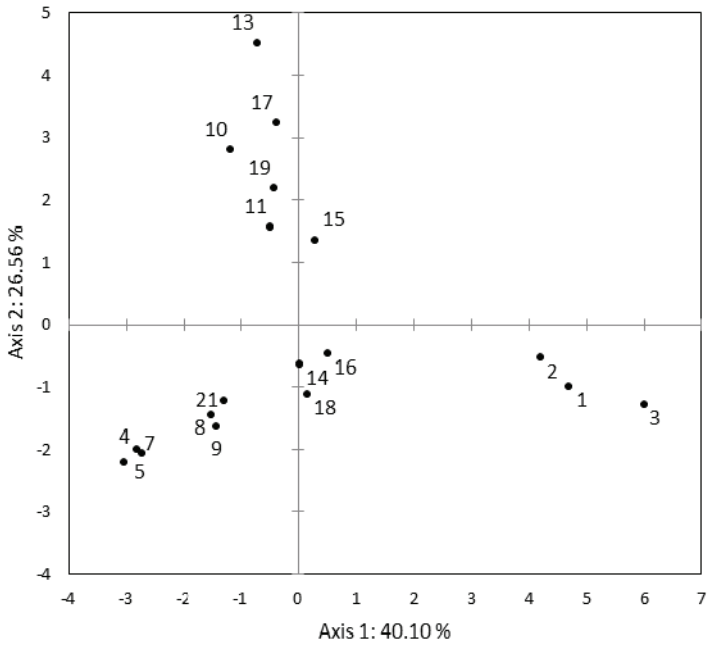


Fig. 1. Principle component analysis (PCA, Score plot) of 16 components (entries 1–16, Table S-II, Supplementary material) in 18 populations (1–5, 7–11, 13–19 and 21) of *Amphoricarpos* taxa.

This situation is clearly illustrated by cluster analysis (Fig. 2). At the higher distance of the cluster (upper gray line), two groups of taxa stand out corresponding to *A. neumayeri* (populations 1–3) and *A. autariatus* (the other 18 populations to the right). At the lower distance (bottom gray line) three groups of taxa stand out corresponding to *A. neumayeri* (populations 1–3), *A. autariatus* ssp. *autariatus* (populations 4, 5 and 7) and *A. autariatus* ssp. *bertisceus* (the other 12 populations).

The groundedness for the formation of the three groups previously suggested by PCA was verified by DA analysis (Fig. 3). The clear formation of the three groups obtained by DA analysis correspond to the defined taxa: *A. neumayeri* (group 1), *A. autariatus* ssp. *autariatus* (group 2) and *A. autariatus* ssp. *bertisceus* (group 3). Over 98 % of discrimination is explained by the first function (dis1), and lactones **4** and **7**, and the contents of sesquiterpene lactones in BEM ($w_{\text{lact BEM}}$) and SL ($w_{\text{lact SL}}$) plant extracts have the greatest influence on this group separation (based on standardized coefficients for canonical variables).

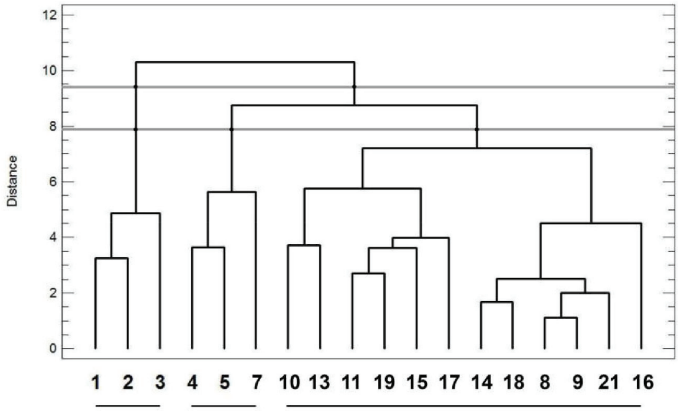


Fig. 2. Cluster analysis (CA) based on a 'furthest-neighbor method' (Euclidean distance) of the 18 studied populations (1–5, 7–11, 13–19 and 21). The numbers on the vertical axis refer to distance level, calculate based on differences between population contents of lactones. The numbers on the horizontal axis refer to the 18 studied populations.

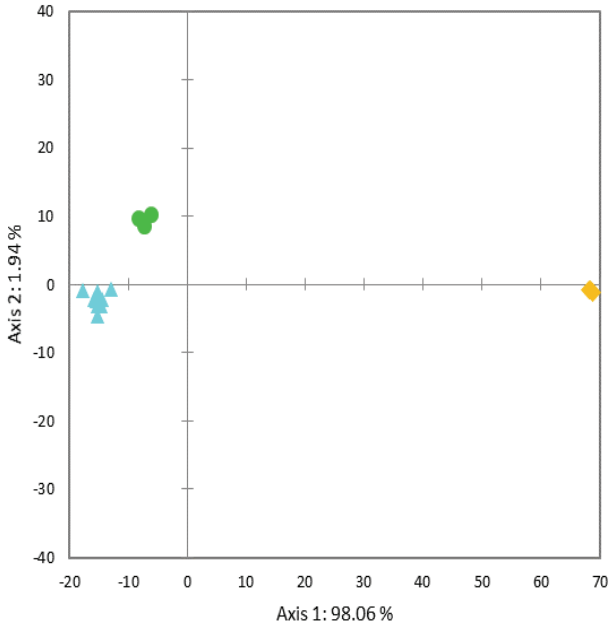


Fig. 3. Discriminant analysis (DA) of 16 components (entries 1–16, Table S-II, Supplementary material) of 18 populations (1–5, 7–11, 13–19 and 21) of *Amphoricarpos* taxa. Populations 1–3 (squares); populations 4, 5 and 7 (circles); populations 8–11, 13–19 and 21 (triangles).

LC–MS analysis, 20 populations. PCA explained 59.84 % of the total variance for the two main axes (axis 1, 40.39 %; axis 2, 19.45 %, Fig. 4).

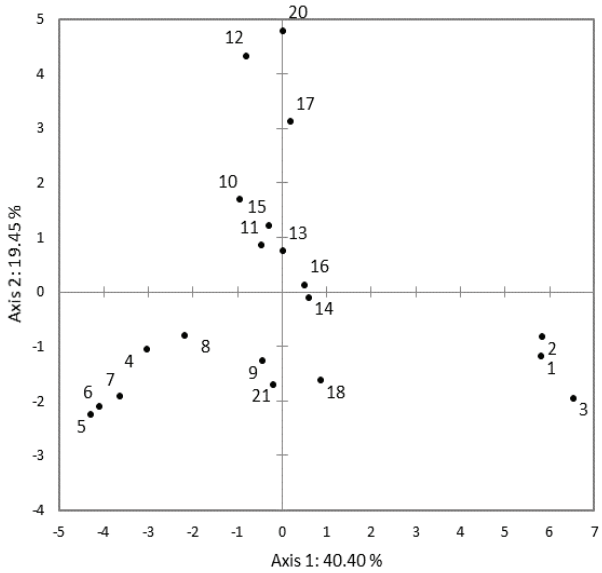


Fig. 4. Principle component analysis (PCA, Score plot) of 23 components (entries 17–39, Table S-II, Supplementary material) in 20 populations (1–18, 20–21) of *Amphoricarpos* taxa.

Lactones **3**, **4** and **8** in both, BEM and SL extracts (in uniform proportions) have the greatest influence on the formation of the first axis (axis 1), and lactone **17** in BEM extracts on the formation of the second axis (axis 2). The plot of factor scores showed a tendency to form three groups. The first group consists of populations 1, 2 and 3 characterized by a higher content of lactones **3**, **4**, **7** and **8** in both, BEM and SL extracts (Table S-II). The second group consists of populations 4–7 characterized by a higher content of lactones **11**, **13** and **14** in BEM, and lactone **13** in SL extracts. The third group is heterogeneous and consists of other populations. This situation is clearly illustrated by cluster analysis (Fig. 5).

Identical to the previous cluster analysis, three groups stand out. The basis of the formation of the three groups previously suggested by PCA was verified by DA analysis (Fig. 6).

The clear formation of the three groups obtained by DA analysis correspond to the defined taxa: *A. neumayeri* (group 1), *A. autariatus* ssp. *autariatus* (group 2) and *A. autariatus* ssp. *bertisceus* (group 3). Over 90 % of discrimination is explained by the first function (dis1) and the greatest influence on this group separation has lactone **11** in BEM extracts (and with this component highly correlated properties **14** in BEM, and **11** and **13** in SL extracts; $r > 0.7$, $P < 0.001$) and **3** in BEM extracts (and with this component highly correlated properties **8** in BEM, and **3** and **8** in SL extracts; $r > 0.7$, $P < 0.001$; based on correlation properties and standardized coefficients for canonical variables, not shown, r = correlation coefficient, P = probability).

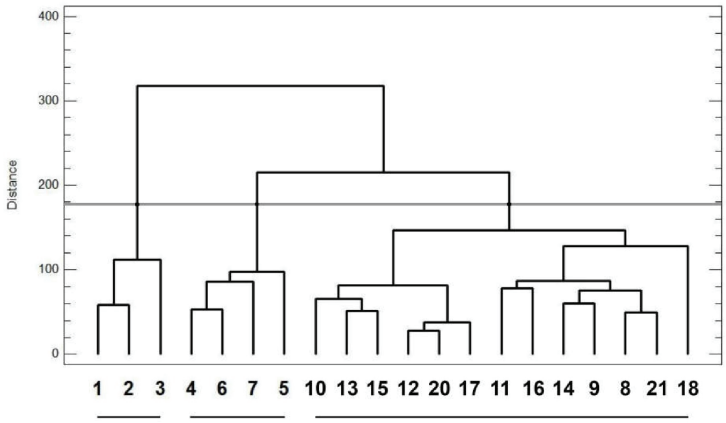


Fig. 5. Cluster analysis (CA) based on a 'furthest-neighbor method' (Euclidean distance) of the 20 studied populations (1–18, 20–21). The numbers on the vertical axis refer to distance level, calculate based on differences between population contents of lactones. The numbers on the horizontal axis refer to the 20 studied populations.

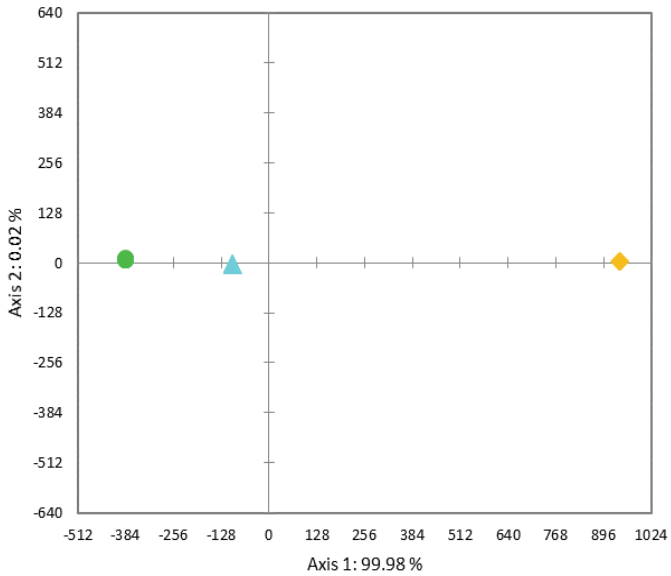


Fig. 6. Discriminant analysis (DA) of 23 components (entries 17–39, see Table S-II, Supplementary material) in 20 populations (1–18, 20 and 21) of *Amphoricarpos* taxa. Populations 1–3 (square); populations 4–7 (circle); populations 8–18, 20 and 21 (triangle).

The chemical analyses of sesquiterpene lactones (^{1}H-NMR, gravimetric, LC/DAD and LC–MS) combined with multivariate data analysis (PCA, DA and CA) discriminated three taxa of *Amphoricarpos* populations: 1) *A. neumayeri* – taxon characterized by higher content of lactones **3**, **4**, **7** and **8**, 2) *A. autariatus* ssp.

autariatus – taxon characterized by higher content of lactones **9**, **11** and **13** and 3) *A. autariatus* ssp. *bertisceus* – the least homogeneous taxon. Lactones **3**, **4**, **7** and **11**, as well as the contents of sesquiterpene lactones in BEM ($w_{\text{lact BEM}}$) and SL ($w_{\text{lact SL}}$) plant extracts have the greatest influence on taxon separation. Based on this, it can be concluded that the chemotaxonomic status of *Amphoricarpos* in Montenegro coincides with the taxonomic status previously defined by Blečić and Mayer[2].

2.5. Metabolomic study of the genus Amphoricarpos

In addition to the above (phytochemical) research, *Amphoricarpos* taxa was the subject of metabolomic analysis. Metabolomics is a new scientific multidisciplinary area that encompasses various aspects of biology, chemistry and mathematics. It uses modern spectroscopic and chromatographic techniques (NMR, IR, MS, GC, LC) and statistical (multivariate) data analysis (PCA, PLS, OPLS-DA, etc.) to measure quantitatively and qualitatively as many metabolites, in the studied organism, as possible and thus obtain a clear metabolic picture under the given conditions. Our metabolic study of the genus *Amphoricarpos* had two objectives: 1) new knowledge about the taxonomic status of the genus and 2) the development of a simple method for identification biologically active compounds in the plant extract.

2.6. Chemotaxonomic study of the genus Amphoricarpos *using metabolomics*

Experimental procedure of this metabolomic study included the following phases: 1) sample collection, 2) rapid sample drying using anhydrous silica gel, 3) preparing the extracts for NMR analysis, 4) recording ^{1}H-NMR spectra and 5) statistical processing of recorded spectra.[10]

1) The leaves specimens (58 in total) of all three taxa (according to Blečić and Mayer[2]) were collected in July 2014 during the flowering period in Montenegro, at seven locations (Sinjajevina, Lovćen, Tara Canyon, Planinica, Visitor, Vratlo, Orjen).

2) Immediately after collection, the samples were hermetically sealed in plastic bags together with anhydrous silica gel, with the aim of removing water as quickly as possible and thus prevent enzymes from chemically altering the sample.

3) Dried leaves were chopped in the laboratory mill under liquid nitrogen and then extracted with a mixture of deuterated methanol and D_2O (1:1) with the addition of buffer and internal NMR standard – TSP-d_4.

4) All ^{1}H-NMR spectra were recorded at 500 MHz under the same conditions. Typical sample spectra of all three examined taxa are shown in Fig. S-1 of the Supplementary material.

Identification of extract components in ^{1}H-NMR spectra was based on comparison with the spectra of reference compounds, previously isolated in our laboratories, and with the spectra of known compounds from the spectrum library.

Overlapping NMR signals were separated using two-dimensional (2D) NMR methods (COSY, TOCSY, ROESY, HSQC and HMBC).

As major chemotaxonomic markers amphoricarpolides hydroxylated in 2*α*-(**8**) and 9*β*-positions were detected (lactones **11** and **13**, Table II).

5) Statistical processing of multivariate NMR spectra analysis, using PCA methods and OPLS/DA, was performed using SIMCA software (version 14, Umetrics, Umeå, Sweden).

The application of these methods made clear differentiation of three supposed taxa – two species of which one is divided into two subspecies, as can be seen from the graph in Fig. 7.

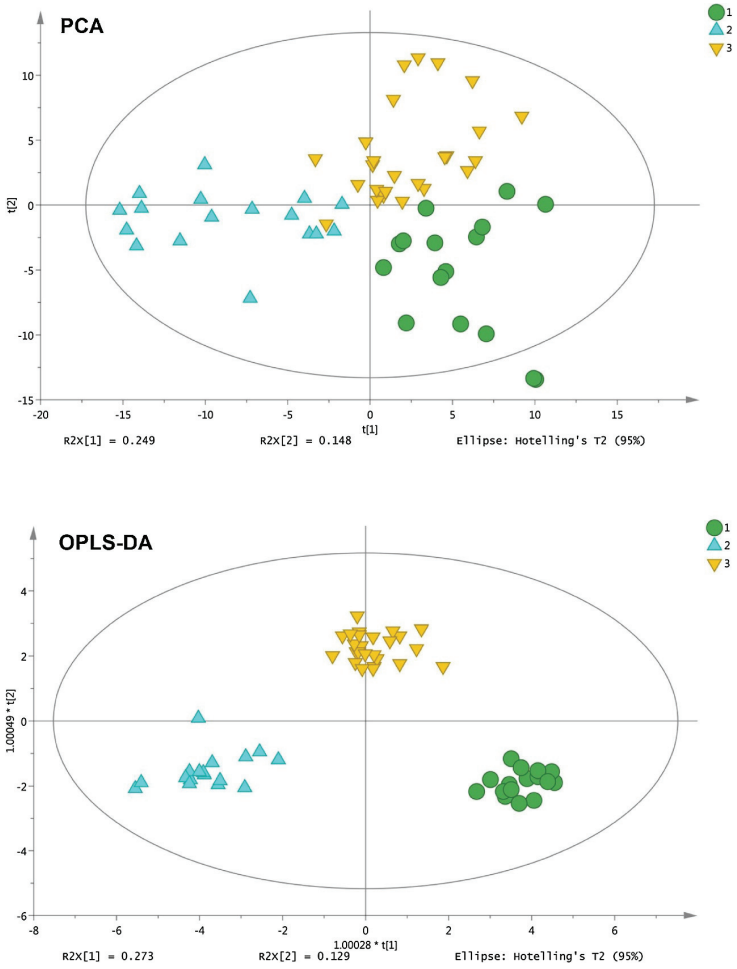


Fig. 7. Results of statistical PCA and OPLS/DA analysis of NMR spectra of extracts of the genus *Amphoricarpos*: 1 – *A. autariatus* ssp. *autariatus*, 2 – *A.autariatus* ssp. *bertisceus*, 3 – *A. neumayeri*.

The score t1 (first component) explains the largest variation of the *X* space, followed by t2 (second component); T2 – significance level of Hotelling's ellipse; R2X[1] – fraction of *X* variation modeled in the component; R2X[2] – cumulative R2X up to the specified component; 1.00028 t[1] – score of the first prediction component scaled proportional to R2X; 1.00049 t[2] – score of the second prediction component scaled proportional to R2X.

Based on the content of major chemotaxonomic markers, that is 2*α*-OH (**8**), 9*β*-OH (**11** and **13**) amphoricarpolides, the differentiation between the two types is demonstrated. Lactone **13** predominates in *A. neumayeri*, while **11** and **8** are characteristic of *A. autariatus.* At the same time different ratio of chlorogenic and malic acid content enabled the separation of two *A. autariatus* subspecies. In the subtype *bertisceus* chlorogenic acid is in excess, while in the subspecies *autariatus* malic acid is dominant. These results are in agreement with the conclusions which were previously given by Blečić and Mayer,[2] on the basis of morphological characteristics of the genus.

2.7. Metabolomic identification of cytotoxic metabolites from A. autariatus *ssp.* autariatus *by application of chromatography/spectroscopy/*in vitro *tests combinations*

A combination of dry-column flash chromatography (DCFC), two spectroscopic methods (NMR and FTIR) and cytotoxicity tests (on HeLa and A549 cervical cancer cells and lung cancer cells) was applied.[11]

Powdered dried leaves of *A. autariatus* ssp. *autariatus* were extracted at room temperature with a mixture of CH_2Cl_2-MeOH (1:1). The extract was separated into 13 fractions by DCFC (SiO_2, eluent CH_2Cl_2/MeOH, gradient 100/0 → 80/20). This chromatographic separation was performed in triplicate. All separated fractions were analyzed using two spectroscopic methods, ^{1}H-NMR and FTIR. At the same time, the *in vitro* cytotoxic effect of each fraction on HeLa and A549 cells was investigated. To correlate chemical composition of fractions, with the results of cytotoxic activity testing, an OPLS analysis was applied. According to the NMR analysis, the highest contribution to the cytotoxic activity was recorded for the fractions containing signals of sesquiterpene *γ*-lactones with characteristic guaianolide skeleton (lactones **11** and **13**, Table IV) which is in line with our previous cytotoxicity study of some amphoricarpolides isolated from the genus *Amphoricarpos*.[12] The results obtained by correlating FTIR data with cytotoxic activity confirmed the results obtained by NMR measurements. These results showed that the sesquiterpene *γ*-lactones may play a major role in cytotoxic activity of the studied extract. This is not unexpected since it is known that the conjugated *α*-methylene-*γ*-lactone group could be responsible for the cytotoxic functions.[13] To finally prove the result obtained from the OPLS models, two identified constituents of the active fractions (lactones **11** and **13**) were tested for cytotoxic activity on HeLa and A549 cell lines. Both reference compounds

exhibited considerable cytotoxic activity, corresponding to the activity obtained from the most effective fractions.

Coupling DCFC chromatography technique to NMR an FTIR based metabolomics and multivariate data analysis revealed the possibility for of the identification of biological active compounds, without prior isolation. This procedure provides a fast method for identification of biologically active compounds combining chromatography and NMR or FTIR spectroscopy techniques with bioassays and multivariate data analysis.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11002, or from the corresponding author on request.

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ИЗВОД

ФИТОХЕМИЈСКО ИСТРАЖИВАЊЕ БИЉНОГ РОДА *Amphoricarpos*

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Фитохемија се бави изучавањем секундарних метаболита које биљке синтетишу из много разлога, укључујући сопствену заштиту од напада биљоједа и од биљних болести. Сматра се да секундарни метаболити представљају адаптацију биљака на различите факторе околине и да су управо они омогућили опстанак врста. Секундарни метаболити биљака могу испољити лековито или токсично дејство на људе и животиње. Лечење биљем има дугу традицију у народној медицини и све до почетка 20. века, када је почела да се развија синтетичка органска хемија, биљке су биле главни извор лекова. Основни циљеви наших фитохемијских истраживања обухватају: изоловање и идентификацију нових (биолошки активних) једињења – потенцијалних лекова, и хемотаксономију (хемосистематика). У тексту су кроз један одабран пример – род *Amphoricarpos* Vis. – приказана оба наведена аспекта наших фитохемијских истраживања.

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SUPPLEMENTARY MATERIAL TO

Phytochemical study of the genus *Amphoricarpos*

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TABLE S-I. Volatile components profiles given as contribution of peak area of compounds relative to the total area of GC/FID signal from plants of genus *Amphoricarpos*

Component	RI[a]	t_R min	Contributions of peak are (relative to the total area), %						
			*OR04	**KD05	**KT05	***VIS05	***VK05	***POP05	***SINJ05
Hex-2*E*-enal	845	3.745	–	–	1.65	2.41	–	–	–
n-Nonane	900	4.102	–	1.17	–	0.79	–	–	–
1-Octen-3-ol	968	4.648	–	–	0.89	0.74	–	–	0.58
Dehydro-1,8-cineole	991	4.884	0.83	0.72	1.46	0.80	–	1.51	1.39
(*2E*,*4E*)-Hepta--2,4-dienal	1010	5.039	–	0.88	1.76	–	2.30	–	1.25
Benzeneacetaldehyde	1042	6.101	4.76	3.70	2.84	0.99	2.09	3.27	4.44
5-Methyl-4--hexen-3-one		6.411	–	–	–	–	0.93	–	–
3,5-Octadiene--2-one		6.767	–	4.07	0.84	–	1.55	–	0.73
n-Nonan-1-al	1090	7.745	1.82	3.02	5.26	–	7.08	5.02	5.00
Phenethyl alcohol	1110	7.956	–	–	0.87	–	–	–	0.83
Camphor	1143	8.833	–	–	–	–	–	3.58	–
Nonan-1-ol	1171	9.741	–	0.43	0.84	–	1.58	0.87	0.73
Decanal	1198	10.722	5.28	12.83	13.58	4.20	11.98	8.52	9.96
2-Decenal	1261	12.908	–	–	–	–	0.73	–	–

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Component	RI[a]	t_R min	Contributions of peak are (relative to the total area), %						
			*OR04	**KD05	**KT05	***VIS05	***VK05	***POP05	***SINJ05
Pelargonic acid	1275	13.070	–	–	1.23	–	1.46	0.90	–
Indole	1288	13.390	–	0.43	0.57	0.70	1.47	1.48	2.83
(*E*,*E*)-*2*,4-Decadienal	1312	14.069	–	0.64	0.97	–	2.67	–	0.84
Capric acid		15.971	–	–	–	–	0.72	–	–
Sativen		16.238	1.13	2.88	3.48	0.49	–	2.13	2.17
β-Caryo-phyllene	1414	17.028	6.80	1.55	0.90	3.38	–	1.57	0.84
Geranyl acetone	1451	18.025	1.34	0.49	0.48	0.83	0.87	–	–
β-Ionone	1486	18.982	–	0.72	0.73	–	1.33	–	1.16
β-Selinene	1490	19.084	1.07	1.55	1.43	0.61	1.93	1.29	1.32
γ-Cadinene	1513	20.060	1.33	0.50	0.55	1.05	0.75	–	–
δ-Cadinene	1524	20.214	1.35	1.41	1.02	1.04	1.53	1.69	2.64
Caryophyllene oxide	1584	21.840	37.68	36.68	28.53	53.07	10.96	24.86	24.07
Longifolenalde hyde		22.309	–	0.51	–	–	–	–	–
Humulene epoxide II	1606	22.443	–	0.47	–	0.70	–	–	–
Diepicedrene-1-oxide		23.246	5.65	6.15	3.70	7.76	1.22	3.45	2.87
cis-6-Do-decen-4-olide		23.771	3.11	1.44	1.65	2.49	1.73	0.94	1.24
Myristic acid		26.781	2.11	–	1.11	–	1.95	1.73	1.26
Hexahydropha rensyl acetone	1849	28.709	2.72	1.96	1.79	1.42	2.83	3.49	4.17
Palmitic acid	2003	31.769	9.93	7.50	8.18	4.33	18.39	17.40	15.52
n-Heneicosane	2100	34.584	2.07	0.62	1.08	0.80	5.68	3.46	1.62
Linolenic acid	2170	35.391	1.56	–	0.73	–	1.07	–	1.32
Oleic acid	2175	35.527	–	–	1.08	–	3.14	4.89	1.62
n-Tricosane	2302	38.771	–	0.62	–	–	–	–	–
n-Heptacosane	2704	46.228	0.97	0.79	0.93	–	1.94	1.54	1.90
n-Nonacosane	2898	49.578	1.01	0.67	1.02	–	2.21	1.64	1.45
Total			92.52	94.71	91.15	90.90	92.09	95.23	94.37

**A neumayeri* Vis., ***A. autariatus* ssp. *autariatus* and ****A. autariatus* ssp. *Bertisceus* (according to Blečić and Mayer[2]); [a]Retention indices (RI) are calculated based on the retention times of the *n*-alkanes analyzed under the same conditions

TABLE S-II. Parameters for statistical analysis obtained by quantitative ^{1}H-NMR, gravimetric, LC-DAD and LC-ESI-MS analyses of extracts of plants of the genus *Amphoricarpos*

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Code		OR04	OR05	OR07	KT04	KT05	KT11	KD05	SINJ04	SINJ05	PR01	ZEL02	ZEL09	VIS04	VIS05	VIS07	VK05	PLK05	POP05	KOT06	KOT011	GR07
Entry	Component	Content of sesquiterpene lactones in crude extracts of aerial parts (BEM) or on leaf-surface (SL) of various species of the genus *Amphoricarpos*, %.																				
1	$w_{\text{lact BEM}}$	1.76	1.60	2.26	0.52	0.57	–	0.52	0.86	0.97	0.61	0.75	–	0.93	1.04	1.06	0.83	0.72	1.12	0.36	–	0.52
2	$w_{\text{lact SL}}$	1.76	2.23	3.36	1.29	1.42	–	1.28	1.60	1.51	1.04	1.64	–	1.48	1.60	1.66	1.04	1.93	1.58	1.20	–	1.02
Content of the most abundant lactones in SL extracts based on the areas of LC/DAD peaks, % of all peaks detected at 210 nm.																						
3	**1**	0.00	0.00	0.00	0.00	0.00	–	0.00	2.25	0.00	4.67	6.85	–	17.28	0.90	15.17	0.90	15.46	0.90	16.10	–	0.90
4	**2**	6.92	12.03	4.41	0.00	0.00	–	0.00	1.79	2.35	4.28	3.98	–	1.54	6.16	4.19	14.95	2.05	3.18	4.86	–	1.87
5	**3**	23.19	33.85	19.99	0.00	0.00	–	0.00	4.59	5.58	3.48	10.57	–	0.90	14.11	10.65	24.42	10.16	11.85	7.87	–	8.29
6	**4**	18.24	7.08	20.30	0.00	0.00	–	0.00	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–	0.00
7	**7**	6.57	2.01	8.85	0.00	0.00	–	0.00	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–	0.00
8	**8**	13.94	11.41	12.36	0.00	0.00	–	0.00	3.45	3.13	0.00	1.12	–	2.75	4.41	3.33	4.26	1.92	8.49	8.85	–	5.43
9	**9**	0.00	0.00	0.00	0.90	21.57	–	21.65	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	0.00	0.00	0.00	0.00	–	0.00
10	**11**	0.00	0.00	0.00	38.59	7.80	–	39.52	22.80	25.10	6.03	5.07	–	7.20	10.94	4.65	9.47	7.23	18.21	2.68	–	20.43
11	**13**	5.16	2.00	4.70	25.55	21.39	–	26.90	27.39	31.55	9.70	18.65	–	5.24	24.56	10.53	17.54	11.54	17.55	14.98	–	19.76
12	**14**	0.00	0.00	0.00	16.23	7.80	–	2.12	10.67	7.38	2.48	1.27	–	0.90	3.25	1.31	6.13	1.18	4.86	2.51	–	8.43
13	**16**	0.00	0.00	0.00	0.00	0.00	–	0.00	0.90	0.00	0.90	0.90	–	6.57	0.90	2.85	0.00	8.07	0.00	0.90	–	0.00
14	**19**	0.00	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	8.76	3.46	–	7.82	0.90	0.90	0.90	2.64	0.00	4.53	–	0.00
15	**20**	0.00	0.00	0.00	0.00	0.00	–	0.00	0.00	0.00	5.14	0.90	–	6.99	0.00	0.90	0.90	0.90	0.00	0.90	–	0.00
16	**22**	0.00	0.00	0.00	0.00	0.00	–	0.00	0.00	0.90	3.48	10.57	–	0.00	0.90	0.00	0.90	10.16	0.00	7.87	–	0.00
Content of the lactones in BEM extracts based on the areas of LC/ESI ToF MS (positive mode) peaks, % of all peaks detected.																						
17	**2**	6.34	5.58	2.43	0.36	0.24	0.29	0.06	1.84	1.44	1.54	3.70	4.42	1.76	3.04	0.05	9.41	1.71	3.63	–	2.65	0.96
18	**3**	24.43	30.65	14.31	0.91	1.46	0.44	0.03	4.94	5.58	6.75	11.66	2.52	6.57	11.31	1.57	17.75	2.47	8.06	–	1.76	7.79
19	**4**	11.40	19.88	39.28	0.95	1.19	0.28	1.32	4.73	4.54	2.90	6.74	0.91	10.49	9.66	5.55	4.45	2.21	13.69	–	1.93	6.61
20	**5**	0.19	0.10	1.45	0.40	0.56	0.85	0.55	3.07	2.06	0.11	1.79	0.85	0.22	0.36	0.04	0.19	0.17	2.26	–	1.04	0.92

Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Code		OR04	OR05	OR07	KT04	KT05	KT11	KD05	SINJ04	SINJ05	PR01	ZEL02	ZEL09	VIS04	VIS05	VIS07	VK05	PLK05	POP05	KOT06	KOT011	GR07
21	**6**	4.46	3.57	7.95	0.23	0.22	0.03	0.14	0.06	4.01	0.19	0.88	0.51	0.64	1.68	0.33	0.64	0.53	0.30	–	0.98	5.24
22	**7**	12.55	3.99	9.87	0.42	0.38	0.06	0.40	1.48	6.49	0.24	0.23	0.12	2.45	2.46	0.17	0.06	0.29	6.12	–	1.39	2.01
23	**8**	14.33	12.67	5.82	0.40	0.29	0.21	0.01	2.01	1.72	1.98	1.54	0.12	1.38	3.12	0.50	2.96	1.61	7.05	–	0.11	3.03
24	**11**	7.26	5.02	3.04	32.26	44.03	32.78	36.05	24.87	10.56	4.70	11.44	5.12	13.97	11.00	2.37	13.52	3.84	23.47	–	1.18	19.90
25	**13**	7.34	11.81	10.70	46.31	23.22	45.72	59.47	31.95	39.38	23.14	14.68	9.67	10.52	31.45	13.58	30.33	7.30	14.11	–	9.16	31.83
26	**14**	0.28	0.25	0.05	10.05	14.48	10.58	0.89	8.36	7.91	0.74	1.83	2.15	2.03	3.35	0.16	5.78	2.54	5.10	–	1.38	3.92
27	**15**	0.19	0.14	0.08	1.12	0.88	0.54	0.42	4.37	1.92	0.97	4.65	5.98	0.62	0.97	0.07	1.75	0.55	0.42	–	5.29	0.24
28	**17**	0.07	0.05	0.04	0.03	0.02	0.03	0.02	0.06	0.02	0.40	0.39	1.67	0.21	0.08	0.31	0.15	0.89	0.10	–	2.35	0.01
		Content of the lactones in SL extracts based on the areas of LC/ESI ToF MS (positive mode) peaks, expressed as a percentage of all peaks detected.																				
29	**2**	6.18	7.67	3.39	2.39	0.27	0.29	0.08	3.02	3.62	0.05	3.90	3.93	1.59	5.47	2.05	11.64	5.10	4.00	–	4.47	1.73
30	**3**	21.14	30.84	15.59	0.91	0.54	0.15	0.10	6.01	6.67	0.59	10.63	4.70	0.77	6.06	10.40	15.92	5.70	9.97	–	7.45	9.42
31	**4**	22.73	20.21	33.05	0.52	1.50	0.43	1.54	3.09	5.01	1.17	4.30	2.15	6.71	5.22	6.36	2.38	2.95	10.23	–	1.50	3.77
32	**5**	0.16	0.63	0.05	5.27	0.24	0.84	0.79	0.13	5.79	0.06	0.30	1.06	0.92	0.28	0.26	0.24	3.79	4.37	–	2.04	1.32
33	**6**	2.64	3.22	9.42	0.70	0.32	0.02	0.33	0.20	3.60	0.04	0.86	0.16	1.46	4.53	1.42	0.10	3.10	0.09	–	3.62	4.95
34	**7**	3.48	4.95	10.37	1.43	0.16	0.11	0.58	2.03	3.79	0.12	0.18	0.16	2.62	1.78	0.21	0.04	4.36	4.02	–	4.27	1.48
35	**8**	10.75	9.71	7.35	2.46	0.16	0.37	0.07	1.95	2.22	0.23	1.08	0.23	2.56	4.21	1.86	3.97	2.97	8.27	–	0.24	4.20
36	**11**	2.19	4.68	4.10	6.95	30.00	36.74	25.48	19.04	5.59	4.67	12.05	0.30	4.53	4.67	6.38	6.05	1.27	19.48	–	0.60	20.74
37	**13**	14.29	9.01	12.42	43.07	45.76	48.73	66.43	32.36	35.67	0.11	35.86	1.17	13.90	29.95	12.16	31.97	0.75	19.02	–	2.16	28.62
38	**14**	0.22	0.15	0.11	5.67	13.27	4.83	3.20	8.54	8.10	0.26	2.14	2.64	1.77	4.45	1.26	8.67	5.74	6.74	–	3.93	6.95
39	**17**	0.19	0.23	0.06	0.32	0.03	0.02	0.03	0.33	0.06	0.47	0.22	2.07	0.69	0.82	0.35	0.12	3.51	0.08	–	2.91	0.06

OR – Orjen, KT – Tara Canyon, KD – Draga Canyon, SINJ – Sinjajevina, PR – Prokletije, ZEL – Zeletin, VIS – Visitor, VKOM – Vasojević's Kom, PLK – Planinica Kom, POP – Popadija, KOT – Kotlovi, GR – Greben; 01, 02, 04, 05, 06, 07, 09, 11 – year (2001, 2002, 2004, 2005, 2006, 2007, 2009, and 2011, respectively); BEM – crude extract of grounded dried aerial parts of plants in the petrol ether–diethylether–methanol (1:1:1) mixture; SL – polar (methanol soluble) fraction of dichloromethane extract of intact air-dried leaves; LC/DAD – liquid chromatography with diode-array detector; LC/ESI ToF MS – liquid chromatography with (electrospray ionization) time-of-flight mass spectrometer as detector

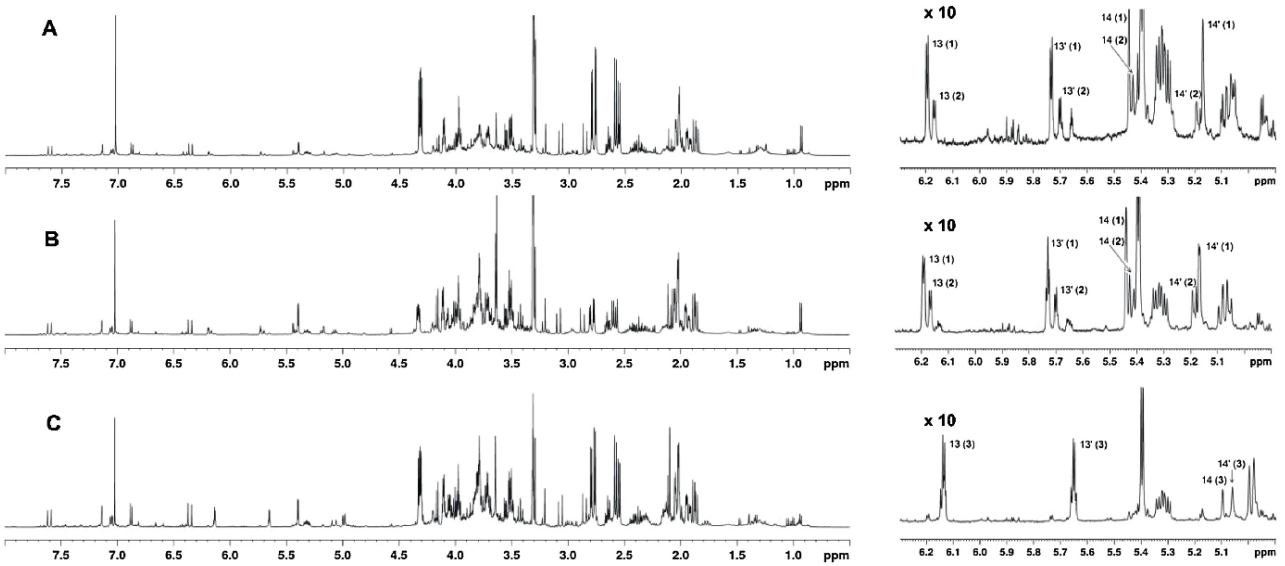

Fig. S-1. Examples of ^{1}H-NMR spectra of the extracts of all three examined taxa; (A) – *A. autariatus* ssp. *autariatus*, (B) – *A. autariatus* ssp. *bertisceus*, (C) – *A. neumayeri*; characteristic signals of amphoricarpolides in the spectral range 4.9–6.3 ppm are magnified 10 times (right part of the picture).

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GC–MS-based metabolomics for the detection of adulteration in oregano samples

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Abstract: Oregano is one of the most used culinary herb and it is often adulterated with cheaper plants. In this study, GC–MS was used for identification and quantification of metabolites from 104 samples of oregano (*Origanum vulgare* and *O. onites*) adulterated with olive (*Olea europaea*), venetian sumac (*Cotinus coggygria*) and myrtle (*Myrtus communis*) leaves, at five different concentration levels. The metabolomics profiles obtained after the two-step derivatization, involving methoxyamination and silanization, were subjected to multivariate data analysis to reveal markers of adulteration and to build the regression models on the basis of the oregano-to-adulterants mixing ratio. Orthogonal partial least squares enabled detection of oregano adulterations with olive, Venetian sumac and myrtle leaves. Sorbitol levels distinguished oregano samples adulterated with olive leaves, while shikimic and quinic acids were recognized as discrimination factor for adulteration of oregano with venetian sumac. Fructose and quinic acid levels correlated with oregano adulteration with myrtle. Orthogonal partial least squares discriminant analysis enabled discrimination of *O. vulgare* and *O. onites* samples, where catechollactate was found to be discriminating metabolite.

Keywords: chromatography; PCA; OPLS; *Origanum vulgare*; *Origanum onites*; *Olea europaea*; *Cotinus coggygria*; *Myrtus communis*.

INTRODUCTION

The global industry that produces herbs and spices is continuously growing, giving a lot of space for motivated adulteration. Thus, developing new analytical methods is highly desirable. Use of spectroscopic techniques combined with

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chemometrics tools, for fraud detection in industry, has increased. Fast, reliable, and efficiant techniques are required to check the authenticity of food.[1,2]

Oregano is one of the most used culinary herbs for pizzas and Mediterranean food.[3] Because of that it is often adulterated with cheaper plants for dilution.[4] Commercially available oregano is usually mixed with other plants, such as cistus, olive tree leaves, myrtle, sumac, and others. Tolerable impurities in oregano, to be sold, of *O. vulgare* and *O. onites* are up to 2 %.

Hitherto, several instrumental techniques utilized to access quality of oregano, such as Fourier transform infrared spectroscopy (FTIR), liquid chromatography–high resolution mass spectrometry (LC–HRMS) and nuclear magnetic resonance spectroscopy (NMR).[5–7] A GC–MS test is also described for detection of oregano adulteration with olive leaves. By injecting of crude ethanolic extracts into GC–MS system, two chromatographic peaks with unknown chemical structures are reported to be markers of the adulteration.[8,9]

In our study, GC–MS was used for identification and quantification of the primary and secondary metabolites, in 104 samples, of oregano adulterated with olive (*Olea europaea*), Venetian sumac (*Cotinus coggygria*) and myrtle (*Myrtus communis*) leaves, at five different concentration levels. The metabolomics profiles obtained after two-step derivatization involving methoxyamination and silanization were subjected to multivariate data analysis to reveal markers of adulteration and to build the regression models on the basis of the oregano-to-adulterants mixing ratio.

EXPERIMENTAL

Plant sample collection

Two grounded samples of *Origanum vulgare*, one of *Origanum onites* and one made of the two mixed species were obtained from commercial sources. Corresponding vouchers of the crude, grounded drugs (BO23ABZOR, BO21ZOR, BO22AAOR and BO6FOR) were deposited in Department of Pharmacy and Biotechnology, University of Bologna (Via Irnerio 42, Bologna, Italy). The leaves of *O. europaea*, *M. communis* and *C. coggygria* were harvested in the Botanical Garden of Bologna (Italy) in September 2019, the samples were identified by Prof. Ferruccio Poli, and voucher specimens (BOLO0602019, BOLO0602020, BOLO0602021) were retained at the Herbarium of Alma Mater Studiorum University of Bologna (SMA, Via Irnerio 42, 40126, Bologna, Italy).

Morphological analysis of the oregano samples was performed following the identification suggested by 9th European Pharmacopoeia[10] and using the oregano taxonomic key presented in Ietswaart, as described in the paper by Mandrone *et al.*[6,11]

Sample preparation

Four samples of oregano were mixed with dried and grounded leaves of three adulterants: Venetian sumac (*C. coggygria*), myrtle (*M. communis*) and olive (*O. europaea*) to make binary mixtures in mass ratios 1, 5, 10 and 20. Two aliquots were taken of each sample, resulting in total of 96 adulterated samples and 8 intact oregano samples, which are all used for further analysis. The samples were then prepared for GC–MS analysis by modified litera-

ture procedure.[12,13] Plant samples were further powdered with electrical mill (IKA, A11 basic, Merck, Italy). Each sample (20 mg) was extracted, with 1 mL mixture of methanol and water (1:1 volume ratio) with addition of 250 μL 2-deoxy-ribose as internal standard (1 mg/mL) on ultrasonic bath (Sonorex Super RK 100, Bandelin, Berlin, Germany) for 5 min. After extraction, samples were centrifuged for 5 min at 15.000 rpm (DLAB D2012 plus, Beijing, China), then 200 μL of supernatant were transferred into eppendorf tube and concentrated under flow of nitrogen up to 100 μL and then stored in the freezer at –20 °C. Samples were lyophilized and then dissolved in pyridine (containing methoxyamine-hydrochloride, concentration 20 mg/mL, that was freshly prepared) and transferred into a glass vial. First step of derivatization was carried out in a sand bath for 2 h at 40 °C. For second step, 100 μL of BSTFA was added, and the mixture was kept for 60 min at 80 °C. Before analyzed on GC–MS, samples were cooled at room temperature.

GC–MS Analysis

The GC–MS analyses of the samples were performed on Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) with a 5975C mass selective detector (MSD) and a FID connected by capillary flow technology through a two-way splitter. A HP-5MSI, non-polar capillary column (30 m×0.25 mm, 0.25 μm film thickness), was used. Column temperature started from 60 to 270 °C at a rate of 3 °C/min, then heated 20 °C/min to 310 °C with 8 min hold at the end. Carrier, auxiliary and make up gas was helium, inlet pressure was 20.6 psi (flow 1.0 mL/min at 210 °C), auxiliary pressure was 3.8 psi and FID make up flow was 25 mL/min. Mass spectra obtained by electron ionization with 70 eV at 200 °C. Quadrupole temperature was set to 150 °C and MS range was 40–900 amu. FID temperature was 300 °C, split ratio was 5:1 and injection volume was 1 μL for all analyses.

Data processing

Library search and compound identification were performed using the MSD ChemStation software, version E02.02 (Agilent Technologies, Santa Clara, CA, USA), the NIST AMDIS software, version 2.70, and the commercially available libraries Wiley07, NIST17 and Adams04.

All the MS chromatograms were converted to AIA format using the MSD ChemStation software. The peak picking, nonlinear peak alignment, and matching of the retention times were then carried out utilizing XCMS online platform which is based on the R software.[14,15] CentWave feature detection algorithm was applied, with 100 ppm maximal tolerated m/z deviation in consecutive scans, 5 s minimum chromatographic peak width, 10 s maximum chromatographic peak width, 0.01 minimum difference in m/z for peaks with overlapping retention times, and value 6 as signal/noise threshold. For the peak alignment, 10 s used as allowable retention time deviations, 0.5 as minimum fraction of samples necessary in at least one of the sample groups for it to be a valid group and 0.5 as width of overlapping m/z slices to use for creating peak density chromatograms and grouping peaks across samples. A total of 104 chromatograms were used for processing which resulted in 557 features detected, and all used in the multivariate analysis.

The data in the table obtained from XCMS online platform were normalized to the content of internal standard (2-deoxy-ribose), and subjected to multivariate data analysis.

Principal component analysis (PCA), orthogonal partial least squares to latent structures (OPLS), and orthogonal partial least squares to latent structures – discriminant analysis (OPLS-DA) methods were performed using SIMCA software (version 15, Sartorius, Göttingen, Germany). The data were mean centered and scaled to unit variance.

RESULTS AND DISCUSSION

Analysis of GC–MS chromatograms of oregano samples

The metabolomes of the intact and adulterated oregano samples were analyzed by GC–MS after two-step derivatization involving methoxyamination and silanization. On this way, even polar metabolites can be introduced in GC–MS system, enabling identification of broad spectrum of the primary and secondary metabolites present therein.

The most abundant compounds in the intact oregano samples were sugars, primarily disaccharide sucrose, and monosaccharides such as glucose and fructose. Next, organic acids such as quinic, citric, malic, γ-aminobutyric, shikimic, rosmarinic, caffeic, succinic acid, ester catechollactate, phosphoric acid, sugar acids such as threonic, erythronic, and glucaric acid, alcohols were present, mainly myo-inositol, sorbitol, xylitol, glycerol and galactinol were detected. Monoterpene carvacrol as main constituent of oregano aroma was also identified. The representative MS chromatograms of oregano sample are depicted on Fig. 1, and in Supplementary material to this paper.

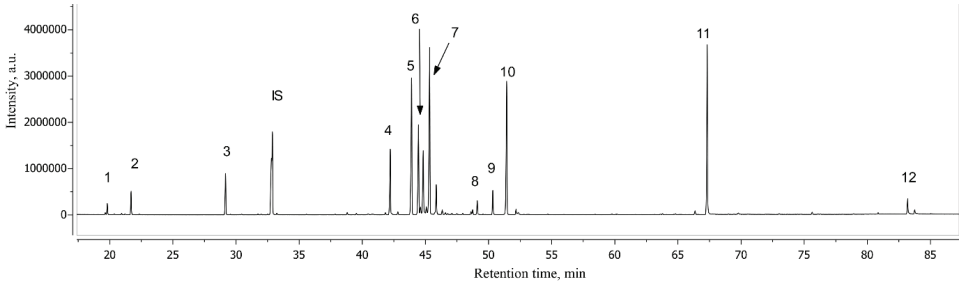

Fig. 1. GC-FID chromatogram of derivatized sample of *Origanum vulgare*. 1 – glycerol, 2 – carvacrol, 3 – malic acid, 4 – citric acid, 5 – quinic acid, 6 – fructose, 7 – glucose, 8 – glucaric acid, 9 – catechollactate, 10 – myo-inositol, 11 – sucrose, 12 – rosmarinic acid and IS – internal standard 2-deoxy-ribose.

Multivariate data analysis

All the 104 samples (8 intact oreganos, 96 samples adulterated with Venetian sumac, myrtle and olive to make binary mixtures in mass ratios 1, 5, 10 and 20) GC–MS metabolomics profiles were subjected to multivariate data analysis. Firstly, PCA as an unsupervised variable reduction technique was performed. On this way, smaller number of novel variables that will account for most of the variation in the observed variables developed. It has resulted in 8 principal components (PCs) model explaining 87.6 % of the total data variance.

Based on PCA score plot of the first two PCs, no grouping was observed according to the adulterations with different plants (Fig. 2a). Interestingly, the

samples were separated in some degree in PC1/PC2 space due to different botanical origin of oregano, regardless of degree of adulteration up to 20 % (Fig. 2b).

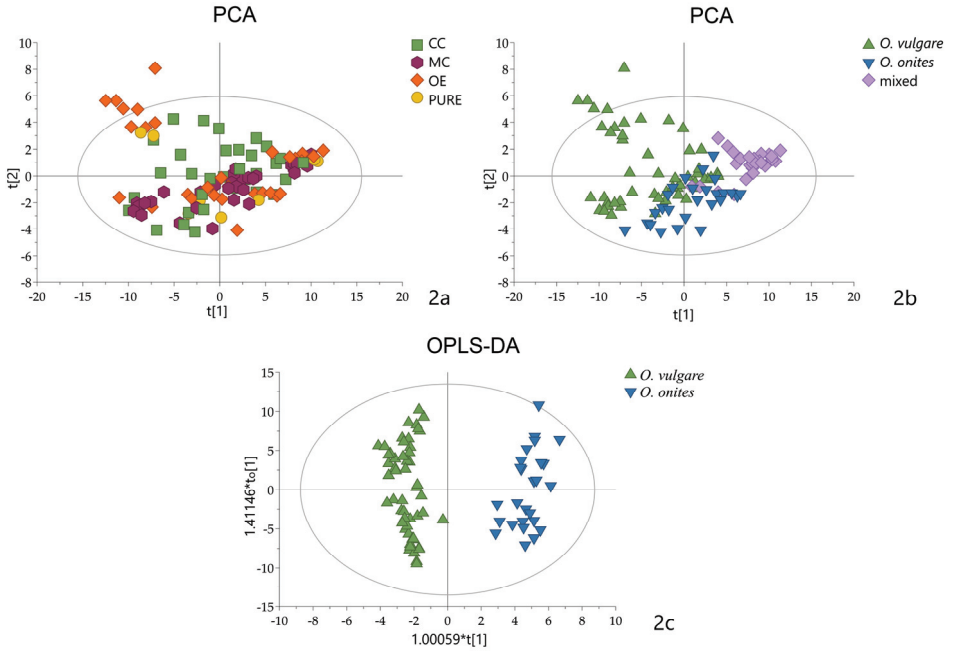

Fig. 2. a) and b) Principal component analysis (PCA) score plot (PC1 *versus* PC2) of all studied samples. The scores are colored according to the (a) adulterants added: OE – olive (*Olea europaea*) leaves, CC – Venetian sumac (*Cotinus coggygria*) leaves, MC – myrtle (*Myrtus communis*) leaves, and PURE – intact oregano samples; b) oregano species: *O. vulgare*, *O. onites*, and mixture of two *O.* species; (**c**) OPLS-DA score plot comprising oregano species *O. vulgare* and *O. onites.*

Next, OPLS-DA as supervised technique was performed. With this technique, novel variables will account for maximum separation between two predefined classes, in this case – *O. vulgare* and *O. onites* as botanical origin of the oregano samples. The samples of mixed origin were set aside. Since in the orthogonal model systematic variation of the variables is divided into two parts: one linearly related to the class information and one orthogonal to it, is the model interpretation is therefore facilitated.[16] Hence, OPLS-DA is suitable for finding variables that have the greatest discriminatory power between two predefined classes.

The quality of the obtained model was assessed by goodness of fit (R^2) indicating how well the variation of variables is explained using the predictive components and predictive ability of the model (Q^2) indicating how well the model predicts new data, as estimated by cross validation. Thus, R^2 and Q^2 values over

0.9 and close to 1 (maximum value) indicated remarkable goodness of fit, and predictive ability (Table I).

The OPLS-DA model was then validated by permutation test and CV-ANOVA. In the permutation test, the R^2 and Q^2 values of the original models were compared with the values of series of models with randomly perturbed class information. The model was considered satisfactory since regressions of Q^2 line intersected the vertical axis at below zero, and all Q^2 and R^2 values of permuted Y vectors were lower than the original ones. Similarly, the model was significant according to CV-ANOVA, with p value less than 0.05 (Table I).

TABLE I. Parameters of the multivariate analysis models

Model name	No. of components	R^2	Q^2	p (CV-ANOVA)	F (CV-ANOVA)
PCA	8	0.876	0.682	–	–
OPLS-DA, oregano species	1+2	0.950	0.926	4×10^{-38}	149
OPLS, adulteration with OE	1+2	0.914	0.830	5×10^{-11}	26
OPLS, adulteration with CC	1+5	0.983	0.910	5×10^{-11}	23
OPLS, adulteration with MC	1+4	0.938	0.753	2×10^{-5}	8

The score plot of the OPLS-DA model indicated good separation between classes along the predictive components (Fig. 2c). The selection of the most influential variables was based on variable influence on projection scores of the predictive components (VIPpred). Variables with the VIPpred score above 1.4 were considered as important for the separation. Thus, catechollactate was found to be discriminating metabolite for *O. onites* samples.

Three OPLS multivariate regression models created corresponding adulteration of oregano with Venetian sumac, myrtle and olive leaves. Five different oregano-to-adulterants mixing ratio levels used in each model. Excellent goodness of fit and predictive ability was obtained according to Q^2 and R^2 values. According to CV-ANOVA, the OPLS models were significant with $p < 0.05$ (Table I). This is also in accordance with the results of the permutation test. According to the score plots of the OPLS models, oregano samples with lower grades of adulteration were clearly separated from those with the higher grades (Fig. 3). The criterion for the selection of the most influential variables in the OPLS models was VIPpred score above 1.4, and loadings scaled as a correlation coefficient above 0.3. Using the above-mentioned criteria sorbitol was found to be the most influential variable in the model of adulteration of oregano with olive leaves. Adulteration of oregano with Venetian sumac resulted in appearance of shikimic and quinic acids as the most influential variable in the model. Similarly, fructose and quinic acid occurred as markers in the model of oregano adulteration with myrtle.

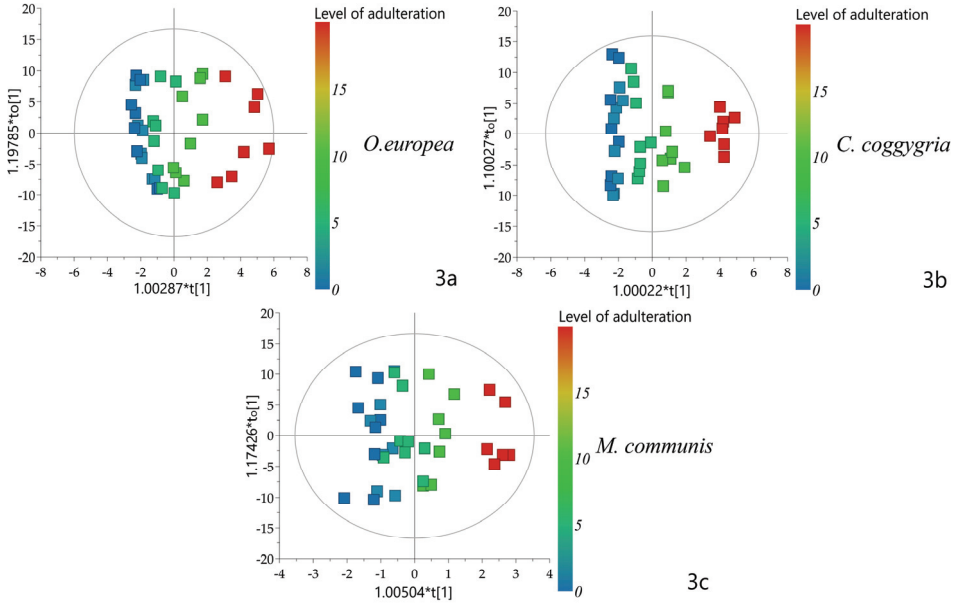

Fig. 3. OPLS score plot comprising oregano adulterated with: a) olive (*Olea europaea*) leaves, b) Venetian sumac (*Cotinus coggygria*) leaves, c) myrtle (*Myrtus communis*) leaves. The scores are colored according to the level of adulteration.

CONCLUSION

The GC–MS-based metabolomics approach has demonstrated to be a very reliable technique for the detection of oregano adulteration. Although the GC–MS technique is primarily intended for nonpolar and volatile samples, the application of the two-step derivatization procedure involving methoxyamination and silanization enabled analysis of more polar metabolites. The utilization of PCA and OPLS multivariate analysis methods on GC–MS profiles, obtained after two-step derivatization of samples, enabled detection of oregano adulterations with olive (*Olea europaea*), Venetian sumac (*Cotinus coggygria*), and myrtle (*Myrtus communis*) leaves.

According to the results, sorbitol levels distinguished oregano samples adulterated with olive leaves, while shikimic and quinic acids were recognized as discrimination factor for adulteration of oregano with Venetian sumac. Fructose and quinic acid levels correlated with oregano adulteration with myrtle.

The proposed workflow confirmed the potential of GC–MS in combination with chemometrics to detect adulteration of food-based products.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11045, or from the corresponding author on request.

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ИЗВОД

МЕТАБОЛОМИКА ЗАСНОВАНА НА ГАСНОЈ ХРОМАТОГРАФИЈИ–МАСЕНОЈ СПЕКТРОМЕТРИЈИ ЗА ДЕТЕКЦИЈУ КРИВОТВОРЕЊА УЗОРАКА ОРИГАНА

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Оригано је једна од најчешће коришћених кулинарских биљака и често се кривотвори јефтинијим биљкама. У овој студији, гасна хроматографија–масена спектрометрија коришћена је за идентификацију и квантификацију метаболита из 104 узорка оригана (*Origanum vulgare* и *O. onites*) кривотвореног маслином (*Olea europea*), венецијанским сумаком (*Cotinus coggygria*) и миртом (*Myrtus communis*), у пет различитих концентрација. Метаболомички профили добијени након двостепене дериватизације, која укључује метоксиаминовање и силанизацију, подвргнути су мултиваријантној анализи података како би се открили маркери кривотворења и направили регресиони модели на основу односа мешања оригана и биљака за кривотворење. Ортогонална делимична анализа најмањих квадрата је омогућила детекцију кривотворења оригана лишћем маслине, венецијанског сумака и мирте. Садржај сорбитола разликовао је узорке оригана кривотворених лишћем маслине, док су шикиминска и кининска киселина препознате као фактор разликовања за кривотворење оригана венецијанским сумаком. Садржај фруктозе и кининске киселине у корелацији су са кривотворењем оригана миртом. Ортогонална делимична анализа најмањих квадрата – дискриминантна анализа је омогућила разликовање узорака *O. vulgare* и *O. onites*, при чему је одређено да је катехоллактат метаболит који разликује ове две биљне врсте.

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Supplementary material

SUPPLEMENTARY MATERIAL TO

GC–MS-based metabolomics for the detection of adulteration in oregano samples

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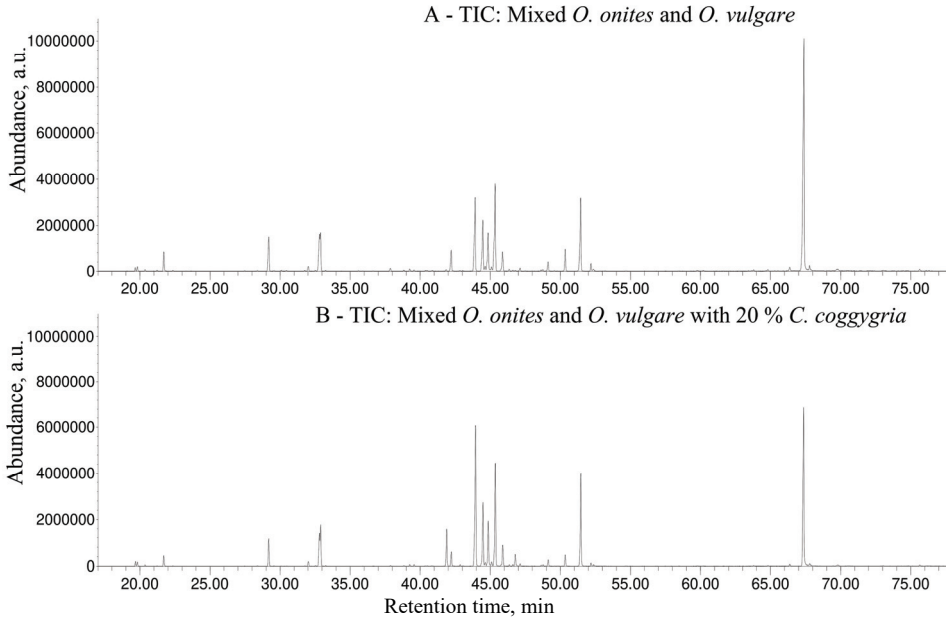

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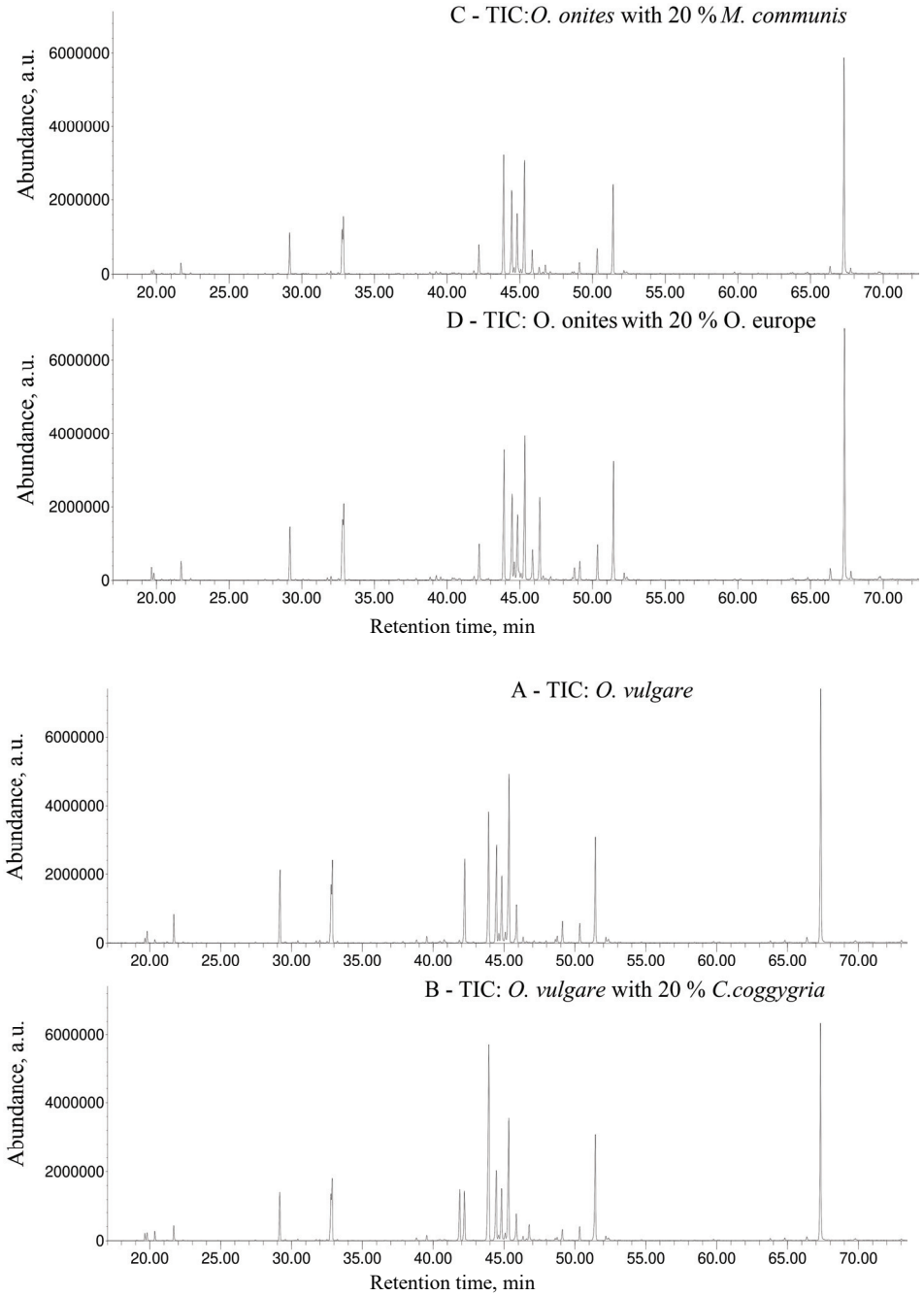
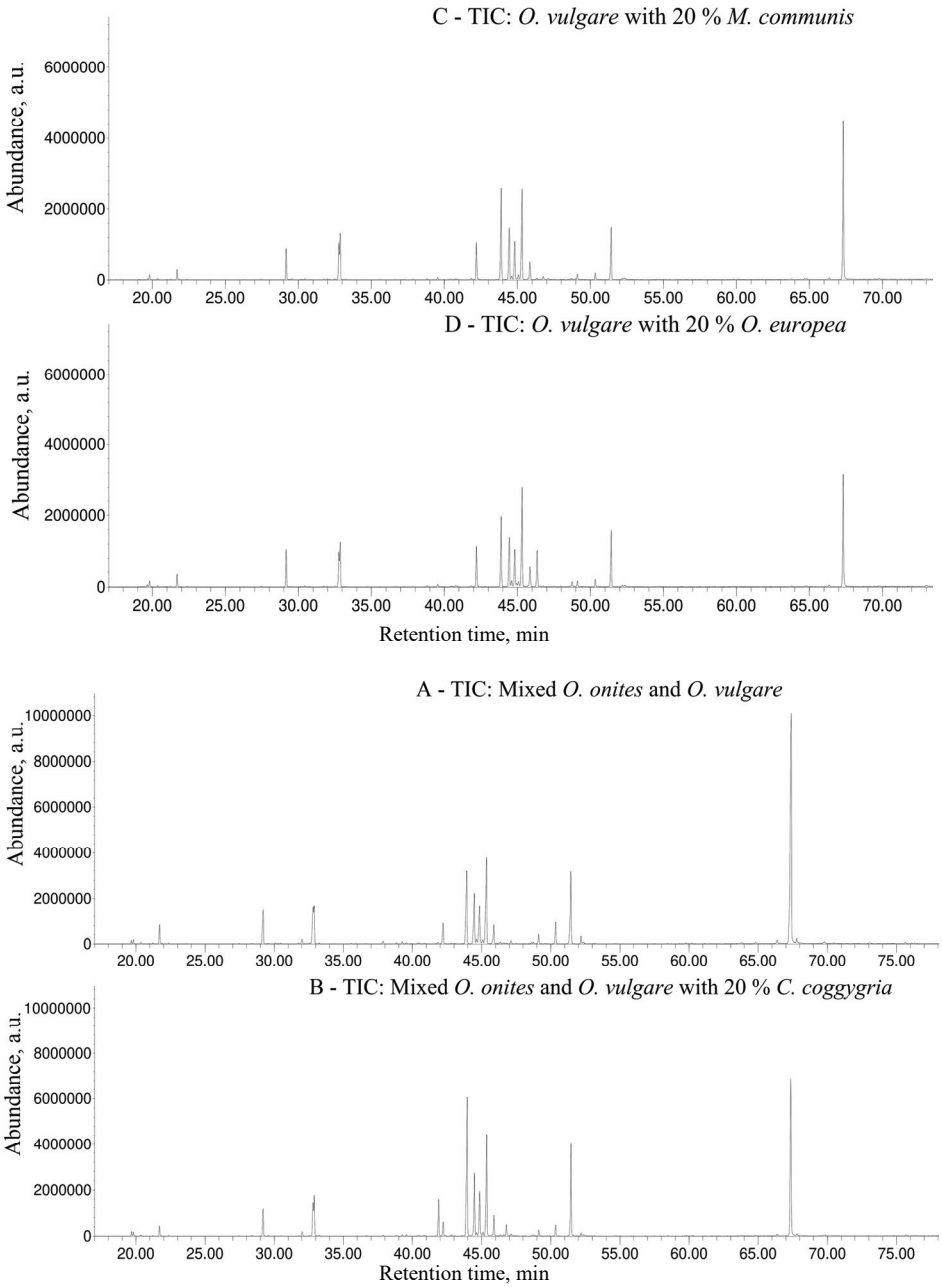

C - TIC:*O. onites* with 20 % *M. communis*
D - TIC: O. onites with 20 % O. europe
A - TIC: *O. vulgare*
B - TIC: *O. vulgare* with 20 % *C.coggygria*
Abundance, a.u.
Retention time, min


C - TIC: O. vulgare with 20 % M. communis
Abundance, a.u.
6000000
4000000
2000000
0
20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00
D - TIC: O. vulgare with 20 % O. europea
Abundance, a.u.
6000000
4000000
2000000
0
20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00
Retention time, min
A - TIC: Mixed O. onites and O. vulgare
Abundance, a.u.
10000000
8000000
6000000
4000000
2000000
0
20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00
B - TIC: Mixed O. onites and O. vulgare with 20 % C. coggygria
Abundance, a.u.
10000000
8000000
6000000
4000000
2000000
0
20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00 60.00 65.00 70.00 75.00
Retention time, min

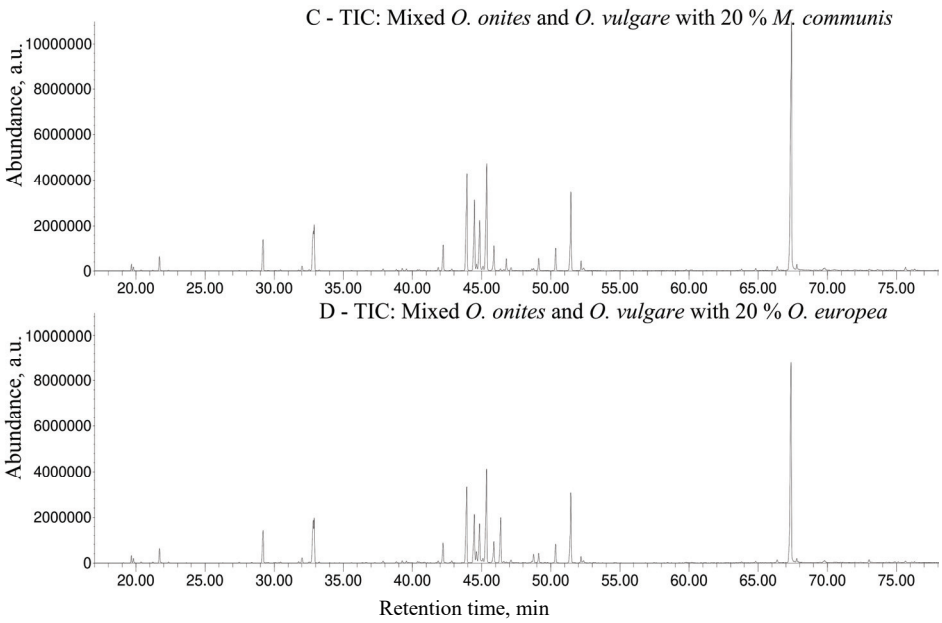


Fig. S-1. GC-FID chromatograms of derivatized samples. TIC – total-ion current.

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Application of LC–MS/MS with ion mobility for chemical analysis of propolis extracts with antimicrobial potential

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Abstract: The objective of this study was to test four-dimensional LC-ESI-MS/ /MS chromatography in analysis of complex mixture such as ethanol extracts of different propolis samples. In total more than 1200 picks were identified and only for 185 literature conformation was found. The given data represent the result of tentative identification, and summarized results are given in the text. Comparing the samples, from different altitudes, 96 components were detected as characteristic in high altitude samples and 18 in samples collected at low altitudes. Antimicrobial activity of ethanol extracts of propolis (EEP) and propylene glycol extracts of propolis (PGEP) were carried out on *S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *P. aeruginosa*, *S. typhimurium*, *E. coli* and *E. cloacae* bacterial strains and compared with broad-spectrum antibiotics, streptomycin and ampicillin. Anti-quorum sensing activity was performed on *P. aeruginosa* by testing the effect of representative propolis extracts on biofilm formation, twitching and motility activity and production of pyocyanin. We demonstrated that the majority of explored propolis extracts have greater or equal minimal inhibitory concentration and minimum bactericidal concentration values compared to antibiotics, independently of the solvent used for the extraction. The samples collected from the highest altitude emerged as least active antimicrobial agents but with the greatest potential as anti-quorum sensing agents.

Keywords: tandem mass spectrometry; parallel accumulation serial fragmentation ion mobility mass spectrometer; poplar propolis; quorum sensing; *P. aeruginosa*.

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INTRODUCTION

Propolis is a mixture of a plenty health beneficial components which manifest antibiotic, antipyretic, antiviral, *etc.* activities. It's a mixture of plant resins with bee's wax. These properties have been known for centuries and are still of interest for scientific researchers all over the world.[1] There are a lot of different types of propolis, typically classified on the basis of geographical origin which is closely related to the present flora. The poplar propolis is the most represented in Europe. Its chemical composition is not unique and depends on the altitude were bees are kept. At altitudes above 500 m the main constituents of propolis resin are phenolic acids and their acetyl glyceryl esters. At lower altitudes (below 400 m) flavonoids and their derivatives are present.[2]

The 70 % ethanol (EEP) and propylene glycol (PGEP) extracts of propolis are most commonly used as auxiliary medicinal products on the prevention of diseases of the respiratory tract, such as influenza and colds, as well as strengthening the immune system.[3,4] It has been reported that EEP shows strong antibacterial activities against gram positive, and gram negative bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Rhodobacter sphaeroides*,[5] *Helicobacter pylori*,[6] *Streptococcus aureus*,[7] *Lactobacillus plantarum* and *Pseudomonas spp.*[8] For this reason, propolis extracts were in high demand during the CoV 19 pandemic, due to the presence of quercetin, a potent antiviral agent.[9] The development of methods for the analysis of complex mixtures, such as propolis extracts, has always been a great challenge. According to the literature data, propolis contains over 300 different organic compounds in a wide range of concentrations and polarities, from waxes to sugars and from mg/g to pg/g.[10] In the case of complex mixtures, two or more different techniques are combined in order to obtain a complete picture of the chemical composition of the analyzed sample. HPLC and HPLC–MS chromatographic techniques, as well as GC and GC–MS, HPTLC, NMR, *etc.*, were used as the most common techniques in the analysis of ethanol extracts of propolis.[11–15] However, HPLC–MS has been shown to be the most suitable for the analysis of polar and medium polar compounds which are responsible for the vast majority of the therapeutic effects of propolis.[16] Over time, mass spectrometry has advanced considerably and there are a huge number of ion sources variations, analyzers and detectors that can be used.

For complete screening of complex samples, such as proplis, mass spectrometers with an ESI ion source and hybrid analyzers (Q TOF or Orbitrap) are usually used[16] as they can measure the precise mass and perform fragmentation of the analyzed molecule, also allow reduction of the chromatographic time and increase the resolution. In this way we can get information about each component: retention time, precise mass and isotopic distributions on the basis of which the molecular formula is obtained, with the pattern of molecule fragmentation. Comparison of these data with spectral libraries and/or with standards of pure

compounds enables identification of a large number of constituents. Because of the huge difference in polarity and concentration of individual components, it is impossible to identify them using single analysis. It is necessary to make modifications, in the sample preparation procedure, with the aim of eliminating the main components, since their high abundance are making analysis of the trace components very difficult. It is necessary to concentrate the sample and/or inject larger volumes which is impossible if the main components are not somehow removed from the sample. In 2016 Bruker has introduced a new generation of mass spectrometers with an ion mobility analyzer of a unique design located behind the ion source and in front of the quadrupoles and time-of-flight (TOF). Trapped ion mobility spectrometry or TIMS instrument introduces a fourth dimension in the analysis of complex mixtures. The separation of molecules based on their ion mobility adds additional information during the molecules identification process. By installing additional segment of analyzing sections to their TIMS cell design enabled development of parallel accumulation serial fragmentation (PASEF®) acquisition method.[17,18]

Herein, for the first time, we present the results obtained using timsTOF spectrometer, with PASEF technology, for analysis of propolis ethanol extracts. Furthermore, we have compered antimicrobial properties of two type of poplar propolis samples (their ethanol and propylene glycol extracts) on selected Gram positive and Gran-negative bacteria.

EXPERIMENTAL

Ethanol (Meilab, Serbia) and propylene glycol (Meilab, Serbia) extracts of poplar propolis samples collected from different altitude in Serbia (Table I) were prepared using 5 mL of solvents and 0.5 g of sample.

The samples were kept in the dark for 72 h at room temperature (25 °C) and filtered through filter paper. The resin content calculation was based on wax residues after extraction with 70 % ethanol. Propolis ethanol extracts profiling has been done on Bruker Elute LC system with Bruker Intensity Solo 1.8 C18-2, 2.1 mm×100 mm. Elution system was H_20 with 0.1 % formic acid (A) and MeCN with 0.1 % formic acid (B). The flow rate was 0.4 mL/min, and gradient program: 0–1 min, 0–2 % B; 1–10 min, 2–100 % B; 10–12 min, 100 % B. MS spectra were measured on timsTOF Pro MS system with ESI positive and negative ionization mode, scan mode with auto MS/MS and PASEF in range of 20-2000 *m*/*z*, and internal calibration. For data processing and interpretation Bruker MetaboScope 4.0 software with T-ReX 4D (time aligned region complete extraction) algorithm was used.

Antibacterial activity

The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Listeria monocytogenes* (NCTC 7973), and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210) and *Enterobacter cloacae* (human isolate), were used. The antibacterial assay was carried out by a microdilution method.[19] The bacterial suspensions were adjusted with sterile saline to a concentration of 10^5 CFU/mL. Compounds were dissolved in 70 % EtOH and PG solution (100 mg/mL) and immediately

added in Tryptic Soy broth (TSB) medium (100 μL) with bacterial inoculum (10^4 CFU per well). The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (*MIC*). The *MIC* obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of INT (*p*-iodonitrotetrazolium violet; 2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride, Sigma) color and compared with positive control for each bacterial strains. The *MBC* were determined by serial sub-cultivation of 2 μL into microtitre plates containing 100 μL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the *MBC*, indicating 99.5 % killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted extracts) and the positive control. Streptomycin (Sigma P 7794) and ampicillin (Panfarma, Belgrade, Serbia) were used as positive controls (1 mg/mL in sterile physiological saline). Five percent 70 % EtOh and PG were used as a negative control.

TABLE I. Samples location, altitude and resin content calculated on wax residues after extraction with 70 % ethanol. Samples from high altitude are bolded

Sample	Sampling place	Altitude, m	Resins content, %
1	Varvarin	150	42.50
2	**Luke, Ivanjica**	**550**	**38.05**
3	Irig	200	57.46
4	**Vukanja, Kruševac**	**500**	**44.70**
5	Vranje	300	33.92
6	Novi Sad	150	66.01
7	Petrovac na Mlavi	300	42.78
8	Rušanj V	200	67.76
9	V. Šiljegovac, Kruševac	200	62.73
10	Belegiš, Stara Pazova	200	51.26
11	Kovin	100	38.60
12	Banatski Karlovac I	150	57.57
13	Banatski Karlovac II	200	75.76
14	Deliblatska peščara	200	47.28
15	**Prijepolje I**	**450**	**61.51**
16	**Prijepolje II**	**900**	**59.06**

RESULTS AND DISCUSSION

LC–MS/MS analysis

The chemical composition of six representative samples, three from low altitude (6, 9 and 12) and three from high altitude (4, 15 and 16), were analyzed using Brukers tims TOF Pro with PASEF LC–MS/MS system on MetaboScope 4.0 platform for the first time. T-ReX 4D algorithm allowed us to combine ions belonging to the same compound into one feature, *i.e.*, isotopes, charge states, adducts or fragments. This non-linear retention time alignment ensure data consistency when chromatographic shifts between LC–MS runs occurre. Also, soft-

ware facilitates confident compound identification based on accurate mass, isotope pattern match, collisional cross sections (CCS) and convenient MS/MS data handling. MetaboScape 4.0 automatically identifies a known compound and characterizes an unknown in details. A fully integrated online data base query in combination with in silico fragmentation[21] for structure assignment and fully automated MS/MS spectral library queries (*e.g.*, Bruker HMDB Metabolite Library and Bruker MetaboBASE® Personal Library) from generated data. Through the use of data-dependent acquisition (DDA)[20] the mass spectrometer constantly cycles through a full scan acquisition (survey scan) and *N* MS/MS scans to ensure a sufficient number of sampling points across a chromatographic peak. This allows high quality MS/MS spectra regardless to the analyte concentration. In Table II the results of LC–MS/MS analysis were summarized in different classes of compounds (for more data see Supplementary material to this paper). It was obvious that the applied LC–MS/MS system detected an incomparably higher number of signals than previously reported in the literature. More than 1200 picks were fully characterized, and tentative identification was performed. The results were compared with literature data to determine if compounds had been previously detected in propolis. Literature data were found for 185 compounds (see Supplementary material) and most of them are: flavonoids, chalcones, phenols, phenolic acids and derivatives as well as terpenes, benzene derivatives, coumarine, saccharides, carboxylic acid, fatty alcohols, aldehyde, acids and their derivatives. Above 1000 detected signals have been reported for the first time as components of EEP. Only 96 components were detected in samples collected from high altitude, while only18 components were detected in samples collected from low altitude.

Results of antimicrobial tests

Uncontrolled and frequent use of antibiotics led to the emergence of resistant strains of bacteria, especially in hospital settings such as methicillin-resistant *S. aureus* (MRSA), *E. coli* and *P. aeruginosa*.[22] Great efforts have been made to solve this problem, by developing new antibiotics and/or testing known natural products. It seems that good candidates are different types of propolis and its extracts. A number of studies showed high efficiency of Brazilian propolis against staphylococci and other pathogenic microorganisms for humans and animals. The Regueira group reported high activity of Brazilian red propolis against *S. aureus*, *E. coli* and *P. aeruginosa*.[23] The Wojtyczka and coworkers published the results on the effect of ethanoic extract of Polish propolis (EEPP) against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) clinical isolates.[24] However, the authors used the propolis from only one location-Kamianna (South Poland). The same group of authors also proved the data of high activity of Polish propolis against biofilm forming *S. epidermidis* strains,[25] and

TABLE II. LC–MS/MS tentative identification of different classes of compounds in propolis samples

Compounds classes	Total number of compounds	
	Detected	Previously reported
Flavonoids and chalcones	182	53
Fatty alcohols, aldehydes, acids and their derivatives	158	11
Phenols, phenolic acids and derivatives	111	50
Carboxylic acids	108	9
Terpenes	88	25
Esters	55	
Benzene derivatives	54	16
Vitamins B and D and their derivatives	31	
Carboxamide	17	
Pyran and furan derivatives	16	2
Alkene and polyene	15	
Coumarine	15	9
Amines	14	1
Prostaglandins	14	
Carbohydrates	9	9
Lactones	8	

what is from a clinical point of view, probably the most beneficial aspect of propolis. The biofilm form of staphylococci has slightly lower susceptibility then planktonic cells with much lower differences compared to other antimicrobial agents, *e.g.*, antibiotics.

In this study, sixteen propolis samples, from different regions of Serbia, were collected and ethanol and propylene glycol extracts prepared. The antibacterial activity of all extracts was verified using four G+ bacterial strains *S. aureus*, *B. cereus*, *M. flavus* and *L. monocytogenes*, and four G– bacterial strains *P. aeruginosa*, *S. typhimurium*, *E. coli* and *E. cloacae.* The minimum inhibitory (*MIC*) and minimum bactericidal concentration (*MBC*) were determined and compared with two controls, broad-spectrum antibiotics streptomycin (protein synthesis inhibitor) and ampicillin (*β*-lactam antibiotic). The results of *MIC* and *MIB* are given in Tables III–VI. All tested bacterial stream had lower *MIC* and *MIB* values for streptomycin than ampicillin. Therefore, *MIC* and *MIB* values of tested extracts, lower or equal to streptomycin, were considered more active than the control. Values higher or equal to ampicillin, were considered less active than control. Values between these two limit values can be considered equivalent to activity of antibiotics. *MIC* and *MBC* for tested EEP and PGEP were different depending on the sample and bacterial culture (Tables III and V). For *E. cloacae* and *S. typhimurium* EEP and PGEP have greater or equal activity in comparison to control antibiotics except for sample 16. PGEP have greater or equal activity for *S. aureus* while EEP have greater or equal activity for *B. cereus.* Both extracts showed the

equal or lower activity compared to the controls for *L. monocytogenes* and *M. flavus*. Both extracts have shown almost the same activity for *E. cloacae* as the antibiotics. Other bacterial cultures (*P. aeruginosa* and *E. coli*) are equally, less or more sensitive to propolis extracts as the antibiotics, depending on the tested sample and used extracts. If we compare different samples and the altitude of collections, there is lower sensitivity of the tested bacterial cultures to propolis samples collected at higher altitudes than at lower ones (Tables III and IV).

TABLE III. Antibacterial activity of EEP (*MIC* in mg/mL). *S.a.* – *Staphylococcus aureus*; *B.c.* – *Bacillus cereus*; *M.f.* – *Micrococcus flavus*; *L.m.* – *Listeria monocytogenes*; *P.a.* – *Pseudomonas aeruginosa*; *E.c.* – *Escherichia coli*; *En.cl.* – *Enterobacter cloacae*; *S.t.* – *Salmonella typhimurium*

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
1	0.25	0.25	0.3	0.25	0.06	1.25	0.125	0.25
2	0.02	0.3	0.125	0.75	0.2	0.5	0.75	0.125
3	0.5	0.125	0.9	0.9	0.6	0.9	0.9	0.7
4	0.3	0.25	1.25	0.25	0.25	0.3	0.25	0.06
5	0.03	0.06	0.06	0.25	0.125	0.6	0.7	0.125
6	0.125	0.06	0.03	0.125	0.03	0.25	0.25	0.03
7	0.03	0.03	0.6	0.5	0.06	0.6	0.6	0.05
8	0.4	0.2	0.4	0.4	0.4	0.4	0.4	0.125
9	0.2	0.125	0.5	0.25	0.2	0.5	0.2	0.125
10	0.2	0.2	0.2	0.25	0.06	0.4	0.125	0.2
11	0.02	0.3	0.2	0.3	0.04	0.3	0.5	0.25
12	0.2	0.25	0.3	0.5	0.3	0.3	0.5	0.2
13	0.125	0.125	0.125	0.6	0.5	0.25	0.25	0.25
14	0.06	0.06	0.06	0.125	0.25	0.125	0.25	0.06
15	0.4	0.4	0.8	0.25	0.8	0.5	0.25	0.4
16	0.8	0.5	0.5	1	0.5	0.5	0.25	0.5
Streptomycin	0.1	0.2	0.05	0.2	0.2	0.3	0.2	0.2
Ampicillin	0.3	0.3	0.3	0.4	0.3	0.4	0.8	0.3

TABLE IV. Antibacterial activity of EEP (*MBC* in mg/mL). *S.a.* – *Staphylococcus aureus*; *B.c.* – *Bacillus cereus*; *M.f.* – *Micrococcus flavus*; *L.m.* – *Listeria monocytogenes*; *P.a.* – *Pseudomonas aeruginosa*; *E.c.* – *Escherichia coli*; *En.cl.* – *Enterobacter cloacae*; *S.t.* – *Salmonella typhimurium*

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
1	0.5	0.5	1	0.5	0.125	2.5	0.25	0.5
2	0.03	0.5	0.5	1	0.25	1	1	0.5
3	1	0.25	1.25	1.25	1.25	1.25	1.25	1
4	0.5	0.5	2.5	1	0.5	0.5	2.5	0.125
5	0.06	0.125	0.125	0.5	0.25	1.25	1	0.25
6	0.25	0.125	0.06	0.25	0.06	0.5	0.5	0.06

TABLE IV. Continued

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
7	0.06	0.06	1.25	1	0.125	1.25	1.25	0.06
8	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.25
9	0.25	0.25	1	0.5	0.25	1	0.25	0.25
10	0.25	0.25	0.25	0.5	0.125	0.5	0.25	0.25
11	0.03	0.5	0.25	0.5	0.06	0.5	1	0.5
12	0.25	1	0.5	1	0.5	0.5	1	0.25
13	0.25	0.25	2.5	1.25	1	0.5	0.5	0.5
14	0.125	0.125	0.3	0.25	0.5	0.25	0.5	0.125
15	0.5	0.5	1	0.5	1	1	0.5	0.5
16	1	1	1	6	1	1	0.5	1
Streptomycin	0.2	0.3	0.1	0.3	0.3	0.5	0.3	0.3
Ampicillin	0.4	0.4	0.4	0.5	0.5	0.8	1.25	0.5

The results for MBC are similar to those for MIC, *S. aureus*, *P. aeruginosa* and *S. typhimurium* were the most sensitive, and *M. flavus* and *L. monocytogenes* the least sensitive to propolis extracts. Ethanol and propylene glycol extracts of propolis showed no obvious difference in their activity, probably due to the similar polarity of used solvents.[26]

EEPs produced from samples collected in different geographical regions, *e.g.*, Albania, Turkey,[27] Russia[28] and Brazil have been observed in other studies.[29] The virulence-related factors of *P. aeruginosa*, such as twitching and motility activity, biofilm formation and pyocyanin production were tested for ethanol and propylene glycol extracts of four representative samples (two low and two high altitudes). Results are presented in Tables VII and VIII and Figs. 1 and 2. Based on the data shown , sample **16** from the highest altitude, inhibits biofilm formation at low concentrations, regardless to the type of extract. Only ethanol extract of sample **5** showed strong activity, better than streptomycin and similar to ampicillin Table VII.

TABLE V. Antibacterial activity of PGEP (*MIC* in mg/mL). *S.a. – Staphylococcus aureus*; *B.c. – Bacillus cereus*; *M.f. – Micrococcus flavus*; *L.m. – Listeria monocytogenes*; *P.a. – Pseudomonas aeruginosa*; *E.c. – Escherichia coli*; *En.cl. – Enterobacter cloacae*; *S.t. – Salmonella typhimurium*

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
1	0.125	0.25	0.25	0.5	0.5	0.75	0.125	0.25
2	0.02	0.5	0.25	0.75	0.06	0.06	0.75	0.015
3	0.25	0.5	0.5	0.125	0.5	0.125	0.6	0.004
4	0.6	0.6	0.2	1.25	0.25	1.25	0.25	0.6
5	0.25	0.25	0.3	0.06	0.03	0.25	0.5	0.125
6	0.125	1.25	1.25	2.5	2.5	1.25	1.25	0.5
7	0.125	0.25	1.25	0.5	0.25	0.25	0.25	0.25

TABLE V. Continued

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
8	0.004	0.5	0.2	0.5	0.015	0.3	0.25	0.008
9	0.025	0.2	0.2	0.2	0.2	0.125	0.2	0.008
10	0.125	0.2	0.2	0.2	0.2	0.25	0.06	0.125
11	0.004	0.2	0.008	0.25	0.012	0.125	0.25	0.006
12	0.125	0.2	0.2	0.25	0.06	0.2	0.125	0.02
13	0.03	0.03	0.03	1.7	0.6	1.7	1.25	0.6
14	0.006	0.3	0.25	0.3	0.2	0.2	0.3	0.008
15	0.012	0.25	0.3	0.75	0.2	0.3	0.5	0.012
16	0.5	1.5	0.3	1	0.25	2	2	1
Streptomycin	0.1	0.2	0.05	0.2	0.2	0.3	0.2	0.2
Ampicillin	0.3	0.3	0.3	0.4	0.3	0.4	0.8	0.3

TABLE VI. Antibacterial activity of PGEP (*MBC* in mg/mL). *S.a.* – *Staphylococcus aureus*; *B.c.* – *Bacillus cereus*; *M.f.* – *Micrococcus flavus*; *L.m.* – *Listeria monocytogenes*; *P.a.* – *Pseudomonas aeruginosa*; *E.c.* – *Escherichia coli*; *En.cl.* – *Enterobacter cloacae*; *S.t.* – *Salmonella typhimurium*

Compound	Bacterium							
	S.a.	*B.c.*	*M.f.*	*L.m.*	*P.a.*	*E.c.*	*En.cl.*	*S.t.*
1	0.25	0.5	0.5	1	2.5	1	0.25	0.5
2	0.03	1	0.5	1	0.125	0.125	1	0.03
3	0.5	1	1.25	1.25	1	0.25	1.25	0.008
4	0.25	0.25	0.25	2.5	0.5	2.5	1.25	1.25
5	0.5	0.5	0.5	0.125	0.125	0.5	1	0.25
6	0.25	2.5	2.5	5	5	2.5	2.5	1
7	0.25	0.5	2.5	1	0.5	0.5	0.5	0.5
8	0.008	1	0.25	1	0.03	0.5	0.5	0.015
9	0.03	0.25	0.25	0.25	0.25	0.25	0.25	0.016
10	0.25	0.25	0.25	0.25	0.25	0.5	0.125	0.25
11	0.008	0.25	0.015	0.5	0.015	0.5	0.5	0.008
12	0.25	0.25	0.25	0.5	0.125	0.25	0.25	0.03
13	0.06	0.06	0.06	2.5	1.25	2.5	2.5	1.25
14	0.008	0.5	0.5	0.5	0.25	0.25	0.25	0.15
15	0.016	0.5	0.5	1	0.25	0.5	1	0.016
16	1	2	0.5	6	0.5	4	4	2
Streptomycin	0.2	0.3	0.1	0.3	0.3	0.5	0.3	0.3
Ampicillin	0.4	0.4	0.4	0.5	0.5	0.8	1.25	0.5

On the other hand, sample **2** completely reduced colony edge protrusions, regardless to the solvent used for extraction. The same sample reduce pyocyanin production for 45 % when prepared as PGEP at the similar level as used controls. Samples **5E**, **5PG**, **8E** and **16E** were less effective, decreasing pyocyanin production for 70, 90, 85 and 75 % respectively.

TABLE VII. Effects of propolis (**2E**, **2PG**; 5E, 5PG; 8E, 8PG; **16E** and **16PG**) on biofilm formation of *P. aeruginosa* (PAO1); biofilm formation values were calculated as: (mean A_{620} treated well/mean A_{620} control well)×100. Values are expressed as means ± *SE*

Agent	Biofilm formation, %		
	0.5*MIC*	0.25*MIC*	0.125*MIC*
5E	83.55±4.73	–	–
5PG	91.43±1.50	91.56±0.97	89.92±0.64
8E	83.94±0.19	88.31±1.83	42.02±0.51
8PG	88.27±2.18	92.29±4.73	93.52±2.06
2E	**94.63±0.51**	**95.23±2.96**	**77.19±1.73**
2PG	**91.20±2.37**	**91.88±0.94**	**91.77±3.76**
16E	**82.47±1.48**	**15.35±0.51**	**–**
16PG	**77.85±1.26**	**8.65±0.65**	**–**
Ampicilin	14.88±1.14	–	–
Streptomicin	86.78±41.72	36.36±1.90	29.42±0.94

TABLE VIII. Twitching and motility activity of representative propolis samples

Agent	Colony diameter ± *SD*, mm	Colony color	Protrusions diameter, μm	Colony edge on microscope
5E	7.33±0.58	White	40-136	Slightly reduced protrusion
5PG	8.00±2.08	White	40-80	Partly reduced protrusion
8E	6.33±0.58	White	24-64	Partly reduced protrusion
8PG	9.33±1.53	Light green	24-80	Partly reduced protrusion
2E	**18.67±4.73**	**Light green**	**-**	**Reduced protrusion**
2PG	**10.33±1.53**	**White**	**-**	**Reduced protrusion**
16E	**6.67±0.58**	**White**	**8-40**	**Partly reduced protrusion**
16PG	**10.33±0.53**	**Light green**	**40-120**	**Partly reduced protrusion**
Streptomycin	7.67±0.58	White	40-120	Slightly reduced protrusion
Ampicillin	8.00±0.00	White	64-160	Slightly reduced protrusion
Control PAO1	21.0±3.60	Green	80-240	Regular protrusion

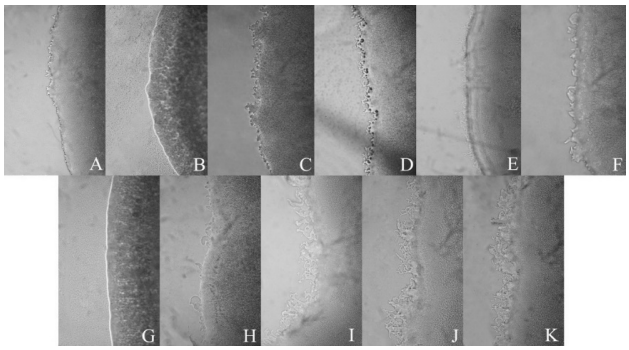

Fig. 1. Light microscopy of colony edges of *P. aeruginosa* in twitching motility, grown in the presence or absence of propolis at a concentration of 0.5 MIC. The colonies from the bacteria grown with: **2E** (A); **2PG** (B); 5E (C); 5PG (D); 8E (E); 8PG (F); **16E** (G); **16P**G (H); streptomycin (I); ampicillin (J); *P. aeruginosa*, control (K); magnification: (A–E)×100.

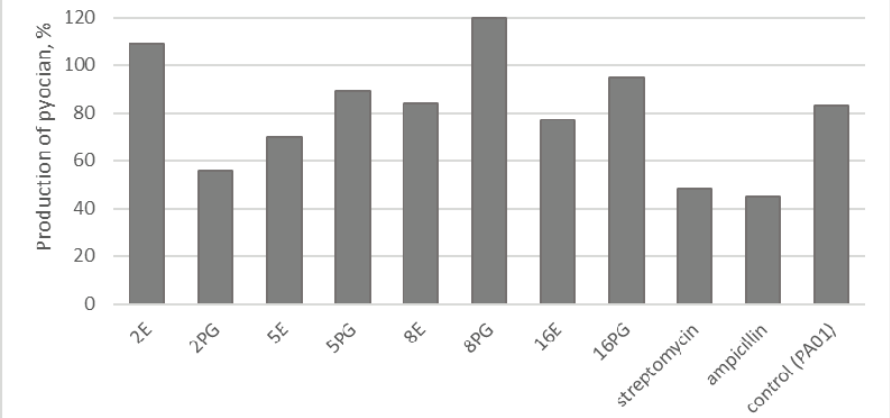

Fig. 2. Effects of propolis extracts (**2E**, **2PG**; 5E, 5PG; 8E, 8PG; **16E** and **16PG**) at 0.5*MIC* on pyocyanin production by *P. aeruginosa* (PAO1). The pyocyanin production was estimated by measuring optical density on 520 nm (OD_{520}) and 600 nm (OD_{600}) and calculated by formula: $I = 100(OD_{520}/OD_{600})$.

CONCLUSION

This paper presents the results of four-dimensional LC–ESI-MS/MS chromatography analysis of propolis samples collected on different locations and altitudes. More than 1200 picks were tentatively identified and for only 185 literature conformation have been found. This indicates the great potential of this technique. The 96 components were obtained only in samples collected from high altitude, while 18 components were obtained only in samples collected from low altitude. Antimicrobial activity of EEP and PGEP extracts for some of the most persistent human Gram-positive and Gram-negative bacteria was equal and/or better compared to broad-spectrum antibiotics used as the control. Comparison of the results, of antimicrobial activity with the collection altitude of the propolis samples, indicate that the sample collected on the highest altitude has the largest anti- quorum sensing potential.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11064, or from the corresponding author on request.

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ИЗВОД

ПРИМЕНА LC–MS/MS ТЕХНИКЕ СА ЈОНСКОМ ПОКРЕТЉИВОШЋУ ЗА ХЕМИЈСКУ АНАЛИЗУ ЕКСТРАКАТА ПРОПОЛИСА СА АНТИМИКРОБНИМ ПОТЕНЦИЈАЛОМ

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Циљ овог истраживања је било тестирање четвородимензионалне LC–ESI-MS/MS хроматографије у анализи комплексних смеша, као што су етанолни екстракти различитих узорака прополиса. Укупно је идентификовано више од 1200 пикова, а само за 185 смо нашли литературну потврду. Приказани подаци представљају тентативну идентификацију и сумирани резултат је дат у тексту. Поређењем узорака са различитих надморских висина, за 96 једињења је утврђено да се налазе само у узорцима са високе надморске висине и 18 само у узорцима прикупљеним на нижим надморским висинама. Антибактеријске активности етанолних и пропиленгликолних екстрактата прополиса (EEP и PGEP, редом) тестиране су на *S. aureus*, *B. cereus*, *M. flavus*, *L. monocytogenes*, *P. aeruginosa*, *S. typhimurium*, *E. coli* и *E. cloacae* бактеријским линијама и поређене са антибиотицима широког спектра деловања, стрептомицином и ампицилином. *Anti-quorum sensing* активност је тестирана на *P. aeruginosa* испитивањем ефекта репрезентативних екстраката прополиса на формирање биофилма, тестовима покретљивости руба колоније (*twitching* и *mobility*) и производње пиоцианина. Показали смо да највећи број, коришћених екстраката прополиса, има исте и/или мање *MIC* и *MBC* вредности, у поређењу са атибиотицима, независно од растварача коришћеног за екстракцију. Узорци прикупљени на високим надморским висинама су се показали као најмање активни антибактерициди али имају велики *anti-quorum sensing* потенцијал.

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SUPPLEMENTARY MATERIAL TO

Application of LC–MS/MS with ion mobility for chemical analysis of propolis extracts with antimicrobial potential

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TABLE S-I. The results of LC-ESI-MSMS analysis of representative ethanol extracts of propolis using MetaboScope 4.0 platform for characterization and identification of separated compounds

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1	85.02828	84.02101	1.09	$C_4H_4O_2$	$[M+H]^+$	4-Hydroxy-2-butynal	Bruker MetaboBASE Personal Library 2.0_in-silico	3118	1288.5	1641	1115.25	0	1965.25	
2	271.09648	270.08921	3.02	$C_{16}H_{14}O_4$	$[M+H]^+$	Cardamonin	Bruker MetaboBASE Personal Library 3.0	2154.75	1013	873.75	2012.5	3633	2223.5	
3	179.07027	178.063	3.61	$C_{10}H_{10}O_3$	$[M+H]^+$	4-Hydroxy-3-methoxy cinnamaldehyde	Bruker MetaboBASE Personal Library 3.0	257	443	450.5	694.5	1021	2349.25	1
4	313.0704	312.06312	5.99	$C_{17}H_{12}O_6$	$[M+H]^+$	4-(3,4-dihydroxyphenyl)-6,7-dihydroxy naphthalene-2-carboxylic acid	MoNA-export-GNPS_QTOF.msp	617.25	0	1986	380.25	141.5	0	
5	163.07533	162.06806	6.01	$C_{10}H_{10}O_2$	$[M+H]^+$	Methyl cinnamate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	58.5	73.75	122.25	122.75	12954.5	2
6	193.08588	192.0786	6.11	$C_{11}H_{12}O_3$	$[M+H]^+$	Methyl trans-p-methoxycinnamate	Bruker MetaboBASE Personal Library 2.0_in-silico	281.25	1383	1408.75	136.25	0	0	3
7	111.04404	110.03677	6.11	$C_6H_6O_2$	$[M+H]^+$	Pyrocatechol	Bruker MetaboBASE Personal Library 2.0	976.25	737.25	1329.75	881.75	0	117.5	
8	355.10262	354.09534	6.16	$C_{16}H_{18}O_9$	$[M+H]^+$	Chlorogenic acid	Bruker MetaboBASE Personal Library 2.0	643.75	213.5	2447	605.75	0	289.25	4
9	201.16381	200.15654	6.29	$C_{15}H_{20}$	$[M+H]^+$	alpha-Corocalene	Bruker MetaboBASE Personal Library 2.0_in-silico	7799.75	1012.5	2954.25	258.5	152	0	5
10	341.10199	340.09471	6.39	$C_{19}H_{16}O_6$	$[M+H]^+$	Ambanol	Bruker MetaboBASE Personal Library 2.0_in-silico	592.25	735.25	1010	2046.25	1654.75	2814	
11	169.04952	168.04225	6.64	$C_8H_8O_4$	$[M+H]^+$ $[M-H]^-$	Isovanillic acid	Bruker HMDB Metabolite Library_2.0	847.75	1075	817.75	1538.25	5315	5236	6
12	147.04399	146.03671	6.65	$C_9H_6O_2$	$[M+H]^+$	Coumarin	Bruker HMDB Metabolite Library_2.0	224	581	542.75	308.5	0	10090	7
13	565.15525	564.14798	6.77	$C_{26}H_{28}O_{14}$	$[M+H]^+$	5,7-dihydroxy-2-(4-hydroxyphenyl)-8-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-6-(3,4,5-trihydroxyoxan-2-yl)chromen-4-one	MoNA-export-GNPS_QTOF.msp	52.75	0	1921.75	0	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
14	447.12919	446.12192	6.83	$C_{22}H_{22}O_{10}$	$[M+H]^+$	3-(4-hydroxyphenyl)-7-methoxy-5-[(3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydro-xymethyl)oxan-2-yl]oxy-chromen-4-one	MoNA-export-GNPS_QTOF.msp	226	1125.25	284.25	0	0	0	
15	325.10719	324.09992	6.87	$C_{19}H_{16}O_5$	$[M+H]^+$	Ambonane	Bruker MetaboBASE Personal Library 2.0_in-silico	0	312.75	104.75	1500.25	3725.75	2516.75	8
16	329.10199	328.09471	6.92	$C_{18}H_{16}O_6$	[M+H]+	Bryacarpene 4	Bruker MetaboBASE Personal Library 2.0_in-silico	375.75	927.25	715.5	1473.25	183.75	3661	9
17	641.17143	640.16415	6.99	$C_{28}H_{32}O_{17}$	$[M+H]^+$ $[M-H]^-$	Rhamnetin 3-sophoroside	Bruker MetaboBASE Personal Library 3.0	1496.75	3054	2442.75	2639	328	449.75	10
18	341.13838	340.13111	7.25	$C_{20}H_{20}O_5$	$[M+H]^+$	Crotin (chalcone)	Bruker MetaboBASE Personal Library 2.0_in-silico	261.75	282.25	260.25	371	830	1964	11
19	300.99903	302.0063	7.36	$C_{14}H_6O_8$	$[M-H]^-$ $[M+H]^+$	Ellagic acid	Bruker MetaboBASE Personal Library 3.0	1057	3724.25	1071.25	9020.5	59333.75	14818.5	12
20	151.11183	150.10456	7.39	$C_{10}H_{14}O$	$[M+H]^+$	Thymol	Bruker HMDB Metabolite Library_2.0	1906.5	747.75	933.75	238.25	0	0	13
21	343.11776	342.11049	7.55	$C_{19}H_{18}O_6$	$[M+H]^+$	1,3-Cyclobutanedicarboxylic acid, 2,4-bis(4-hydroxy-phenyl)-monomethyl ester (Thesine)	MoNA-export-GNPS_QTOF.msp	0	749.5	0	1750.5	0	18200	
22	175.11181	174.10454	7.56	$C_{12}H_{14}O$	$[M+H]^+$	2,4,6,8,10-dodecapentaenal	Bruker MetaboBASE Personal Library 2.0_in-silico	715.25	1272.5	1189	1151.75	0	0	
23	595.16623	594.15895	7.57	$C_{27}H_{30}O_{15}$	$[M+H]^+$ $[M-H]^-$	kaempferol-3-O-robinobioside	MoNA-export-GNPS_QTOF.msp	223.75	609.5	356.25	1427.5	18157.5	0	14
24	149.0608	150.06807	7.69	$C_9H_{10}O_2$	$[M-H]^-$ $[M+H]^+$	4-Vinylguaiacol	Bruker MetaboBASE Personal Library 2.0	3164	5378.25	3546.75	11503	11572.75	19940.5	15
25	231.0653	230.05803	7.73	$C_{13}H_{10}O_4$	$[M+H]^+$	Coriandrin	Bruker MetaboBASE Personal Library 2.0_in-silico	567.25	771.25	1106.5	1853.75	1118	1831.75	16
26	287.0914	286.08412	8.15	$C_{16}H_{14}O_5$	$[M+H]^+$	5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	1520.25	1139	1171.5	509	213.75	5163.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
27	133.06472	132.05744	8.38	C_9H_8O	$[M+H]^+$	Cinnamaldehyde	Bruker HMDB Metabolite Library_2.0	438	423.75	483	949.25	2264.25	1831	17
28	491.1196	492.12687	8.45	$C_{23}H_{24}O_{12}$	$[M-H]^-$ $[M+H]^+$	5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	MoNA-export-GNPS_QTOF.msp	0	318.75	0	0	4522.5	0	
29	549.16112	548.15384	8.54	$C_{26}H_{28}O_{13}$	$[M+H]^+$	Daidzein 7-O-apiosyl-(1->6)-glucoside	Bruker MetaboBASE Personal Library 2.0_in-silico	712	1603	1092.25	463.25	0	0	18
30	219.10166	218.09438	8.57	$C_{13}H_{14}O_3$	$[M+H]^+$	(R)-Bitalin A	Bruker MetaboBASE Personal Library 2.0_in-silico	4220.5	3023	4025.25	4857.75	4840	3668.25	19
31	345.09702	344.08975	8.68	$C_{18}H_{16}O_7$	$[M+H]^+$	Eupatorin	Bruker MetaboBASE Personal Library 3.0	20986.25	35769.5	18506.5	11833	1293.25	361.75	20
32	249.11196	248.10468	8.71	$C_{14}H_{16}O_4$	$[M+H]^+$	Prenyl caffeate	Bruker MetaboBASE Personal Library 2.0_in-silico	1213.25	776.25	1729.75	647.75	0	0	21
33	237.07591	236.06863	8.81	$C_{12}H_{12}O_5$	$[M+H]^+$	Radicinin	Bruker MetaboBASE Personal Library 2.0_in-silico	413	1032.25	402.25	5550.5	1983.5	10875.75	22
34	331.08137	330.0741	8.92	$C_{17}H_{14}O_7$	$[M+H]^+$	5,7-dihydroxy-2-(4-hydroxyphenyl)-3,6-dimethoxy-4H-chromen-4-one	MoNA-export-GNPS_QTOF.msp	87239.75	123792.5	91144.25	57934.25	14235	1922.25	
35	255.0652	254.05792	9.04	$C_{15}H_{10}O_4$	$[M+H]^+$	Chrysin	Bruker MetaboBASE Personal Library 2.0	2282.5	5062.25	4932	1547.5	461.25	0	23
36	433.11341	432.10613	9.11	$C_{21}H_{20}O_{10}$	$[M+H]^+$	5-hydroxy-2-(4-hydroxyphenyl)-7-[(2S,3R, 4S,5S, 6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	MoNA-export-GNPS_QTOF.msp	4290.75	6801.5	8962.5	3998	134	0	
37	477.13962	476.13234	9.17	$C_{23}H_{24}O_{11}$	$[M+H]^+$	5-hydroxy-6,7-dimethoxy-2-[4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]chromen-4-one	MoNA-export-GNPS_QTOF.msp	0	0	161.25	0	3676.75	4067.25	
38	329.10207	328.09479	9.28	$C_{18}H_{16}O_6$	$[M+H]^+$	Isotectorigenin 7-methyl ether	Bruker MetaboBASE Personal Library 3.0	3666.25	5592.25	2658.25	2153	451.75	290.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
39	475.12404	474.11676	9.32	$C_{23}H_{22}O_{11}$	$[M+H]^+$, $[M-H]^-$	Genistin 6"-O-acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2095.5	1476.75	1852.5	532	159.25	796.5	24
40	221.08084	220.07356	9.36	$C_{12}H_{12}O_4$	$[M+H]^+$	Tubaic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	936.75	156.5	5743.25	4498.5	17156	25
41	317.0657	316.05842	9.39	$C_{16}H_{12}O_7$	$[M+H]^+$, $[M-H]^-$	3-O-methylquercetin	MoNA-export-GNPS_QTOF.msp	206779.25	247768	210248.25	177486.25	28120.75	18800.5	26
42	153.1274	152.12012	9.58	$C_{10}H_{16}O$	$[M+H]^+$	2,4-decadienal	Bruker MetaboBASE Personal Library 2.0_in-silico	1551.5	1024	2153	1296.5	0	82.25	27
43	343.15354	342.14627	9.77	$C_{20}H_{22}O_5$	$[M+H]^+$	Austrobailignan 7	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	2643.75	28
44	199.07536	198.06808	10.06	$C_{13}H_{10}O_2$	$[M+H]^+$	Splitomicin	Bruker MetaboBASE Personal Library 2.0	7513.5	6604	7935	5479.5	813.25	0	29
45	347.07626	346.06899	10.12	$C_{17}H_{14}O_8$	$[M+H]^+$	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,6-dimethoxychromen-4-one	MoNA-export-GNPS_QTOF.msp	2426.75	1434.25	1342.75	2517.5	17685.75	9739.25	30
46	489.15543	490.16271	10.46	$C_{28}H_{26}O_8$	$[M-H]^-$ $[M+H]^+$	Edulisin I	Bruker MetaboBASE Personal Library 2.0_in-silico	0	281.75	400.5	1432	7338.5	3874.5	31
47	201.16386	200.15659	10.5	$C_{15}H_{20}$	$[M+H]^+$	(S)-gamma-Calacorene	Bruker MetaboBASE Personal Library 2.0_in-silico	10148.25	2748.25	4191.75	833.5	350.5	295	32
48	151.11187	150.10459	10.5	$C_{10}H_{14}O$	$[M+H]^+$	2-trans-4-trans-7-cis-Decatrienal	Bruker MetaboBASE Personal Library 2.0_in-silico	3457.75	714	1351.75	491.75	315.5	105.5	33
49	241.08597	240.0787	10.73	$C_{15}H_{12}O_3$	$[M+H]^+$	Dihydroflavonol	Bruker MetaboBASE Personal Library 2.0_in-silico	2829.25	2366.75	2599.25	2035.5	251.75	132.75	34
50	273.11204	272.10476	10.79	$C_{16}H_{16}O_4$	$[M+H]^+$	6-O-Methylequol	Bruker MetaboBASE Personal Library 2.0_in-silico	517.25	686.75	686.25	2029.75	3326.25	5674	
51	361.09193	360.08466	11.19	$C_{18}H_{16}O_8$	$[M+H]^+$	Centaureidin	Bruker MetaboBASE Personal Library 3.0	4101.75	0	768	0	19306.25	9183.25	35
52	219.13805	218.13077	11.28	$C_{14}H_{18}O_2$	$[M+H]^+$	2-(3-Hydroxy-4-methyl-phenyl)-5-methyl-4-hexen-3-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2331.25	1619.5	1646.75	923.5	113.75	0	36
53	295.13315	294.12587	11.36	$C_{19}H_{18}O_3$	$[M+H]^+$	Phenol, 2-methoxy-4-[3-methyl-5-[(1E)-1-propen-1-yl]-2-benzo furanyl]	MoNA-export-GNPS_QTOF.msp	0	43	1703.75	557.5	4094	0	37
54	175.14815	174.14087	11.37	$C_{13}H_{18}$	$[M+H]^+$	5,7alpha-Dihydro-1,4,4,7a-tetramethyl-4H-indene	Bruker MetaboBASE Personal Library 2.0_in-silico	3538.5	6239	4638.5	3959.25	161.25	1093.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
55	135.11684	134.10956	11.38	$C_{10}H_{14}$	$[M+H]^+$	p-Mentha-1,3,8-triene	Bruker MetaboBASE Personal Library 2.0_in-silico	4350.75	6340	7754.75	5238.5	823.5	1761	38
56	133.10118	132.0939	11.52	$C_{10}H_{12}$	$[M+H]^+$	p-Mentha-1,3,5,8-tetraene	Bruker MetaboBASE Personal Library 2.0	1622.25	4084	1761	1881	329.5	501	
57	119.08561	118.07834	11.52	C_9H_{10}	$[M+H]^+$	4-Methylstyrene	Bruker MetaboBASE Personal Library 3.0	1538.5	3714.5	1716.75	1916	840.75	737.75	
58	251.16412	250.15685	11.54	$C_{15}H_{22}O_3$	$[M+H]^+$	13-Hydroxymarasmene	Bruker MetaboBASE Personal Library 2.0_in-silico	534.25	1089.5	1109.5	783.25	2551.5	0	39
59	203.07034	202.06307	11.6	$C_{12}H_{10}O_3$	$[M+H]^+$	3,4',5-Biphenyltriol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	258.5	75.25	1561.25	4688.5	6208.5	40
60	79.05428	78.047	11.9	C_6H_6	$[M+H]^+$	Benzene	Bruker MetaboBASE Personal Library 2.0_in-silico	7792.25	5747.5	9238.75	5565.5	139	693.75	41
61	193.19508	192.1878	11.91	$C_{14}H_{24}$	$[M+H]^+$	5-Ethyl-7-methyl-3E,5E,7E-undecatriene	Bruker MetaboBASE Personal Library 2.0_in-silico	2166.25	3951.5	2653.25	2910.75	0	940	
62	361.09179	360.08452	12.07	$C_{18}H_{16}O_8$	$[M+H]^+$	2-(3,4-dihydroxyphenyl)-5-hydroxy-3,6,7-trimetho-xychromen-4-one	MoNA-export-GNPS_QTOF.msp	28319	610.75	5248.5	0	0	0	42
63	283.09647	282.0892	12.21	$C_{17}H_{14}O_4$	$[M+H]^+$	Castillene E	Bruker MetaboBASE Personal Library 2.0_in-silico	41852.25	25152.25	61949.25	28318.75	15957	18777.5	43
64	191.07022	190.06295	12.22	$C_{11}H_{10}O_3$	$[M+H]^+$	Hymecromone methyl ether (7-Methoxy-4-methylcoumarin)	Bruker MetaboBASE Personal Library 2.0_in-silico	0	802.75	601	4017	7542.5	7603	44
65	369.13336	368.12608	12.3	$C_{21}H_{20}O_6$	$[M+H]^+$	3'-Angeloyloxy-2',4'-dihy-droxy-6'-methoxychalcone	Bruker MetaboBASE Personal Library 2.0_in-silico	25749.25	21703.25	19218	10470.5	832.25	861.75	
66	345.09692	344.08965	12.34	$C_{18}H_{16}O_7$	$[M+H]^+$	5,7-dihydroxy-3,6-dime-thoxy-2-(4-methoxyphenyl)-4H-chromen-4-one	MoNA-export-GNPS_QTOF.msp	93970.75	125836.25	93336.25	61789.25	331526.75	209218.5	45
67	231.13812	230.13084	13.21	$C_{15}H_{18}O_2$	$[M+H]^+$	Dehydromyodesmone	Bruker MetaboBASE Personal Library 2.0_in-silico	908.5	871.75	2242	693.25	114.75	365.75	46
68	165.05462	164.04734	13.21	$C_9H_8O_3$	$[M+H]^+$	p-Coumaric acid	MoNA-export-GNPS_QTOF.msp	1776.75	2772.25	1604.25	2030.25	1779	1502.75	47
69	239.23715	238.22987	13.23	$C_{16}H_{30}O$	$[M+H]^+$	2-hexadecenal	Bruker MetaboBASE Personal Library 2.0	2686.75	4905	2603.75	1669.25	199.75	542.25	48
70	323.12871	324.13598	13.28	$C_{20}H_{20}O_4$	$[M-H]^-$ $[M+H]^+$	Moracin I	Bruker MetaboBASE Personal Library 2.0_in-silico	9958.75	6981.25	11319.5	5498.5	378.25	3281.25	49

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
71	273.07573	272.06846	13.39	$C_{15}H_{12}O_5$	$[M+H]^+$	Alternariol monomethyl ether_120111	MoNA-export-GNPS_QTOF.msp	52986	31580.75	33005.5	26242.25	4935.5	2133.25	50
72	389.12294	388.11566	13.48	$C_{20}H_{20}O_8$	$[M+H]^+$	7-Hydroxy-3',4',5,6,8-pentamethoxyflavone	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	3677.25	
73	223.20576	222.19848	13.53	$C_{15}H_{26}O$	$[M+H]^+$	(-)-Tamariscol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	120.5	3089	0	51
74	177.05458	176.0473	13.99	$C_{10}H_8O_3$	$[M+H]^+$	1,4,5-Naphthalenetriol	Bruker MetaboBASE Personal Library 2.0_in-silico	1237.75	1421.25	333.5	4992.75	11567.75	18795	52
75	377.30548	376.2982	14.16	$C_{24}H_{40}O_3$	$[M+H]^+$	Allolithocholic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	3709.75	867.25	53
76	329.10195	328.09468	14.24	$C_{18}H_{16}O_6$	$[M+H]^+$	5-hydroxy-3,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (Kaempferol 3,7,4'-trimethyl ether)	MoNA-export-GNPS_QTOF.msp	4698.25	3790	4918.5	1728.25	1992.5	55090.25	54
77	323.12783	322.12056	14.42	$C_{20}H_{18}O_4$	$[M+H]^+$	7-hydroxy-3-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]chromen-4-one	MoNA-export-GNPS_QTOF.msp	10884.5	6439.5	12544.5	4473.75	277.5	391.5	
78	287.09118	286.08391	15.03	$C_{16}H_{14}O_5$	$[M+H]^+$	(2R,3R)-3,7-dihydroxy-6-methoxy-2-phenyl-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	3273.25	2563	3505	2544	889.5	262.5	
79	237.18477	236.17749	15.74	$C_{15}H_{24}O_2$	$[M+H]^+$	(2E,6E)-1-Hydroxy-2,6,10-farnesatrien-9-one	Bruker MetaboBASE Personal Library 2.0_in-silico	1846.25	2721.75	1753	1420.25	0	2428.5	55
80	173.13249	172.12521	15.8	$C_{13}H_{16}$	$[M+H]^+$	1,2-Dihydro-1,1,6-trimethylnaphthalene	Bruker MetaboBASE Personal Library 2.0_in-silico	590.5	482.25	1106.5	2614	6101.25	14924.5	56
81	275.20052	274.19324	16.53	$C_{18}H_{26}O_2$	$[M+H]^+$	Empenthrin	Bruker MetaboBASE Personal Library 2.0_in-silico	1652	7013.25	2341	2783.5	223	571.25	
82	263.2006	262.19332	16.64	$C_{17}H_{26}O_2$	$[M+H]^+$	Acetylenic acids; 10,16-Heptadecadien-8-ynoic acid, (E)-	Bruker MetaboBASE Personal Library 2.0_in-silico	3041.5	12657.75	4168.5	4698	493	481.5	57
83	163.14814	162.14086	16.73	$C_{12}H_{18}$	$[M+H]^+$	1,3-Diisopropylbenzene	Bruker MetaboBASE Personal Library 2.0_in-silico	1160.75	2025.75	2356	1756.5	0	1546.75	58
84	339.15905	338.15178	17.06	$C_{21}H_{22}O_4$	$[M+H]^+$	Bergamottin	Bruker MetaboBASE Personal Library 2.0_in-silico	1399	2300.75	1058	1682	0	0	59
85	151.1117	150.10443	17.23	$C_{10}H_{14}O$	$[M+H]^+$	(-)-Isopiperitenone	Bruker MetaboBASE Personal Library 2.0_in-silico	1783.75	3137.25	2668.5	2112.75	0	421.25	60

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
86	71.04925	70.04197	17.27	C_4H_6O	$[M+H]^+$	Vinyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	108	401.25	146.25	0	9764	4998	
87	315.25326	314.24598	17.46	$C_{18}H_{34}O_4$	$[M+H]^+$	Octadecanedioic acid	Bruker HMDB Metabolite Library_2.0	2136	1373	2033.25	3330.5	1153	5496.75	61
88	449.28998	448.2827	17.53	$C_{26}H_{40}O_6$	$[M+H]^+$	16-Feruloyloxypalmitate	Bruker MetaboBASE Personal Library 2.0_in-silico	57.5	1175.5	0	6586.75	0	14028.75	
89	203.17939	202.17211	17.87	$C_{15}H_{22}$	$[M+H]^+$	alpha-curcumene	Bruker MetaboBASE Personal Library 2.0_in-silico	2369.5	4385.25	2421.75	3379.25	22803.25	15644.75	17
90	591.42537	590.41809	18.23	$C_{35}H_{58}O_7$	$[M+H]^+$	Hebevinoside IX	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	6024	62
91	469.40289	468.39561	18.29	$C_{32}H_{52}O_2$	$[M+H]^+$	Lupeol acetate	Bruker MetaboBASE Personal Library 3.0	351.75	0	385.5	1324.75	0	20650.25	63
92	297.27893	296.27166	18.43	$C_{19}H_{36}O_2$	$[M+H]^+$	Methyl oleate	Bruker MetaboBASE Personal Library 2.0	512.25	518.5	554	939.75	5546.75	2591.5	.
93	219.17416	218.16689	19.28	$C_{15}H_{22}O$	$[M+H]^+$	(E)-10,11-Dihydro-al-phaatlantone ((Z)-.alpha.-Atlantone)	Bruker MetaboBASE Personal Library 2.0_in-silico	896	0	241.5	213.5	74.25	2414.25	64
94	257.081	256.07372	19.58	$C_{15}H_{12}O_4$	$[M+H]^+$	5,7-dihydroxy-2-phenyl-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	724	1279.5	1113	683	0	0	
95	441.37281	440.36553	20.82	$C_{30}H_{48}O_2$	$[M+H]^+$	Sebiferic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	107	440.5	258.25	2392.25	30197	4287.75	65
96	271.09633	270.08905	0.77	$C_{16}H_{14}O_4$	$[M+H]^+$	(E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (Pinostrobin chalcone)	MoNA-export-GNPS_QTOF.msp	1812.5	600.75	1349.75	971	2672	1924.25	17
97	203.05256	180.06333	1.04	$C_6H_{12}O_6$	$[M+Na]^+$, $[M-H]^-$ $[M-H-H_2O]^-$ $[M+K]^+$	D-Tagatose	Bruker HMDB Metabolite Library_2.0	116120.75	60642.75	85301.25	72996.25	15154.75	103534	23
98	163.06004	162.05278	1.1	$C_6H_{10}O_5$	$[M+H]^+$ $[M-H_2O+H]^+$	3-hydroxy-3-methyl-Glutaric acid	Bruker MetaboBASE Personal Library 2.0	13633	6088.25	7559.5	4921.5	628.75	8749.75	66
99	127.03893	126.03166	1.1	$C_6H_6O_3$	$[M+H]^+$	Allomaltol	Bruker MetaboBASE Personal Library 2.0	3845.75	2565.5	2327.75	1971.75	88.5	3005.75	67
100	139.03898	138.0317	6.12	$C_7H_6O_3$	$[M+H]^+$	3,4-Dihydroxy-benzaldehyde	Bruker MetaboBASE Personal Library 2.0	6531	5672	15470.5	8060	2630.25	3936.75	68

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
101	167.07028	166.06301	6.28	$C_9H_{10}O_3$	$[M+H]^+$	Ethyl salicylate	Bruker MetaboBASE Personal Library 2.0_in-silico	77.5	203	0	0	3321.75	2193	69
102	163.0389	162.03163	6.29	$C_9H_6O_3$	$[M+H]^+$	Umbelliferone	Bruker MetaboBASE Personal Library 2.0_in-silico	843.75	961.25	2437	2566	516.75	1392.25	70
103	137.05967	136.05239	6.42	$C_8H_8O_2$	$[M+H]^+$ $[M-H]^-$	2-Hydroxyacetophenone	Bruker MetaboBASE Personal Library 3.0	956.75	1408.75	935.5	1207.75	0	1305.25	71
104	207.06506	206.05778	6.43	$C_{11}H_{10}O_4$	$[M+H]^+$	Scoparone	Bruker MetaboBASE Personal Library 2.0_in-silico	1012.75	1492.5	1346.5	1114.25	239.5	1228.75	72
105	177.01937	178.02664	6.56	$C_9H_6O_4$	$[M-H]^-$, $[M+H]^+$	Caffeoquinone	Bruker MetaboBASE Personal Library 2.0_in-silico	11144.5	12246	25554.5	13148.25	3580.25	9604.25	73
106	203.09151	202.08423	6.56	$C_9H_{14}O_5$	$[M+H]^+$	Diethyl Oxalpropionate	Bruker MetaboBASE Personal Library 2.0_in-silico	416	1897.75	343.75	1995.5	0	0	74
107	197.08083	196.07355	6.59	$C_{10}H_{12}O_4$	$[M+H]^+$	Orsellinic acid, ethyl ester	Bruker MetaboBASE Personal Library 2.0_in-silico	295.25	189.5	221.5	124	6869	5851.25	75
108	179.03504	180.04231	6.61	$C_9H_8O_4$	$[M-H]^-$ $[M+H]^+$	Caffeate	Bruker MetaboBASE Personal Library 3.0	145903.5	138837	209601.5	165106.25	51185.5	84103	76
109	135.04402	134.03675	6.6	$C_8H_6O_2$	$[M+H]^+$	6E-Octene-2,4-diynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	4680.25	4309	6643.25	5702	1224.25	2416	
110	137.05969	136.05242	6.64	$C_8H_8O_2$	$[M+H]^+$	p-Anisaldehyde	Bruker MetaboBASE Personal Library 3.0	228.25	0	0	291	0	16055	77
111	227.09143	226.08416	6.75	$C_{11}H_{14}O_5$	$[M+H]^+$	Ethyl syringate	Bruker MetaboBASE Personal Library 3.0	113.25	62.25	474.5	79.75	1253.25	2820	78
112	345.13344	344.12616	6.89	$C_{19}H_{20}O_6$	$[M+H]^+$	Dihydromilletenone methyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	1346.5	1225.75	2264	1829.25	2183.75	2665.75	
113	137.05967	136.05239	6.96	$C_8H_8O_2$	$[M+H]^+$	3,4-Dihydroxystyrene	Bruker MetaboBASE Personal Library 2.0_in-silico	693	1124.5	766	2702.5	2940.5	8381.25	79
114	121.02957	122.03685	7	$C_7H_6O_2$	$[M-H]^-$ $[M+H]^+$	Salicylaldehyde	Bruker MetaboBASE Personal Library 3.0	16703.25	24585.75	19838.5	20790.5	53852.5	32263	13
115	355.1176	354.11032	7.04	$C_{20}H_{18}O_6$	$[M+H]^+$	Elliptinol	Bruker MetaboBASE Personal Library 2.0_in-silico	886.5	951.5	1363	2617	3942.5	5353.75	80
116	239.0913	238.08403	7.18	$C_{12}H_{14}O_5$	$[M+H]^+$	3,4,5-Trimethoxycinnamic acid	Bruker HMDB Metabolite Library_2.0	145.5	306.75	0	1403.25	1890.75	2175.75	81

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
117	611.16111	610.15383	7.21	$C_{27}H_{30}O_{16}$	$[M+H]^+$ $[M-H]^-$	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	MoNA-export-GNPS_QTOF.msp	347	1943	4755	2687	12752.25	623	
118	343.08243	344.08971	7.23	$C_{18}H_{16}O_7$	$[M-H]^-$, $[M+H]^+$	Lathycarpin	Bruker MetaboBASE Personal Library 2.0_in-silico	4912.75	4801.75	6672	6491.5	0	1243	[8]
119	237.07587	236.0686	7.31	$C_{12}H_{12}O_5$	$[M+H]^+$	6H-2-Benzopyran-5-carboxaldehyde, 7,8-dihydro-7,8-dihydroxy-3,7-dimethyl-6-oxo-, (7R-trans)-	Bruker MetaboBASE Personal Library 3.0	274.5	239.5	102	537.5	4152.75	5301.5	[82]
120	207.0652	206.05792	7.33	$C_{11}H_{10}O_4$	$[M+H]^+$ $[M-H]^-$	Citropten	Bruker MetaboBASE Personal Library 2.0_in-silico	491.25	733.5	250.75	1012	2314.25	1866.25	[83]
121	119.04911	118.04183	7.38	C_8H_6O	$[M+H]^+$	Benzofuran	Bruker MetaboBASE Personal Library 2.0_in-silico	5279.5	5772.25	6221.25	7468.75	10497.25	9091.5	[84]
122	237.18501	236.17773	7.41	$C_{15}H_{24}O_2$	$[M+H]^+$	4E,7Z,10Z-Tridecatrienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	17271	6792	6369.75	3482.75	239.5	420.25	
123	303.05001	302.04273	7.42	$C_{15}H_{10}O_7$	$[M+H]^+$	2-(2,6-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	MoNA-export-GNPS_QTOF.msp	682	1321.25	1807.75	1825.5	19146.75	4381.75	[85]
124	125.05965	124.05237	7.44	$C_7H_8O_2$	$[M+H]^+$	Guaiacol	Bruker HMDB Metabolite Library_2.0	3200.5	3440.25	6446.5	6574.25	20192.25	18348.5	[86]
125	153.05465	152.04738	7.44	$C_8H_8O_3$	$[M+H]^+$ $[M-H]^-$	Vanillin	MoNA-export-GNPS_QTOF.msp	16475.75	17521.5	33053.75	29388.5	92165.25	86765.5	[13]
126	611.16128	610.154	7.45	$C_{27}H_{30}O_{16}$	$[M+H]^+$	3-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxychromen-4-one	MoNA-export-GNPS_QTOF.msp	0	911.25	0	738.75	12408.5	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
127	333.2062	350.20931	7.47	$C_{20}H_{30}O_5$	$[M-H_2O+H]^+$ $[M+H]^+$	Andrographolide	Bruker MetaboBASE Personal Library 2.0_in-silico	5553.5	7187.25	3534	3631.75	0	143.5	87
128	315.19549	314.18821	7.47	$C_{20}H_{26}O_3$	$[M+H]^+$	19-Oxo-9-cis-retinoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	3226.75	3524	1793.25	1844	0	0	88
129	287.0914	286.08412	7.49	$C_{16}H_{14}O_5$	$[M+H]^+$	Calythropsin	Bruker MetaboBASE Personal Library 2.0_in-silico	887.5	1742.75	1652	432.75	122	0	89
130	217.15877	216.1515	7.54	$C_{15}H_{20}O$	$[M+H]^+$	(R)-ar-Turmerone	Bruker MetaboBASE Personal Library 2.0_in-silico	1716	3833.25	2901.75	3391.5	179.5	0	90
131	235.16932	234.16204	7.54	$C_{15}H_{22}O_2$	$[M+H]^+$	(Z)-alpha-Bergamotenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2238	3654	3428.5	3448.5	213.25	295.75	
132	255.19551	254.18823	7.57	$C_{15}H_{26}O_3$	$[M+H]^+$	Prohydrojasmon	Bruker MetaboBASE Personal Library 2.0_in-silico	1758.25	44	723.5	0	0	0	91
133	247.1329	246.12562	7.56	$C_{15}H_{18}O_3$	$[M+H]^+$	2-Oxo-5,11(13)-eudesmadien-12,8-olide	Bruker MetaboBASE Personal Library 2.0_in-silico	1124.5	1906	1889.75	1410	0	282.75	
134	233.1537	232.14651	7.58	$C_{15}H_{20}O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	Glechomanolide	Bruker MetaboBASE Personal Library 2.0_in-silico	2498.25	5517.5	3188	2374.25	242.5	723.75	92
135	449.10819	448.10091	7.57	$C_{21}H_{20}O_{11}$	$[M+H]^+$	(2R,3S)-2-[(3,4-dihydroxyphenyl)methyl]-2-hydroxy-3-[(E)-3-(3-hydroxy-4-methoxyphenyl)prop-2-enoyl]oxybutanedioic acid	MoNA-export-GNPS_QTOF.msp	149	175	380.75	98.5	2569	130	
136	183.06539	182.05811	7.58	$C_9H_{10}O_4$	$[M+H]^+$	3,4-Dihydroxy-hydrocinnamic acid	Bruker HMDB Metabolite Library_2.0	1323.25	1187	2589	1988.5	707	895.5	93
137	251.16421	250.15693	7.59	$C_{15}H_{22}O_3$	$[M+H]^+$	Procurcumadiol	Bruker MetaboBASE Personal Library 2.0_in-silico	2921.5	6340.75	3452.5	3159	161.25	683	94
138	303.08654	302.07927	7.59	$C_{16}H_{14}O_6$	$[M+H]^+$	Folerogenin	Bruker MetaboBASE Personal Library 3.0	258	842.75	183.25	0	0	3127.25	
139	195.06527	194.05799	7.71	$C_{10}H_{10}O_4$	$[M+H]^+$	Trans-Ferulic acid	Bruker HMDB Metabolite Library_2.0	18305.75	25645	35628	57289.75	63900.5	95214.25	47
140	177.05467	176.04739	7.7	$C_{10}H_8O_3$	$[M+H]^+$	4-Methylumbelliferone	Bruker MetaboBASE Personal Library 2.0_in-silico	76843.75	89762.75	123799.25	190414	223947.75	359675	72
141	303.05102	304.0583	7.73	$C_{15}H_{12}O_7$	$[M-H]^-$ $[M+H]^+$	Dihydroquercetin	MoNA-export-GNPS_QTOF.msp	4961.25	8615.25	6836	8188.25	19824	4442.75	95
142	285.04057	286.04785	7.72	$C_{15}H_{10}O_6$	$[M-H]^-$ $[M+H]^+$	Kaempferol	Bruker HMDB Metabolite Library_2.0	1484.5	2286.5	1711	2310	5235.75	902.5	96

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
143	285.0762	284.06892	7.77	$C_{16}H_{12}O_5$	$[M+H]^+$	Maackiain	Bruker MetaboBASE Personal Library 2.0_in-silico	1224.75	1744.75	1293.5	1314	739.75	1424	97
144	167.07046	166.06318	7.77	$C_9H_{10}O_3$	$[M+H]^+$	Apocynin	Bruker MetaboBASE Personal Library 2.0	853.25	1095	768.5	1988.75	1468.5	1448.5	98
145	359.11276	358.10548	7.79	$C_{19}H_{18}O_7$	$[M+H]^+$	Hypolaetin 7,8,3',4'-tetramethyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	1379.25	1241	2316	4196.75	2536.25	4015.25	8
146	447.09323	448.1005	7.85	$C_{21}H_{20}O_{11}$	$[M-H]^-$ $[M+H]^+$	kaempferol-7-O-hexoside	MoNA-export-GNPS_QTOF.msp	1689.75	3465.5	3292.25	3194.75	9452.25	1225	99
147	219.10161	218.09434	7.83	$C_{13}H_{14}O_3$	$[M+H]^+$	Propranolol glycol	Bruker MetaboBASE Personal Library 3.0	3528	2285.25	7847	1977.5	71.75	3182.25	100
148	237.11191	236.10463	7.83	$C_{13}H_{16}O_4$	$[M+H]^+$	3-Dimethylallyl-4-hydroxymandelic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	929.5	619.25	2555.5	134.25	0	599	101
149	177.05466	194.05821	7.85	$C_{10}H_{10}O_4$	$[M-H_2O+H]^+$ $[M+H]^+$ $[M+Na]^+$	Isoferulic acid	Bruker HMDB Metabolite Library_2.0	99099.75	132875.5	238944	227145.75	25695.5	36095.25	102
150	149.05972	148.05245	7.85	$C_9H_8O_2$	$[M+H]^+$	(E)-3-(2-Hydroxyphenyl)-2-propenal	Bruker MetaboBASE Personal Library 2.0_in-silico	3613	4298.25	9495.25	7247.75	0	0	103
151	479.11881	478.11149	7.86	$C_{22}H_{22}O_{12}$	$[M+H]^+$ $[M-H]^-$ $[M+Na]^+$	Isorhamnetin 3-glucoside	Bruker MetaboBASE Personal Library 3.0	1855.75	2116	1735	5250.25	57852.25	1662	104
152	317.06572	316.05844	7.87	$C_{16}H_{12}O_7$	$[M+H]^+$	Isorhamnetin	Bruker MetaboBASE Personal Library 3.0	1934.5	2434.5	1698.75	4284.25	41106.5	1351.25	105
153	341.10209	340.09481	7.87	$C_{19}H_{16}O_6$	$[M+H]^+$	Methylophiopogonone A	Bruker MetaboBASE Personal Library 2.0_in-silico	2405.25	2089.25	4003.5	5562.25	3563	4411.25	
154	279.08675	278.07947	7.93	$C_{14}H_{14}O_6$	$[M+H]^+$	planchol A	MoNA-export-GNPS_QTOF.msp	1928.75	3854.75	2345.25	16179.75	11597.25	37474.25	106
155	331.08129	330.07402	7.94	$C_{17}H_{14}O_7$	$[M+H]^+$	2-(3,4-dihydroxyphenyl)-5-hydroxy-6,7-dimethoxychromen-4-one	MoNA-export-GNPS_QTOF.msp	12464.75	18297	10350.25	5544.25	157.75	0	
156	431.13399	430.12672	7.92	$C_{22}H_{22}O_9$	$[M+H]^+$	3-(4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydro-xymethyl)oxan-2-yl]oxy-chromen-4-one	MoNA-export-GNPS_QTOF.msp	1875.5	3982	2588	1224	0	0	107
157	435.12885	434.12157	8.01	$C_{21}H_{22}O_{10}$	$[M+H]^+$	Naringenin-7-O-Glucoside	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	8401.5	3856.5	108

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
158	273.07585	272.06857	8	$C_{15}H_{12}O_5$	$[M+H]^+$	Naringenin	Bruker HMDB Metabolite Library_2.0	223	355.75	275.5	469.5	7308.5	3322.5	109
159	147.04513	148.05241	8.11	$C_9H_8O_2$	$[M-H]^-$ $[M+H]^+$ $[M-H_2O+H]^+$	4-Hydroxycinnamyl aldehyde	Bruker MetaboBASE Personal Library 2.0	3179	3017.5	3209.25	6091.25	69635	40150.5	110
160	249.1119	248.10462	8.15	$C_{14}H_{16}O_4$	$[M+H]^+$	Pyriculol	Bruker MetaboBASE Personal Library 2.0_in-silico	1884.25	1458.25	1623	1240.5	0	126.5	111
161	343.11746	342.11019	8.18	$C_{19}H_{18}O_6$	$[M+H]^+$	1,3-Cyclobutanedicarboxylic acid, 2,4-bis(4-hydroxyphenyl)-, monomethyl ester (Thesin)	MoNA-export-GNPS_QTOF.msp	0	429.5	145.75	1780	6249	4560.75	112
162	273.07547	272.06819	8.26	$C_{15}H_{12}O_5$	$[M+H]^+$	6,8-dihydroxy-3-(4-hydroxyphenyl)-3,4-dihydroisochromen-1-one	MoNA-export-GNPS_QTOF.msp	1071.25	3553.25	2362.75	2091	0	0	113
163	449.10878	448.10151	8.27	$C_{21}H_{20}O_{11}$	$[M+H]^+$	petunidin-3-O-arabinoside	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	3277	0	114
164	313.10709	312.09981	8.28	$C_{18}H_{16}O_5$	$[M+H]^+$ $[M-H]^-$	Bryacarpene 5	Bruker MetaboBASE Personal Library 2.0_in-silico	359.75	1430.25	1849.75	2481.25	320	2269.5	9
165	285.07583	284.06856	8.29	$C_{16}H_{12}O_5$	$[M+H]^+$ $[M-H]^-$	7-hydroxy-3-(4-hydroxyphenyl)-5-methoxychromen-4-one	MoNA-export-GNPS_QTOF.msp	112955	91094.5	69549.75	58817.75	4544	1491.75	
166	169.08716	170.09444	8.3	$C_9H_{14}O_3$	$[M-H]^-$ $[M+H]^+$	cis-3-Hexenyl pyruvate	Bruker MetaboBASE Personal Library 2.0_in-silico	896.25	1808	1433.75	1235	761.5	987.75	
167	391.139	390.13173	8.37	$C_{20}H_{22}O_8$	$[M+H]^+$	Piceid	Bruker MetaboBASE Personal Library 3.0	0	182.25	0	1301.75	5125.75	4676.5	115
168	243.06517	242.0579	8.36	$C_{14}H_{10}O_4$	$[M+H]^+$	3-(2-Carboxyvinyl)naphthalene-2-carboxylic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1232.75	1637.25	1889	1245.75	130	977.5	116
169	287.05616	288.06343	8.41	$C_{15}H_{12}O_6$	$[M-H]^-$ $[M+H]^+$	2,4,6-trihydroxy-2-[(4-hydroxyphenyl)methyl]-1-benzofuran-3-one	MoNA-export-GNPS_QTOF.msp	28191.5	36298	30146	26388	11574	25650	117
170	283.09643	282.08915	8.48	$C_{17}H_{14}O_4$	$[M+H]^+$	3,8-dimethoxyflavone	Bruker MetaboBASE Personal Library 2.0_in-silico	0	81.75	227.25	698.5	2972.25	5303	118
171	301.1073	300.10002	8.48	$C_{17}H_{16}O_5$	$[M+H]^+$	Astraciceran	Bruker MetaboBASE Personal Library 2.0_in-silico	170.75	120.75	183.5	753	2027.5	4079.5	119
172	433.1132	432.1059	8.5	$C_{21}H_{20}O_{10}$	$[M+H]^+$ $[M+Na]^+$	Apigetrin	MoNA-export-GNPS_QTOF.msp	28480.25	38989	34258.75	19703	168.75	0	120

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
173	271.06016	270.05288	8.49	$C_{15}H_{10}O_5$	$[M+H]^+$	Genistein	MoNA-export-GNPS_QTOF.msp	50217	70268	62400.5	37040	1512.25	560.25	121
174	315.08647	314.0792	8.53	$C_{17}H_{14}O_6$	$[M+H]^+$	Odoratin	MoNA-export-GNPS_QTOF.msp	27124	27298	16845.25	9530.25	1157	1267.5	122
175	163.07531	162.06803	8.53	$C_{10}H_{10}O_2$	$[M+H]^+$	Safrole	Bruker MetaboBASE Personal Library 2.0_in-silico	339.5	374	476.5	1600	1864	2731.75	123
176	221.18978	220.1825	8.6	$C_{15}H_{24}O$	$[M+H]^+$	Apritone	Bruker MetaboBASE Personal Library 2.0_in-silico	1404	2009.75	2122.25	893.5	1965.25	1475.75	124
177	279.08749	280.09477	8.62	$C_{14}H_{16}O_6$	$[M-H]^-$ $[M+Na]^+$ $[M-H_2O+H]^+$ $[M+H]^+$	Gravolenic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2003	14314.5	1058.5	66663.25	147602.5	162272.5	
178	311.22186	310.21459	8.68	$C_{18}H_{30}O_4$	$[M+H]^+$	9S-hydroxy-12R,13S-epoxy-10E,15Z-octadecadienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	780.75	1482.5	1074.5	892.25	0	0	
179	319.08146	318.07418	8.68	$C_{16}H_{14}O_7$	$[M+H]^+$	Demethylsulochrin	Bruker MetaboBASE Personal Library 2.0_in-silico	878	1596	1364.5	2273.5	340.5	0	125
180	151.11185	150.10457	8.68	$C_{10}H_{14}O$	$[M+H]^+$	Verbenone	Bruker MetaboBASE Personal Library 2.0	731	1123.25	831.25	777	0	0	77
181	301.03542	302.04269	8.68	$C_{15}H_{10}O_7$	$[M-H]^-$ $[M+H]^+$	Tricetin	Bruker MetaboBASE Personal Library 2.0	1154.5	1459	1551.75	575	0	0	126
182	255.19545	254.18818	8.71	$C_{15}H_{26}O_3$	$[M+H]^+$	5-Acetoxydihydrothe-aespirane	Bruker MetaboBASE Personal Library 2.0_in-silico	1270.25	1900.25	1631.5	1126	202.5	0	
183	207.10144	206.09416	8.75	$C_{12}H_{14}O_3$	$[M+H]^+$	3-Dimethylallyl-4-hydroxybenzoate	Bruker MetaboBASE Personal Library 2.0_in-silico	410.25	567.75	905.5	525.5	0	1828.5	127
184	315.1229	314.11562	8.76	$C_{18}H_{18}O_5$	$[M+H]^+$	Matteucinol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	286.5	0	991.5	2259.25	2181.5	128
185	149.05958	148.05231	8.8	$C_9H_8O_2$	$[M+H]^+$	Di-2-furanylmethane	Bruker MetaboBASE Personal Library 2.0_in-silico	273.75	524.5	0	1854.5	3490.5	4656.75	
186	301.07074	300.06347	8.78	$C_{16}H_{12}O_6$	$[M+H]^+$	Isokaempferide	MoNA-export-GNPS_QTOF.msp	101025.25	122693.25	82812.5	65568.5	7919	1901	129
187	167.07032	166.06305	8.82	$C_9H_{10}O_3$	$[M+H]^+$	3,4-Dimethoxy-benzaldehyde	Bruker MetaboBASE Personal Library 2.0	1977.5	1033.25	3661.75	1660.75	0	173.5	130
188	239.20067	238.19339	8.84	$C_{15}H_{26}O_2$	$[M+H]^+$	7Z,11Z-Tridecadienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	3111.75	4720.5	5675.75	3829.25	0	280.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
189	247.09655	246.08927	8.84	$C_{14}H_{14}O_4$	$[M+H]^+$	Prenyletin	Bruker MetaboBASE Personal Library 2.0_in-silico	5070.25	5537	3947.75	3807.25	0	524.75	131
190	191.07033	208.07385	8.85	$C_{11}H_{12}O_4$	$[M-H_2O+H]^+$ $[M+H]^+$ $[M+Na]^+$	3-(3,4-Dimethoxyphenyl)-2-propenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	305202.25	206977.25	497484.25	271738	31520	52259.5	71
191	163.07542	162.06814	8.85	$C_{10}H_{10}O_2$	$[M+H]^+$	8Z-Decene-4,6-diynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	9578.75	6580.5	15123.25	8586.25	1705	2105.5	132
192	315.08641	314.07914	8.86	$C_{17}H_{14}O_6$	$[M+H]^+$	3-O-Methylalnusin	MoNA-export-GNPS_QTOF.msp	1783.25	1869.75	1012.25	759.5	0	351	133
193	301.10712	300.09984	8.87	$C_{17}H_{16}O_5$	$[M+H]^+$	Heliannone A	Bruker MetaboBASE Personal Library 2.0_in-silico	583.25	746	1043.25	3338.75	16102	21129.25	134
194	219.17447	218.16719	8.9	$C_{15}H_{22}O$	$[M+H]^+$	6,8,10,12-pentadecatetraenal	Bruker MetaboBASE Personal Library 2.0_in-silico	7711.25	9636.75	3743.75	4008.5	341.5	1974	135
195	229.12236	228.11508	8.86	$C_{15}H_{16}O_2$	$[M+H]^+$	(S)-Curzeone	Bruker MetaboBASE Personal Library 2.0_in-silico	1570.75	2300.5	1762	1283	0	119.25	136
196	327.12304	326.11576	8.86	$C_{19}H_{18}O_5$	$[M+H]^+$	1,5-bis(4-hydroxy-3-methoxyphenyl)-1,4-pentadien-3-one	Bruker MetaboBASE Personal Library 2.0_in-silico	1125	0	1772	451.5	0	1072.75	136
197	233.15369	232.14642	8.87	$C_{15}H_{20}O_2$	$[M+H]^+$	Eremofrullanolide	Bruker MetaboBASE Personal Library 2.0_in-silico	7825.75	16518.75	7100	7688.5	641.5	1886.25	137
198	215.14315	214.13587	8.9	$C_{15}H_{18}O$	$[M+H]^+$	Farfugin A	Bruker MetaboBASE Personal Library 2.0_in-silico	1697	2698.75	1538.5	1703.75	145.5	281.75	138
199	187.14825	186.14098	8.9	$C_{14}H_{18}$	$[M+H]^+$	7-Ethyl-5,6-dihydro-1,4-dimethylazulene	Bruker MetaboBASE Personal Library 2.0_in-silico	1158	2257.5	1097.75	1499.25	0	224	139
200	243.10163	242.09436	8.98	$C_{15}H_{14}O_3$	$[M+H]^+$	Thunalbene	MoNA-export-GNPS_QTOF.msp	0	0	0	112.25	3023	1443	140
201	287.05598	288.06325	9.06	$C_{15}H_{12}O_6$	$[M-H]^-$ $[M+H]^+$	Eriodictyol	Bruker MetaboBASE Personal Library 3.0	4011.5	4036.5	5490.75	8610.5	13085.25	13089.5	141
202	417.11825	416.11098	9.04	$C_{21}H_{20}O_9$	$[M+H]^+$ $[M-H]^-$	Daidzin	Bruker MetaboBASE Personal Library 3.0	29981.75	51343.5	48809.75	23610.5	2152.5	0	
203	247.13293	246.12566	9.06	$C_{15}H_{18}O_3$	$[M+H]^+$	α-Santonin	Bruker MetaboBASE Personal Library 2.0_in-silico	3187.5	1891.75	3255.75	2440.25	1791.5	1682.5	142
204	275.20074	274.19346	9.04	$C_{18}H_{26}O_2$	$[M+H]^+$	13-Octadecene-9,11-diyno-ic acid, (Z)- (Bolekic acid)	Bruker MetaboBASE Personal Library 2.0_in-silico	2008	3967.25	938.5	2225.25	0	132.25	143

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
205	219.13809	218.13081	9.05	$C_{14}H_{18}O_2$	$[M+H]^+$	C14:5n-1,3,5,7,9 (5,7,9, 11,13-tetradecapentaenoic acid)	Bruker MetaboBASE Personal Library 2.0_in-silico	3729	2970.75	1688.75	2960.25	299	399.5	144
206	311.22178	310.21451	9.06	$C_{18}H_{30}O_4$	$[M+H]^+$	trans-EKODE-(E)-Ib	Bruker MetaboBASE Personal Library 2.0	1771.5	2436	1271.25	1861.25	0	0	145
207	257.0808	256.07353	9.1	$C_{15}H_{12}O_4$	$[M+H]^+$	Pinocembrin	MoNA-export-GNPS_QTOF.msp	3376.75	4441.25	3260	2865.75	0	202.25	109
208	301.03546	302.04273	9.13	$C_{15}H_{10}O_7$	$[M-H]^-$ $[M+H]^+$	Quercetin	Bruker HMDB Metabolite Library_2.0	68108.75	79927.25	88469.5	77594.5	42977.5	51133.25	109
209	357.16969	356.16241	9.17	$C_{21}H_{24}O_5$	$[M+H]^+$	Fragransol C	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	5459.75	146
210	209.15367	208.14639	9.14	$C_{13}H_{20}O_2$	$[M+H]^+$	(3R,8E)-3-Hydroxy-5,8-megastigmadien-7-one	Bruker MetaboBASE Personal Library 2.0_in-silico	1140	1216.75	997.75	961	752	970	147
211	375.18038	374.1731	9.16	$C_{21}H_{26}O_6$	$[M+H]^+$	Hexahydrocurcumin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	3220.5	148
212	399.144	398.13673	9.17	$C_{22}H_{22}O_7$	$[M+H]^+$	Dulxanthone E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	193	224.25	2355	149
213	275.20068	274.1934	9.19	$C_{18}H_{26}O_2$	$[M+H]^+$	9-Octadecene-12,14,16-triynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2141.5	5950.5	1225	2412.75	450.75	624.5	150
214	195.06521	194.05794	9.2	$C_{10}H_{10}O_4$	$[M+H]^+$	Dimethyl phthalate	Bruker MetaboBASE Personal Library 2.0_in-silico	492	1168.75	355.25	4715.75	332.5	10881.5	
215	293.2113	292.20402	9.22	$C_{18}H_{28}O_3$	$[M+H]^+$	13-keto-9Z,11E,15Z-octadecatrienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	9871.75	32818.75	5121	12330.25	433.75	606	
216	303.08634	302.07907	9.29	$C_{16}H_{14}O_6$	$[M+H]^+$	2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	216.75	582.5	156.5	2474	32327.25	1601	151
217	419.13398	418.1267	9.37	$C_{21}H_{22}O_9$	$[M+H]^+$	Liquiritin	Bruker MetaboBASE Personal Library 3.0	198.25	124.5	2802.25	316.5	0	0	152
218	241.086	240.07872	9.43	$C_{15}H_{12}O_3$	$[M+H]^+$ $[M-H]^-$	Flavidin	Bruker MetaboBASE Personal Library 2.0_in-silico	65675.75	58843.75	56223.25	39584.5	3768.75	1521.25	153
219	213.09105	212.08378	9.43	$C_{14}H_{12}O_2$	$[M+H]^+$	Benzyl benzoate	Bruker MetaboBASE Personal Library 2.0_in-silico	7289.25	6415.75	6575.5	4693.5	819	0	13
220	177.05465	176.04738	9.47	$C_{10}H_8O_3$	$[M+H]^+$	Herniarin	Bruker MetaboBASE Personal Library 2.0_in-silico	434.75	3777.75	0	19047.75	26161.75	52215.25	72
221	247.0964	246.08913	9.49	$C_{14}H_{14}O_4$	$[M+H]^+$	Torachrysone	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	224.5	671	2162.25	154

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
222	315.08641	314.07914	9.5	$C_{17}H_{14}O_6$	$[M+H]^+$	Gnaphaliin	Bruker MetaboBASE Personal Library 3.0	1563.5	2228.25	2471.75	1333.5	560.75	313.75	155
223	281.08081	280.07353	9.52	$C_{17}H_{12}O_4$	$[M+H]^+$	Neodunol	Bruker MetaboBASE Personal Library 2.0_in-silico	3074.75	3222	3136	2413.25	0	1242.25	156
224	255.19557	254.1883	9.55	$C_{15}H_{26}O_3$	$[M+H]^+$	5-Acetoxydihydrothe-aespirane (6-acet-oxydihydrotheaspirane)	Bruker MetaboBASE Personal Library 2.0_in-silico	1514	1311.75	1382.25	1722	593.5	892.75	
225	207.06627	208.07355	9.58	$C_{11}H_{12}O_4$	$[M-H]^-$ $[M+H]^+$	2,5-Dimethoxycinnamic acid	Bruker MetaboBASE Personal Library 3.0	74502.5	29851.75	65855.75	22564.25	99.75	18228.5	157
226	231.10161	230.09433	9.62	$C_{14}H_{14}O_3$	$[M+H]^+$	Osthenol	Bruker MetaboBASE Personal Library 2.0_in-silico	1613	3897.25	604.5	1579.25	0	0	158
227	131.04913	148.05243	9.67	$C_9H_8O_2$	$[M-H_2O+H]^+$ $[M+H]^+$ $[M-H]^-$	Cinnamic acid	Bruker HMDB Metabolite Library_2.0	16430	23421	26672.25	15542.25	6365.25	6843.5	47
228	233.15363	232.14635	9.66	$C_{15}H_{20}O_2$	$[M+H]^+$	Furoeremophilone 1	Bruker MetaboBASE Personal Library 2.0_in-silico	2181.5	5868	2417	2412.75	0	317.75	159
229	195.06526	194.05799	9.65	$C_{10}H_{10}O_4$	$[M+H]^+$ $[M-H]^-$	Methyl 2,5-dihydroxycinnamate	Bruker MetaboBASE Personal Library 2.0_in-silico	2167.25	2112.75	2216	3275.25	748.25	4942.5	160
230	293.17444	292.16717	9.66	$C_{17}H_{24}O_4$	$[M+H]^+$	Acetylvalerenolic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	544.75	601	1138.5	1403.25	3439.75	161
231	481.11332	480.10604	9.68	$C_{25}H_{20}O_{10}$	$[M+H]^+$	2,3-Dehydrosilychristin	Bruker MetaboBASE Personal Library 2.0_in-silico	2848.25	2289.75	3521.25	1506	120.75	0	162
232	161.05971	178.06301	9.7	$C_{10}H_{10}O_3$	$[M-H_2O+H]^+$ $[M+H]^+$	4-Methoxycinnamic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	47067.5	49463.75	111749	65930.75	8739.25	42694.75	163
233	261.11214	260.10486	9.73	$C_{15}H_{16}O_4$	$[M+H]^+$	Kanzonol Q	Bruker MetaboBASE Personal Library 2.0_in-silico	1992.5	2782.75	2121.75	1453.75	491	350	164
234	269.08086	268.07359	9.81	$C_{16}H_{12}O_4$	$[M+H]^+$ $[M-H]^-$	6-hydroxy-5-methoxy-2-phenylchromen-4-one	MoNA-export-GNPS_QTOF.msp	465366.5	447774.75	351345.5	249969.25	21251.5	13094.5	165
235	447.12938	446.1221	9.92	$C_{22}H_{22}O_{10}$	$[M+H]^+$	Prunetin 5-O-glucoside	Bruker MetaboBASE Personal Library 3.0	4838.5	5085	5688.5	3016.5	0	0	166
236	231.06513	230.05786	9.93	$C_{13}H_{10}O_4$	$[M+H]^+$	4-methoxy-7-methyl-furo[3,2-g]chromen-5-one	MoNA-export-GNPS_QTOF.msp	0	238.25	40	561	1971	4090.5	167
237	367.1178	384.12113	9.96	$C_{21}H_{20}O_7$	$[M-H_2O+H]^+$ $[M+H]^+$	Calebin A	Bruker MetaboBASE Personal Library 2.0_in-silico	323.5	7345.25	361	38081.75	77101.5	97277	
238	279.23198	278.22471	9.95	$C_{18}H_{30}O_2$	$[M+H]^+$	4E,6E,11Z-Hexadecatrienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2624	2570.25	2497.25	2006	1067.25	114.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
239	401.15963	400.15235	10.02	$C_{22}H_{24}O_7$	$[M+H]^+$	Isoyatein	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	13133.5	168
240	223.16936	222.16209	10	$C_{14}H_{22}O_2$	$[M+H]^+$	Isokobusone	Bruker MetaboBASE Personal Library 3.0	1644.75	2217.25	4910.25	2529	461.5	815.25	169
241	121.06484	120.05756	9.99	C_8H_8O	$[M+H]^+$	Phenylacetaldehyde	Bruker HMDB Metabolite Library_2.0	2083	1532.75	2671.25	1826.5	0	181	170
242	549.17583	548.16855	10.01	$C_{30}H_{28}O_{10}$	$[M+H]^+$	3,4-Dihydroxyrottlerin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	249.25	84.25	1423.75	4459.75	6240.5	
243	227.07032	226.06305	10.05	$C_{14}H_{10}O_3$	$[M+H]^+$	Oroselone	Bruker MetaboBASE Personal Library 2.0_in-silico	35070.25	29699	36637.25	24497	1510	874.5	171
244	253.17982	252.17255	10.04	$C_{15}H_{24}O_3$	$[M+H]^+$	Dendrobane A	Bruker MetaboBASE Personal Library 2.0_in-silico	1083.25	940	644.75	680	3799.75	1862	172
245	177.05464	176.04736	10.11	$C_{10}H_8O_3$	$[M+H]^+$	2-Propenal, 3-(1,3-benzodioxol-5-yl)-	Bruker MetaboBASE Personal Library 3.0	4469.75	15559.5	3392.25	45620.25	59998	137147.25	173
246	373.12839	372.12112	10.09	$C_{20}H_{20}O_7$	$[M+H]^+$	6,7,2',4',5'-Penta-methoxyisoflavone	Bruker MetaboBASE Personal Library 2.0_in-silico	43626	32210	33053	18046	3034	18148.5	174
247	287.22182	286.21455	10.13	$C_{16}H_{30}O_4$	$[M+H]^+$	Hexadecanedioic acid	Bruker HMDB Metabolite Library_2.0	0	0	0	0	0	5566.5	175
248	271.0965	270.08922	10.15	$C_{16}H_{14}O_4$	$[M+H]^+$	Alpinetin	MoNA-export-GNPS_QTOF.msp	273466.25	208158.5	265600.75	156823.5	17843.25	7103.5	176
249	359.11263	358.10536	10.13	$C_{19}H_{18}O_7$	$[M+H]^+$	Penduletin 4'-methyl ether	MoNA-export-GNPS_QTOF.msp	2828.75	6429	2561	2505	347	156.75	177
250	287.09156	286.08429	10.31	$C_{16}H_{14}O_5$	$[M+H]^+$	Quinquangulin	Bruker MetaboBASE Personal Library 2.0_in-silico	347	817	263.75	650	3019	2193.5	178
251	285.112	284.10473	10.33	$C_{17}H_{16}O_4$	$[M+H]^+$	DL-Propylene glycol dibenzoate	Bruker MetaboBASE Personal Library 2.0_in-silico	359.75	340	325	762	2305.5	2265.5	
252	271.06011	270.05284	10.32	$C_{15}H_{10}O_5$	$[M+H]^+$	Naringenin	MoNA-export-GNPS_QTOF.msp	9018.5	9872	15664.75	5953.75	0	0	109
253	291.15889	290.15161	10.33	$C_{17}H_{22}O_4$	$[M+H]^+$ $[M-H]^-$	1-Dehydro-[6]-gingerdione	Bruker MetaboBASE Personal Library 2.0_in-silico	82.75	0	67.25	971.5	1306.75	3839.75	179
254	205.15872	204.15144	10.34	$C_{14}H_{20}O$	$[M+H]^+$	4'-tert-Butyl-2',6'-dimethylacetophenone	Bruker MetaboBASE Personal Library 3.0	1080.75	1714	1469	1262.25	0	381.5	
255	313.23724	312.22997	10.36	$C_{18}H_{32}O_4$	$[M+H]^+$	8,13-dihydroxy-9,11-octadecadienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	938.25	1465.25	1026.5	982.5	0	69.75	
256	195.0653	194.05803	10.38	$C_{10}H_{10}O_4$	$[M+H]^+$	Ferulic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1635.5	1165.75	1462.25	1186.5	0	0	47

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
257	345.09653	344.08926	10.43	$C_{18}H_{16}O_7$	$[M+H]^+$	Pachypodol	MoNA-export-GNPS_QTOF.msp	6955	16294.25	8405.25	5036.25	2822.25	1609	180
258	251.20062	250.19332	10.55	$C_{16}H_{26}O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	3E,8Z,11Z-Tetradecatrienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	87.5	0	0	126	13806	
259	409.12721	408.11993	10.56	$C_{23}H_{20}O_7$	$[M+H]^+$	Dehydroamorphigenin	Bruker MetaboBASE Personal Library 2.0_in-silico	2099	1274.5	1718.25	1135.5	315.75	157	181
260	165.05454	164.04727	10.58	$C_9H_8O_3$	$[M+H]^+$	Caffeic aldehyde	Bruker MetaboBASE Personal Library 2.0_in-silico	1298.25	840	0	655.25	266.25	3247.25	182
261	193.08601	192.07873	10.6	$C_{11}H_{12}O_3$	$[M+H]^+$	Ethyl-*p*-coumarate	Bruker MetaboBASE Personal Library 2.0	4270.75	2629.75	4105.25	3841.25	880.25	11262.5	108
262	87.04395	86.03668	10.64	$C_4H_6O_2$	$[M+H]^+$	Diacetyl (2,3-Butanedione)	Bruker MetaboBASE Personal Library 2.0_in-silico	709.5	1108.75	1308.5	752.5	123.75	0	183
263	383.11275	382.10548	10.69	$C_{21}H_{18}O_7$	$[M+H]^+$	Sarothranol	Bruker MetaboBASE Personal Library 2.0_in-silico	796.25	1374	397.75	9324.25	11388.5	25365	184
264	329.10207	328.09488	10.72	$C_{18}H_{16}O_6$	$[M+H]^+$ $[M+Na]^+$	5,8-Dihydroxy-7-methoxyflavanone 8-O-acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	101632	94462.5	87372	66007.5	7402.75	3287.75	185
265	297.18515	296.17787	10.73	$C_{20}H_{24}O_2$	$[M+H]^+$	Crocetindial	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	162.5	2292.25	5387	
266	357.2645	358.27178	10.75	$C_{20}H_{38}O_5$	$[M-H]^-$ $[M+H]^+$	13,14-dihydro Prostaglandin F1α	Bruker MetaboBASE Personal Library 3.0	1463	2216	1074.75	605.25	0	0	
267	223.09659	222.08931	10.77	$C_{12}H_{14}O_4$	$[M+H]^+$	Ferulic acid ethylester	Bruker MetaboBASE Personal Library 3.0	1351.25	916.75	1497	1320.25	338.5	4838.75	186
268	387.14405	386.13677	10.77	$C_{21}H_{22}O_7$	$[M+H]^+$ $[M-H]^-$	Peucenidin	Bruker MetaboBASE Personal Library 2.0_in-silico	23391	18287.5	13442.5	7938.5	649	513.5	187
269	223.16945	222.16217	10.79	$C_{14}H_{22}O_2$	$[M+H]^+$	7E,9Z,11-Dodecatrienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	157.25	0	0	399	2588.75	1415.75	188
270	271.2268	270.21953	10.87	$C_{16}H_{30}O_3$	$[M+H]^+$	7-keto palmitic acid (2-oxopalmitic acid)	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	190.25	9009.5	189
271	317.06567	316.05839	10.85	$C_{16}H_{12}O_7$	$[M+H]^+$	Rhamnetin	Bruker MetaboBASE Personal Library 2.0	87221.5	109741.5	128084.25	103041.75	21993	19674.25	105
272	315.08642	314.07914	10.91	$C_{17}H_{14}O_6$	$[M+H]^+$	Luteolin 3',4'-dimethyl ether	Bruker MetaboBASE Personal Library 3.0	17728.25	19961	12428.75	7591.25	84355	52928.25	190
273	157.06479	174.0681	10.95	$C_{11}H_{10}O_2$	$[M-H_2O+H]^+$ $[M-H]^-$ $[M+H]^+$	Juarezic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	113591.5	95722.25	146095.5	53698	7774.5	19764.75	191

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
274	309.20615	308.19886	10.99	$C_{18}H_{28}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	Corchorifatty acid D	Bruker MetaboBASE Personal Library 2.0_in-silico	11290.75	21903.5	12001	9016.75	496.5	661.25	192
275	231.13795	230.13067	11.02	$C_{15}H_{18}O_2$	$[M+H]^+$	3-(1,2,3,4-Tetrahydro-6-hydroxy-2-naphthyl)-cyclopentanone	Bruker MetaboBASE Personal Library 2.0_in-silico	671	1923.25	807.25	843.5	214	309.75	
276	249.14852	248.14124	10.99	$C_{15}H_{20}O_3$	$[M+H]^+$	1,2-Dihydrosantonin	Bruker MetaboBASE Personal Library 2.0_in-silico	1404	5118	1934.25	2090.25	2301.75	877.75	
277	325.20207	326.20935	11	$C_{18}H_{30}O_5$	$[M-H]^-$ $[M+H]^+$	2,3-dinor Prostaglandin E1	Bruker MetaboBASE Personal Library 2.0	4095	8497.5	4008.5	3957.25	1186.5	80.5	
278	293.21112	292.20385	11.03	$C_{18}H_{28}O_3$	$[M+H]^+$	9-hydroxy-10E,14Z-octadecadien-12-ynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	4282.5	4276.75	4766	2702.25	3055	257.75	
279	181.12237	180.1151	11.03	$C_{11}H_{16}O_2$	$[M+H]^+$	Norecasantalic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1187.25	865.5	1472.25	1097	2290.5	1691.25	193
280	233.15368	232.1464	11.04	$C_{15}H_{20}O_2$	$[M+H]^+$	Turmeronol A	Bruker MetaboBASE Personal Library 2.0_in-silico	2665.5	4612	3074	1987.25	3111	1028	194
281	339.25331	338.24604	11.05	$C_{20}H_{34}O_4$	$[M+H]^+$	Aphidicolin	Bruker MetaboBASE Personal Library 2.0_in-silico	797.5	1451.75	1425.25	438	0	0	
282	357.26388	356.25661	11.09	$C_{20}H_{36}O_5$	$[M+H]^+$ $[M-H]^-$	13,14-dihydro-15(R)-Prostaglandin E1	Bruker MetaboBASE Personal Library 2.0	975	2143.25	1030.75	651	0	0	
283	345.09692	344.08965	11.09	$C_{18}H_{16}O_7$	$[M+H]^+$	Eupatilin	Bruker MetaboBASE Personal Library 3.0	2103.25	1645	1332	497	44284.5	24472.5	195
284	317.21116	316.20388	11.14	$C_{20}H_{28}O_3$	$[M+H]^+$	19-Hydroxy-13-cis-retinoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	106.5	320.75	102	1963.75	3338	10962.25	
285	303.08642	302.07914	11.14	$C_{16}H_{14}O_6$	$[M+H]^+$	Hesperetin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1300.75	3066.25	3189.75	196
286	301.10708	300.09981	11.19	$C_{17}H_{16}O_5$	$[M+H]^+$	Coelogin	Bruker MetaboBASE Personal Library 2.0_in-silico	14468.25	13902	9621.5	7337.5	3965.5	4530.75	197
287	297.24247	296.23517	11.23	$C_{18}H_{32}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$	7-Methoxy-9-methyl-hexadeca-4E,8E-dienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	17697.25	39579	15160	18966	1028.5	611.25	
288	313.23845	314.24572	11.24	$C_{18}H_{34}O_4$	$[M-H]^-$ $[M+H]^+$ $[M+Na]^+$	(±)12,13-DiHOME	Bruker MetaboBASE Personal Library 3.0	23790.5	51379.5	18733.75	23159.75	2960.25	492	
289	261.22154	260.21427	11.23	$C_{18}H_{28}O$	$[M+H]^+$	6-[5]-ladderane-1-hexanol	Bruker MetaboBASE Personal Library 2.0_in-silico	3364.25	8161.25	3032.5	3065.5	805	274.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
290	291.19564	290.18836	11.21	$C_{18}H_{26}O_3$	$[M+H]^+$	8-hydroxy-17-octadecene-10,12-diynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	4511.75	10565.25	3611.25	5179	1923	1803.5	198
291	189.09101	188.08373	11.23	$C_{12}H_{12}O_2$	$[M+H]^+$	Trigoforin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	363	2241.75	199
292	311.22179	310.21447	11.32	$C_{18}H_{30}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$ $[M-H]^-$	9(S)-HpOTrE	Bruker MetaboBASE Personal Library 2.0	17679.5	31960.5	15819.75	17693	1544.25	646.75	
293	263.09147	262.08419	11.32	$C_{14}H_{14}O_5$	$[M+H]^+$	Dorsteniol	Bruker MetaboBASE Personal Library 2.0_in-silico	3440.5	56604.5	1898.25	245847.75	645616.25	610795.75	
294	205.19518	204.18791	11.35	$C_{15}H_{24}$	$[M+H]^+$ $[M+NH_4]^+$	Aristolene	Bruker MetaboBASE Personal Library 3.0	37211	48455	69051.5	49193	4608.5	12638.5	.
295	231.13804	230.13073	11.37	$C_{15}H_{18}O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	8,12-Epoxy-4(15),7,11-eudesmatrien-1-one	Bruker MetaboBASE Personal Library 2.0_in-silico	25575	43918.25	30510	26586	1868.75	5092	
296	221.15369	220.14641	11.37	$C_{14}H_{20}O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	Oblongolide	Bruker MetaboBASE Personal Library 2.0_in-silico	5901	9529.75	6386.5	6150.25	942	1412.75	200
297	249.14858	248.1413	11.38	$C_{15}H_{20}O_3$	$[M+H]^+$	Istanbulin B	Bruker MetaboBASE Personal Library 2.0_in-silico	11476.25	21131.25	12900.5	12286.25	984.25	3170.75	201
298	149.1325	148.12523	11.36	$C_{11}H_{16}$	$[M+H]^+$	Ectocarpen	Bruker MetaboBASE Personal Library 2.0_in-silico	15296.25	21912.5	32938.25	21525	2026.5	5623	
299	175.07533	174.06805	11.37	$C_{11}H_{10}O_2$	$[M+H]^+$	Menadiol	Bruker MetaboBASE Personal Library 2.0_in-silico	2385	3447.75	2664	2261.25	419	737.5	202
300	121.06478	120.05751	11.37	C_8H_8O	$[M+H]^+$ $[M-H]^-$	Lentialexin	Bruker MetaboBASE Personal Library 2.0_in-silico	2984.5	5539	4096.75	3117.75	261.25	430.75	203
301	123.11681	122.10953	11.36	C_9H_{14}	$[M+H]^+$	Santene	Bruker MetaboBASE Personal Library 2.0_in-silico	8411.75	11813.75	12105.5	10404.25	1440.25	3577.5	204
302	205.15873	204.15146	11.37	$C_{14}H_{20}O$	$[M+H]^+$ $[M-H]^-$	2,4-di-tert-butylphenol	Bruker MetaboBASE Personal Library 2.0_in-silico	1439.5	2016	1634	1516	202	106.25	205
303	315.19586	314.18858	11.39	$C_{20}H_{26}O_3$	$[M+H]^+$	all-trans-4-oxoretinoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	202.25	1418	3127	
304	357.13319	356.12591	11.43	$C_{20}H_{20}O_6$	$[M+H]^+$	5-Deoxykievitol	Bruker MetaboBASE Personal Library 2.0_in-silico	608.75	542.25	922.75	881.5	2472.25	3405.75	206
305	355.1178	354.11053	11.41	$C_{20}H_{18}O_6$	$[M+H]^+$	Albanin A	Bruker MetaboBASE Personal Library 2.0_in-silico	6348.75	3979.25	3969.75	2276.5	2252.5	2364.5	207
306	315.15914	314.15186	11.44	$C_{19}H_{22}O_4$	$[M+H]^+$	7-[(6-Hydroxy-3,7-dimethyl-2,7-octadienyl)oxy]-2H-1-benzopyran-2-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2771.75	826.25	2256.75	286.5	0	0	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
307	299.09143	298.08416	11.47	$C_{17}H_{14}O_5$	$[M+H]^+$	Coumafuryl	Bruker MetaboBASE Personal Library 2.0_in-silico	1345.25	2762.5	911.5	9536.25	17611.75	21435.5	
308	181.04957	180.04227	11.49	$C_9H_8O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	Caffeic acid	Bruker HMDB Metabolite Library_2.0	75988.75	45336.5	88463.5	52345.75	4813	6696.25	208
309	161.05969	160.05242	11.53	$C_{10}H_8O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	2,6-Dihydroxynaphthalene	Bruker MetaboBASE Personal Library 2.0	38529.5	18602.5	50752.5	27188.25	10676.25	15272.5	
310	469.33155	468.3242	11.52	$C_{30}H_{44}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	3-oxoglycyrrhetinic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	7452.25	41188.75	5981	7819.25	116.75	149.75	209
311	271.09628	270.08913	11.53	$C_{16}H_{14}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	Isoliquiritigenin 4-methyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	12518.5	4302.25	16948.25	7391	2636.25	2656.5	210
312	487.34234	486.33507	11.52	$C_{30}H_{46}O_5$	$[M+H]^+$	Bridgesigenin A	MoNA-export-GNPS_QTOF.msp	1566.75	11526.75	1208.5	1053.75	388	202.75	211
313	225.09102	224.08374	11.53	$C_{15}H_{12}O_2$	$[M+H]^+$	2-Propenoic acid, 2,3-diphenyl- (α-Phenylcinnamic acid)	Bruker MetaboBASE Personal Library 3.0	2899.5	1390.75	3729.25	2361.25	876.75	1261.75	
314	381.13341	380.12613	11.5	$C_{22}H_{20}O_6$	$[M+H]^+$	Glabrachromene I	Bruker MetaboBASE Personal Library 2.0_in-silico	0	535.5	89	2302.75	5268	5194.25	212
315	373.12846	372.12119	11.54	$C_{20}H_{20}O_7$	$[M+H]^+$	Sigmoidin D	Bruker MetaboBASE Personal Library 2.0_in-silico	3053.75	1569.75	2260.75	1136.5	374	0	213
316	329.10198	328.09471	11.56	$C_{18}H_{16}O_6$	$[M+H]^+$	Betagarin	Bruker MetaboBASE Personal Library 2.0_in-silico	1719.75	978.5	2194	3084	9089.5	13849.5	214
317	255.06522	254.05794	11.59	$C_{15}H_{10}O_4$	$[M+H]^+$	5,7-dihydroxy-2-phenylchromen-4-one	MoNA-export-GNPS_QTOF.msp	225150.25	345864.5	234362.25	1227562	230067.25	314435	
318	277.21623	276.20895	11.63	$C_{18}H_{28}O_2$	$[M+H]^+$	Stearidonic Acid	Bruker MetaboBASE Personal Library 2.0	18042.75	34894.5	14979.25	18556.5	0	0	215
319	285.07576	284.06848	11.62	$C_{16}H_{12}O_5$	$[M+H]^+$	Acacetin	Bruker MetaboBASE Personal Library 3.0	107822.25	123263.25	82459.5	149121	0	0	208
320	309.20612	308.19885	11.61	$C_{18}H_{28}O_4$	$[M+H]^+$	Corchorifatty acid A	Bruker MetaboBASE Personal Library 2.0_in-silico	3609.25	6124	4309	5106.25	152.5	91.5	
321	139.07535	138.06808	11.67	$C_8H_{10}O_2$	$[M+H]^+$	1,2-Dimethoxybenzene	Bruker MetaboBASE Personal Library 2.0_in-silico	2807.5	2167.5	2209	3294.75	109.75	748.75	77
322	203.10667	202.09939	11.68	$C_{13}H_{14}O_2$	$[M+H]^+$	3-(3-Methylbutylidene)-1(3H)-isobenzofuranone	Bruker MetaboBASE Personal Library 2.0_in-silico	23016	23081	22344.75	35745	1915.25	4451.5	
323	345.09688	344.0896	11.75	$C_{18}H_{16}O_7$	$[M+H]^+$	Morin 3,2',4'-trimethyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	130.75	175.75	701.5	78	813	29678.5	216

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
324	311.09142	310.08415	11.74	$C_{18}H_{14}O_5$	$[M+H]^+$	Hoslundal	Bruker MetaboBASE Personal Library 2.0_in-silico	4123.75	3079.5	6233.75	4134.5	3518.25	5566.5	217
325	263.20068	280.20398	11.73	$C_{17}H_{28}O_3$	$[M-H_2O+H]^+$ $[M+H]^+$	Methyl (2E,6E,10R,11S)-10,11-epoxy-3,7,11-trimethyltrideca-2,6-dienoate	Bruker MetaboBASE Personal Library 2.0_in-silico	1473	3727.5	678	2595	0	0	
326	363.21694	362.20966	11.74	$C_{21}H_{30}O_5$	$[M+H]^+$	Hydrocortisone	MoNA-export-GNPS_QTOF.msp	0	456.25	0	2721.75	882.5	1429.75	
327	285.07571	284.06843	11.75	$C_{16}H_{12}O_5$	$[M+H]^+$	Genkwanin	Bruker MetaboBASE Personal Library 2.0	237289.5	134332.5	123476	222583	0	0	218
328	325.10707	324.09979	11.78	$C_{19}H_{16}O_5$	$[M+H]^+$	Neoraunone	Bruker MetaboBASE Personal Library 2.0_in-silico	893	865.25	1463	2514	9950	6380	219
329	325.23745	324.23014	11.83	$C_{19}H_{32}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$ $[M+Na]^+$	Methyl-10-hydroperoxy-8E,12Z,15Z-octadecatrienoate	Bruker MetaboBASE Personal Library 2.0_in-silico	2526.5	7700.25	2101.25	3658.25	0	0	
330	375.10755	374.10027	11.84	$C_{19}H_{18}O_8$	$[M+H]^+$	Chrysosplenol E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	5772.25	220
331	271.06	270.05273	11.83	$C_{15}H_{10}O_5$	$[M+H]^+$ $[M-H]^-$	3,5,7-trihydroxy-2-phenylchromen-4-one	MoNA-export-GNPS_QTOF.msp	674429	605289.75	672793.5	544246.75	70230.25	19863.75	47
332	205.1948	204.18753	11.84	$C_{15}H_{24}$	$[M+H]^+$	(+)-endo-beta-Bergamotene	Bruker MetaboBASE Personal Library 2.0_in-silico	2662.25	3624	5065	4656	1057.25	2306.5	
333	381.20627	380.199	11.85	$C_{24}H_{28}O_4$	$[M+H]^+$	Conferone	Bruker MetaboBASE Personal Library 2.0_in-silico	2053.25	4197.25	1617.5	1983.25	0	0	221
334	253.21631	252.20901	11.87	$C_{16}H_{28}O_2$	$[M+H]^+$ $[M-H_2O+H]^+$	11-Methyl-9Z,12-tridecadienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	1007	851	809.5	4338.75	2629.75	11222	
335	271.22657	288.22999	11.87	$C_{16}H_{32}O_4$	$[M-H_2O+H]^+$ $[M+H]^+$	9,10-dihydroxy-hexadecanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	208.75	0	3802.5	2374.25	9279.25	
336	285.11211	284.1048	11.91	$C_{17}H_{16}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	Phenethyl Caffeiate	Bruker MetaboBASE Personal Library 3.0	53763	30541	68561.75	40815.25	2349.5	4783.75	222
337	105.06964	104.06236	11.9	C_8H_8	$[M+H]^+$	Styrene	Bruker MetaboBASE Personal Library 3.0	63997	49360.5	73423.25	36581	1280.5	2735.25	13
338	95.08547	94.07819	11.91	C_7H_{10}	$[M+H]^+$	2-Methyl-1,3-cyclohexadiene	Bruker MetaboBASE Personal Library 2.0_in-silico	1585	3104.25	2226.5	1520	0	216.25	223
339	239.20051	238.19323	11.92	$C_{15}H_{26}O_2$	$[M+H]^+$	14-Pentadecynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1816	6286	4724.5	3462.5	230.5	722.25	
340	269.08083	268.07356	11.94	$C_{16}H_{12}O_4$	$[M+H]^+$	3-hydroxy-6-methoxy-2-phenylchromen-4-one	MoNA-export-GNPS_QTOF.msp	69233.75	26738	24979	18406.25	2306.25	2314.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
341	293.21129	292.20396	11.94	$C_{18}H_{28}O_3$	$[M+H]^+$ $[M-H]^-$ $[M-H_2O+H]^+$	9-OxoOTrE	Bruker MetaboBASE Personal Library 3.0	47871.25	52686	33370.75	39098.25	3679.5	5753.75	224
342	167.03385	166.02657	11.96	$C_8H_6O_4$	$[M+H]^+$	Benzoquinoneacetic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	79640.5	35959.75	26321	30847	5718.25	3875.25	
343	287.09141	286.08414	11.97	$C_{16}H_{14}O_5$	$[M+H]^+$	5-Methoxynaringenin	MoNA-export-GNPS_QTOF.msp	121416.25	65478	60597.75	57999	10267.5	8006.5	225
344	331.08125	330.07398	12	$C_{17}H_{14}O_7$	$[M+H]^+$	Remerin	Bruker MetaboBASE Personal Library 2.0_in-silico	82366.25	96813.75	90317	61600.5	143491.75	77535.5	226
345	139.04008	140.04736	11.98	$C_7H_8O_3$	$[M-H]^-$ $[M+H]^+$	2-methoxyresorcinol	Bruker MetaboBASE Personal Library 3.0	8932.75	4061.25	3314	4614	1901.25	1213	227
346	313.10709	312.09982	12.02	$C_{18}H_{16}O_5$	$[M+H]^+$	1,4,5-Trihydroxy-3-prenylxanthone	Bruker MetaboBASE Personal Library 2.0_in-silico	1804.75	1344.25	1625.25	0	294	0	228
347	453.33651	452.32924	12.09	$C_{30}H_{44}O_3$	$[M+H]^+$	3-Oxo-12,18-ursadien-28-oic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	5874.75	15705	11001	7966.75	1994.5	2759.25	
348	335.21948	312.23007	12.12	$C_{18}H_{32}O_4$	$[M+Na]^+$ $[M+H]^+$	10S,11S-epoxy-9S-hydroxy-12Z-octadecenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2917.5	3784	2477	2584.25	167.25	559	
349	299.20056	298.19328	12.13	$C_{20}H_{26}O_2$	$[M+H]^+$	19-Norethindrone-68-22-4	MoNA-export-GNPS_QTOF.msp	77.5	467	362.25	3250.25	16416.25	43587.5	
350	293.21129	292.20401	12.11	$C_{18}H_{28}O_3$	$[M+H]^+$	(-)-8-hydroxy-11E,17-octadecadien-9-ynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	37525	61963	21773.25	26666.5	1416.5	1152.75	
351	285.07568	284.0684	12.15	$C_{16}H_{12}O_5$	$[M+H]^+$	Izalpinin	Bruker MetaboBASE Personal Library 3.0	558337.5	415818.75	480346.25	87217.25	29282.75	20028.25	229
352	325.20093	324.19365	12.15	$C_{18}H_{28}O_5$	$[M+H]^+$	Dinor-PGE2	Bruker MetaboBASE Personal Library 2.0_in-silico	950.25	1720.25	1290.5	962.5	108.75	65.25	
353	341.23225	340.22498	12.15	$C_{19}H_{32}O_5$	$[M+H]^+$	Idebenone Metabolite (1,4-Benzenediol, 2-(10-hydroxydecyl)-5,6-dimethoxy-3-methyl-)	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1011	203.5	2479.75	230
354	353.26869	352.26142	12.18	$C_{21}H_{36}O_4$	$[M+H]^+$	Ebelactone B	Bruker MetaboBASE Personal Library 3.0	1027.75	3109.5	0	2364	0	0	
355	249.11309	250.12037	12.22	$C_{14}H_{18}O_4$	$[M-H]^-$ $[M+H]^+$	Di-n-propylphthalate	Bruker MetaboBASE Personal Library 2.0_in-silico	44432	41899.5	46063.75	102275.5	3228.75	10641.75	
356	177.09104	176.08376	12.24	$C_{11}H_{12}O_2$	$[M+H]^+$	Prenylbenzoquinone	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	84.5	142.75	4169.75	1636.5	231

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
357	309.24255	308.23527	12.28	$C_{19}H_{32}O_3$	$[M+H]^+$	Methyl 12,13-epoxy-9,15-octadecadienoate	Bruker MetaboBASE Personal Library 2.0_in-silico	1082.25	2470.75	698.25	1074.75	134	274.5	
358	293.08179	294.08906	12.15	$C_{18}H_{14}O_4$	$[M-H]^-$ $[M+H]^+$	6-Methoxy-[2",3":7,8]furanoflavanone	Bruker MetaboBASE Personal Library 2.0_in-silico	9551.25	6149	17100	10969.25	13026.25	7250	232
359	291.23185	290.22457	12.28	$C_{19}H_{30}O_2$	$[M+H]^+$	11beta-Hydroxy-5alpha-androstan-17-one	Bruker MetaboBASE Personal Library 2.0_in-silico	1067.25	3261.75	910.75	1318.25	0	107.25	
360	159.08054	158.07326	12.27	$C_{11}H_{10}O$	$[M+H]^+$	2-Methoxynaphthalene	Bruker MetaboBASE Personal Library 2.0	0	189.5	118	0	2978.75	809	
361	353.1019	352.09462	12.32	$C_{20}H_{16}O_6$	$[M+H]^+$	Bavacoumestan B	Bruker MetaboBASE Personal Library 2.0_in-silico	4216.25	1370	4235.75	2918.75	513.5	529.5	233
362	233.15343	232.14615	12.35	$C_{15}H_{20}O_2$	$[M+H]^+$	Alantolactone	Bruker MetaboBASE Personal Library 2.0_in-silico	2604.25	4690	3182.75	4026.75	351.5	0	234
363	295.09739	296.10467	12.43	$C_{18}H_{16}O_4$	$[M-H]^-$ $[M-H_2O+H]^+$ $[M+H]^+$	Demethoxyegonol	Bruker MetaboBASE Personal Library 2.0_in-silico	351209	414601.5	405024.75	369880	52206.75	48108.75	235
364	251.10662	250.09934	12.41	$C_{17}H_{14}O_2$	$[M+H]^+$	Flindersiachromone	Bruker MetaboBASE Personal Library 2.0_in-silico	4800.5	4270.75	6036.75	3332	338	0	
365	253.08685	254.09413	12.43	$C_{16}H_{14}O_3$	$[M-H]^-$ $[M-H_2O+H]^+$ $[M+H]^+$	Obtusaquinone	Bruker MetaboBASE Personal Library 2.0_in-silico	69949	72008.5	63262.5	179358.75	789810.25	554479.25	
366	299.0914	298.08412	12.45	$C_{17}H_{14}O_5$	$[M+H]^+$	3-hydroxy-3',4'-dimethoxyflavone	Bruker MetaboBASE Personal Library 3.0	31658.5	35193	24468.25	11657.5	2793.25	2196	236
367	209.09611	208.08884	12.43	$C_{15}H_{12}O$	$[M+H]^+$	1-Methoxyphenanthrene	Bruker MetaboBASE Personal Library 2.0_in-silico	129	101.25	317.5	1063	5181.25	3295.25	
368	295.22687	294.21959	12.48	$C_{18}H_{30}O_3$	$[M+H]^+$	9-OxoODE	Bruker MetaboBASE Personal Library 2.0	90412	182657.75	45522.25	114690.25	2494.75	4487	
369	231.10248	232.10976	12.58	$C_{14}H_{16}O_3$	$[M-H]^-$ $[M+H]^+$	Encecalin	Bruker MetaboBASE Personal Library 2.0_in-silico	119632	156849.75	87849	100313.25	6327.5	26258.25	1
370	157.08592	156.07864	12.48	$C_8H_{12}O_3$	$[M+H]^+$	5-oxo-7-octenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	8297.5	18574.75	4431.5	11307.25	0	323	
371	197.15368	196.1464	12.48	$C_{12}H_{20}O_2$	$[M+H]^+$	Allyl cyclohexylpropionate	Bruker MetaboBASE Personal Library 2.0_in-silico	3706	7728	1882.5	5045	0	111.25	237
372	219.17437	236.17774	12.52	$C_{15}H_{24}O_2$	$[M-H_2O+H]^+$ $[M+Na]^+$	Capsidiol	Bruker MetaboBASE Personal Library 3.0	9496.25	12708.5	11266	8960	5764	4912.75	238
373	227.20077	226.19349	12.53	$C_{14}H_{26}O_2$	$[M+H]^+$	Myristoleic acid	Bruker MetaboBASE Personal Library 2.0	0	206.25	0	1663.75	2862	6368	239

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
374	119.04903	118.04176	12.56	C_8H_6O	$[M+H]^+$ $[M-H]^-$	2,4,6-Octatriyn-1-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	7560.25	8098	5466.75	4491	788.5	1943.25	
375	187.11164	186.10437	12.61	$C_{13}H_{14}O$	$[M+H]^+$	(all-E)-3,5,7-Tridecatriene-9,11-diyn-1-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	6939	15113.25	3591.75	6461.75	131	1189.25	
376	151.03882	150.03155	12.61	$C_8H_6O_3$	$[M+H]^+$	Piperonal	Bruker MetaboBASE Personal Library 2.0	6152	4722	8008.75	12116	21563.5	19683.75	240
377	283.0974	284.10468	12.61	$C_{17}H_{16}O_4$	$[M-H]^-$ $[M+H]^+$ $[M-H_2O+H]^+$	3,4-dimethoxydalbergione	Bruker MetaboBASE Personal Library 2.0_in-silico	11615	6642	16017.5	36550.75	132702.75	109671.25	241
378	267.19552	266.18825	12.68	$C_{16}H_{26}O_3$	$[M+H]^+$	4-Hydroxy-3-methoxy-2,10-bisaboladien-9-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2542.75	4554.5	1389	1950.75	1393.75	1903.75	
379	249.18486	248.17758	12.69	$C_{16}H_{24}O_2$	$[M+H]^+$	C16:4n-2,5,9,12	Bruker MetaboBASE Personal Library 2.0_in-silico	1933	3517	1198	2186.75	109.5	430	
380	343.24786	342.24058	12.69	$C_{19}H_{34}O_5$	$[M+H]^+$	methyl 9,12-dihydroxy-13-oxo-10-octadecenoate	Bruker MetaboBASE Personal Library 2.0_in-silico	389	528.25	0	1524.25	13245.5	0	
381	457.33147	456.32419	12.76	$C_{29}H_{44}O_4$	$[M+H]^+$	Callystatin A	Bruker MetaboBASE Personal Library 2.0_in-silico	2165.25	94.75	671.5	497	5173	13424.75	
382	245.11721	244.10994	12.78	$C_{15}H_{16}O_3$	$[M+H]^+$	Osthol	Bruker MetaboBASE Personal Library 3.0	4728.25	4397.75	4178	3669.75	1438.5	648	
383	287.09135	286.08407	12.85	$C_{16}H_{14}O_5$	$[M+H]^+$	Naringenin 5-methyl ether	Bruker MetaboBASE Personal Library 3.0	10643.5	12211.75	11200.5	5549.5	1268.75	0	242
384	291.19544	290.18816	12.82	$C_{18}H_{26}O_3$	$[M+H]^+$	8-oxo-9,11-octadecadiynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	6754.25	9470	6006	6226	4847.75	3543.75	243
385	271.09645	270.08918	12.87	$C_{16}H_{14}O_4$	$[M+H]^+$	Alpinetin	Bruker MetaboBASE Personal Library 3.0	36115.5	37739.25	17011.25	13291.5	1540.5	1209.5	244
386	203.14312	202.13585	12.87	$C_{14}H_{18}O$	$[M+H]^+$	(±)-Anisoxide	Bruker MetaboBASE Personal Library 2.0_in-silico	1592.25	478	1011.25	746.25	0	0	245
387	329.10197	328.09469	12.91	$C_{18}H_{16}O_6$	$[M+H]^+$	Isotectorigenin, 7-methyl ether	Bruker MetaboBASE Personal Library 3.0	3591.5	5544.5	3548.75	2181.5	22196.75	11091.5	
388	299.1278	298.1205	12.94	$C_{18}H_{18}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	5,7-Dimethoxy-6-C-methylflavanone	Bruker MetaboBASE Personal Library 2.0_in-silico	23170.5	15710.25	27780.75	11509.75	3989.25	4190.75	246
389	335.25821	352.26148	13.02	$C_{21}H_{36}O_4$	$[M-H_2O+H]^+$ $[M+H]^+$	Montanol	Bruker MetaboBASE Personal Library 2.0_in-silico	3853	19532.25	591	10212	0	0	247
390	303.23183	302.22456	12.99	$C_{20}H_{30}O_2$	$[M+H]^+$	Sandaracopimaric acid	Bruker MetaboBASE Personal Library 3.0	1007	283	7417.5	1201.25	144	1318.25	248

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
391	247.09665	246.08938	12.99	$C_{14}H_{14}O_4$	$[M+H]^+$	Aegelinol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	97.5	0	173.5	3096.5	1818.25	249
392	345.09689	344.08962	13.02	$C_{18}H_{16}O_7$	$[M+H]^+$	3,5-Dihydroxy-6,7,8-trimethoxyflavone	Bruker MetaboBASE Personal Library 2.0_in-silico	33140.75	10018.5	11790.5	5288.5	5924.75	12209.75	250
393	263.12865	264.13593	13.03	$C_{15}H_{20}O_4$	$[M-H]^-$ $[M+H]^+$	2-Methyl-1-[2,4,6-trihydroxy-3-(3-methyl-2-butenyl)phenyl]-1-propanone	Bruker MetaboBASE Personal Library 2.0_in-silico	17846.25	19299.25	16131.75	13265.25	1455.75	5867.25	
394	341.10207	340.09479	13.04	$C_{19}H_{16}O_6$	$[M+H]^+$	Methyl 2-(5-hydroxy-4-oxo-2-phenylchromen-7-yl)oxypropanoate	Bruker MetaboBASE Personal Library 3.0	5610.5	4364	4357.25	4753.75	359.25	0	
395	181.04956	180.04228	13.03	$C_9H_8O_4$	$[M+H]^+$	trans-2,3-Dihydroxycinnamate	Bruker MetaboBASE Personal Library 2.0_in-silico	2993	2828.75	2911.75	2184	0	1055.5	109
396	329.2687	328.26143	13.04	$C_{19}H_{36}O_4$	$[M+H]^+$	MG(16:1(9Z)/0:0/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	1805	1973	1030	1163.25	1841	10401.5	
397	357.13333	356.12605	13.06	$C_{20}H_{20}O_6$	$[M+H]^+$	Kenusanone J	Bruker MetaboBASE Personal Library 2.0_in-silico	4419	6898.5	4669.5	3089.25	773.75	675	251
398	191.03385	190.02657	13.08	$C_{10}H_6O_4$	$[M+H]^+$	8H-1,3-Dioxolo[4,5-h][1]benzopyran-8-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2464.5	1737.5	3586	1793.75	1000.75	1400.5	
399	353.23255	352.22528	13.17	$C_{20}H_{32}O_5$	$[M+H]^+$ $[M-H]^-$	Prostaglandin I2	Bruker MetaboBASE Personal Library 3.0	3723.5	7061.25	3642.75	4528.75	665.5	0	
400	217.15861	216.15134	13.14	$C_{15}H_{20}O$	$[M+H]^+$	Furanodiene	Bruker MetaboBASE Personal Library 2.0_in-silico	0	644	0	107.5	5168.5	2009	252
401	313.10706	312.09979	13.17	$C_{18}H_{16}O_5$	$[M+H]^+$	Helilandin A	Bruker MetaboBASE Personal Library 2.0_in-silico	4125.75	2593.25	3570.75	2589.75	2136.25	4553.5	253
402	443.35207	442.3448	13.21	$C_{29}H_{46}O_3$	$[M+H]^+$	Camellenodiol	Bruker MetaboBASE Personal Library 2.0_in-silico	1961	0	518.25	203.25	1249.5	16875.25	254
403	335.22147	334.2142	13.21	$C_{20}H_{30}O_4$	$[M+H]^+$	15(S)-HpEPE	Bruker MetaboBASE Personal Library 2.0_in-silico	1519.75	2617.75	1631.75	1798	555.75	1909	
404	291.23193	308.23523	13.23	$C_{19}H_{32}O_3$	$[M-H_2O+H]^+$ $[M+H]^+$	methyl 9,10-epoxy-12,15-octadecadienoate	Bruker MetaboBASE Personal Library 2.0_in-silico	3425.5	14648	2099.75	6246.75	0	63	
405	279.10252	280.10979	13.27	$C_{18}H_{16}O_3$	$[M-H]^-$, $[M+H]^+$	6-hydroxy-7Z,9E-Octadecadiene-11,13,15,17-tetraynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	166468.25	207941.5	137062.75	190695	15425.5	29875.75	
406	239.20059	238.19332	13.29	$C_{15}H_{26}O_2$	$[M+H]^+$	(Z)-3-Nonenyl (E)-2-hexenoate	Bruker MetaboBASE Personal Library 2.0_in-silico	2148	8871	3759.5	2945	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
407	253.21634	252.20906	13.32	$C_{16}H_{28}O_2$	$[M+H]^+$	(E)-3,7-Dimethyl-2,6-octadienyl hexanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	197.25	905.5	307	1144.25	6893.75	63081	
408	271.22687	270.2196	13.33	$C_{16}H_{30}O_3$	$[M+H]^+$	7-keto palmitic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	616.25	0	638.5	2753.75	42270.5	
409	133.10109	132.09382	13.31	$C_{10}H_{12}$	$[M+H]^+$	Dicyclopentadiene	Bruker MetaboBASE Personal Library 2.0_in-silico	520.75	1331.5	1039	264.25	672.25	2610.25	255
410	179.14317	178.1359	13.33	$C_{12}H_{18}O$	$[M+H]^+$	Geijerone	Bruker MetaboBASE Personal Library 2.0_in-silico	497.25	511.75	165.25	401	740.5	3237.5	
411	235.16926	234.16199	13.33	$C_{15}H_{22}O_2$	$[M+H]^+$	Confertifoline	Bruker MetaboBASE Personal Library 2.0_in-silico	24972.75	36046.25	28823.5	21101.25	5572.75	3393.75	256
412	165.1273	164.12002	13.35	$C_{11}H_{16}O$	$[M+H]^+$	5-Phenyl-1-pentanol	Bruker MetaboBASE Personal Library 2.0_in-silico	358	589	502	288	654.25	2950.25	
413	369.26379	368.25651	13.38	$C_{21}H_{36}O_5$	$[M+H]^+$	gamma-Eudesmol rhamnoside	Bruker MetaboBASE Personal Library 2.0_in-silico	1006.5	3064	0	6059.75	42992	1929	257
414	195.06518	194.0579	13.38	$C_{10}H_{10}O_4$	$[M+H]^+$	Piperonyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	1509.5	1410.25	1550.25	2185.25	2835.5	2213.5	
415	187.07535	186.06808	13.43	$C_{12}H_{10}O_2$	$[M+H]^+$ $[M-H2O+H]^+$	2-Naphthylacetic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	6622	6881.75	10574.5	9636	1509	1850.75	
416	207.06518	206.05788	13.42	$C_{11}H_{10}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	3-Methoxy-4,5-methyl-enedioxycinnamaldehyde	Bruker MetaboBASE Personal Library 2.0_in-silico	4152.75	4047	6062.75	6295	1402.25	1174.75	258
417	355.11755	354.11027	13.43	$C_{20}H_{18}O_6$	$[M+H]^+$	Isolicoflavonol	Bruker MetaboBASE Personal Library 2.0_in-silico	46419	27671.75	28968	19384.25	2430.25	2483.5	259
418	293.21053	292.20325	13.41	$C_{18}H_{28}O_3$	$[M+H]^+$	12,13S-epoxy-9Z,11,15Z-octadecatrienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	9895	8708.75	4764.75	6004.5	2792.75	15246.75	
419	249.18498	248.17771	13.45	$C_{16}H_{24}O_2$	$[M+H]^+$	16:4(6Z,9Z,12Z,15Z) (6Z,9Z,12Z,15Z-hexadecatetraenoic acid)	Bruker MetaboBASE Personal Library 2.0_in-silico	4372.75	15648.25	3429	5151.25	341.25	338.75	
420	311.22182	310.21454	13.46	$C_{18}H_{30}O_4$	$[M+H]^+$	13S-HpOTrE	Bruker MetaboBASE Personal Library 2.0_in-silico	14064	17119.75	8402.75	9950.5	2049.25	1341.5	
421	383.22187	382.21459	13.5	$C_{24}H_{30}O_4$	$[M+H]^+$	Gummosin	Bruker MetaboBASE Personal Library 3.0	18399	32873.5	18036.25	13826.75	0	507.25	260
422	269.21136	268.20396	13.53	$C_{16}H_{28}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$	(1S,2S)-3-oxo-2-pentyl-cyclopentanehexanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	342.75	178.75	1501.25	14758.5	1958	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
423	357.2789	374.28203	13.55	$C_{24}H_{38}O_3$	$[M-H2O+H]^+$ $[M+H]^+$	1α,24-dihydroxy-25,26,27-trinorvitamin D3 / 1α,24-dihydroxy-25,26,27-trinorcholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	433.5	299.75	0	252.25	13720.25	8563	
424	301.10699	300.09971	13.52	$C_{17}H_{16}O_5$	[M+H]+	Kukulkanin A	Bruker MetaboBASE Personal Library 2.0_in-silico	30089	35206.25	39087	18667	7904.75	32705.25	261
425	457.25868	456.25152	13.6	$C_{27}H_{36}O_6$	$[M+H]^+$ $[M-H_2O+H]^+$	Lucidenic acid F	Bruker MetaboBASE Personal Library 2.0_in-silico	9192.25	9213.75	6414.5	8555.5	1268	88.25	262
426	313.14341	312.13613	13.58	$C_{19}H_{20}O_4$	$[M+H]^+$	Gancaonin V	Bruker MetaboBASE Personal Library 2.0_in-silico	1046.75	2498.75	2142.25	2148.25	210.5	1002.75	263
427	291.23188	290.22461	13.61	$C_{19}H_{30}O_2$	$[M+H]^+$	Epietiocholanolone	Bruker HMDB Metabolite Library_2.0	2440.75	3350	1868.5	2098.25	325.5	967.75	
428	305.24765	304.24037	13.61	$C_{20}H_{32}O_2$	$[M+H]^+$	(-)-Cladielline	Bruker MetaboBASE Personal Library 2.0_in-silico	1169	164.5	3746	1550.75	5404.75	4457.25	
429	321.24343	322.25071	13.63	$C_{20}H_{34}O_3$	$[M-H]^-$ $[M+H]^+$	8(S)-HETrE	Bruker MetaboBASE Personal Library 2.0_in-silico	3021	1603.75	11002.25	4411.25	13934	11232.5	
430	373.29512	372.28785	13.67	$C_{21}H_{40}O_5$	$[M+H]^+$	1-Glyceryl ricinoleate	Bruker MetaboBASE Personal Library 3.0	7033.5	2456	6647.5	3362	0	375.5	
431	271.09633	270.08905	13.67	$C_{16}H_{14}O_4$	$[M+H]^+$	(E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one	MoNA-export-GNPS_QTOF.msp	611881.5	661345.25	655435	464812.5	73173.75	215456	
432	337.23707	336.22979	13.69	$C_{20}H_{32}O_4$	$[M+H]^+$	15-epi-PGA1	Bruker MetaboBASE Personal Library 2.0	2543	6931.5	3669.5	3397	0	179.5	
433	355.24857	354.24129	13.68	$C_{20}H_{34}O_5$	$[M+H]^+$ $[M-H]^-$	11β-13,14-dihydro-15-keto PGF2α	Bruker MetaboBASE Personal Library 2.0_in-silico	0	3554	2587.5	0	0	0	
434	355.28422	354.27695	13.69	$C_{21}H_{38}O_4$	$[M+H]^+$	2-Linoleoyl Glycerol	Bruker MetaboBASE Personal Library 3.0	7055.5	4531.25	6458.25	5539.75	10069	0	
435	339.25296	338.2458	13.74	$C_{20}H_{34}O_4$	$[M+H]^+$, $[M+Na]^+$	(±)8,9-DHET	Bruker MetaboBASE Personal Library 2.0_in-silico	6688.5	17903.5	12599	7016.25	0	169.5	
436	191.07024	190.06296	13.71	$C_{11}H_{10}O_3$	$[M+H]^+$	Hymecromone methyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	12630.5	9924.25	11446.75	8133.75	2223.75	1544.25	
437	209.08085	208.07357	13.72	$C_{11}H_{12}O_4$	$[M+H]^+$	DL-Benzylsuccinic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	3040.25	2164.25	3015.25	2275.75	838.25	208	
438	281.24749	280.24022	13.73	$C_{18}H_{32}O_2$	$[M+H]^+$	11-Hexadecynyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	3286.25	2074.75	3458.75	2556.75	2971.25	2700.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
439	205.12232	204.11504	13.77	$C_{13}H_{16}O_2$	$[M+H]^+$	Cinnamyl butyrate	Bruker MetaboBASE Personal Library 2.0_in-silico	1038	1425.75	5278.5	638.75	0	4042	264
440	231.1379	230.13063	13.76	$C_{15}H_{18}O_2$	$[M+H]^+$	Eremanthin	MoNA-export-GNPS_QTOF.msp	3848.75	5095.75	4032.25	3001.25	1021	1097	265
441	159.08033	158.07306	13.77	$C_{11}H_{10}O$	$[M+H]^+$	(2-Naphthyl)methanol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	571.75	2130.5	0	0	1523.25	
442	319.2259	318.21863	13.78	$C_{20}H_{30}O_3$	$[M+H]^+$	Galanal A	Bruker MetaboBASE Personal Library 2.0_in-silico	2777.5	4779.75	3097.25	2754	0	936.75	266
443	279.23183	278.22456	13.79	$C_{18}H_{30}O_2$	$[M+H]^+$	Pinolenic Acid	Bruker MetaboBASE Personal Library 3.0	11388.75	15414	9622	12137.25	0	0	267
444	295.22775	296.23502	13.83	$C_{18}H_{32}O_3$	$[M-H]^-$ $[M+H]^+$	Leukotoxin a (9,10-eode)	Bruker MetaboBASE Personal Library 3.0	20789	34391.5	17638.75	16000.25	5060.25	0	
445	387.27472	386.26744	13.85	$C_{21}H_{38}O_6$	$[M+H]^+$	3-methoxy Prostaglandin F1α	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	2947.25	
446	473.32652	472.31925	13.86	$C_{29}H_{44}O_5$	$[M+H]^+$	Hecogenin acetate	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	2577.5	1554.75	
447	315.2531	314.24583	13.95	$C_{18}H_{34}O_4$	$[M+H]^+$	9,13-dihydroxy-11-octadecenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1837.25	1925.75	1128.5	1205.75	0	304	268
448	271.22774	272.23502	13.97	$C_{16}H_{32}O_3$	$[M-H]^-$ $[M+H]^+$	2-hydroxyhexadecanoic acid	Bruker MetaboBASE Personal Library 3.0	31419.75	30652.5	21920.5	19471	8718.5	47883.75	269
449	237.22139	254.22472	13.97	$C_{16}H_{30}O_2$	$[M-H_2O+H]^+$ $[M+H]^+$	cis-7-Hexadecenoic Acid	Bruker MetaboBASE Personal Library 2.0	28260	29836.25	22857.25	19799	9481.25	73522.25	270
450	219.21081	218.20353	13.97	$C_{16}H_{26}$	$[M+H]^+$	(3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	11873.25	13280.5	9300.5	6403.5	4546.5	32821.25	271
451	171.13792	170.13065	13.99	$C_{10}H_{18}O_2$	$[M+H]^+$	delta-Decalactone	Bruker MetaboBASE Personal Library 3.0	1323.25	1295.5	1063	953.75	439.5	2855.5	272
452	301.23758	300.23031	13.99	$C_{17}H_{32}O_4$	$[M+H]^+$	MG(0:0/14:1(9Z)/0:0) (2-myristoleoyl-glycerol)	Bruker MetaboBASE Personal Library 2.0_in-silico	0	624.75	0	2768.75	10846	12397.25	
453	199.16935	198.16208	14.01	$C_{12}H_{22}O_2$	$[M+H]^+$	cis-5-dodecenoic acid	Bruker MetaboBASE Personal Library 3.0	1445.75	1174	1070.25	1398.5	762.25	3140	273
454	313.27393	312.26659	14.04	$C_{19}H_{36}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$	Ricinoleic Acid methyl ester	Bruker MetaboBASE Personal Library 3.0	4022.25	6992.5	3931.5	3102.25	757.75	1352.5	274
455	253.25277	252.2455	14.03	$C_{17}H_{32}O$	$[M+H]^+$	7-Ethyl-4-pentadecen-6-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2343.75	5090.25	1584.25	1584.75	0	87	275
456	545.38374	544.37646	14.05	$C_{33}H_{52}O_6$	$[M+H]^+$	Ganoderic acid Mi	Bruker MetaboBASE Personal Library 2.0_in-silico	117	880.75	0	108.5	6072	3724	276

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
457	293.10211	292.09484	14.03	$C_{15}H_{16}O_6$	$[M+H]^+$	trans-Grandmarin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1442.25	2590.25	3522.5	277
458	165.05445	164.04717	14.03	$C_9H_8O_3$	$[M+H]^+$	m-Coumaric acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1255.25	1721	787.5	812.25	203.5	978	218
459	387.12264	386.11536	14.05	$C_{24}H_{18}O_5$	$[M+H]^+$ $[M-H]^-$	8-Cinnamoyl-3,4-dihydro-5,7-dihydroxy-4-phenylcoumarin	Bruker MetaboBASE Personal Library 2.0_in-silico	1458	2291.5	1511.5	1099	0	0	
460	317.2688	316.26135	14.08	$C_{18}H_{36}O_4$	$[M+H]^+$ $[M-H_2O+H]^+$	7,8-dihydroxy stearic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	198	0	1926.75	1536	10149.25	
461	199.07535	198.06808	14.08	$C_{13}H_{10}O_2$	$[M+H]^+$	Dehydrosafynol	Bruker MetaboBASE Personal Library 2.0_in-silico	2571.75	1872	2135	1579.5	265.5	107.5	
462	297.24244	296.23517	14.1	$C_{18}H_{32}O_3$	$[M+H]^+$	12S,13R-EpOME	Bruker MetaboBASE Personal Library 2.0_in-silico	304	1626.5	0	1866.25	1075.25	2041.25	
463	505.35249	504.34522	14.11	$C_{30}H_{48}O_6$	$[M+H]^+$	Theasapogenol E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	8336.5	5945.5	278
464	329.23338	330.24065	14.14	$C_{18}H_{34}O_5$	$[M-H]^-$ $[M+H]^+$	9,10,18-trihydroxy-12-octadecenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1431	2665.75	5036.5	279
465	323.25816	322.25085	14.14	$C_{20}H_{34}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$	Austroinulin	Bruker MetaboBASE Personal Library 2.0_in-silico	5356	16110.5	12120.75	6611.75	0	0	280
466	351.25315	368.25664	14.14	$C_{21}H_{36}O_5$	$[M-H_2O+H]^+$ $[M+Na]^+$ $[M+H]^+$	PGF2α methyl ester	Bruker MetaboBASE Personal Library 2.0_in-silico	11192.75	12064.25	6957.75	9237	2056	810.75	
467	275.20074	274.19346	14.14	$C_{18}H_{26}O_2$	$[M+H]^+$	3,6,9,12,15-octadeca-pentaenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	5812.5	24393.5	4674.75	9307	2140.75	658.5	
468	341.26877	340.2615	14.13	$C_{20}H_{36}O_4$	$[M+H]^+$	PGF2α Alcohol	Bruker MetaboBASE Personal Library 2.0_in-silico	4844.5	13979.75	10695.5	5720.75	0	0	
469	219.21085	218.20357	14.16	$C_{16}H_{26}$	$[M+H]^+$	7-Ethyl-3,11-dimethyl-dodeca-1,3,6,10-tetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	412.75	291	1426.25	1893.5	4577	23155.5	
470	475.24807	474.24079	14.15	$C_{30}H_{34}O_5$	$[M+H]^+$	Poinsettifolin B	Bruker MetaboBASE Personal Library 2.0_in-silico	1939	5328	1049.25	2061.75	0	0	281
471	237.22149	236.21422	14.16	$C_{16}H_{28}O$	$[M+H]^+$	5-Cyclohexadecen-1-one	Bruker MetaboBASE Personal Library 3.0	3442	1557.5	1223.75	3272.25	11102.75	18571.5	
472	279.23182	278.22454	14.2	$C_{18}H_{30}O_2$	$[M+H]^+$	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl propionate	Bruker MetaboBASE Personal Library 2.0_in-silico	9248.5	14604.25	8284.75	7727.25	543	3301	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
473	389.13821	388.13093	14.16	$C_{24}H_{20}O_5$	$[M+H]^+$	Calomelanol D-1	Bruker MetaboBASE Personal Library 2.0_in-silico	1512.5	1893	720.5	1327.25	0	0	282
474	317.21078	316.2035	14.17	$C_{20}H_{28}O_3$	$[M+H]^+$	Pisiferic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	436.5	1456.25	1186.5	4833.25	5211.75	17448.5	283
475	405.13333	404.12606	14.24	$C_{24}H_{20}O_6$	$[M+H]^+$	Calomelanol C	Bruker MetaboBASE Personal Library 2.0_in-silico	55863.25	64157.5	64036.5	81470.75	5087.5	1873.25	284
476	261.22133	260.21405	14.26	$C_{18}H_{28}O$	$[M+H]^+$	5-(1-oxopropan-2-yl)isolongifol-5-ene	Bruker MetaboBASE Personal Library 2.0_in-silico	2086	1763	2157	924.5	506.75	986.75	
477	301.21625	300.20898	14.3	$C_{20}H_{28}O_2$	$[M+H]^+$	6,9,12-Eicosatriynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	84	973	547.25	1945.75	2892.75	13098.5	
478	401.30556	400.29829	14.3	$C_{26}H_{40}O_3$	$[M+H]^+$	(17E)-1α,25-dihydroxy-17,20-didehydro-21-norvitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	6507.5	843.25	1210.25	0	1419.75	5331.75	
479	299.0914	298.08413	14.29	$C_{17}H_{14}O_5$	$[M+H]^+$	7-hydroxy-8,4'-dimethoxyisoflavone	Bruker MetaboBASE Personal Library 3.0	101773.75	84437.5	90295.25	53274.25	6401.25	4000.75	285
480	299.25819	298.25091	14.36	$C_{18}H_{34}O_3$	$[M+H]^+$	4-keto stearic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1374	153.5	1433.25	1201.75	1159.25	2079.75	
481	221.1536	220.14632	14.4	$C_{14}H_{20}O_2$	$[M+H]^+$	2-Methyl-1-phenyl-2-propanyl butyrate	Bruker MetaboBASE Personal Library 2.0_in-silico	1387.75	851.5	830.25	969.75	334	339.25	
482	455.352	454.34475	14.42	$C_{30}H_{46}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$	Dehydro (11,12) ursolic acid lactone	Bruker MetaboBASE Personal Library 3.0	9505.75	11411.75	9150	7792.75	14946.25	13766.5	
483	321.24216	338.2457	14.44	$C_{20}H_{34}O_4$	$[M-H_2O+H]^+$ $[M+Na]^+$ $[M-H]^-$	11-deoxy-PGE1	Bruker MetaboBASE Personal Library 3.0	10526.75	26903.75	20005.75	9793	0	3325	
484	293.2475	292.24023	14.49	$C_{19}H_{32}O_2$	$[M+H]^+$	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl butyrate	Bruker MetaboBASE Personal Library 2.0_in-silico	2164.25	5881.75	1563.75	2328.25	291.5	465.5	
485	207.17434	206.16706	14.53	$C_{14}H_{22}O$	$[M+H]^+$	9Z,11E,13-Tetradecatrienal	Bruker MetaboBASE Personal Library 2.0_in-silico	4714.75	6511.75	3255.5	3971.5	12071.5	4850.75	
486	387.28909	386.28181	14.52	$C_{25}H_{38}O_3$	$[M+H]^+$	Testosterone isocaproate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	19794	3598.5	
487	387.12269	386.11541	14.51	$C_{24}H_{18}O_5$	$[M+H]^+$	Calomelanol J	Bruker MetaboBASE Personal Library 2.0_in-silico	972	2146	0	1322	0	0	286
488	339.25321	338.24594	14.53	$C_{20}H_{34}O_4$	$[M+H]^+$	(±)14,15-DHET	Bruker MetaboBASE Personal Library 2.0_in-silico	3308.75	7598.5	3133	3116	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
489	415.31994	414.31266	14.52	$C_{27}H_{42}O_3$	$[M+H]^+$	(22E)-(24R)-24,25-dihydroxy-22,23-didehydrovitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	165	61	84.25	0	3156.25	1893	
490	253.21622	270.21953	14.52	$C_{16}H_{30}O_3$	$[M-H_2O+H]^+$ $[M+H]^+$	9-Hexadecenoic acid, 12-hydroxy-, (Z)-(+)-	Bruker MetaboBASE Personal Library 2.0_in-silico	1066.75	1098.5	632.75	397.75	389.25	10939.25	
491	225.2213	224.21402	14.54	$C_{15}H_{28}O$	$[M+H]^+$	8E,10Z-Pentadecadien-1-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	131.25	0	311.25	184.5	362.25	2645	
492	257.2113	256.20403	14.58	$C_{15}H_{28}O_3$	$[M+H]^+$	Lyngbic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2386	3531.5	1221.25	1564.75	2574.25	1801.5	
493	545.38391	544.37663	14.6	$C_{33}H_{52}O_6$	$[M+H]^+$	11α-Hemiglutaryloxy-1,25-dihydroxyvitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	241	359.25	61.75	0	11396.75	5801.75	
494	263.23706	262.22976	14.61	$C_{18}H_{30}O$	$[M+H]^+$ $[M-H_2O+H]^+$	Farnesyl acetone	Bruker MetaboBASE Personal Library 3.0	78895.5	89342.75	67287.5	71669.75	7502.25	3171.75	
495	281.24763	280.24035	14.61	$C_{18}H_{32}O2$	$[M+H]^+$	Linoleic acid	Bruker HMDB Metabolite Library_2.0	55538.75	61807.75	48993.5	49519.75	5253.75	3305	17
496	297.2434	298.25067	14.79	$C_{18}H_{34}O_3$	$[M-H]^-$ $[M+H]^+$	6R,7S-epoxy-octadecanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	15956.75	27655	13092.25	18014	72278.5	6672.75	
497	197.15358	196.1463	14.62	$C_{12}H_{20}O_2$	$[M+H]^+$	Artemisia alcohol acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	1701.25	2612.25	2232	2581.75	212.75	0	287
498	221.22649	220.21921	14.62	$C_{16}H_{28}$	$[M+H]^+$	4,8,12-Trimethyl-1,3E,7E,11-tridecatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	1893.5	1830.5	1892.75	1968.75	0	0	288
499	211.16934	210.16206	14.62	$C_{13}H_{22}O_2$	$[M+H]^+$	2-Propenyl cyclohexanebutanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	2986.25	3156.25	2733.75	2716.25	146.5	115.75	
500	185.1536	184.14632	14.61	$C_{11}H_{20}O_2$	$[M+H]^+$	6E-Nonenyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2250.25	2485	2032.5	2030	454	0	
501	473.36273	472.35545	14.63	$C_{30}H_{48}O_4$	$[M+H]^+$	26,27-diethyl-1α,25-dihydroxy-20,21-didehydro-23-oxavitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	1827.25	3060.5	3222	2834.5	47559.75	24519.25	
502	325.10703	324.09976	14.65	$C_{19}H_{16}O_5$	$[M+H]^+$	2,3-dehydro-UWM6	Bruker MetaboBASE Personal Library 2.0_in-silico	1700.75	1679.5	2717.25	494.25	281.5	757.25	
503	157.10109	156.09381	14.66	$C_{12}H_{12}$	$[M+H]^+$	2,6-Dimethylnaphthalene	Bruker MetaboBASE Personal Library 2.0	1062.5	2786.25	2678	1812.75	291	647.75	
504	307.22679	306.21958	14.67	$C_{19}H_{30}O_3$	$[M+H]^+$ $[M-H_2O+H]^+$ $[M-H]^-$	Androst-5-ene-3beta,17beta,19-triol	Bruker MetaboBASE Personal Library 2.0_in-silico	2053.75	3435.25	1143	1169	0	87.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
505	475.37787	474.3706	14.68	C30H50O4	[M+H]+	Priverogenin B	Bruker MetaboBASE Personal Library 2.0_in-silico	0	541	205.25	585.5	32349.25	19314.75	289
506	179.14301	178.13574	14.69	C12H18O	[M+H]+	Quinceoxepine	Bruker MetaboBASE Personal Library 2.0_in-silico	2087.75	3136.5	1170.25	1806	2987.75	1383.75	
507	443.35165	442.34437	14.72	C29H46O3	[M+H]+	(22E)-1α,25-dihydroxy-26,27-dimethyl-22,23-didehydrocholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	11990.25	1527.25	2692.25	623.25	4148.75	7526.75	
508	269.24768	268.24033	14.77	C17H32O2	[M+H]+ [M-H2O+H]+	cis-7-Hexadecenoic Acid methyl ester	Bruker MetaboBASE Personal Library 3.0	7687.75	9465.75	8771.5	6481.75	1443.5	618.75	
509	283.22689	282.21953	14.78	C17H30O3	[M+H]+ [M-H]- [M-H2O+H]+	Acetylenic acids; 10-Heptadecen-8-ynoic acid, 7-hydroxy-	Bruker MetaboBASE Personal Library 2.0_in-silico	1784	5726	1276.25	2521.75	0	108.25	290
510	233.22638	232.2191	14.78	C17H28	[M+H]+	6,8-Diethyl-4-methyl-3E,5E,7E,9E-dodecatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	2875.5	3420.25	3416.5	2561.5	560.25	129.25	
511	281.24762	298.25095	14.8	C18H34O3	[M-H2O+H]+ [M+H]+	(9Z,12R)-12-Hydroxyoctadec-9-enoic acid	Bruker MetaboBASE Personal Library 3.0	10438.5	14659.5	7699.5	23381.75	108412.25	8476	
512	327.28956	326.28227	14.84	C20H38O3	[M+H]+ [M-H2O+H]+	2-oxophytanic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	13642	17346	16108	9312.5	834.25	1030.5	
513	345.30004	344.29277	14.84	C20H40O4	[M+H]+	11,12-dihydroxy arachidic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2514.25	4260.75	3194.25	2130.75	0	317.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
514	225.18496	224.17768	14.84	C14H24O2	[M+H]+	Neryl butyrate	Bruker MetaboBASE Personal Library 2.0_in-silico	2958.75	11184.5	5025	5079	1786.75	1131	291
515	263.23704	262.22977	14.88	C18H30O	[M+H]+	6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one	Bruker MetaboBASE Personal Library 2.0_in-silico	11384.25	25190.25	7640.5	8510.25	46412	8467.25	292
516	391.28446	390.27719	14.92	C24H38O4	[M+H]+	Nutriacholic acid	Bruker HMDB Metabolite Library_2.0	0	0	0	0	6021.75	1949.75	293
517	277.21629	294.21968	14.94	C18H30O3	[M-H2O+H]+ [M+H]+ [M+Na]+ [M+NH4]+	9(S)-HOTrE	Bruker MetaboBASE Personal Library 3.0	648286	513315.25	535754.5	637242.25	35031.75	93474.25	
518	207.13791	206.13064	14.94	C13H18O2	[M+H]+	Etrogol	Bruker MetaboBASE Personal Library 2.0_in-silico	3201.25	4627	2683.75	3497.5	401.75	475	294
519	457.36769	456.36041	15.06	C30H48O3	[M+H]+	Soyasapogenol E	Bruker MetaboBASE Personal Library 2.0_in-silico	1087.5	1130.75	0	2232.5	76346.75	35733.75	295
520	499.37788	498.37061	15.07	C32H50O4	[M+H]+	Oleanolic acid acetate	Bruker MetaboBASE Personal Library 3.0	0	0	0	99.25	14361.25	7457.5	296
521	587.39461	586.38733	15.13	C35H54O7	[M+H]+	Ganoderic acid Md	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	5366.25	2397.5	276
522	253.2528	252.24552	15.24	C17H32O	[M+H]+	13-heptadecyn-1-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	5102.25	1040.75	6303.75	2270.75	0	1065.75	297
523	331.26321	330.25593	15.24	C22H34O2	[M+H]+	7Z, 10Z, 13Z, 16Z, 19Z-docosapentaenoic acid (n-3 docosapentaenoic acid)	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	23640.5	5024.5	298

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
524	205.19508	204.1878	15.22	C15H24	[M+H]+	sesquisabinene	Bruker MetaboBASE Personal Library 2.0_in-silico	14646.25	28761.75	27858	23212.25	3711.75	8128.75	299
525	279.15909	278.15181	15.25	C16H22O4	[M+H]+	Diisobutyl phthalate	Bruker MetaboBASE Personal Library 3.0	4777.25	3078.25	4029	3987.25	5521.5	3996	17
526	205.08592	204.07865	15.27	C12H12O3	[M+H]+	3-Butylidene-7-hydroxyphthalide	Bruker MetaboBASE Personal Library 2.0_in-silico	7458	5367.75	6084	6428.75	4686.5	5094.25	300
527	411.27438	410.26713	15.3	C23H38O6	[M+H]+ [M+Na]+ [M-H2O+H]+	PGF2α-11-acetate methyl ester	Bruker MetaboBASE Personal Library 2.0_in-silico	2274.75	7480.5	1569	3216.25	0	0	
528	109.10107	108.09379	15.27	C8H12	[M+H]+	4-Vinylcyclohexene	Bruker MetaboBASE Personal Library 2.0_in-silico	1445	3554.5	3093.75	2656.5	2328.25	1181.75	
529	177.09095	176.08368	15.34	C11H12O2	[M+H]+	3-(4-Methoxyphenyl)-2-methyl-2-propenal	Bruker MetaboBASE Personal Library 2.0_in-silico	0	5165.5	60.5	2030.5	0	0	
530	485.36252	484.35524	15.35	C31H48O4	[M+H]+	1-Hydroxyprevitamin D3 diacetate	Bruker MetaboBASE Personal Library 2.0_in-silico	951.5	73	0	0	11659.75	5852.75	
531	255.23203	254.22475	15.38	C16H30O2	[M+H]+	11Z-hexadecenoic acid	Bruker MetaboBASE Personal Library 2.0	2443.75	3995.75	3068	2075	0	4290.5	301
532	477.39371	476.38643	15.42	C30H52O4	[M+H]+	Panaxatriol	Bruker MetaboBASE Personal Library 2.0_in-silico	80	1123.75	245.5	4229	0	16218.25	302
533	357.27945	356.27287	15.43	C24H36O2	[M+H]+, [M-H2O+H]+	4,8,12,15,19,21-tetracosahexaenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	323	0	334	1549	344337.5	106819.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
534	297.24251	296.23518	15.5	C18H32O3	[M+H]+ [M+NH4]+ [M+Na]+ [M-H2O+H]+	13(R)-HODE	Bruker MetaboBASE Personal Library 3.0	124031.5	225428.25	99084.75	109842.25	2551.25	15166.25	
535	473.36265	472.35537	15.52	C30H48O4	[M+H]+	Momordicin I	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	439.5	47103.5	29170.25	303
536	283.26333	282.25602	15.59	C18H34O2	[M+H]+ [M-H2O+H]+	Petroselinic acid	Bruker MetaboBASE Personal Library 3.0	31455	28278.75	34576.25	26185.5	5227.25	7044.25	304
537	273.24251	272.23524	15.58	C16H32O3	[M+H]+	3-hydroxy-hexadecanoic acid	Bruker MetaboBASE Personal Library 2.0	0	138.25	0	1853.25	0	9290.25	
538	431.31563	430.30933	15.68	C27H42O4	[M+H]+ [M+HCOOH-H]- [M-H]-, [M+Na]+	3β-Hydroxy-5α,6α-epoxy-9-oxo-9,10-seco-5-cholest-7-en-11-al	Bruker MetaboBASE Personal Library 2.0_in-silico	163	458.5	138.25	727	28708	14998.5	
539	201.185	200.17772	15.66	C12H24O2	[M+H]+	Lauric acid	Bruker MetaboBASE Personal Library 3.0	701	888	575.25	3259.5	6733.75	7190.25	305
540	205.19511	204.18784	15.69	C15H24	[M+H]+	sesquisabinene	Bruker MetaboBASE Personal Library 2.0_in-silico	68292	3238	25012.75	4385	4962.25	1022	299
541	471.38323	470.37595	15.67	C31H50O3	[M+H]+	Momoridcin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	9020.5	1353.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
542	371.14896	370.14169	15.67	C21H22O6	[M+H]+	2',4'-Dihydroxy-3'-isovaleryloxy-6'-methoxychalcone	Bruker MetaboBASE Personal Library 2.0_in-silico	12874.75	10340.25	13338	7559.75	1603	470.5	
543	253.25272	252.24545	15.66	C17H32O	[M+H]+	7-Ethyl-4-pentadecen-6-one	Bruker MetaboBASE Personal Library 2.0_in-silico	5842.75	6072.75	4508.25	3489	0	442.75	
544	149.1325	148.12523	15.69	C11H16	[M+H]+	Ectocarpen	Bruker MetaboBASE Personal Library 2.0_in-silico	9810	718.25	3883.75	448	1907	0	
545	475.37794	474.37067	15.67	C30H50O4	[M+H]+	26,27-diethyl-1α,25-dihydroxy-22-oxavitamin D3 / 26,27-diethyl-1α,25-dihydroxy-22-oxacholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	1723	624.75	1024.25	492.25	38543	23248.25	
546	457.36771	456.36043	15.72	C30H48O3	[M+H]+	(20S)-20-butyl-1α,25-dihydroxy-16,17-didehydro-21-norvitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	3388.75	485.25	1075	1150	43163	12480.5	
547	191.17943	190.17215	15.76	C14H22	[M+H]+	7-Ethyl-3,5-dimethyl-2E,4E,6E,8E-decatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	0	112.75	0	0	10943	6126	
548	329.26879	328.26152	15.81	C19H36O4	[M+H]+	MG(0:0/16:1(9Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	840.5	1207	422.75	2406.75	6769.5	8330.5	
549	301.21638	300.2091	15.8	C20H28O2	[M+H]+ [M-H]-	Dehydroabietic acid	Bruker MetaboBASE Personal Library 3.0	772.25	544.5	1019.75	2176.75	5772	12527.5	129
550	491.37337	490.36583	15.86	C30H50O5	[M+H]+ [M-H2O+H]+	Alisol A	Bruker MetaboBASE Personal Library 3.0	5363.5	5056.5	10342.25	4389	220866.75	164891.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
551	309.24258	308.23531	15.87	C19H32O3	[M+H]+ [M+Na]+ [M-H2O+H]+	3-Methyl-5-pentyl-2-furannonanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	11538	42539	9443.5	13573	0	756.5	
552	321.2423	320.23502	15.86	C20H32O3	[M+H]+	Crispane	Bruker MetaboBASE Personal Library 2.0_in-silico	1703	2877.75	2908	2022	2234	371	306
553	281.2472	280.23992	15.86	C18H32O2	[M+H]+	Stearolic acid	Bruker MetaboBASE Personal Library 3.0	2196.5	2869.25	2761.75	2551.25	422.25	0	307
554	339.25353	338.24626	15.89	C20H34O4	[M+H]+	Zoapatanol	Bruker MetaboBASE Personal Library 2.0_in-silico	3022	5329.5	5605.25	0	0	0	308
555	381.30005	380.29277	15.95	C23H40O4	[M+H]+	Isopersin	Bruker MetaboBASE Personal Library 2.0_in-silico	1957.75	901.25	1631.25	958	0	10337	309
556	269.24757	268.24029	15.99	C17H32O2	[M+H]+	12E-Pentadecenyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2468	2849	2954.75	1875.25	0	0	
557	457.3676	456.36029	16.02	C30H48O3	[M+H]+ [M-H2O+H]+	Olean-12-en-28-oic acid, 3-hydroxy-, (3beta,5xi,9xi,18xi)-	MoNA-export-GNPS_QTOF.msp	4248.25	2866	2556.25	15711	250894.25	190914.5	310
558	369.3	368.29273	16.04	C22H40O4	[M+H]+ [M+Na]+ [M-H2O+H]+ [M-H]-	FAHFA 22:1; FAHFA 2:0/20:1; [M-H]-	MSDIAL-LipidDBs-VS34.msp	6477.75	9944.25	4346.75	4026.25	0	0	
559	441.37275	440.36554	16.05	C30H48O2	[M+H]+ [M-H2O+H]+	3beta-3-Hydroxy-18-lupen-21-one	Bruker MetaboBASE Personal Library 2.0_in-silico	12034.75	5814.75	3817.75	3592.75	177216	88245.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
560	355.2845	354.2772	16.08	C21H38O4	[M+H]+ [M+Na]+	PGF2α Alcohol methyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	4802.25	13520	1328.5	8661	0	0	
561	337.2738	336.26652	16.06	C21H36O3	[M+H]+	3-Methyl-5-pentyl-2-furanundecanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	4516	13228.25	1106.75	7734.75	0	0	311
562	267.26837	266.26111	16.08	C18H34O	[M+H]+ [M-H2O+H]+	13E-Octadecenal	Bruker MetaboBASE Personal Library 2.0_in-silico	52272.5	22727.25	59715.25	35956.25	2510.25	5024	
563	327.28967	326.28229	16.08	C20H38O3	[M+H]+ [M-H2O+H]+	11-Eicosenoic acid, 14-hydroxy-, [R-(E)]-	Bruker MetaboBASE Personal Library 2.0_in-silico	44347	19552	51148	28179.75	1997.25	4313.25	
564	309.24268	308.23534	16.09	C19H32O3	[M+H]+ [M-H2O+H]+	methyl 12,13-epoxy-9,15-octadecadienoate	Bruker MetaboBASE Personal Library 2.0_in-silico	244.75	616.5	0	621.5	43694.25	0	
565	345.30023	344.29295	16.08	C20H40O4	[M+H]+	11,12-dihydroxy arachidic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	15089.25	3331.75	16359.5	5896	0	484	312
566	291.26831	290.26103	16.08	C20H34O	[M+H]+	(-)-Dilophol	Bruker MetaboBASE Personal Library 2.0_in-silico	7352.25	3184	8061.75	4528	550.25	1012.25	313
567	327.25356	326.24629	16.09	C19H34O4	[M+H]+	Avocadyne 4-acetate	Bruker MetaboBASE Personal Library 3.0	0	0	0	117	4655.25	0	314
568	531.36826	530.36099	16.09	C32H50O6	[M+H]+	Acinospesigenin A	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	24427.75	18135	315
569	323.12787	322.12059	16.12	C20H18O4	[M+H]+	7-hydroxy-3-[4-hydroxy-3-(3-methylbut-2-enyl)-phenyl]chromen-4-one	MoNA-export-GNPS_QTOF.msp	5730.75	2960	5262.5	1997.75	668	151.5	316

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
570	277.21624	276.20896	16.09	C18H28O2	[M+H]+	Stearidonic Acid	Bruker MetaboBASE Personal Library 2.0	5344.5	10270.5	3889	5483.25	21101	751.5	215
571	315.25306	314.24578	16.13	C18H34O4	[M+H]+	Octadecanedioic acid	Bruker HMDB Metabolite Library_2.0	0	2682.25	0	1029	0	0	317
572	271.1904	270.18313	16.15	C15H26O4	[M+H]+	Ethylene brassylate	Bruker MetaboBASE Personal Library 3.0	0	59	0	1620.25	376.75	4827	318
573	475.37819	474.37091	16.15	C30H50O4	[M+H]+	Priverogenin B	Bruker MetaboBASE Personal Library 2.0_in-silico	7894.5	8293	11819	5481.75	663127.25	371166.5	289
574	301.21613	300.20885	16.15	C20H28O2	[M+H]+	ent-6,16-Kauradien-19-oic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	544.75	212.75	2140.5	566.5	530	376.25	
575	359.2943	358.28702	16.17	C24H38O2	[M+H]+	Hyrtial	Bruker MetaboBASE Personal Library 2.0_in-silico	883.5	114	0	0	61239.5	13378.5	
576	429.33605	428.32877	16.19	C28H44O3	[M+H]+	Ergosterol Peroxide_120246	MoNA-export-GNPS_QTOF.msp	226.5	90	0	1532	8421.5	7456.75	
577	311.25812	310.25084	16.2	C19H34O3	[M+H]+ [M-H]-	Methoprene (S)	Bruker MetaboBASE Personal Library 2.0	2778.25	8869.5	2068.25	3781	0	0	
578	357.30007	356.2926	16.24	C21H40O4	[M+H]+ [M-H2O+H]+	MG(0:0/18:1(9Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	9714	3792.25	0	20120.75	545.75	11569	
579	283.26321	282.25594	16.23	C18H34O2	[M+H]+	Petroselinic acid	Bruker MetaboBASE Personal Library 3.0	4653.75	4688.25	8319.75	8766	740.5	754.25	319
580	247.24218	246.2349	16.22	C18H30	[M+H]+	5,7-Diethyl-9-methyl-3E, 5E, 7E,9E-tridecatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	1502.5	1353	2892.75	2537	106.25	391	
581	349.27387	348.26659	16.24	C22H36O3	[M+H]+	CP 47,497-C8-homolog C-8-hydroxy metabolite	Bruker MetaboBASE Personal Library 2.0	1070.75	4415.25	482.5	2694	0	514.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
582	269.24764	268.24036	16.26	C17H32O2	[M+H]+	cis-7-Hexadecenoic Acid methyl ester	Bruker MetaboBASE Personal Library 2.0	7510.5	4588.5	1591.25	2123.5	6279.25	9982.25	
583	237.22138	236.2141	16.27	C16H28O	[M+H]+	10,12-hexadecadienal	Bruker MetaboBASE Personal Library 2.0_in-silico	3037	1609	399.5	1362.75	2802.25	4187	320
584	297.24202	296.23474	16.28	C18H32O3	[M+H]+	Avenoleic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	268.25	1457.25	1451	3678	1722.5	2423.25	321
585	297.27902	296.27174	16.34	C19H36O2	[M+H]+	Methyl oleate	Bruker MetaboBASE Personal Library 2.0	1354.75	1548.5	1387.5	2496	0	136.75	322
586	475.248	474.24075	16.36	C30H34O5	[M+H]+ [M-H2O+H]+	Rubraflavone B	Bruker MetaboBASE Personal Library 2.0_in-silico	5054.5	15254.25	5289.5	6978.75	0	1497.5	
587	487.37839	504.38167	16.36	C31H52O5	[M-H2O+H]+ [M+H]+	1α,25-dihydroxy-2β-(4-hydroxybutoxy)vitamin D3 / 1α,25-dihydroxy-2β-(4-hydroxybutoxy)cholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	60	0	0	48218.25	5892.75	
588	611.45178	610.44451	16.36	C35H62O8	[M+H]+	Trilobalicin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1459.5	4920.5	5465.25	323
589	345.27897	344.27169	16.37	C23H36O2	[M+H]+	plastoquinol-1	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	190.75	8725.75	1013.75	324
590	279.2683	278.26102	16.36	C19H34O	[M+H]+	9S,10R-Epoxy-3Z,6Z-nonadecadiene	Bruker MetaboBASE Personal Library 2.0_in-silico	807	1404.75	1101.25	504.5	0	275.25	
591	307.22691	306.21963	16.37	C19H30O3	[M+H]+	10-HODTA methyl ester	Bruker MetaboBASE Personal Library 2.0_in-silico	3632.75	3874.25	535.5	1088.25	0	0	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
592	469.33133	468.32406	16.46	C30H44O4	[M+H]+	3-oxoglycyrrhetinic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	830.25	690	0	1217	15990	30746.25	325
593	261.22128	260.214	16.44	C18H28O	[M+H]+	5-(1-oxopropan-2-yl)isolongifol-5-ene	Bruker MetaboBASE Personal Library 2.0_in-silico	1468.5	3005.75	3147.75	1834.25	348.5	0	
594	587.39251	586.38524	16.44	C35H54O7	[M+H]+	Ganoderic acid Md	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	9099	1534.5	326
595	469.36743	468.36015	16.47	C31H48O3	[M+H]+	Methyl 3-oxo-12-oleanen-28-oate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	35666	3857.75	
596	415.32092	414.31365	16.49	C27H42O3	[M+H]+	16-Dehydroepicalcitriol	Bruker MetaboBASE Personal Library 2.0_in-silico	2374.5	676.25	0	185.5	27721.5	16827.75	
597	489.22738	488.2201	16.49	C30H32O6	[M+H]+ [M-H]-	Epilumaflavanone B	Bruker MetaboBASE Personal Library 2.0_in-silico	7877.25	19103.25	5627.75	10460.25	123	1088	327
598	357.27925	374.28231	16.53	C24H38O3	[M-H2O+H]+ [M+H]+	1α,24-dihydroxy-25,26,27-trinorvitamin D3 / 1α,24-dihydroxy-25,26,27-trinorcholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	50609	5773.5	
599	325.23754	324.23026	16.54	C19H32O4	[M+H]+	Avocadynone acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	121.75	916	0	5549.75	12970	26770.5	
600	471.34658	470.3393	16.54	C30H46O4	[M+H]+	Dicyclopenta[a,i]phenanthrene-3a(1H)-carboxylic acid, 10-formyloctadecahydro-9-hydroxy-5a,5b,8,8,10a-pentamethyl-1-(1-methylethenyl)-	MoNA-export-GNPS_QTOF.msp	8331	1123.75	1624.5	0	9601.75	5520.25	328

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
601	307.26319	306.25591	16.54	C20H34O2	[M+H]+	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl pentanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	14412.75	19527.5	15873.75	7655	124	372.5	
602	399.31124	398.30396	16.55	C23H42O5	[M+H]+	1-Oleoyl-2-acetyl-sn-glycerol	Bruker MetaboBASE Personal Library 2.0	2204.25	1454.75	2325.75	3962	0	0	
603	455.35201	472.35514	16.62	C30H48O4	[M-H2O+H]+ [M+H]+	(2alpha,3beta,5xi,9xi,18xi)-2,3-Dihydroxyolean-12-en-28-oic acid	MoNA-export-GNPS_QTOF.msp	2574.25	3517.75	2229.5	18358.25	190859.25	57649.5	
604	491.37345	490.36618	16.66	C30H50O5	[M+H]+	Camelliagenin C	Bruker MetaboBASE Personal Library 2.0_in-silico	5611.25	3681.25	4761.75	3105.5	257776	170100.75	329
605	263.23706	262.22978	16.68	C18H30O	[M+H]+	6,10,14-Trimethyl-5,9,13-pentadecatrien-2-one	Bruker MetaboBASE Personal Library 2.0_in-silico	8381.75	6441.5	1838.25	2977.75	6873.25	906.25	
606	323.25811	322.25083	16.67	C20H34O3	[M+H]+	Austroinulin	Bruker MetaboBASE Personal Library 2.0_in-silico	3964.5	6589.25	9234.5	13968.5	39051.5	3636	330
607	337.14351	336.13623	16.7	C21H20O4	[M+H]+	Licoagrochalcone B	Bruker MetaboBASE Personal Library 2.0_in-silico	7495.5	3872.5	9438.25	2287	0	0	331
608	277.21625	276.20898	16.72	C18H28O2	[M+H]+	(9Z,14Z)-octadeca-9,14-dien-6-ynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	61308	69921.75	13529	25800.75	5454.25	3037	
609	453.33669	452.32941	16.75	C30H44O3	[M+H]+	(22E,24E)-3-Oxo-9,19-cyclolanosta-22,24-dien-26-oic acid	MoNA-export-GNPS_QTOF.msp	4460	1577	827.75	10021	28585.75	39199.75	
610	185.11713	184.10986	16.74	C10H16O3	[M+H]+	4,5-Dihydro-5,5-dimethyl-4-(3-oxobutyl)furan-2(3H)-one	Bruker MetaboBASE Personal Library 2.0_in-silico	3671	3828	757.5	1424.75	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
611	337.23742	336.23015	16.73	C20H32O4	[M+H]+	1-Naphthalenepentanoic acid, 5-carboxydecahydro-beta,5,8a-trimethyl-2-methylene-	MoNA-export-GNPS_QTOF.msp	1252.5	3783.5	6140	2116.5	0	0	332
612	291.19539	290.18811	16.73	C18H26O3	[M+H]+	8-hydroxy-17-octadecene-10,12-diynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1051.75	3622.75	4130	2104.25	152.5	98.25	198
613	289.25273	288.24545	16.74	C20H32O	[M+H]+	3,7,11,15-tetramethyl-2E,6E,10E,14-hexadecatetraenal	Bruker MetaboBASE Personal Library 2.0_in-silico	426.5	268.75	701.75	777.5	4190.75	4271.75	
614	295.2633	294.25603	16.78	C19H34O2	[M+H]+	Methyl linoleate	Bruker MetaboBASE Personal Library 2.0	16968.75	13960.75	6767.5	7815.5	4407	1666	1
615	297.24179	296.23452	16.78	C18H32O3	[M+H]+	7-Methoxy-9-methyl-hexadeca-4E,8E-dienoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2337.5	2686.5	1642	1740.5	0	847.5	
616	473.36264	472.35528	16.83	C30H48O4	[M+H]+ [M-H2O+H]+	Momordicin I	Bruker MetaboBASE Personal Library 2.0_in-silico	2817.25	2839.25	8253.75	4992.25	255972.5	112234.75	
617	471.34738	470.3401	16.8	C30H46O4	[M+H]+	Pomonic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	15381.5	1784.75	3209.5	1381.5	11142.75	4851.5	333
618	219.17433	218.16705	16.84	C15H22O	[M+H]+ [M-H2O+H]+	beta-Atlantone	Bruker MetaboBASE Personal Library 2.0_in-silico	1397	837.25	1305.5	341.5	2497.75	24485	334
619	237.18478	236.17751	16.84	C15H24O2	[M+H]+	4E,7Z,10Z-Tridecatrienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	1175.5	191.5	268.25	314.75	1834.25	17001.5	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
620	441.37265	440.36537	16.82	C30H48O2	[M+H]+	11-Oxo-beta-amyrin	Bruker MetaboBASE Personal Library 2.0_in-silico	6860.5	3765.75	2223	13492.75	102694.75	55772.25	335
621	281.24767	280.24039	16.83	C18H32O2	[M+H]+	10E,12Z-Octadecadienoic acid	Bruker MetaboBASE Personal Library 3.0	0	0	171.75	373	14792	851.5	
622	313.27387	312.2666	16.85	C19H36O3	[M+H]+	Ricinoleic Acid methyl ester	Bruker MetaboBASE Personal Library 3.0	2301.75	2939.5	550	1249.75	21319.75	0	
623	323.29446	322.28718	16.88	C21H38O2	[M+H]+ [M-H]-	Isopropyl linoleate	Bruker MetaboBASE Personal Library 3.0	5088.25	1445.25	4146.25	2152.5	0	448.75	336
624	357.30088	358.30815	16.9	C21H42O4	[M-H]- [M+H]+	1-Stearoyl-rac-glycerol	Bruker MetaboBASE Personal Library 3.0	37563.75	9715.75	39094.25	17723.5	1036.25	4751	337
625	305.28392	304.27664	16.9	C21H36O	[M+H]+	3-pentadecylphenol	Bruker MetaboBASE Personal Library 2.0_in-silico	2635.5	674.25	2272	1138.75	0	0	338
626	281.2841	280.27682	16.89	C19H36O	[M+H]+	2-methyl-7R,8S-Epoxy-17-octadecene	Bruker MetaboBASE Personal Library 2.0_in-silico	20088	5430.75	19600.25	8857	125	2044.5	
627	467.33781	468.34509	16.91	C27H48O6	[M-H]- [M+H]+	5β-Cholestane-2β,3α,7α,12α,26,27-hexol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	244.75	6457	108.5	
628	283.26329	300.26661	16.9	C18H36O3	[M-H2O+H]+ [M+H]+	(R)-10-hydroxystearic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	52354.25	59507	90198.5	37758.75	1205.75	268400	
629	457.36728	456.36001	16.92	C30H48O3	[M+H]+ [M-H]-	(22E)-1α,25-dihydroxy-26,27-dimethyl-22,23-didehydro-24a-homo-20-epivitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	2784.25	3458.25	4315.25	4768.75	68375.75	45245.25	
630	439.35708	438.3498	16.98	C30H46O2	[M+H]+	Thujyl 19-trachylobanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	8946.5	5483	12021.25	9388.75	65748.25	39396.25	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
631	517.38905	516.38178	16.96	C32H52O5	[M+H]+	22-Acetylpriverogenin B	Bruker MetaboBASE Personal Library 2.0_in-silico	1066.75	302.75	0	2183	59165.75	28581.5	
632	265.25261	264.24533	16.97	C18H32O	[M+H]+	9S,10R-Epoxy-6Z-octadecene	Bruker MetaboBASE Personal Library 2.0_in-silico	468	110.5	166	0	1658	18028.5	
633	397.3101	396.30277	17.01	C27H40O2	[M+H]+ [M-H2O+H]+	alpha-Micropteroxanthin B	Bruker MetaboBASE Personal Library 2.0_in-silico	155	161.5	0	0	42251	16087.75	
634	447.34698	446.3397	17.02	C28H46O4	[M+H]+ [M-H2O+H]+	(20S)-1α,25-dihydroxy-20-methoxyvitamin D3 / (20S)-1α,25-dihydroxy-20-methoxycholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	169.75	233.25	0	0	30572.25	13179.75	
635	415.32072	414.31344	17.02	C27H42O3	[M+H]+ [M-H]-	25-hydroxy-23-oxovitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	118	0	0	168	15026.5	7594.5	
636	321.27862	320.27134	17.04	C21H36O2	[M+H]+	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl hexanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	1062.5	3395.75	1997.25	1568.25	0	0	
637	387.2894	386.28212	17.07	C25H38O3	[M+H]+	Testosterone isocaproate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	10460	471.25	
638	299.25817	298.251	17.11	C18H34O3	[M+H]+ [M+Na]+	14-oxo-octadecanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	3422.25	3506.25	5975.75	2445	0	31677.75	339
639	253.21617	252.20889	17.11	C16H28O2	[M+H]+	(Z)-3-Decenyl (E)-3-hexenoate	Bruker MetaboBASE Personal Library 2.0_in-silico	1871.75	2966	4876.75	2352.75	259.5	18663.75	
640	271.2267	270.21942	17.11	C16H30O3	[M+H]+	7-keto palmitic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	982.75	1079	2114.25	1193.25	0	7438.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
641	397.33156	396.32429	17.11	C24H44O4	[M+H]+ [M-H]-	FAHFA 24:1; FAHFA 2:0/22:1; [M-H]-	MSDIAL-LipidDBs-VS34.msp	1801.75	3523.5	1651.25	1506.75	0	0	
642	477.39378	476.38651	17.14	C30H52O4	[M+H]+	Panaxatriol	Bruker MetaboBASE Personal Library 2.0_in-silico	2329.75	2161.75	3123	1980.75	213039.25	105034.5	
643	487.34176	486.33448	17.11	C30H46O5	[M+H]+ [M-H]-	Olean-12-en-28-oic acid, 3,15,21,29-tetrahydroxy-, 28,15-lactone, (3β,15β,20β,21β)- (9CI)	MoNA-export-GNPS_QTOF.msp	0	0	0	0	5781.25	4239	
644	255.23187	254.22461	17.15	C16H30O2	[M+H]+ [M-H2O+H]+	11Z-hexadecenoic acid	Bruker MetaboBASE Personal Library 2.0	564.5	899.75	1314	3055.5	565.75	10863	340
645	499.37808	498.3708	17.24	C32H50O4	[M+H]+	Oleanolic acid acetate	Bruker MetaboBASE Personal Library 3.0	1076.25	387	461.75	1204.75	36239.25	41548.5	
646	249.22141	248.21413	17.23	C17H28O	[M+H]+	Avocadenofuran	Bruker MetaboBASE Personal Library 2.0_in-silico	4234.25	7764	6309	5638.25	112.5	1049.75	
647	281.24748	280.24021	17.26	C18H32O2	[M+H]+	14-Octadecynoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	773	3282.25	3224.25	39564	
648	309.27903	308.27175	17.26	C20H36O2	[M+H]+	Linoleic Acid ethyl ester	Bruker MetaboBASE Personal Library 2.0	66082	68459.75	112866.5	56232.5	123	6456.75	341
649	125.09585	124.08857	17.25	C8H12O	[M+H]+	5,7-octadienal	Bruker MetaboBASE Personal Library 2.0_in-silico	668.75	1057	231.25	748.75	10991.75	6628.75	
650	453.37244	452.36517	17.25	C31H48O2	[M+H]+	Phyllohydroquinone	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	224.75	167	63024.5	7258.5	
651	143.10656	142.09929	17.25	C8H14O2	[M+H]+	3Z-Hexenyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	455.5	192.5	0	378.25	42699.75	17169.75	342

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
652	425.3271	424.31982	17.31	C25H44O5	[M+H]+	Unoprostone isopropyl ester	Bruker MetaboBASE Personal Library 2.0	1678.5	397	1276.75	1064	0	0	
653	425.37646	424.36918	17.33	C30H48O	[M+H]+	Lupenone	Bruker MetaboBASE Personal Library 3.0	4905.25	1878.75	3470	5165.25	9708	12946	343
654	439.35693	438.34965	17.33	C30H46O2	[M+H]+	alpha,gamma-Onoceradienedione	Bruker MetaboBASE Personal Library 2.0_in-silico	26076.5	8460	8722.25	6198	637816.75	371487	344
655	309.27895	326.28239	17.38	C20H38O3	[M-H2O+H]+ [M+Na]+ [M+H]+	2-oxophytanic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	33507.5	68752.75	51599	35728.5	0	2926.5	
656	397.3101	396.30282	17.39	C27H40O2	[M+H]+	beta-Micropteroxanthin	Bruker MetaboBASE Personal Library 2.0_in-silico	516.25	0	144.25	420.25	19494.25	14352.5	
657	475.3782	474.37093	17.4	C30H50O4	[M+H]+	1α,25-dihydroxy-26,27-dimethyl-24a,24b-dihomo-22-oxa-20-epivitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	13388.75	9693	20105.5	5467	1109816.75	617379.25	
658	281.2475	280.24023	17.41	C18H32O2	[M+H]+	11-Hexadecynyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2210.75	2524.5	3988	9875.75	0	3585.5	
659	455.35184	454.34455	17.45	C30H46O3	[M+H]+ [M-H2O+H]+	(3beta,5xi,9xi,13alpha,17alpha,18xi)-3-Hydroxy-13,28-epoxyurs-11-en-28-one	MoNA-export-GNPS_QTOF.msp	32173	3267.5	7585.5	12530.75	74560.5	79868	
660	285.22123	284.21395	17.47	C20H28O	[M+H]+	Retinal	Bruker HMDB Metabolite Library_2.0	1166.25	522.25	1092	1069.5	1440.5	4295	
661	255.23201	254.22474	17.44	C16H30O2	[M+H]+	cis-7-Hexadecenoic Acid	Bruker MetaboBASE Personal Library 3.0	715	502.75	1105.5	1785.5	1809.25	6827.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
662	109.10103	108.09375	17.52	C8H12	[M+H]+	4-Vinylcyclohexene	Bruker MetaboBASE Personal Library 2.0_in-silico	98	0	0	56	4929	1397.75	
663	257.21107	256.20379	17.49	C15H28O3	[M+H]+	Lyngbic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1136.25	3511.25	994	1326.25	0	0	
664	441.37161	440.36433	17.49	C30H48O2	[M+H]+ [M-H]-	Sebiferic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	18835	10182.25	9822	37920.25	67979	77833.75	
665	383.3158	382.30853	17.61	C23H42O4	[M+H]+	MG(0:0/20:2(11Z,14Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	8583.75	11541.5	9595	9399.75	0	2474.25	
666	233.22642	232.21915	17.6	C17H28	[M+H]+	Aplotaxene	Bruker MetaboBASE Personal Library 2.0_in-silico	943.5	1359	2020.25	813.75	75.5	0	
667	297.27903	296.27175	17.61	C19H36O2	[M+H]+	Phytomonic Acid	Bruker MetaboBASE Personal Library 3.0	13589.75	20131	35688.75	9951.5	2223.5	1903.75	
668	251.23705	250.22977	17.59	C17H30O	[M+H]+	8,11-Heptadecadienal	Bruker MetaboBASE Personal Library 2.0_in-silico	1269	2722	4396.5	1585.25	0	175.5	345
669	335.29455	334.28728	17.57	C22H38O2	[M+H]+	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl heptanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	4759.75	4156.25	2697	2072.75	0	108	
670	325.27395	324.26534	17.63	C20H36O3	[M+H]+ [M+Na]+ [M+NH4]+	14,15-EE-5(Z)-E	Bruker MetaboBASE Personal Library 3.0	118781.25	273020.75	183072.5	130213	0	17797	
671	355.32092	354.31355	17.64	C22H42O3	[M+H]+ [M-H2O+H]+	3-oxo-docosanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	58889.25	22061.75	56118.5	43230	2766.75	4138.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
672	295.29976	294.29249	17.64	C20H38O	[M+H]+ [M-H2O+H]+	9R,10S-Epoxy-6Z-eicosene	Bruker MetaboBASE Personal Library 2.0_in-silico	54867.75	18772	48164.5	37184.5	1302.25	2380.25	
673	279.23154	278.22443	17.63	C18H30O2	[M+H]+ [M-H2O+H]+	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl propionate	Bruker MetaboBASE Personal Library 2.0_in-silico	20079.25	49464.75	31729.25	21391.75	862.25	3739.25	
674	373.33162	372.32435	17.63	C22H44O4	[M+H]+	13,14-dihydroxy-docosanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	12237	2949.75	10316.75	5613.25	0	0	
675	311.29509	310.28781	17.67	C20H38O2	[M+H]+	14(Z)-Eicosenoic Acid	Bruker MetaboBASE Personal Library 2.0	2208.5	999.25	2332.75	1733.75	0	0	346
676	305.24746	304.24018	17.69	C20H32O2	[M+H]+	omega-3 Arachidonic Acid	Bruker MetaboBASE Personal Library 2.0	7019	19768	16564.25	8136.75	1026.75	235.75	
677	409.34614	408.33887	17.69	C29H44O	[M+H]+	24-Norursa-3,12-dien-11-one	Bruker MetaboBASE Personal Library 3.0	412	0	0	580.75	2989.25	9326	
678	235.16927	234.16199	17.69	C15H22O2	[M+H]+	Dihydroisoalantolactone	Bruker MetaboBASE Personal Library 2.0_in-silico	2589.25	4120.75	2599.75	3164.25	3202.75	5110.75	347
679	267.2318	266.22453	17.7	C17H30O2	[M+H]+	8E,10E-Pentadecadienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	696.5	748	1724	819.25	0	102.25	
680	461.39811	460.39083	17.72	C30H52O3	[M+H]+	Taraxastane-3beta,16beta,20beta-triol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	1353	213.5	5837	0	7856	
681	385.2737	384.26643	17.75	C25H36O3	[M+H]+	Persicachrome	Bruker MetaboBASE Personal Library 2.0_in-silico	10782	9052.25	12429	7271.75	2078.75	1535.5	348
682	291.23192	290.22465	17.75	C19H30O2	[M+H]+	11beta-Hydroxy-5alpha-androstan-17-one	Bruker MetaboBASE Personal Library 2.0_in-silico	7539.75	32841.75	13542.5	10597	0	1096.75	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
683	277.25246	276.24519	17.81	C19H32O	[M+H]+	5-(1-hydroxybutan-2-yl)isolongifol-5-ene	Bruker MetaboBASE Personal Library 2.0_in-silico	916.5	2323.5	1960.75	1187.75	118.75	193.5	
684	337.30605	336.29877	17.81	C22H40O2	[M+H]+	Butyl 9,12-octadecadienoate	Bruker MetaboBASE Personal Library 3.0	0	1762.25	4894.25	0	0	0	
685	405.35161	404.34434	17.88	C30H44	[M+H]+	4,4'-Diapo-zeta-carotene	Bruker MetaboBASE Personal Library 2.0_in-silico	271.5	323	134	0	7182.75	5335.5	
686	205.15864	204.15136	17.88	C14H20O	[M+H]+	2-Benzylidene-1-heptanol	Bruker MetaboBASE Personal Library 2.0_in-silico	258.75	477.5	192.25	117.75	6950	4877	349
687	245.1899	244.18263	17.89	C17H24O	[M+H]+	Falcarinol	Bruker MetaboBASE Personal Library 2.0_in-silico	131	213.25	0	0	5151	3645	350
688	263.20052	262.19324	17.96	C17H26O2	[M+H]+	Acetylenic acids; 10,16-Heptadecadien-8-ynoic acid, (E)-	Bruker MetaboBASE Personal Library 2.0_in-silico	374.5	2142.5	339.75	1274.5	1979	974.5	
689	459.38225	458.37498	17.97	C30H50O3	[M+H]+	Longispinogenin	Bruker MetaboBASE Personal Library 2.0_in-silico	2222.75	2087.5	770.75	3117.75	51857.25	29365.75	351
690	513.39318	512.3859	18.01	C33H52O4	[M+H]+	Methyl 3b-hydroxy-13(18)-oleanen-28-oate	Bruker MetaboBASE Personal Library 2.0_in-silico	531	1920.75	197.75	1206.75	17136.75	2332.75	
691	355.26374	372.26685	17.96	C24H36O3	[M-H2O+H]+ [M+H]+	cholacalcioic acid / 25,26,27-trinorvitamin D3 24-carboxylic acid / 25,26,27-trinorcholecalciferol 24-carboxylic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	6912.25	352
692	415.32157	416.32885	17.99	C27H44O3	[M-H]- [M+H]+	Octadecyl cis-p-coumarate	Bruker MetaboBASE Personal Library 2.0_in-silico	3955.75	1626.75	1360	4564	1516	953.75	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
693	397.30997	414.31354	18	C27H42O3	[M-H2O+H]+ [M+H]+	Calicoferol B	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	4673	2386	
694	519.40465	518.39692	18.05	C32H54O5	[M+H]+ [M-H2O+H]+	(20R)-24-Hydroxygemini-vitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	2070.5	3009.75	12638.25	1735.25	1345.25	142285.25	
695	459.34743	458.34016	18.04	C29H46O4	[M+H]+	3aH-Cyclopenta[a]chrysene-3a-carboxylic acid, 1-acetyleicosahydro-9-hydroxy-5a,5b,8,8,11a-pentamethyl-, (1R,3aS,5aR,5bR,9S,11aR)-	MoNA-export-GNPS_QTOF.msp	0	0	0	0	10973	6517.5	
696	287.23693	286.22965	18.09	C20H30O	[M+H]+	Helicallenal	Bruker MetaboBASE Personal Library 2.0_in-silico	447.5	851.25	790	3237	1531.75	6729.75	
697	425.37749	442.3809	18.16	C30H50O2	[M-H2O+H]+ [M+H]+	9,19-Cyclolanost-25-ene-3,24-diol	Bruker MetaboBASE Personal Library 3.0	3778.25	2901.25	3222.75	692.5	139714.25	78258.25	
698	407.36715	406.35987	18.16	C30H46	[M+H]+	4,4'-Diapophytofluene	Bruker MetaboBASE Personal Library 2.0_in-silico	1785.5	991.5	1217	666.25	37875.5	21362	353
699	281.24743	280.24016	18.21	C18H32O2	[M+H]+	11Z,14E-Hexadecadienyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	3577.25	3347.25	6244.25	3996.75	3344.25	4226	
700	205.19503	204.18775	18.2	C15H24	[M+H]+	(2Z,4E,6E)-2,4,6,10-Farnesatetraene	Bruker MetaboBASE Personal Library 2.0_in-silico	753.25	257.75	496.5	477.25	4364.25	180	
701	379.28496	378.27768	18.27	C23H38O4	[M+H]+	[12]-Gingerol	Bruker MetaboBASE Personal Library 2.0_in-silico	3623.5	683.75	4811.25	234.5	704	474.5	354

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
702	277.21629	294.21961	18.33	C18H30O3	[M-H2O+H]+ [M+H]+	13(S)-HOTrE	Bruker MetaboBASE Personal Library 3.0	20554.5	78203.5	15903.5	39136.5	2268.25	1819.25	355
703	613.4677	612.46043	18.3	C35H64O8	[M+H]+	Annomuricin A	Bruker MetaboBASE Personal Library 2.0_in-silico	0	978	0	5352	4570.5	6601.5	356
704	309.31529	308.30802	18.32	C21H40O	[M+H]+	9R,10S-Epoxy-6Z-heneicosene	Bruker MetaboBASE Personal Library 2.0_in-silico	10840.75	3832	10129.75	4481.5	0	0	
705	281.24866	282.25593	18.53	C18H34O2	[M-H]- [M+H]+	Elaidic acid	Bruker MetaboBASE Personal Library 3.0	4309	2979.25	5115.5	4770.25	2084	1140.75	357
706	487.37847	486.37119	18.39	C31H50O4	[M+H]+ [M-H]-	3,7-Dihydroxy-25-methoxycucurbita-5,23-dien-19-al	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	66304	5296	358
707	441.39403	440.38675	18.41	C27H52O4	[M+H]+	MG(0:0/24:1(15Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	21889.5	7240	15629.25	0	12488.5	2742.25	
708	309.27897	308.2717	18.48	C20H36O2	[M+H]+	5(Z),14(Z)-Eicosadienoic Acid	Bruker MetaboBASE Personal Library 2.0	2409	3674.75	6687	3308	0	301.75	
709	339.32578	338.3185	18.51	C22H42O2	[M+H]+	13(Z)-Docosenoic Acid	Bruker MetaboBASE Personal Library 2.0	2688.25	1989.5	2074.25	2031.5	0	195.75	359
710	369.33663	368.32935	18.65	C23H44O3	[M+H]+	22-oxo-tricosanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	7397.5	1217.75	1830.5	2449	365.25	0	
711	339.29051	340.29779	18.67	C21H40O3	[M-H]-, [M+H]+	Glycidyl stearate	Bruker MetaboBASE Personal Library 2.0_in-silico	2840.25	3807.75	3789.5	3641.75	0	462	
712	441.37269	440.36542	18.85	C30H48O2	[M+H]+	30:6(12Z,15Z,18Z,21Z,24Z,27Z)	Bruker MetaboBASE Personal Library 2.0_in-silico	2588.75	1525.75	3453	7635	4411	7964.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
713	385.33151	384.32424	18.94	C23H44O4	[M+H]+	MG(0:0/20:1(11Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	27849	25580.25	39234.5	30390.25	1015	1632.5	
714	457.38977	456.38208	20.05	C27H52O5	[M+H]+ [M+Na]+	DG(12:0/12:0/0:0)[iso2]	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	776	9701	1510	
715	367.35726	366.34998	20.06	C24H46O2	[M+H]+ [M-H2O+H]+	Nervonic acid	Bruker HMDB Metabolite Library_2.0	6362.25	3320.5	5892.25	3230.75	337	2219.5	239
716	413.36288	412.3556	20.48	C25H48O4	[M+H]+	MG(0:0/22:1(13Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	16691.5	12202.75	18932.5	15185.5	1157.5	1484.25	
717	511.43658	510.42931	21.06	C31H58O5	[M+H]+	DG(12:0/16:1(9Z)/0:0)[iso2]	Bruker MetaboBASE Personal Library 2.0_in-silico	1107.75	0	390.5	0	453.25	225.75	
718	271.09642	270.08915	22.27	C16H14O4	[M+H]+	Alpinetin	Bruker MetaboBASE Personal Library 3.0	2668	2306.75	2312.75	2284.5	3127.25	1891.5	244
719	177.05462	176.04734	22.27	C10H8O3	[M+H]+	Herniarin	Bruker MetaboBASE Personal Library 2.0_in-silico	1628.25	1105.25	1462.25	1631.5	2065.25	864.25	360
720	271.09643	270.08915	22.66	C16H14O4	[M+H]+	(E)-1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one	MoNA-export-GNPS_QTOF.msp	2720.5	2558	2448	1959.25	2369	2452.5	361
721	177.05461	176.04734	22.87	C10H8O3	[M+H]+	4-Methylumbelliferone	Bruker MetaboBASE Personal Library 2.0_in-silico	1139	928.75	1055.75	1841.5	2098	972	360
722	109.02947	110.03674	5.37	C6H6O2	[M-H]-	Resorcinol	Bruker MetaboBASE Personal Library 2.0	1081	1309	1516.75	1369	0	672.25	362
723	125.02452	126.0318	10.39	C6H6O3	[M-H]-	Hydroxyhydroquinone	Bruker MetaboBASE Personal Library 3.0	4020.25	4114.75	1799	2939.75	211.5	0	363

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
724	137.02445	138.03172	8.73	C7H6O3	[M-H]-	p-Salicylic acid	Bruker MetaboBASE Personal Library 3.0	1041.25	1208.75	1460.5	780.25	288.5	866.5	364
725	137.02446	138.03173	6.12	C7H6O3	[M-H]-	3,4-Dihydroxybenzaldehyde	Bruker HMDB Metabolite Library_2.0	46195.75	37476.25	79621	53754.25	27807.25	37671.25	68
726	143.03493	144.04221	5.3	C6H8O4	[M-H]-	4-Ethoxy-4-oxobut-2-enoic acid	Bruker MetaboBASE Personal Library 3.0	357	1167	1494.25	1061.5	0	97.5	
727	145.01419	146.02147	1.15	C5H6O5	[M-H]-	Oxoglutaric acid	Bruker HMDB Metabolite Library_2.0	5296.5	1506	3094	2427.75	0	3576	
728	149.06074	150.06801	5.99	C9H10O2	[M-H]-	2-Methoxy-4-vinylphenol	Bruker MetaboBASE Personal Library 3.0	1286.75	5047	1067	2224	0	354.25	17
729	151.04014	152.04742	6.56	C8H8O3	[M-H]-	4-acetoxyphenol	Bruker MetaboBASE Personal Library 2.0	4803.25	3136	2140.5	8163.25	0	160.5	
730	153.01922	154.0265	20.75	C7H6O4	[M-H]-	2,3-Dihydroxybenzoic acid	Bruker MetaboBASE Personal Library 3.0	570.75	947	396.75	1027.5	381.5	742.25	365
731	153.01939	154.02666	20.42	C7H6O4	[M-H]-	gentisic acid	Bruker MetaboBASE Personal Library 3.0	629.5	571.25	370	491.5	235.25	0	366
732	153.0194	154.02668	6.29	C7H6O4	[M-H]-	Protocatechuic acid	Bruker HMDB Metabolite Library_2.0	1068	779	3229.75	1078.25	1052.75	1952	47
733	163.03997	164.04724	11.18	C9H8O3	[M-H]-	m-Coumaric acid	Bruker HMDB Metabolite Library_2.0	0	104.25	81	307	35570.25	1819.75	367
734	167.0349	168.04218	6.47	C8H8O4	[M-H]-	Isovanillic acid	Bruker HMDB Metabolite Library_2.0	886.25	1237.75	1384.5	2855.5	2463.25	4439.75	6
735	173.08198	174.08926	7.44	C8H14O4	[M-H]-	Suberic acid	Bruker MetaboBASE Personal Library 3.0	5585.5	8069.5	5905	4637.5	2131.75	1861.5	141

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
736	177.05569	178.06297	9.67	C10H10O3	[M-H]-	Coumarinic acid methyl ether	Bruker MetaboBASE Personal Library 3.0	902.75	1353	2840.25	1262.75	4800.25	1319	163
737	179.03472	180.04199	11.64	C9H8O4	[M-H]-	Caffeic acid	Bruker HMDB Metabolite Library_2.0	11440.25	8938.5	13006.75	13262.25	1161.5	2837.5	366
738	181.05059	182.05787	5.08	C9H10O4	[M-H]-	Hydroxyphenyllactic acid	Bruker MetaboBASE Personal Library 2.0	1952.5	2157.75	2268	1309	0	0	
739	185.11835	186.12563	8.68	C10H18O3	[M-H]-	10-hydroxy-2E-decenoic acid	Bruker MetaboBASE Personal Library 3.0	1646.75	3012.25	1494	806.75	0	0	
740	187.09762	188.1049	8.3	C9H16O4	[M-H]-	Azelaic acid	Bruker HMDB Metabolite Library_2.0	14523.5	23949.75	17900.75	12517	11651.75	9852.5	
741	191.01969	192.02697	2.08	C6H8O7	[M-H]-	Citric acid	Bruker HMDB Metabolite Library_2.0	4767	4266.25	2776.25	3922.75	0	2956.25	368
742	191.05605	192.06333	1.12	C7H12O6	[M-H]-	Quinic acid	Bruker MetaboBASE Personal Library 3.0	2495	2821.5	6915.75	7150.75	865.5	7192.75	23
743	191.07131	192.07859	10.6	C11H12O3	[M-H]-	Ethyl-p-coumarate	Bruker MetaboBASE Personal Library 3.0	12047.25	7529.25	5269.75	6598.5	983	33542.25	
744	193.03532	194.0426	1.06	C6H10O7	[M-H]-	2-keto-D-Gluconic acid	Bruker MetaboBASE Personal Library 3.0	8382.5	2158	5357.25	3458.75	0	6961.75	369
745	193.05043	194.05771	11.21	C10H10O4	[M-H]-	Scytalone	Bruker MetaboBASE Personal Library 3.0	0	0	0	160.5	8083.75	3104.75	
746	193.05052	194.0578	8.62	C10H10O4	[M-H]-	Erbstatin Analog	Bruker MetaboBASE Personal Library 3.0	4884.25	3321	3748.25	3711.5	3313.25	2226.5	
747	193.05061	194.05789	7.86	C10H10O4	[M-H]-	3-Hydroxy-4-methoxycinnamic aicd	Bruker HMDB Metabolite Library_2.0	24832.5	26541.5	64955.5	44785.25	13186.75	19764.5	6

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
748	193.05064	194.05791	7.68	C10H10O4	[M-H]-	ferulic acid	Bruker MetaboBASE Personal Library 3.0	32544.75	48260.75	32990	105824.25	105377.25	160990.25	366
749	195.05094	196.05822	1.08	C6H12O7	[M-H]-	D-Gluconic acid	Bruker MetaboBASE Personal Library 3.0	6799.75	4385	11533.5	2647.75	0	8388	322
750	201.11326	202.12054	9.12	C10H18O4	[M-H]-	Sebacic acid	Bruker HMDB Metabolite Library_2.0	1366	799.75	1213.75	837.5	1367.75	1772	
751	203.03503	204.04231	7.6	C11H8O4	[M-H]-	1,4-Dihydroxy-2-naphthoic acid	Bruker MetaboBASE Personal Library 3.0	744.75	731.5	1246.75	1666.25	1510.75	1771.5	
752	207.06615	208.07342	7.94	C11H12O4	[M-H]-	DL-Benzylsuccinic acid	Bruker MetaboBASE Personal Library 2.0	828.5	767.25	1745.75	117	0	0	
753	207.0662	208.07347	8.86	C11H12O4	[M-H]-	Dimethylcaffeic acid	Bruker MetaboBASE Personal Library 3.0	40736	22518.5	71166.25	27571.5	2369.75	4132.5	370
754	215.12894	216.13622	9.89	C11H20O4	[M-H]-	Undecanedioic acid	Bruker HMDB Metabolite Library_2.0	448	383.75	694.75	0	0	3391	
755	215.16513	216.1724	10.9	C12H24O3	[M-H]-	12-Hydroxydodecanoic acid	Bruker MetaboBASE Personal Library 3.0	945	926.75	178.75	2876.25	2056.75	5190.5	
756	235.06114	236.06841	7.27	C12H12O5	[M-H]-	Radicinin	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	1639.25	2612.25	22
757	235.09744	236.10472	11.42	C13H16O4	[M-H]-	Dihydro-β-tubaic acid	Bruker MetaboBASE Personal Library 2.0	20312	14944.5	27622.5	17346.75	1098	3584	
758	239.03488	240.04216	12.16	C14H8O4	[M-H]-	1,4-Dihydroxyanthracene-9,10-dione	Bruker MetaboBASE Personal Library 3.0	2526.75	2116.75	1716.25	1679	0	0	
759	243.06619	244.07347	8.73	C14H12O4	[M-H]-	Cearoin	Bruker MetaboBASE Personal Library 2.0	142.75	193.25	0	0	1632.5	412.75	122

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
760	243.12381	244.13109	8.73	C12H20O5	[M-H]-	4-Oxododecanedioic acid	Bruker MetaboBASE Personal Library 3.0	1222.25	1675	1160	1151.5	773.25	392.25	
761	243.19635	244.20363	14.9	C14H28O3	[M-H]-	3-hydroxy-tetradecanoic acid	Bruker MetaboBASE Personal Library 3.0	2799.5	2188.25	882	11485	46431.75	64030.5	
762	243.19642	244.20369	12.54	C14H28O3	[M-H]-	2-Hydroxymyristic Acid	Bruker MetaboBASE Personal Library 3.0	0	0	0	4272.25	5929	11482	371
763	245.04563	246.05291	7.41	C13H10O5	[M-H]-	Hispidin	Bruker MetaboBASE Personal Library 3.0	114	1852.25	0	181	0	0	
764	253.05052	254.0578	12.67	C15H10O4	[M-H]-	Chrysin	Bruker MetaboBASE Personal Library 3.0	10376.75	8718	7255	4784.5	1292.5	1017.75	109
765	253.05065	254.05792	10.07	C15H10O4	[M-H]-	3,4'-dihydroxyflavone	Bruker MetaboBASE Personal Library 3.0	2013.5	2097.5	2189	1959	0	0	
766	255.06618	256.07346	11.47	C15H12O4	[M-H]-	Hydrangenol	Bruker MetaboBASE Personal Library 3.0	1903.75	2743.75	0	250	0	0	372
767	255.06624	256.07352	11.75	C15H12O4	[M-H]-	Pinocembrin	Bruker MetaboBASE Personal Library 3.0	802148	659586	865157.5	724809.75	106266.25	110034.75	109
768	257.1756	258.18288	12.09	C14H26O4	[M-H]-	Tetradecanedioic acid	Bruker MetaboBASE Personal Library 2.0	198.75	211	136	589	1192	2247.5	
769	261.11306	262.12033	12.73	C15H18O4	[M-H]-	1(3H)-Isobenzofuranone, 4-methoxy-5-methyl-6-((3-methyl-2-butenyl)oxy)-	Bruker MetaboBASE Personal Library 3.0	8132.5	5535.5	7560.75	5019.25	1644.5	682	373
770	265.1444	266.15182	11.38	C15H22O4	[M-H]- [M-H-H2O]-	1,4-Dihydroxy-6,6,9a-trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c]furan-3(1H)-one	Bruker MetaboBASE Personal Library 3.0	32405.25	57884.5	42038.25	29505.5	1734.5	4724.75	374

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
771	269.04567	270.05295	9.79	C15H10O5	[M-H]-	Apigenin	Bruker MetaboBASE Personal Library 3.0	153248.5	165897.75	192717.25	182190.75	150930.75	124021.75	109
772	269.0819	270.08917	11.55	C16H14O4	[M-H]-	4'-O-Methylhydrangenol	Bruker MetaboBASE Personal Library 3.0	581984.75	397708	711544.75	475011.75	218162.75	248618.25	
773	271.06115	272.06843	8.01	C15H12O5	[M-H]-	(±)-Naringenin	Bruker MetaboBASE Personal Library 2.0	95.25	0	249	0	3114.75	1259.25	109
774	271.22772	272.23499	16.63	C16H32O3	[M-H]-	3-hydroxy-hexadecanoic acid	Bruker MetaboBASE Personal Library 3.0	13050.25	4691.5	4272.75	12700.5	32415.5	53048	375
775	271.22773	272.23501	14.16	C16H32O3	[M-H]-	2-hydroxyhexadecanoic acid	Bruker MetaboBASE Personal Library 3.0	3347	1699	2158.25	4196.5	9731.5	99343.25	
776	275.09291	276.10019	9.82	C15H16O5	[M-H]-	Ursinic acid	Bruker MetaboBASE Personal Library 2.0	912	199	851	360	0	0	376
777	281.08203	282.08931	13.45	C17H14O4	[M-H]-	3-benzyl-4-hydroxy-5-(4-hydroxyphenyl)furan-2(5H)-one	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	2577.75	740	
778	283.06114	284.06841	11.74	C16H12O5	[M-H]-	Genkwanin	Bruker MetaboBASE Personal Library 3.0	83331.75	72535.25	66338	68661	96908	50549.5	269
779	283.06116	284.06843	13.89	C16H12O5	[M-H]-	4',6-Dihydroxy-3'-methoxyaurone	Bruker MetaboBASE Personal Library 3.0	3975.25	3399	3492.25	2199	165.75	0	377
780	283.06118	284.06845	12.16	C16H12O5	[M-H]- [M+Cl]-	Izalpinin	Bruker MetaboBASE Personal Library 3.0	71637.25	217961.5	238739	193024.75	13852.75	9266.25	
781	283.0975	284.10478	11.91	C17H16O4	[M-H]-	Phenethyl Caffeate	Bruker MetaboBASE Personal Library 2.0	486980	358213.25	573995.75	483051.5	75003.5	138749.25	378
782	283.09754	284.10481	19.58	C17H16O4	[M-H]-	Alpinetin methyl ether	Bruker MetaboBASE Personal Library 3.0	856.25	443.25	542.5	564	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
783	285.0405	286.04777	9.08	C15H10O6	[M-H]-	Luteolin	Bruker MetaboBASE Personal Library 3.0	52169.25	47423.75	45494	37311	17039	16109.5	379
784	285.07668	286.08396	11.68	C16H14O5	[M-H]-	Naringenin 5-methyl ether	Bruker MetaboBASE Personal Library 3.0	13629.75	19730	13104	47916.75	141637	141347.5	222
785	285.2435	286.25078	17.41	C17H34O3	[M-H]-	Avocadene	Bruker MetaboBASE Personal Library 3.0	503	1115.75	715	703.25	0	115.75	
786	287.05598	288.06325	8.72	C15H12O6	[M-H]-	Eriodictyol	Bruker MetaboBASE Personal Library 3.0	7242.75	7778.5	7059	4087.75	1279.5	829	380
787	287.09254	288.09982	7.44	C16H16O5	[M-H]-	Iriflophenone trimethyl ether	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	0	1708.25	381
788	291.19641	292.20369	14.77	C18H28O3	[M-H]-	(10E,12Z,15Z)-9-oxooctadeca-10,12,15-trienoic acid	Bruker MetaboBASE Personal Library 3.0	5730.75	11004.5	4434.75	5010.75	0	0	
789	293.21194	294.21921	13.37	C18H30O3	[M-H]-	9S-hydroxy-10E,12Z,15Z-octadecatrienoic acid	Bruker MetaboBASE Personal Library 3.0	1959	4365.25	2528	2602.25	0	135.25	141
790	293.21208	294.21936	14.94	C18H30O3	[M-H]-	9-oxo-10E,12Z-octadecadienoic acid	Bruker MetaboBASE Personal Library 3.0	208351.5	303991.5	176024	227794.5	10217.75	27968.5	
791	295.22767	296.23494	14.43	C18H32O3	[M-H]-	(±)12(13)epoxy-9Z-octadecenoic acid	Bruker MetaboBASE Personal Library 2.0	722.5	1676.5	687.5	387	11294.25	3073.75	
792	297.04042	298.0477	11.38	C16H10O6	[M-H]-	3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid	Bruker MetaboBASE Personal Library 3.0	745.25	1339.25	1586.5	279.25	0	0	
793	297.14957	298.15685	11.36	C19H22O3	[M-H]-	Acerogenin G	Bruker MetaboBASE Personal Library 3.0	0	269.75	3443.5	2197.75	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
794	297.24336	298.25064	15.01	C18H34O3	[M-H]-	(9Z,12R)-12-Hydroxyoctadec-9-enoic acid	Bruker MetaboBASE Personal Library 3.0	58693.25	65943	38363.25	51493	1529.75	9682.25	
795	299.05601	300.06329	11.84	C16H12O6	[M-H]-	Chrysoeriol (Luteolin 3'-methyl ether)	MoNA-export-GNPS_QTOF.msp	9314.75	7977.75	8127.25	30876.5	82297.5	132917.25	
796	299.05613	300.0634	10.84	C16H12O6	[M-H]-	5,7-dihydroxy-2-(4-hydroxyphenyl)-3-methoxy-4H-chromen-4-one	MoNA-export-GNPS_QTOF.msp	89260.5	76569	78138.75	47353.75	4793.75	5675	
797	299.05627	300.06354	8.78	C16H12O6	[M-H]- [M+HCOOH -H]-	Tectorigenin	Bruker MetaboBASE Personal Library 3.0	38218.25	48072.75	26702.5	19492.5	2591.25	205.75	382
798	299.09233	300.0996	11.43	C17H16O5	[M-H]-	3,9-Dihydroeucomin	Bruker MetaboBASE Personal Library 3.0	3612.5	2171.5	6413.25	3292.5	327.25	777	
799	299.25895	300.26623	15.59	C18H36O3	[M-H]-	DL-2-hydroxy stearic acid	Bruker MetaboBASE Personal Library 3.0	19943.75	19606.5	22735.25	19366.25	3219.25	8346.75	383
800	301.07174	302.07902	9.28	C16H14O6	[M-H]-	3',5,7-Trihydroxy-3'-methoxyflavanone, Homoeriodictyol	Bruker MetaboBASE Personal Library 3.0	0	116.5	182.75	190.25	2630	2084.25	
801	309.20695	310.21423	12.76	C18H30O4	[M-H]-	9S-hydroperoxy-10E,12Z,15Z-octadecatrienoic acid	Bruker MetaboBASE Personal Library 2.0	18979.25	32017.75	11950	22903.75	1376.75	363.5	
802	309.20697	310.21425	13.49	C18H30O4	[M-H]-	9-oxo-11-(3-pentyl-2-oxiranyl)-10E-undecenoic acid	Bruker MetaboBASE Personal Library 3.0	6207.25	8173.25	4128.5	6441.5	5742.75	1246.25	384

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
803	311.22267	312.22977	14.19	C18H32O4	[M-H]- [M-H-H2O]-	(9S,10E,12Z)-9-hydroperoxyoctadeca-10,12-dienoic acid	Bruker MetaboBASE Personal Library 3.0	467.25	2082.25	753.5	12075.5	179523.5	3544.25	
804	311.29528	312.30256	17.83	C20H40O2	[M-H]-	Stearic Acid ethyl ester	Bruker MetaboBASE Personal Library 3.0	1200.5	627.25	940.25	1015	0	0	
805	313.03511	314.04238	11.42	C16H10O7	[M-H]-	Wedelolactone	Bruker MetaboBASE Personal Library 3.0	941.5	1617	1470	862.75	0	0	
806	313.07161	314.07888	12.14	C17H14O6	[M-H]-	Gnaphaliin	Bruker MetaboBASE Personal Library 3.0	29750.5	30743.5	23234	20202.5	23238.5	30235.25	
807	313.07169	314.07897	13.71	C17H14O6	[M-H]-	Koparin 2'-methyl ether	Bruker MetaboBASE Personal Library 2.0	1653.5	1598.75	1433.75	691	365.75	246	
808	313.10809	314.11537	8.35	C18H18O5	[M-H]-	Deoxysappanone b 7,3'-dimethyl ether	Bruker MetaboBASE Personal Library 2.0	0	0	90.5	135.25	1851.25	545.5	155
809	315.05102	316.0583	10.1	C16H12O7	[M-H]-	Quercetin 3-methyl ether	Bruker MetaboBASE Personal Library 3.0	33929	39805.75	36200	31323.5	60765.25	13809	385
810	315.05105	316.05833	10.86	C16H12O7	[M-H]-	Pinoquercetin	Bruker MetaboBASE Personal Library 3.0	79824.75	83800.25	113369	58999.75	10572.75	6917.5	386
811	323.09224	324.09951	12.91	C19H16O5	[M-H]-	4'-Hydroxywarfarin	Bruker MetaboBASE Personal Library 3.0	1176.5	567.25	1648	1369	520.75	105.75	387
812	327.08736	328.09464	6.93	C18H16O6	[M-H]-	Isotectorigenin, 7-methyl ether	Bruker MetaboBASE Personal Library 3.0	0	652.75	276.75	235.75	0	2543.25	388
813	329.06662	330.07389	12.02	C17H14O7	[M-H]-	Quercetin 3,7-dimethyl ether	MoNA-export-GNPS_QTOF.msp	3288.25	4814.5	4328	4038.5	40662.25	19227.25	389

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
814	329.06674	330.07403	10.36	C17H14O7	[M-H]- [M+Cl]-	5,7-dihydroxy-2-(4-hydroxyphenyl)-3,6-dimethoxy-4H-chromen-4-one	MoNA-export-GNPS_QTOF.msp	123838	131332.5	102108.25	84086.5	45407.25	13838.25	
815	341.10303	342.1103	7.55	C19H18O6	[M-H]-	Mundoserone	Bruker MetaboBASE Personal Library 2.0	0	72.75	0	427.25	187.25	5521.5	4
816	345.13393	346.14121	6.37	C19H22O6	[M-H]-	1,7-Bis(3,4-dihydroxyphenyl)-6-hydroxy-3-heptanone	Bruker MetaboBASE Personal Library 3.0	0	99.75	0	1045.75	826.5	2004	
817	353.08772	354.095	6.17	C16H18O9	[M-H]-	Chlorogenic acid	Bruker MetaboBASE Personal Library 2.0	672	566.75	2640.5	644	0	756.75	390
818	353.08821	354.09548	5.61	C16H18O9	[M-H]-	Cryptochlorogenic acid	Bruker MetaboBASE Personal Library 3.0	731.25	1194	1388.25	593.25	0	368.75	391
819	357.10358	358.11086	1.07	C12H22O12	[M-H]-	Lactobionic acid	Bruker MetaboBASE Personal Library 3.0	1143.5	625.5	1540.5	732.75	0	871	
820	359.07686	360.08414	12.08	C18H16O8	[M-H]-	Irigenin	Bruker MetaboBASE Personal Library 3.0	5453	0	1165	0	0	0	
821	359.07713	360.0844	6.64	C18H16O8	[M-H]-	Rosmarinate	Bruker MetaboBASE Personal Library 2.0	947.75	740	998	1319.5	874	940.25	
822	359.15024	360.15752	8.58	C20H24O6	[M-H]-	Lariciresinol	Bruker MetaboBASE Personal Library 2.0	1015.25	944	851.25	1151.75	1252.75	585.75	392
823	367.21275	368.22002	7.48	C20H32O6	[M-H]-	Thromboxane B3	Bruker MetaboBASE Personal Library 2.0	2864	3296.5	1784.25	2105.75	0	0	
824	371.11355	372.12083	11.59	C20H20O7	[M-H]-	2-ethoxycarbonyl-2-hydroxy-5,7-dimethoxyisoflavanone	Bruker MetaboBASE Personal Library 3.0	13057.75	9670.5	7846	4898.75	0	151.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
825	373.09334	374.10061	11.54	C19H18O8	[M-H]-	Casticin	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	1705.75	882.75	393
826	373.12889	374.13617	8.36	C20H22O7	[M-H]-	Diffractaic acid	Bruker MetaboBASE Personal Library 3.0	628.5	690.25	1055.5	1270.25	1692.5	2292.75	394
827	373.12923	374.1365	9.62	C20H22O7	[M-H]-	Nortrachelogenin	Bruker MetaboBASE Personal Library 3.0	204.75	365.75	302	1094.75	22827.5	2757.5	395
828	375.10854	376.11581	7.61	C19H20O8	[M-H]-	Dihydroatranorin	Bruker MetaboBASE Personal Library 3.0	2180.5	3792	2571	9542.75	19301.25	34720	
829	385.12919	386.13647	10.57	C21H22O7	[M-H]-	3H-Furo[4,3,2-de]indeno [4,5-h]-2-benzopyran-3,6,9-trione, 1,6b,7,8, 9a,10,11,11b-octahydro-11-hydroxy-1-(methoxy-methyl)-9a,11b-dimethyl-, (1S,6bR,9aS,11R,11bR)-	MoNA-export-GNPS_QTOF.msp	11906.75	7161.75	4517.75	3252	406.75	1330.5	
830	397.20193	398.2092	14.35	C24H30O5	[M-H]-	Taprostene	Bruker MetaboBASE Personal Library 2.0	4071.25	5345.25	4252.25	4076.75	638.5	884.5	
831	431.09824	432.10551	7.92	C21H20O10	[M-H]-	Apigenin 7-O-glucoside	Bruker MetaboBASE Personal Library 3.0	1659	1702.25	1752.75	985.25	0	0	396
832	431.09845	432.10572	9.12	C21H20O10	[M-H]-	Emodin 8-glucoside	Bruker MetaboBASE Personal Library 3.0	1885	2868	3750.75	1807.75	0	0	397
833	431.09849	432.10577	8.5	C21H20O10	[M-H]-	Genistein 4'-O-glucoside	Bruker MetaboBASE Personal Library 2.0	45100.75	63727.25	53789	32744	953	0	398

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
834	433.11412	434.1214	8.26	C21H22O10	[M-H]-	7-hydroxy-2-(4-hydroxyphenyl)-5-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	2432.75	5707	3994	4348.5	0	0	
835	439.16102	440.1683	7.74	C21H28O10	[M-H]-	Grandidentoside	Bruker MetaboBASE Personal Library 3.0	224.25	956.75	613.75	422.5	0	0	
836	447.09316	448.10043	8.25	C21H20O11	[M-H]-	petunidin-3-O-arabinoside	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	3819.75	0	114
837	459.38364	460.39092	18.23	C30H52O3	[M-H]-	Protopanaxadiol	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	1843.25	1428	399
838	463.08799	464.09527	8.21	C21H20O12	[M-H]-	Spiraeoside	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	2665.5	0	
839	485.3267	486.33398	15.44	C30H46O5	[M-H]-	(1R,2S,5aR,5bR,7aS,10R,12bR)-2-Hydroxy-10-isopropenyl-3,3,5a,5b,12b-pentamethyl-octadecahydrodicyclopenta[a,i]phenanthrene-1,7a(1H)-dicarboxylic acid	MoNA-export-GNPS_QTOF.msp	1088.25	0	0	0	2193	7475.25	
840	487.3426	488.34987	12.1	C30H48O5	[M-H]-	Arjunolic acid	Bruker MetaboBASE Personal Library 3.0	2199.75	4697.5	3741	2920.75	1148.75	1407.5	
841	495.20189	496.20917	11.53	C28H32O8	[M-H]-	Trichotetronine	Bruker MetaboBASE Personal Library 3.0	5663.75	4520.5	7539.25	4707.25	0	1039.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
842	215.05489	214.04762	8.29	C10H6N4O2	[M+H]+	2-(1h-1,2,4-triazol-5-yl)-1h-isoindole-1,3(2h)-dione	Bruker MetaboBASE Personal Library 2.0_in-silico	0	221.75	0	1393.75	856	2478.5	
843	333.06065	332.05338	8.55	C10H13N4O7P	[M+H]+	2'-Deoxyinosine 5'-monophosphate	Bruker MetaboBASE Personal Library 2.0_in-silico	927.5	1928	2380.25	1082.75	0	0	
844	361.25847	360.25119	8.7	C20H32N4O2	[M+H]+	1-[(3aS,7aR)-5-cyclopentyl-7a-(hydroxymethyl)-1,3,3a,4,6,7-hexahydropyrrolo[3,4-c]pyridin-2-yl]-3-(4-methylpyrazol-1-yl)propan-1-one	Bruker MetaboBASE Personal Library 3.0	0	205.75	0	1828.75	382.5	2402	
845	229.14354	228.13626	9	C13H16N4	[M+H]+	4-(3-phenyl-1,2,4-triazol-4-yl)piperidine	Bruker MetaboBASE Personal Library 3.0	676.5	2171.25	147.25	2817.5	0	0	
846	221.08092	220.07364	9.96	C10H11F3O2	[M+H]+	Trifluoromethylphenylpropanediol	Bruker MetaboBASE Personal Library 2.0_in-silico	267	7048.25	528	37249.75	75843.5	97213	
847	347.07629	346.06901	10.84	C14H14N6OS2	[M+H]+	Glucokinase Activator, Cpd A	Bruker MetaboBASE Personal Library 3.0	1725.5	0	358.75	0	0	0	
848	325.15871	324.15144	12.42	C19H20N2O3	[M+H]+	Bindarit	MoNA-export-GNPS_QTOF.msp	3555.75	4105.25	3631.75	6260.75	0	0	
849	425.30537	424.29809	13.25	C25H41FO4	[M+H]+	2α-Fluoro-19-nor-22-oxa-1α,25-dihydroxyvitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	247.5	4243.75	0	0	0	0	
850	393.22749	392.22022	13.99	C23H28N4O2	[M+H]+	PAC-1	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	2172	4261	9098	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
851	449.25346	448.24618	14.1	C23H36N4O3S	[M+H]+	5-(diethylsulfamoyl)-1-methyl-N-[3-(3-methylpiperidin-1-yl)propyl]-indole-2-carboxamide	Bruker MetaboBASE Personal Library 3.0	0	0	0	596.75	1377.5	5788.75	
852	201.16381	200.15653	17.53	C10H20N2O2	[M+H]+	N-methyl-4-propoxypiperidine-1-carboxamide	Bruker MetaboBASE Personal Library 3.0	1216	1653.5	502.25	653.75	2582.5	2141	
853	459.25302	458.24574	18.55	C26H30N6O2	[M+H]+	N-[2-[benzyl(methyl)amino]ethyl]-2-(3,7-dimethyl-4-oxo-2-phenylpyrazolo[3,4-d]pyridazin-5-yl)propanamide	Bruker MetaboBASE Personal Library 3.0	3280.25	3656.5	4212.5	1411	0	0	
854	297.13323	296.12595	8.06	C16H16N4O2	[M+H]+	N-[(2-ethoxypyridin-3-yl)methyl]-3H-benzimidazole-5-carboxamide	Bruker MetaboBASE Personal Library 3.0	1034.25	1037.75	1318.25	0	0	0	
855	177.05465	176.04738	8.35	C5H8N2O5	[M+H]+ [M-H]-	Ureidosuccinic acid	Bruker HMDB Metabolite Library_2.0	22888.5	14252	18807	22052.5	45901.5	47325.25	
856	417.11835	416.11104	8.8	C18H20N6O2S2	[M+H]+ [M-H2O+H]+	N-(5-ethylsulfanyl-1,3,4-thiadiazol-2-yl)-1-[6-(furan-2-yl)pyridazin-3-yl]piperidine-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	740.25	1073.25	323.5	4890.75	1900.25	10583.25	
857	387.18046	386.17319	9.2	C20H26N4O2S	[M+H]+	[1-(3-ethoxy quinoxalin-2-yl)piperidin-3-yl]-thiomorpholin-4-yl methanone	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	0	2722.75	
858	271.06012	270.05284	9.33	C15H11ClN2O	[M+H]+	N-Desmethyldiazepam (Nordazepam)	Bruker MetaboBASE Personal Library 3.0	1455	1899	1735.5	1300.25	0	0	400

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
859	277.10813	278.1154	9.47	C13H18N4OS	[M-H]- [M+H]+	N-[2-(dimethylamino) ethyl]-4-methyl-2-pyrrol-1-yl-1,3-thiazole-5-carboxamide	Bruker MetaboBASE Personal Library 3.0	20280.25	10219	19513.25	8859	839	1098.75	
860	429.11912	430.1264	9.51	C20H22N4O5S	[M-H]- [M-H2O+H]+ [M+H]+	5-(5-cyclobutyl-1,3,4-oxadiazol-2-yl)-N-(4-morpholin-4-ylphenyl)furan-2-sulfonamide	Bruker MetaboBASE Personal Library 3.0	1685.75	13515.5	600.25	66523.5	48622.75	134277	
861	451.10265	450.09537	10.01	C23H19ClN4O2S	[M+H]+ [M-H]-	2-(4-chlorophenyl)-N-[[3-[3-(2-cyclopropyl-1,3-thiazol-4-yl)phenyl]-1,2,4-oxadiazol-5-yl]methyl]acetamide	Bruker MetaboBASE Personal Library 3.0	1860.25	1474.25	2477.75	1288.5	0	0	
862	187.14814	186.14087	10	C9H18N2O2	[M+H]+	N-(5-acetamidopentyl)acetamide	Bruker MetaboBASE Personal Library 3.0	755.75	1243.75	1684.25	1339.75	0	130.25	
863	463.13913	462.13185	10.08	C24H19FN4O5	[M+H]+	ethyl 4-[[2-[3-[3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl]-2-oxopyridin-1-yl]acetyl]amino]benzoate	Bruker MetaboBASE Personal Library 3.0	4964.5	3524	4739.75	2308.75	3933.5	9185.75	
864	469.14953	468.14225	10.45	C23H24N4O5S	[M+H]+	N-[(2-methoxyphenyl) methyl]-2-methyl-5-{2-methyl-5,8-dioxo-4H,5H,6H,7H,8H-pyrazolo[1,5-a][1,3]diazepin-3-yl}benzene-1-sulfonamide	Bruker MetaboBASE Personal Library 3.0	0	89.5	0	1477.25	1973.25	2545.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
865	449.15948	448.15221	10.58	C23H21FN6OS	[M+H]+	N-(2-cyanophenyl)-2-[6-[4-(2-fluorophenyl) piperazin-1-yl]pyrimidin-4-yl]sulfanylacetamide	Bruker MetaboBASE Personal Library 3.0	3214.5	2186.25	4724	2168	1194	1704.5	
866	311.12755	310.12027	10.75	C14H19ClN4O2	[M+H]+	Cl-Amidine	Bruker MetaboBASE Personal Library 3.0	108.25	250.5	141.5	306.25	3288.5	2487	
867	329.13843	328.13115	10.78	C20H16N4O	[M+H]+	3-(8-benzoyl-6,7-dihydro-5H-imidazo[1,2-a]py-rimidin-2-yl)benzonitrile	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	222.5	4240.25	
868	323.1279	322.12062	10.85	C12H17F3N4O3	[M+H]+	N-(2-methoxyethyl)-4-[5-(trifluoromethyl)-1,3,4-oxadiazol-2-yl]piperidine-1-carboxamide	Bruker MetaboBASE Personal Library 3.0	1094.5	512.25	1632.5	2049.75	3359	3745.75	
869	115.0541	114.04682	10.93	C4H6N2O2	[M+H]+	Muscimol	Bruker MetaboBASE Personal Library 3.0	3139.75	2887	4325.5	1977	348.5	2824	
870	431.14902	430.14174	11.05	C22H18N6O4	[M+H]+	1-(4-methoxyphenyl)-5-[[3-(2-methoxyphenyl)-1,2,4-oxadiazol-5-yl]methyl]pyrazolo[3,4-d]pyrimidin-4-one	Bruker MetaboBASE Personal Library 3.0	8815.5	6052.75	7667	4906.5	2045.5	2067.5	
871	391.13901	390.13173	11.08	C21H19ClN6	[M+H]+	2-(4-chlorophenyl)-4-(4-pyridin-2-ylpiperazin-1-yl)pyrazolo[1,5-a]pyrazine	Bruker MetaboBASE Personal Library 3.0	2925	2678.75	2713	1534.5	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
872	397.12841	396.12113	11.47	C20H17FN4O4	[M+H]+ [M-H]-	2-[[3-(3,4-dimethoxyphenyl)-1,2,4-oxadiazol-5-yl]methyl]-5-(3-fluoro-4-methylphenyl)-1,3,4-oxadiazole	Bruker MetaboBASE Personal Library 3.0	97.75	3353.25	95	15743	28980	32584.5	
873	117.06978	116.0625	11.53	C4H8N2O2	[M+H]+	HA-966	Bruker MetaboBASE Personal Library 3.0	42813	23554.25	61644	30225.75	8457.75	12709.25	
874	131.04914	130.04186	11.55	C6H7FO2	[M+H]+	1-Fluorocyclohexadiene-cis,cis-1,2-diol	Bruker MetaboBASE Personal Library 2.0_in-silico	210.75	361.5	243.25	711.5	13926.25	1327.25	
875	417.13336	416.12609	11.7	C23H17FN4O3	[M+H]+, [M-H]-	N-(4-acetamidophenyl)-1-(4-fluorophenyl)-4-oxo-cinnoline-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	9194.5	9531	6467.25	3761.5	339.75	0	
876	377.12323	376.11595	12.35	C17H20N4O4S	[M+H]+	methyl 5-ethyl-2-[[2-(3-oxo-5,6,7,8-tetrahydro-cinnolin-2-yl)acetyl] amino]-1,3-thiazole-4-carboxylate	Bruker MetaboBASE Personal Library 3.0	0	0	0	2235.75	2704.5	3414	
877	143.04914	142.04186	12.42	C5H6N2O3	[M+H]+	5-Methylbarbiturate	Bruker MetaboBASE Personal Library 2.0_in-silico	1274.25	1298.75	1284	3060.75	5665.75	7751	
878	341.13841	340.13114	12.5	C21H16N4O	[M+H]+	(10R)-9,10,11,12-tetrahydro-10-methyl-3-(6-methyl-3-pyridinyl)-8H-[1,4]diazepino[5′,6′:4,5] thieno[3,2-f]quinolin-8-one hydrate	Bruker MetaboBASE Personal Library 3.0	1760.75	1280.25	2443.25	3022.75	4509.5	12470.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
879	403.21168	402.20441	12.59	C21H30N4O2S	[M+H]+	1-methyl-3-(pyrrolidin-1-ylmethyl)-5-(2,4,6-trimethylphenyl)sulfonyl-6,7-dihydro-4H-pyrazolo[4,3-c]pyridine	Bruker MetaboBASE Personal Library 3.0	1152	2676.25	5237.75	3412.75	0	0	
880	239.10665	238.09938	12.6	C11H14N2O4	[M+H]+	Felbamate	Bruker MetaboBASE Personal Library 2.0_in-silico	10780.25	7200.75	14828.75	18738	32464.75	26927	
881	401.10208	400.09481	12.8	C19H14F2N4O4	[M+H]+	2-(3,5-difluorophenyl)-5-[[3-(3,4-dimethoxyphenyl)-1,2,4-oxadiazol-5-yl]methyl]-1,3,4-oxadiazole	Bruker MetaboBASE Personal Library 3.0	1369.5	1628.25	1516	985.25	0	0	
882	277.10712	276.09984	12.88	C10H16N2O7	[M+H]+	Thymidine glycol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	366.5	0	1910.5	5281.5	4084	
883	447.14403	446.13675	12.98	C24H22N4O3S	[M+H]+	1-[4-(furan-2-carbonyl)piperazin-1-yl]-2-[2-(4-phenyl-1,3-thiazol-2-yl)pyrrol-1-yl]ethanone	Bruker MetaboBASE Personal Library 3.0	30661.25	25967.5	35017.75	21671.5	14415.25	11483.25	
884	327.0864	326.07913	13.02	C18H15ClN2O2	[M+H]+	N-[2-(3-chlorophenyl)-4-methoxyquinolin-6-yl]acetamide	Bruker MetaboBASE Personal Library 3.0	2210.5	3560.5	1452.25	1943.5	333.25	0	
885	401.13831	400.13104	13	C21H16N6O3	[M+H]+	2-[4-oxo-3-[(3-phenyl-1,2,4-oxadiazol-5-yl)methyl]pyrimido[5,4-b]indol-5-yl]acetamide	Bruker MetaboBASE Personal Library 3.0	1120.75	1130.75	1238.75	655.5	0	320.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
886	419.14903	418.14175	13.06	C21H26N2O3S2	[M+H]+	Thioridazine-2-sulfone-5-sulfoxide	Bruker MetaboBASE Personal Library 2.0_in-silico	18970.5	17305	25764.25	11954.75	1952.5	11213.75	
887	471.18045	470.17317	13.42	C27H26N4O2S	[M+H]+	N-(2,3-dihydro-1H-inden-1-yl)-1-(4-oxo-7-phenyl-1H-thieno[3,2-d]pyrimidin-2-yl)piperidine-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	7019	5300.75	5668	2944	0	0	
888	233.1326	232.12532	13.42	C13H16N2O2	[M+H]+	N-ethyl-5-methyl-3-phenyl-4H-1,2-oxazole-5-carboxamide	Bruker MetaboBASE Personal Library 3.0	4387.75	5456.5	2114.5	2799.5	0	0	
889	371.14903	370.14175	13.74	C19H22N4O2S	[M+H]+	2-[3-(5-ethyl-1,3,4-oxadiazol-2-yl)-2,5-dimethylpyrrol-1-yl]-N-(3-methylsulfanylphenyl)acetamide	Bruker MetaboBASE Personal Library 3.0	4169.75	5180.75	4292.75	2943.25	228.25	82.5	
890	343.11764	342.11036	14.36	C20H14N4O2	[M+H]+	7-(1-benzofuran-2-yl)-2-(2-methoxyphenyl)-[1,2,4]triazolo[1,5-a]pyrimidine	Bruker MetaboBASE Personal Library 3.0	16110.25	15775.25	11151.75	10153.75	2327.5	1122.25	
891	189.16377	188.15649	14.59	C9H20N2O2	[M+H]+	Propamocarb	Bruker MetaboBASE Personal Library 2.0_in-silico	2959	3584.75	2257.5	2331.75	428.5	312	
892	431.1492	430.14192	14.61	C24H19FN4O3	[M+H]+ [M-H]-	2-(4-fluorophenyl)-5-[[2-(4-methoxyphenyl)-5-methyl-1,3-oxazol-4-yl]methyl]pyrazolo[1,5-a]pyrazin-4-one	Bruker MetaboBASE Personal Library 3.0	19652.75	16040	30401.5	12994	661.75	5080.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
893	337.2531	336.24582	14.93	C19H32N2O3	[M+H]+	N-[7-(2-cyclohexylacetyl)-7-azaspiro[3.5]nonan-3-yl]-2-methoxyacetamide	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	2972.5	729.5	
894	343.2481	342.24083	15.26	C20H30N4O	[M+H]+	N-butan-2-yl-1-[2-(1-methylpyrrolo[2,3-b]pyridin-3-yl)ethyl]piperidine-4-carboxamide	Bruker MetaboBASE Personal Library 3.0	0	921.5	0	5116	35161	44420	
895	419.33696	418.33002	15.42	C27H43FO2	[M+H]+ [M+Na]+ [M-H]-	(5E,10E)-19-fluoro-1α-hydroxyvitamin-D3 / (5E,10E)-19-fluoro-1α-hydroxycholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	17111.75	5103.75	20521.75	10252	0	0	
896	273.12741	272.12013	15.87	C12H20N2O3S	[M+H]+	N-[4-[1-hydroxy-2-[{1-methylethyl}amino]ethyl]phenyl]methanesulphonamide	Bruker MetaboBASE Personal Library 3.0	2167.75	2532.5	3042.5	2495.75	0	864.25	
897	319.28454	318.27727	16.8	C21H36NO	[M+H]+	Tridihexethyl	Bruker MetaboBASE Personal Library 2.0_in-silico	9010.5	1922	7940.25	3351.5	2297.25	3328.5	
898	273.22132	272.21404	17.77	C16H29FO2	[M+H]+	14-Fluoro-11E-tetradecenyl acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	422.5	1335	499	1868.75	2528	6526.5	
899	194.11751	193.11023	0.86	C11H15NO2	[M+H]+	Methedrone	Bruker MetaboBASE Personal Library 2.0_in-silico	3824.5	3037.75	3614	5345.75	4651	4786.25	
900	69.03357	68.02629	1.09	C4H4O	[M+H]+	Furan	Bruker MetaboBASE Personal Library 2.0_in-silico	3254.25	1314.5	1784.25	1288	0	1943.5	
901	138.05488	137.0476	1.11	C7H7NO2	[M+H]+	Trigonelline	Bruker MetaboBASE Personal Library 2.0	3668.75	1495	2162.25	1924.25	313.25	2841	401

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
902	174.07609	173.06881	1.12	C6H12N3OP	[M+H]+	Tris(1-aziridinyl) phosphine oxide	Bruker MetaboBASE Personal Library 2.0_in-silico	1883	1067.5	1945.75	1591.75	0	2172.75	
903	440.17654	439.16926	1.15	C23H31Cl2NO3	[M+H]+	Estramustine	Bruker MetaboBASE Personal Library 2.0_in-silico	1377.75	1203.5	947.5	797.5	0	679.75	
904	233.06316	232.05588	1.21	C13H12O2S	[M+H]+	(2E,11Z)-5-[5-(Methylthio)-4-penten-2-ynyl]-2-furanacrolein	Bruker MetaboBASE Personal Library 2.0_in-silico	11474.25	4439	6902	4109	602.5	4219.5	
905	169.03555	168.02828	2.03	C5H4N4O3	[M+H]+, [M-H]-	Uric acid	Bruker HMDB Metabolite Library_2.0	2330	3703.5	5771.75	4097.25	854.75	1356.5	
906	139.05029	138.04302	2.04	C6H6N2O2	[M+H]+	Nicoxamat	Bruker MetaboBASE Personal Library 3.0	2221.25	661	1426.25	608.5	114.75	0	
907	130.04991	129.04263	2.15	C5H7NO3	[M+H]+, [M-H]-	(R)-(+)-2-Pyrrolidone-5-carboxylic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1239.5	1458.25	997	913.25	0	578	
908	137.04582	136.03854	4.41	C5H4N4O	[M+H]+	Hypoxanthine	Bruker HMDB Metabolite Library_2.0	1115.25	624.25	1468.25	509.25	0	286.5	402
909	194.11761	193.11033	4.64	C11H15NO2	[M+H]+	Salsoline	Bruker MetaboBASE Personal Library 2.0_in-silico	4108.25	3164.5	4242.25	6169.75	6068.25	5666.75	
910	218.08119	217.07391	4.87	C12H11NO3	[M+H]+	Cotarnine	Bruker MetaboBASE Personal Library 2.0	0	0	511.25	0	0	17056	
911	206.13863	205.13135	5.23	C9H19NO4	[M+H]+, [M-H]-	Panthenol	Bruker MetaboBASE Personal Library 3.0	7498.75	3300.25	1607.5	2257.5	0	2171	403
912	343.12506	342.11779	5.28	C12H22O11	[M+H]+	Fagopyritol A1	Bruker MetaboBASE Personal Library 2.0_in-silico	1619	0	257	0	0	0	
913	192.13833	191.13106	5.32	C12H17NO	[M+H]+	4-Ethylmethcathinone	Bruker MetaboBASE Personal Library 2.0_in-silico	1193	926	1327.75	1985	2152.75	1949.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
914	208.13323	207.12595	5.39	C12H17NO2	[M+H]+	3-(Dimethylamino)propyl benzoate	Bruker MetaboBASE Personal Library 2.0_in-silico	219	111	12027.25	0	119.75	0	
915	212.0919	211.08462	5.6	C10H13NO4	[M+H]+	L-3,4-Dihydroxyphe-nylalanine methyl ester hydrochloride	Bruker MetaboBASE Personal Library 3.0	1803.5	332.25	1096	185.5	0	871.5	
916	146.06083	145.05355	5.94	C9H7NO	[M+H]+	Oxyquinoline	Bruker MetaboBASE Personal Library 3.0	2047.75	932	693.25	706	161.5	505.5	
917	190.04983	189.04256	6.02	C10H7NO3	[M+H]+	Kynurenic acid	Bruker HMDB Metabolite Library_2.0	3272	870.25	2413.75	1111.75	0	1263.5	404
918	208.13321	207.12593	6.12	C12H17NO2	[M+H]+	Salsolidine	Bruker MetaboBASE Personal Library 2.0_in-silico	362.75	1221.25	310	438.25	102.5	94.75	
919	376.16045	375.15317	6.21	C16H25NO9	[M+H]+	Simmondsin	Bruker MetaboBASE Personal Library 3.0	1444	625.25	0	1242.75	0	423.25	
920	332.11315	331.10587	6.72	C17H17NO6	[M+H]+	Citbrasine	Bruker MetaboBASE Personal Library 2.0_in-silico	985.25	1375.75	1498.25	984.75	0	0	
921	595.16575	594.15847	6.81	C27H30O15	[M+H]+	Pelargonin	Bruker MetaboBASE Personal Library 3.0	163.75	125	2560	0	0	0	
922	625.14101	626.14829	6.8	C27H30O17	[M-H]- [M+H]+	5,7-dihydroxy-2-[3-hydro-xy-4-[(2S,3R, 4S,5S,6R)-3,4,5-trihydroxy-6-(hydro-xymethyl)oxan-2-yl]oxy-phenyl]-3-[(2S,3R,4S, 5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-4-one	MoNA-export-GNPS_QTOF.msp	0	999.25	123.25	70.5	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
923	136.07557	135.0683	6.84	C8H9NO	[M+H]+	Phenacylamine	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	0	5113	
924	392.13439	391.12711	7.37	C21H15F2N5O	[M+H]+	PharmaGSID_47330	Bruker MetaboBASE Personal Library 3.0	0	245.5	233.5	1607.75	1672.5	1544.5	
925	491.17316	490.16588	7.4	C28H26O8	[M+H]+	Edulisin I	Bruker MetaboBASE Personal Library 2.0_in-silico	1599.25	544.75	890.75	163.25	0	0	
926	435.09251	434.08523	7.72	C20H18O11	[M+H]+ [M-H]-	Quercetin-3-O-pentoside	MoNA-export-GNPS_QTOF.msp	0	215.25	121.5	0	2870	2023	
927	342.13388	341.1266	8.02	C19H19NO5	[M+H]+	Cassythine	Bruker MetaboBASE Personal Library 2.0_in-silico	1820	1838	2688.75	1615	0	0	
928	254.11363	253.10635	8.02	C11H15N3O4	[M+H]+	Pyricarbate	Bruker MetaboBASE Personal Library 2.0_in-silico	1238.5	408.75	828.75	356.25	0	1167.25	
929	149.04506	148.03778	8.1	C5H8O5	[M+H]+	D-erythro-3-Methylmalate	Bruker MetaboBASE Personal Library 2.0_in-silico	1142.25	854	1325.25	1896.25	1246.25	1677.75	
930	330.26399	329.25671	8.13	C18H35NO4	[M+H]+	4,8 dimethylnonanoyl carnitine	Bruker MetaboBASE Personal Library 2.0_in-silico	0	323.75	2009	0	0	0	
931	632.26052	631.25324	8.29	C34H37N3O9	[M+H]+ [M-H]-	N1,N5,N10-Tricaffeoyl spermidine	Bruker MetaboBASE Personal Library 2.0_in-silico	1572.25	585.5	1563.25	1116.25	469.75	363.5	
932	327.15922	326.15194	8.35	C20H22O4	[M+H]+	2-Methoxy-4-{(2S,3R)-7-methoxy-3-methyl-5-[(1E)-1-propen-1-yl]-2,3-dihydro-1-benzofuran-2-yl}phenol	MoNA-export-GNPS_QTOF.msp	1172.5	1093	1844.5	1187.25	298.25	655.5	
933	284.09184	283.08456	8.42	C16H13NO4	[M+H]+ [M-H]-	5,7-dimethoxy-2-pyridin-3-ylchromen-4-one	Bruker MetaboBASE Personal Library 3.0	19142.75	74255	30114	21922.75	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
934	381.18116	380.17389	8.55	C22H24N2O4	[M+H]+ [M-H]-	Di-4-coumaroylputrescine	Bruker MetaboBASE Personal Library 2.0_in-silico	4229.5	804	2382	281	463.75	1366.5	405
935	441.20254	440.19526	8.78	C24H28N2O6	[M+H]+	Diferuloylputrescine	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	1616.25	936.75	1672	3490.5	406
936	89.03847	88.03119	8.81	C4H8S	[M+H]+	cis-2,3-Dimethylthiirane	Bruker MetaboBASE Personal Library 2.0_in-silico	0	437.5	341.25	1210	1308.75	3241.75	
937	639.19259	638.18531	8.82	C29H34O16	[M+H]+	6-Hydroxyluteolin 6,4'-dimethyl ether 7-rutinoside	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	3287.75	0	
938	593.13056	594.13784	8.9	C30H26O13	[M-H]- [M+H]+	[(2R,3S,4S,5R,6S)-6-[5,7-dihydroxy-2-(4-hydroxy-phenyl)-4-oxochromen-3-yl]oxy-3,4,5-trihydroxy-oxan-2-yl]methyl (E)-3-(4-hydroxyphenyl)prop-2-enoate	MoNA-export-GNPS_QTOF.msp	225.25	2613.75	804.25	7047.25	2040.25	1592.75	
939	438.23927	437.232	9.24	C25H31N3O4	[M+H]+	N1,N10-Dicoumaroylspermidine	Bruker MetaboBASE Personal Library 2.0_in-silico	2130.75	1611.75	2863.25	1721.5	923.25	1448.75	
940	584.2761	583.26882	9.28	C34H37N3O6	[M+H]+ [M-H]-	N1,N5,N10-Tricoumaroyl spermidine	Bruker MetaboBASE Personal Library 2.0_in-silico	10682.25	8553.5	13990.75	7433.25	3158.5	7768.5	
941	341.1386	340.13132	9.38	C20H20O5	[M+H]+	Euchrenone a7	Bruker MetaboBASE Personal Library 2.0_in-silico	1375.75	1220.75	1982.75	2993.5	6043.25	11242.25	
942	347.07632	346.06904	9.46	C17H14O8	[M+H]+	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3,6-dimethoxychromen-4-one	MoNA-export-GNPS_QTOF.msp	1727.75	812	740.25	2928	516	2996.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
943	406.25942	405.25214	9.6	C24H31N5O	[M+H]+	5-[2-[4-[(2,5-dimethylphenyl)methyl]-1,4-diazepan-1-yl]pyridin-3-yl]-3-propan-2-yl-1,2,4-oxadiazole	Bruker MetaboBASE Personal Library 3.0	386.75	1126.75	0	0	0	0	
944	787.37089	786.36361	9.66	C46H50N4O8	[M+H]+	(E)-3-(4-hydroxyphenyl)-N-[3-[[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]-[4-[[(E)-3-(4-hydroxyphenyl)prop-2-enoyl]-[3-[[(E)-3-(4-hydroxyphenyl)prop-2-enoyl] amino] propyl]amino]butyl]amino] propyl]prop-2-enamide	MoNA-export-GNPS_QTOF.msp	1492.25	608	1886.75	743.25	458.25	1831	
945	579.15089	578.14362	9.7	C30H26O12	[M+H]+	Apigenin 7-(4"-Z-p-coumarylglucoside)	Bruker MetaboBASE Personal Library 2.0_in-silico	435.5	1286.5	598.5	1246	1022.75	1757.75	47
946	386.16037	385.1531	9.72	C21H23NO6	[M+H]+	Desmethylcolchicine	Bruker MetaboBASE Personal Library 3.0	905	1770.75	0	0	0	0	
947	295.13301	294.12573	10.57	C19H18O3	[M+H]+	Phenol, 2-methoxy-4-[3-methyl-5-[(1E)-1-propen-1-yl]-2-benzofuranyl]-	MoNA-export-GNPS_QTOF.msp	0	0	0	0	3981.5	801.75	
948	373.22214	372.21487	10.65	C20H28N4O3	[M+H]+	2-(dimethylamino)-7-[(4-ethoxy-3-methoxy-phenyl)methyl]-5,6,8,9-tetrahydro-1H-pyrimido[4,5-d]azepin-4-one	Bruker MetaboBASE Personal Library 3.0	0	0	0	672.75	1230.75	2519.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
949	369.17189	368.16461	11.23	C22H24O5	[M+H]+	Quercetol C	Bruker MetaboBASE Personal Library 2.0_in-silico	1149.25	1562.75	1200.5	1670.5	0	318.25	
950	343.29571	342.28844	11.79	C19H38N2O3	[M+H]+	Cocamidopropyl Betaine	MoNA-export-GNPS_QTOF.msp	6571.5	2972.5	8739.5	5810.25	3885.25	5038.75	
951	214.25313	213.24585	12.32	C14H31N	[M+H]+	N,N-Dimethyldodecan-1-amine	Bruker MetaboBASE Personal Library 3.0	1394.5	1157.25	4596.5	787.25	189	574.5	
952	230.24804	229.24076	12.5	C14H31NO	[M+H]+	N,N-Dimethyldode-cylamine-N-oxide	Bruker MetaboBASE Personal Library 3.0	11465	4452.5	23888.5	5910.75	4640.75	5769.75	
953	317.17243	316.16515	13.55	C19H24O4	[M+H]+	Bisphenol A bis(2-hydroxyethyl)ether	Bruker MetaboBASE Personal Library 2.0_in-silico	4660.75	7443	5363.5	5792.75	0	2390	
954	223.0636	222.05633	13.57	C9H10N4OS	[M+H]+	9H-Purine-6-thiol, 9-(tetrahydro-2-furyl)-	Bruker MetaboBASE Personal Library 3.0	1641.5	1156.25	1299.5	3147	3222.5	1905	
955	242.28439	241.27712	13.84	C16H35N	[M+H]+	1-Hexadecylamine	Bruker MetaboBASE Personal Library 2.0	1044.75	693.25	6179.25	636	621.5	288.25	
956	304.29995	303.29267	13.86	C21H37N	[M+H]+	Benzalkonium	Bruker MetaboBASE Personal Library 2.0_in-silico	5107.75	1769	1493.75	1886.5	979.5	2860.25	
957	256.30006	255.29278	15.05	C17H37N	[M+H]+	N-Methyldioctylamine	Bruker MetaboBASE Personal Library 3.0	999.5	1045	38470.25	806.25	113.5	0	
958	411.19534	410.18807	15.08	C22H28F2O5	[M+H]+	Tafluprost (free acid)	Bruker MetaboBASE Personal Library 2.0	0	4013.75	0	520	0	0	
959	505.22215	504.21488	15.48	C30H32O7	[M+H]+ [M-H]-	Artocommunol CC	Bruker MetaboBASE Personal Library 2.0_in-silico	1197	1853.25	975.25	1078.25	0	0	
960	268.08236	267.07509	15.73	C13H14FNO2S	[M+H]+	4-fluoro-3-methoxy-N-pro-pan-2-yl-1-benzo-thiophene-2-carboxamide	Bruker MetaboBASE Personal Library 3.0	2708.75	1874.5	2833	3976	3794.25	3710.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
961	284.33124	283.32396	16.37	C19H41N	[M+H]+	Cetrimonium	Bruker MetaboBASE Personal Library 2.0_in-silico	1064.75	1018	25224	800.75	361	0	
962	251.04665	250.03937	16.57	C12H11O4P	[M+H]+	Diphenyl phosphate	Bruker MetaboBASE Personal Library 3.0	314.75	377.25	4377.5	328.25	0	110.25	
963	339.15921	338.15193	17.6	C21H22O4	[M+H]+	9-[(3,7-Dimethyl-2,6-octadienyl)oxy]-7H-furo[3,2-g][1]benzopyran-7-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2185	2939.25	2026.75	1584	0	0	
964	409.38251	408.37524	18.9	C30H48	[M+H]+	Dehydrosqualene	Bruker MetaboBASE Personal Library 2.0_in-silico	1046.75	497	966.5	424	1804.5	2006	
965	569.44119	568.43392	19.26	C33H60O7	[M+H]+	Muricin E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	57.25	0	1526.5	2102.25	1815	
966	339.32591	338.31864	19.41	C22H42O2	[M+H]+	5,7-Docosanedione	Bruker MetaboBASE Personal Library 2.0_in-silico	1258.75	487.25	2807	803	0	0	
967	489.39415	488.38687	19.59	C31H52O4	[M+H]+	(22S)-1α,22,25-trihydroxy-26,27-dimethyl-24a,24b-dihomovitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	2795.25	575.75	
968	367.32164	368.32891	19.85	C23H44O3	[M-H]-, [M+H]+	3-oxo-tricosanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	1656.75	1249.75	1124	1799	0	0	
969	413.37766	412.37038	19.92	C29H48O	[M+H]+	β-Sitostenone	Bruker MetaboBASE Personal Library 2.0	954.75	159	1036	1689.75	780.5	814.25	
970	504.19249	503.18522	1.04	C18H33NO15	[M+H]+	beta-D-Galactopyranosyl-(1->4)-2-amino-2-deoxy-beta-D-glucopyranosyl-(1->6)-D-mannose	Bruker MetaboBASE Personal Library 2.0_in-silico	727.5	1893.25	261.5	1028.75	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
971	140.06823	139.06096	1.05	C7H9NO2	[M+H]+	Gabaculine	Bruker MetaboBASE Personal Library 2.0_in-silico	3851	1338.5	4258	1816.75	1320.5	1941.5	
972	290.07616	289.06888	1.03	C13H12ClN5O	[M+H]+	N-[[5-(3-chlorophenyl) furan-2-yl]methyl]-1-methyltetrazol-5-amine	Bruker MetaboBASE Personal Library 3.0	5560.5	0	3664.25	1711	0	0	
973	104.10683	103.09955	1.04	C5H13NO	[M+H]+	Choline	Bruker MetaboBASE Personal Library 2.0	1836.25	1111	2046.75	1485.75	227.25	1545.75	
974	160.09678	159.08951	1.1	C7H13NO3	[M+H]+	4-hydroxystachydrine	Bruker MetaboBASE Personal Library 2.0_in-silico	3103	1851	2652	1173.5	0	724.25	
975	118.08622	117.07895	1.09	C5H11NO2	[M+H]+	Betaine	Bruker MetaboBASE Personal Library 3.0	7145.25	2040	8627.5	2307.75	1507.25	2115.25	
976	258.10997	257.10269	1.09	C8H20NO6P	[M+H]+	Glycerophosphocholine	Bruker HMDB Metabolite Library_2.0	9121	2915.75	6596.75	2456.5	73.25	5631.75	
977	277.0893	276.08202	1.14	C13H13ClN4O	[M+H]+	(2E)-2-{1-[4-Chloro-3-(1H-pyrrol-1-yl)phenyl]ethylidene} hydrazinecarboxamide	Bruker MetaboBASE Personal Library 3.0	4942.75	820	6336.75	4237	2110	4895.5	
978	278.12337	277.1161	1.15	C12H15N5O3	[M+H]+ [M-H2O+H]+	Entecavir	Bruker MetaboBASE Personal Library 2.0_in-silico	16448.75	17721	14413.5	9392.25	88.5	9058.75	
979	144.10185	143.09458	1.14	C7H13NO2	[M+H]+	Proline betaine	Bruker MetaboBASE Personal Library 3.0	5403	3621.5	22174.5	2212.5	0	1999.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
980	360.14992	359.14265	1.15	C20H17N5O2	[M+H]+	3H-benzimidazol-5-yl-[3-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrrolidin-1-yl]methanone	Bruker MetaboBASE Personal Library 3.0	4698.5	3088.75	2869.25	4288	589.25	5417	
981	280.13917	279.13189	2.02	C15H21NO2S	[M+H]+	(3-ethyl-3-hydroxy-7-azaspiro[3.5]nonan-7-yl)-thiophen-3-ylmethanone	Bruker MetaboBASE Personal Library 3.0	1126.25	1506.25	1424.25	367.25	0	0	
982	268.10402	267.09675	3.92	C10H13N5O4	[M+H]+	Adenosine	Bruker MetaboBASE Personal Library 3.0	856.75	631	961.75	582	0	439.25	
983	246.11251	245.10524	5.62	C14H15NO3	[M+H]+	Ethyl (6-methyl-4-oxo-1,4-dihydro-2-quinolinyl)acetate	Bruker MetaboBASE Personal Library 3.0	760.5	1164	0	110.5	0	0	
984	196.0968	195.08953	5.8	C10H13NO3	[M+H]+	n-acetyldopamine	Bruker MetaboBASE Personal Library 2.0_in-silico	1681.25	1033	713.5	737.5	0	825	
985	207.06537	206.0581	6.13	C11H10O4	[M+H]+	Scoparone	Bruker MetaboBASE Personal Library 2.0_in-silico	789	1081.75	1133.5	1058	112.5	920	
986	276.12316	275.11588	6.14	C15H17NO4	[M+H]+	(±)-Ribaline	Bruker MetaboBASE Personal Library 2.0_in-silico	2111	3384.5	847.75	3629.25	704	2813	
987	495.14762	494.14035	6.41	C23H26O12	[M+H]+	7,8,4'-Trihydroxy-3',5'-dimethoxyflavanone 4'-O-glucoside	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	2088.5	
988	277.06841	276.06113	6.59	C14H12O6	[M+H]+	O-Demethylfonsecin	Bruker MetaboBASE Personal Library 2.0_in-silico	7994.5	6132.5	10229	16245.25	2151.5	7412.5	
989	327.1226	326.11533	6.98	C19H18O5	[M+H]+	Sappanone a trimethyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	124	247.5	0	1558.75	1450.25	2310.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
990	170.11743	169.11015	7.1	C9H15NO2	[M+H]+	Piperidione	Bruker MetaboBASE Personal Library 2.0_in-silico	1171.75	1162	1457	1578.25	0	1981.75	
991	316.11841	315.11113	7.23	C17H17NO5	[M+H]+	Citpressine II	Bruker MetaboBASE Personal Library 2.0_in-silico	518	1384.75	424.5	506.75	0	75.25	
992	359.11265	358.10537	7.31	C19H18O7	[M+H]+	Altisin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	326.5	0	1072.75	3536.75	3632.75	
993	449.14448	448.1372	7.48	C22H24O10	[M+H]+	Dihydrowogonin 7-O-glucoside	Bruker MetaboBASE Personal Library 2.0_in-silico	1701.5	3036.25	2976	1048.25	0	0	
994	309.16738	308.1601	7.58	C17H24O5	[M+H]+	ACRL Toxin II	Bruker MetaboBASE Personal Library 2.0_in-silico	2004.25	4418	2755.25	2259.75	0	986	
995	435.12877	434.12149	7.74	C21H22O10	[M+H]+	Naringenin-7-O-Glucoside	Bruker MetaboBASE Personal Library 3.0	124	1007	1502.75	0	0	0	208
996	217.04762	216.04035	7.74	C12H8O4	[M+H]+	Isobergaptene	Bruker MetaboBASE Personal Library 2.0_in-silico	978	2056.5	1967	5347.25	3852.5	7111.75	
997	323.14677	322.1395	7.74	C18H18N4O2	[M+H]+	N,N-diethyl-2-(4-nitrophenyl)quinazolin-4-amine	Bruker MetaboBASE Personal Library 3.0	0	462	0	1788.75	0	3270.75	
998	429.11648	428.10921	7.81	C18H22ClFN4O3S	[M+H]+	N-(3-chloro-4-fluorophenyl)-1-(1-propan-2-ylimidazol-4-yl)sulfonylpiperidine-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	0	80	0	1754	757.25	1052	
999	325.06849	324.06121	7.82	C18H12O6	[M+H]+	Grevilline A	Bruker MetaboBASE Personal Library 2.0_in-silico	1569.75	1336.75	1375.75	1009.5	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
1000	357.15208	356.1448	7.9	C16H24N2O5S	[M+H]+	1-(3,4-dimethoxyphenyl) sulfonyl-N-ethyl-piperidine-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	0	0	2191	0	0	0	
1001	267.15734	266.15007	8	C15H22O4	[M+H]+	4-Gingerol	Bruker MetaboBASE Personal Library 2.0_in-silico	228.25	133.75	622.75	434.75	2220	1577.5	
1002	289.10491	288.09763	8.15	C16H16O5	[M+H]+	Marmesin acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	2114.75	1096.75	1969.25	1238.5	0	108.25	
1003	431.13628	430.129	8.3	C22H22O9	[M+H]+	Torosaflavone B	Bruker MetaboBASE Personal Library 2.0_in-silico	346.75	2233	1247	339.25	320	202.5	
1004	319.11539	318.10812	8.33	C16H18N2O3S	[M+H]+	N-(4-tert-butyl-1,3-thiazol-2-yl)-2,3-dihydro-1,4-benzodioxine-6-carboxamide	Bruker MetaboBASE Personal Library 3.0	5346.25	2611	4974	2735	897	0	
1005	384.14429	383.13702	8.48	C21H21NO6	[M+H]+	Rhoeadine	Bruker MetaboBASE Personal Library 2.0_in-silico	517.5	0	1917.25	0	0	0	
1006	356.14931	355.14203	8.6	C20H21NO5	[M+H]+	4-(3,4-dimethoxyphenyl)-1-(3-methoxyphenyl) piperidine-2,6-dione	Bruker MetaboBASE Personal Library 3.0	1352.25	1089	1375.75	627	0	0	
1007	309.16755	308.16028	8.61	C16H24N2O2S	[M+H]+	2-N,2-N,5-N-triethyl-4,5,6,7-tetrahydro-1-benzothiophene-2,5-dicarboxamide	Bruker MetaboBASE Personal Library 3.0	1129.5	1419.5	910.5	828	0	285.25	
1008	313.10715	312.09987	8.83	C18H16O5	[M+H]+	Leridal	Bruker MetaboBASE Personal Library 2.0_in-silico	2270.75	2085.25	4118	2514.25	135.25	157.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1009	314.13911	313.13184	8.91	C16H19N5S	[M+H]+	4-[2-(dimethylamino) ethylamino]-2-methylsulfanyl-6-phenylpyrimidine-5-carbo-nitrile	Bruker MetaboBASE Personal Library 3.0	943.75	1294.5	853	336.5	0	0	
1010	401.15969	400.15241	8.91	C22H24O7	[M+H]+	Kenusanone E	Bruker MetaboBASE Personal Library 2.0_in-silico	758.5	546	1598.5	686.5	0	2275	
1011	369.22505	368.21777	9.05	C21H28N4O2	[M+H]+	N-(2,5-dimethylphenyl)-2-[4-methyl-2-(4-methyl-piperidin-1-yl)-6-oxo-pyrimidin-1-yl]acetamide	Bruker MetaboBASE Personal Library 3.0	2191.75	2040.25	1642.25	1462	0	0	
1012	356.14943	355.14215	9.08	C20H21NO5	[M+H]+	Gravacridonediol methyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	6903.75	2774	4592.75	2738.25	0	0	13
1013	293.13638	292.1291	9.14	C14H17FN4O2	[M+H]+	2-(4-fluorophenyl)-5-(hydroxymethyl)-N-(2-methylpropyl)triazole-4-carboxamide	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	0	3144.5	
1014	351.21454	350.20727	9.23	C20H30O5	[M+H]+	(E)-4-acetoxy-8-(3-oxo-2-(pent-2-en-1-yl)cyclopent-1-en-1-yl)octanoic acid	Bruker MetaboBASE Personal Library 3.0	1816.5	3543	654	2125	0	0	
1015	150.09132	149.08404	9.27	C9H11NO	[M+H]+	2,3,6,7-Tetrahydrocyclo-pent[b]azepin-8(1H)-one	Bruker MetaboBASE Personal Library 2.0_in-silico	1183	549.75	312	141.75	604	1872.25	
1016	463.12469	464.13197	9.32	C22H24O11	[M-H]-, [M+H]+	Lanceolin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	420.25	1892.75	2724.75	29823.5	276.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
1017	326.13889	325.13161	9.32	C19H19NO4	[M+H]+	Nornantenine	Bruker MetaboBASE Personal Library 2.0_in-silico	12115	11622.5	12782.75	5366	0	0	
1018	368.1606	367.15332	9.48	C20H21N3O4	[M+H]+	2-(2-ethyl-6,7-dihydro-[1,4]dioxino[2,3-f]benzimidazol-3-yl)-N-(4-methoxyphenyl)acetamide	Bruker MetaboBASE Personal Library 3.0	356.75	1435	274.5	593.5	0	0	
1019	394.1896	393.18232	9.64	C21H23N5O3	[M+H]+	N-benzyl-2-[2-(cyclo-pentylamino)-2-oxoethyl]-3-oxo-[1,2,4]triazolo[4,3-a]pyridine-6-carboxamide	Bruker MetaboBASE Personal Library 3.0	2088.25	897.5	2174.75	1417.75	944	2139.5	
1020	399.14221	398.13493	9.63	C22H22O7	[M+H]+	Dulxanthone E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	120	0	2448	0	1290.75	
1021	340.15448	339.14721	9.63	C20H21NO4	[M+H]+	4-(3,4-dimethoxyphenyl)-1-(4-methylphenyl) piperidine-2,6-dione	Bruker MetaboBASE Personal Library 3.0	13249.5	11905.75	13704	6580	121.75	910.5	
1022	291.15678	290.14951	9.66	C17H22O4	[M+H]+	[6]-Dehydrogingerdione	Bruker MetaboBASE Personal Library 2.0_in-silico	3522.5	7666.5	3021.5	3981	152.5	0	407
1023	254.05717	253.0499	9.82	C10H11N3O3S	[M+H]+	Sulfamethoxazole	Bruker MetaboBASE Personal Library 2.0_in-silico	1393	1398.5	1230.75	795.75	0	0	408
1024	357.22497	356.21769	9.87	C19H32O6	[M+H]+	5,7-Megastigmadien-9-ol glucoside	Bruker MetaboBASE Personal Library 2.0_in-silico	0	529.5	0	4672.25	4464.75	8377.25	
1025	340.1545	339.14722	9.96	C18H21N5S	[M+H]+	4-methyl-5-[[3-[(5-methyl-2-pyridin-4-ylimidazol-1-yl)methyl]azetidin-1-yl]methyl]-1,3-thiazole	Bruker MetaboBASE Personal Library 3.0	18656.5	15910.75	20970.75	8167.25	463	1426.5	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1026	303.08533	302.07806	10.08	C16H14O6	[M+H]+	Hesperetin	Bruker MetaboBASE Personal Library 3.0	8310.75	11476.25	11409.5	13972.75	5129.75	3241.75	409
1027	309.20434	308.19707	10.13	C18H28O4	[M+H]+	Corchorifatty acid D	Bruker MetaboBASE Personal Library 2.0_in-silico	768.25	1005.75	456	498	187	2824	
1028	293.07856	292.07128	10.15	C18H12O4	[M+H]+	4-Methoxyfurano [2",3":6,7]aurone	Bruker MetaboBASE Personal Library 2.0_in-silico	5339.75	3175.75	4741.75	3049.75	0	0	
1029	233.18999	232.18271	10.13	C16H24O	[M+H]+	1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-1,6-heptadien-3-one	Bruker MetaboBASE Personal Library 2.0_in-silico	0	176.25	0	0	0	2908	
1030	259.09652	258.08924	10.13	C15H14O4	[M+H]+	10-methoxy-2,2-dimethy-lpyrano[3,2-g]chromen-8-one	MoNA-export-GNPS_QTOF.msp	378.5	548.75	636.75	928.75	8038.5	2802.5	
1031	419.11287	418.10559	10.27	C24H18O7	[M+H]+	8-Caffeoyl-3,4-dihydro-5,7-dihydroxy-4-phenylcoumarin	Bruker MetaboBASE Personal Library 2.0_in-silico	2449.5	1225.75	2288.5	1663	0	141.25	
1032	342.17	341.16272	10.34	C20H23NO4	[M+H]+	5,8,13,13a-Tetrahydrocolumbamine	Bruker MetaboBASE Personal Library 2.0_in-silico	1556.5	2176.5	1246.5	398.5	0	0	
1033	457.14971	456.14243	10.37	C22H24N4O5S	[M+H]+	6-(methanesulfonamido)-2-[4-(2-methoxyphenyl) piperazin-1-yl]quinoline-4-carboxylic acid	Bruker MetaboBASE Personal Library 3.0	0	69	0	223.5	2709.25	2638.25	
1034	373.12823	372.12095	10.46	C20H20O7	[M+H]+	6-Methoxyprosogerin B Diethyl Ether	Bruker MetaboBASE Personal Library 2.0_in-silico	110.5	564	0	1998	4968.5	7864	
1035	487.16045	486.15318	10.48	C25H26O10	[M+H]+	Aquayamycin	Bruker MetaboBASE Personal Library 2.0_in-silico	0	431.75	0	1679.75	2341	3528.5	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1036	275.1632	274.15593	10.54	C17H22O3	[M+H]+	Carbestrol	Bruker MetaboBASE Personal Library 3.0	1215	903.75	1092.5	872.5	476	507.25	
1037	251.16418	250.15691	10.62	C15H22O3	[M+H]+	Lactaronecatorin A	Bruker MetaboBASE Personal Library 2.0_in-silico	1725	2036.25	2361	1486.75	1858.5	1174.5	
1038	285.07578	262.08541	10.68	C14H14O5	[M+Na]+ [M+H]+	Dorsteniol	Bruker MetaboBASE Personal Library 2.0_in-silico	532505.25	498316.75	437182.25	316904.75	68443.75	20850	
1039	324.21747	323.21019	10.73	C18H29NO4	[M+H]+	Lycofawcine	Bruker MetaboBASE Personal Library 2.0_in-silico	0	1109.25	0	376.25	0	0	
1040	409.12749	408.12021	10.74	C23H20O7	[M+H]+, [M-H]-	Dehydroamorphigenin	Bruker MetaboBASE Personal Library 2.0_in-silico	1839.75	1218.5	1804	1451.75	194.5	143	
1041	369.13167	368.12439	10.85	C21H20O6	[M+H]+	Curcumin	Bruker MetaboBASE Personal Library 2.0_in-silico	778.25	766	979.25	1539	470.25	7203.75	
1042	527.15503	526.14776	11.14	C27H26O11	[M+H]+	6-Desmethoxy hormothamnione triacetate	Bruker MetaboBASE Personal Library 3.0	0	0	0	1335.25	2259.25	3363	
1043	351.21445	350.20718	11.21	C20H30O5	[M+H]+	PGK2	Bruker MetaboBASE Personal Library 2.0	2775.25	3406.5	2800.5	2002.75	0	477.5	
1044	321.24271	320.23544	11.27	C20H32O3	[M+H]+	(±)15-HETE	Bruker MetaboBASE Personal Library 2.0_in-silico	497.25	2214.5	519.5	1236.75	120.75	542.5	
1045	569.18072	568.17344	11.45	C33H28O9	[M+H]+	Asticolorin C	Bruker MetaboBASE Personal Library 2.0_in-silico	1845.25	1268	2443.75	867.75	0	105.75	
1046	275.16186	274.15458	11.52	C17H22O3	[M+H]+	Panaquinquecol 4	Bruker MetaboBASE Personal Library 2.0_in-silico	2511.75	5052.25	1844.25	3933	0	135.75	
1047	240.23233	239.22505	11.79	C15H29NO	[M+H]+	(2R,6R,7S,8S)-7-ethyl-2-propyl-1-azaspiro [5.5]undecan-8-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	1691.75	905.5	2418.75	1296.75	921.25	1536	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1048	299.09137	298.0841	11.8	C17H14O5	[M+H]+	Lawinal	Bruker MetaboBASE Personal Library 2.0_in-silico	8348.5	5477	18656	3794.5	1068.25	4392.5	
1049	307.09439	306.08712	11.91	C19H14O4	[M+H]+	(3S)-8-hydroxy-3-methyl-3,4-dihydro-2H-benzo[a]anthracene-1,7,12-trione	MoNA-export-GNPS_QTOF.msp	2394.25	1100.25	2261.75	3175.5	262	0	
1050	427.17546	426.16818	11.95	C24H26O7	[M+H]+ [M-H]-	Mangostenol	Bruker MetaboBASE Personal Library 2.0_in-silico	2404.5	1762.75	3409	4590.25	1222	1374.25	
1051	343.11761	342.11033	12.35	C19H18O6	[M+H]+	Norartocarpetin 5,7,2',4'-tetramethyl ether	Bruker MetaboBASE Personal Library 2.0_in-silico	16115	17022	11374.25	8387.75	2296.75	1407	
1052	339.12285	338.11558	12.65	C18H18N4OS	[M+H]+	N-(4,5-dihydrobenzo[e][1,3]benzothiazol-2-yl)-1-ethyl-5-methylpyrazole-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	245	440.75	608.25	1496.75	6750.5	8396	
1053	493.28	492.27272	12.75	C28H36N4O4	[M+H]+	ethyl 6-[(4-benzyl-piperazin-1-yl)methyl]-2-oxo-4-(4-propan-2-yloxy-phenyl)-3,4-dihydro-1H-pyrimidine-5-carboxylate	Bruker MetaboBASE Personal Library 3.0	0	817	0	4492.5	4050.25	7746	
1054	393.13188	392.1246	12.84	C23H20O6	[M+H]+	(E)-1-[3-[(2,3-dihydroxy-phenyl)methyl]-2,4-dihydroxy-6-methoxy-phenyl]-3-phenylprop-2-en-1-one	MoNA-export-GNPS_QTOF.msp	2000.5	1747.25	1982	1366.5	0	196.5	
1055	307.22707	306.21979	12.88	C19H30O3	[M+H]+	5-Androstenetriol	Bruker MetaboBASE Personal Library 2.0_in-silico	1217	2604.5	158.25	989.5	0	198.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1056	457.3313	456.32402	12.97	C29H44O4	[M+H]+	Callystatin A	Bruker MetaboBASE Personal Library 2.0_in-silico	871	425.25	504.25	2079.25	2212	10826.5	
1057	383.24114	382.23387	13	C22H30N4O2	[M+H]+	6-[(3-methoxy-4-propan-2-yloxyphenyl)methyl]-2-pyrrolidin-1-yl-7,8-dihydro-5H-pyrido[4,3-d]pyrimidine	Bruker MetaboBASE Personal Library 3.0	0	0	0	1219.5	2138	2493.5	
1058	295.09646	272.10604	13.08	C16H16O4	[M+Na]+[M+H]+	1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenylpropan-1-one	MoNA-export-GNPS_QTOF.msp	60062.75	43313.75	81773.75	39307	4827.75	9763.25	410
1059	288.2535	287.24623	13.15	C16H33NO3	[M+H]+	Lauroyl diethanolamide	Bruker MetaboBASE Personal Library 2.0_in-silico	12888.75	4396.5	3139.25	4858	1936	4532	
1060	299.20052	298.19325	13.15	C20H26O2	[M+H]+	All-Trans-3,4-Didehydro-Retinoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	55.5	73.75	106	293.75	1975.25	3541.25	
1061	267.19546	266.18818	13.21	C16H26O3	[M+H]+	4-Hydroxy-3-methoxy-2,10-bisaboladien-9-one	Bruker MetaboBASE Personal Library 2.0_in-silico	0	186.25	0	0	0	2737.25	
1062	233.13258	232.12531	13.31	C12H21ClO2	[M+H]+	TRIMEDLURE	Bruker MetaboBASE Personal Library 2.0_in-silico	4729.25	9535.25	7166.25	4989.25	0	0	
1063	161.13243	160.12515	13.34	C7H16N2O2	[M+H]+	Bethanechol	Bruker MetaboBASE Personal Library 2.0	1981	2861.25	1820	840.25	742.25	3781.5	
1064	459.38354	458.37627	13.34	C30H50O3	[M+H]+	Heliantriol F	Bruker MetaboBASE Personal Library 2.0_in-silico	1936	925.25	414.25	696.25	346.5	1874	
1065	275.20062	274.19334	13.46	C18H26O2	[M+H]+	13-Octadecene-9,11-diynoic acid, (Z)-	Bruker MetaboBASE Personal Library 2.0_in-silico	2401.75	2762	1892.25	2095	2569.5	444.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1066	421.12846	420.12118	13.46	C20H24N2O4S2	[M+H]+	5-(cyclobutanecarbonyl)-N-[(2-methoxyphenyl) methyl]-6,7-dihydro-4H-thieno[3,2-c]pyridine-2-sulfonamide	Bruker MetaboBASE Personal Library 3.0	1385.5	1102.5	1185	992.75	164.5	104.75	
1067	228.2687	227.26142	13.58	C15H33N	[M+H]+	n-Pentadecylamine	Bruker MetaboBASE Personal Library 3.0	1064.75	1238.25	33439.25	619	160.75	0	
1068	353.13845	352.13117	13.59	C21H20O5	[M+H]+	Neorautane	Bruker MetaboBASE Personal Library 2.0_in-silico	2829	1828.25	2288.5	825	0	0	
1069	355.26601	354.25873	13.7	C24H34O2	[M+H]+	5β-Chola-3,8(14),11-trien-24-oic Acid	Bruker MetaboBASE Personal Library 2.0_in-silico	3114.75	2435	3621	4124.75	5394.75	4301.75	
1070	401.10214	400.09486	13.81	C20H15F3N4S	[M+H]+	2-(4-methylsulfanylphe-nyl)-N-[3-(trifluoromethyl) phenyl]imidazo[1,2-a]pyrazin-3-amine	Bruker MetaboBASE Personal Library 3.0	1420.5	1169.75	1835	166	0	0	
1071	485.32623	484.31895	13.93	C30H44O5	[M+H]+ [M-H]-	Liquoric acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	2253.5	5299	
1072	185.15375	184.14648	13.97	C11H20O2	[M+H]+	5-Hexyldihydro-4-methyl-2(3H)-furanone	Bruker MetaboBASE Personal Library 2.0_in-silico	1438.5	1555.75	1120	1289.75	603	1449.25	
1073	437.23234	436.22506	14	C27H32O5	[M+H]+	Lespedezaflavanone F	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	524.25	0	3358.75	
1074	277.25263	276.24535	14.03	C19H32O	[M+H]+	5-(1-hydroxybutan-2-yl)isolongifol-4-ene	Bruker MetaboBASE Personal Library 2.0_in-silico	1148.5	1742.5	1435.5	1362	0	0	
1075	455.31567	454.30839	14.1	C29H42O4	[M+H]+	Coenzyme Q4	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	1159	1950.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1076	363.25072	362.24344	14.13	C22H34O4	[M+H]+	16,16-dimethyl-PGA2	Bruker MetaboBASE Personal Library 2.0_in-silico	1444.5	2743.25	2382.25	1652.75	0	0	
1077	457.25871	456.25144	14.2	C27H36O6	[M+H]+	Lucidenic acid F	Bruker MetaboBASE Personal Library 2.0_in-silico	3038.75	3684.25	2682	2124.5	153.5	0	
1078	371.26127	370.254	14.22	C24H34O3	[M+H]+	Rimexolone	Bruker MetaboBASE Personal Library 2.0_in-silico	1409.75	953	1310.5	2070.5	2773.75	1981.5	
1079	200.20102	199.19374	14.24	C12H25NO	[M+H]+	Dodecanamide	Bruker MetaboBASE Personal Library 2.0_in-silico	2272.75	1582.25	2763	3393.75	3444.75	2907.5	
1080	413.30444	412.29716	14.29	C27H40O3	[M+H]+	Valenciachrome	Bruker MetaboBASE Personal Library 2.0_in-silico	127.25	0	0	0	5051.25	3329.5	
1081	455.31628	456.32356	14.41	C29H44O4	[M-H]- [M+H]+	1α-hydroxy-18-(5-hydro-xy-5-methyl-2-hexyny-loxy)-23,24,25,26,27-pentanorvitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	2823.5	0	268.25	111	1438.25	6759	
1082	453.33647	452.32919	14.45	C30H44O3	[M+H]+	3-oxoursan (28-13)olide	Bruker MetaboBASE Personal Library 2.0_in-silico	2961	254.5	585.75	251.5	3286.5	3246.5	
1083	459.34675	458.33947	14.6	C29H46O4	[M+H]+	(3beta,5alpha,9alpha,22E,24R)-3,5,9-Trihydroxy-23-methylergosta-7,22-dien-6-one	Bruker MetaboBASE Personal Library 2.0_in-silico	3260.75	1348.5	1906.75	1007	1281.25	0	
1084	471.3454	470.33812	14.67	C30H46O4	[M+H]+	Colubrinic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	17664.75	1932.25	3780.5	3401.75	12839.75	17495	
1085	329.24776	328.24048	14.63	C22H32O2	[M+H]+	(4Z,7Z,10Z,13Z,16Z,19Z)-4,7, 10,13,1 6,19-Docosahexaenoic acid	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	2701.5	751.5	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1086	431.31561	430.30833	14.75	C27H42O4	[M+H]+	(24S,25R)-25,26-epoxy-1α,24-dihydroxyvitamin D3 / (24S,25R)-25,26-epoxy-1α,24-dihydroxycholecalciferol	Bruker MetaboBASE Personal Library 2.0_in-silico	1482.5	0	97.5	0	5236.75	1790	
1087	301.17755	300.17027	14.75	C18H24N2S	[M+H]+	4-(4-cyclohexylphenyl)-N-propyl-1,3-thiazol-2-amine	Bruker MetaboBASE Personal Library 3.0	1577.25	1515.75	2038.25	2010.25	112.5	976.25	
1088	473.32682	472.31954	14.76	C29H44O5	[M+H]+	(3beta,17alpha,23R)-17,23-Epoxy-3,29-dihydroxy-27-norlanost-8-ene-15,24-dione	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	2824.75	1561	
1089	425.34136	424.33409	14.8	C29H44O2	[M+H]+	4,4'-Methylenebis(2,6-di-tert-butylphenol)	Bruker MetaboBASE Personal Library 2.0_in-silico	444	0	289	507.25	8792.75	5684.25	
1090	509.38365	508.37637	14.83	C30H52O6	[M+H]+	4,4,10,13,14-pentamethyl-17-(1,5,6-trihydroxy-6-methylheptan-2-yl)-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthrene-2,3,12-triol	MoNA-export-GNPS_QTOF.msp	0	0	0	416.75	11410	5121.25	
1091	489.35808	488.3508	14.84	C30H48O5	[M+H]+	Glyyunnansapogenin B	Bruker MetaboBASE Personal Library 2.0_in-silico	2896	136	1129	0	13192.75	8711.5	
1092	193.12227	192.115	14.94	C12H16O2	[M+H]+	2E,6E,8E,10E-dodecatetraenoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	2087	2976.25	1930.5	2228.25	0	582.5	
1093	561.34316	560.33589	14.98	C32H48O8	[M+H]+ [M-H]-	Propanedioic acid,	MoNA-export-GNPS_QTOF.msp	0	0	0	0	5553.5	3288.5	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1094	459.38243	458.37515	15.06	C30H50O3	[M+H]+	Heliantriol C	Bruker MetaboBASE Personal Library 2.0_in-silico	458.5	1152.25	574.5	3625.5	19884	15982.25	
1095	489.3581	488.35083	15.08	C30H48O5	[M+H]+	Camelliagenin B	Bruker MetaboBASE Personal Library 2.0_in-silico	183.25	383	0	2236.75	19781.5	4289.5	
1096	469.29506	468.28778	15.1	C29H40O5	[M+H]+	(17alpha,23S)-17,23-Epoxy-29-hydroxy-27-norlanosta-1,8-diene-3,15,24-trione	Bruker MetaboBASE Personal Library 2.0_in-silico	3068	3610	2457.5	1946.5	0	0	
1097	383.24067	382.23339	15.25	C22H30N4O2	[M+H]+	PharmaGSID_47333	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	3068.5	3670.25	
1098	331.08115	330.07387	15.26	C13H18N2O4S2	[M+H]+	Penicillin O	Bruker MetaboBASE Personal Library 2.0_in-silico	359	302.5	343	59.25	4031.5	2153.5	
1099	477.26338	476.2561	15.26	C30H36O5	[M+H]+	Hydroxysophoranone	Bruker MetaboBASE Personal Library 2.0_in-silico	2624.75	6735.75	3166	2742.5	0	0	
1100	489.2274	488.22012	15.32	C23H32N6O4S	[M+H]+	Vardenafil	Bruker MetaboBASE Personal Library 2.0_in-silico	3273	5922	2924.5	2020.75	0	0	
1101	379.28401	396.28754	15.35	C23H40O5	[M-H2O+H]+ [M+H]+	10-F2-dihomo-IsoP	Bruker MetaboBASE Personal Library 2.0_in-silico	2434.25	5414.25	1262.75	3136.25	542.5	873	
1102	455.2794	454.27213	15.38	C28H38O5	[M+H]+	Euglobal V	Bruker MetaboBASE Personal Library 2.0_in-silico	787.75	1975	1185	979.25	0	0	
1103	489.28497	488.27769	15.42	C28H40O7	[M+H]+	2,3-Dihydrowithanolide E	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	1482	3534.75	3696	
1104	411.32533	410.31805	15.46	C28H42O2	[M+H]+	Calicoferol D	Bruker MetaboBASE Personal Library 2.0_in-silico	1393	0	0	0	433.25	2892.25	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1105	338.34206	337.33478	15.51	C22H43NO	[M+H]+	13E-Docosenamide	Bruker MetaboBASE Personal Library 3.0	1150.75	321.5	1009.25	455.5	4198	1755	
1106	359.21934	358.21206	15.59	C22H30O4	[M+H]+	Piperoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	3266.25	4313	3169.5	3412	0	0	
1107	467.31554	466.30826	15.66	C30H42O4	[M+H]+	Pristimerol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	858.5	1153.25	2878	
1108	121.10112	120.09384	15.69	C9H12	[M+H]+	1,2,4-Tris(methylene) cyclohexane	Bruker MetaboBASE Personal Library 2.0_in-silico	8830.75	269.25	3037.5	340.25	870	642	
1109	283.22672	282.21944	15.67	C17H30O3	[M+H]+	Acetylenic acids; 10-Heptadecen-8-ynoic acid, 7-hydroxy-	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	9029	0	
1110	413.29034	412.2829	15.85	C23H40O6	[M+H]+ [M+NH4]+ [M+Na]+	3-methoxy Limaprost	Bruker MetaboBASE Personal Library 2.0_in-silico	2186	6147.5	1211.5	1983.25	0	2945.5	
1111	455.35207	454.34479	15.86	C30H46O3	[M+H]+	Dehydro (11,12) ursolic acid lactone	Bruker MetaboBASE Personal Library 3.0	2556	1511.25	1802	3096	45446	30418.75	
1112	411.32148	410.31421	16.02	C23H42N2O4	[M+H]+	N-oleoyl glutamine	Bruker MetaboBASE Personal Library 2.0_in-silico	653	1813.5	2971	1002.25	0	223.25	
1113	507.27381	506.26653	16.12	C31H38O6	[M+H]+	Isoamoritin	Bruker MetaboBASE Personal Library 2.0_in-silico	921.25	1607	2094	713.5	0	442.75	
1114	433.23514	432.22786	16.17	C24H28N6O2	[M+H]+	5-cyclopropyl-N-[1-[3-(3,4-dimethylphenyl)-1H-pyrazole-5-carbonyl] piperidin-4-yl]-1H-pyrazole-3-carboxamide	Bruker MetaboBASE Personal Library 3.0	1256.25	2220.25	1600.25	1781.25	0	0	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1115	393.26369	392.25642	16.17	C23H36O5	[M+H]+ [M-H]-	Acetoxy-10-gingerol	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	1337	2418.75	179
1116	485.35945	484.35217	16.22	C31H48O4	[M+H]+	26-Methyl nigranoate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	6434.75	
1117	443.38689	442.37961	16.24	C30H50O2	[M+H]+	gamma-Taraxastanonol	Bruker MetaboBASE Personal Library 2.0_in-silico	3257.5	1521.5	669	6211	0	12881.25	
1118	461.39816	460.39089	16.28	C30H52O3	[M+H]+	(3beta,11alpha,13beta)-3,11,13-Oleananetriol	Bruker MetaboBASE Personal Library 2.0_in-silico	0	1415.5	561.5	7059	4358.75	14145.5	
1119	323.29454	322.28726	16.27	C21H38O2	[M+H]+	(3S,4R)-(6R,7S)-Diepoxy-9Z-heneicosene	Bruker MetaboBASE Personal Library 2.0_in-silico	349.5	1177.25	1552	414.75	0	0	
1120	339.27157	338.2643	16.3	C24H34O	[M+H]+	3-(2,4-Cyclopentadien-1-ylidene)-5alpha-androstan-17beta-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	9723.5	6645.25	10519	16005.5	14840	12904.25	
1121	293.24743	292.24016	16.33	C19H32O2	[M+H]+	D-Homo-17a-oxa-5alpha-androstan-3beta-ol	Bruker MetaboBASE Personal Library 2.0_in-silico	2696.75	3150.75	1511	1783.75	3299.75	419.5	
1122	249.2578	248.25052	16.46	C18H32	[M+H]+	6Z,9Z,12Z-Octadecatriene	Bruker MetaboBASE Personal Library 2.0_in-silico	2220	2625.25	2092.25	1291.75	304.5	460.5	
1123	453.33703	452.32975	16.49	C30H44O3	[M+H]+	1α,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a-homovitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	3531	364.75	497.5	915.5	8672.25	4293.5	
1124	301.21632	318.21943	16.73	C20H30O3	[M-H2O+H]+ [M+H]+	(5xi,9xi)-12-Hydroxyabieta-7,13-dien-18-oic acid	MoNA-export-GNPS_QTOF.msp	2649	201.25	12156	195.25	288.5	5498.75	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1125	513.3567	512.34943	16.72	C32H48O5	[M+H]+	11a,12a-Epoxy-3b-hydroxy-28,13-oleananolide 3-acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	237.25	0	9326.5	6975	411
1126	343.22465	342.21738	16.88	C17H30N2O5	[M+H]+	E-64d	Bruker MetaboBASE Personal Library 2.0_in-silico	1894.75	3499.5	4118.25	2221.25	0	0	
1127	555.36561	554.35833	16.93	C34H50O6	[M+H]+	Ganoderic acid R	Bruker MetaboBASE Personal Library 2.0_in-silico	1062.25	549.25	1335.5	390.5	13730.75	9746.25	
1128	228.23235	227.22507	16.97	C14H29NO	[M+H]+	Halaminol A	Bruker MetaboBASE Personal Library 2.0_in-silico	2376	929.5	2147.5	1306.5	1221.25	1045.75	
1129	395.38861	394.38133	17.05	C26H50O2	[M+H]+	5,7-Hexacosanedione	Bruker MetaboBASE Personal Library 2.0_in-silico	3726.5	1466	2654	4987.5	4627.75	2240.5	
1130	193.12223	192.11496	17.08	C12H16O2	[M+H]+	11,12,13-Trinor-1,3,5-bisabolatrien-10-oic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	94	56.75	331.75	0	360.75	9739.25	
1131	549.28494	548.27767	17.06	C33H40O7	[M+H]+ [M-H]-	(+)-Myristinin A	Bruker MetaboBASE Personal Library 2.0_in-silico	5689.75	10907	4744.25	6013.75	0	0	
1132	269.21102	268.20374	17.17	C16H28O3	[M+H]+	(1S,2S)-3-oxo-2-pentyl-cyclopentanehexanoic acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	0	8238.25	
1133	60.04465	59.03738	17.23	C2H5NO	[M+H]+	Acetamide	Bruker MetaboBASE Personal Library 2.0_in-silico	7755.25	6014.75	7710	11133.75	15539	9747	
1134	263.27344	262.26617	17.23	C19H34	[M+H]+	6Z-Nonadecen-9-yne	Bruker MetaboBASE Personal Library 2.0_in-silico	3686.5	2848.25	3639	2449.5	101.25	0	
1135	337.31017	336.30289	17.35	C22H40O2	[M+H]+	(E)-3,7-Dimethyl-2,6-octadienyl dodecanoate	Bruker MetaboBASE Personal Library 2.0_in-silico	1369.5	2157.25	905.75	1193.25	0	0	
1136	615.48331	614.47603	17.49	C35H66O8	[M+H]+	Donhexocin	Bruker MetaboBASE Personal Library 2.0_in-silico	1400.25	538.75	1183.75	7612.75	426.75	9378.25	

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								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1137	399.2893	398.28202	17.52	C26H38O3	[M+H]+	17beta-Hydroxyestr-4-en-3-one cyclopentanepropionate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	3950.75	0	
1138	639.48203	638.47475	17.58	C37H66O8	[M+H]+	Purpureacin-1	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	2089	3972.75	8741	412
1139	425.3772	424.36992	17.61	C30H48O	[M+H]+	Beta-Amyrone	Bruker MetaboBASE Personal Library 2.0	3057.25	2278.5	4287	7302.25	93622.75	60067	413
1140	307.26301	306.25573	17.64	C20H34O2	[M+H]+	8,11,14-Eicosatrienoic acid	Bruker HMDB Metabolite Library_2.0	1818	4084	2527.5	2393	79.25	891.75	
1141	459.38157	458.37429	17.69	C30H50O3	[M+H]+	Soyasapogenol B	Bruker MetaboBASE Personal Library 2.0_in-silico	3458	3440.5	3307	6138.5	201713	119789.5	
1142	443.38604	442.37876	17.71	C30H50O2	[M+H]+	Uvaol	Bruker HMDB Metabolite Library_2.0	0	3904	469.5	16099.25	22740	36947.25	414
1143	282.27943	281.27215	17.96	C18H35NO	[M+H]+	Oleamide	Bruker MetaboBASE Personal Library 3.0	7310.25	19031.5	10764.25	9874.5	4674.75	9110.75	
1144	256.26366	255.25638	17.96	C16H33NO	[M+H]+	Palmitic amide	Bruker MetaboBASE Personal Library 2.0	41875.75	55281.25	56535	77737.75	15628.5	60557.5	
1145	413.34055	412.33328	18	C28H44O2	[M+H]+	25-Hydroxyvitamin D2	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	2838.75	0	
1146	471.34699	470.33972	18.08	C30H46O4	[M+H]+	Ganoderiol B	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	0	0	4204.25	1827.25	
1147	124.08686	123.07959	18.08	C6H9N3	[M+H]+	3,3'-Iminobispropanenitrile	Bruker MetaboBASE Personal Library 3.0	3592.25	2574	3871	5050.75	4646.5	3840.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1148	357.28125	356.27397	18.21	C24H36O2	[M+H]+	(1S)-1-hydroxy-23,24-didehydro-25,26,27-trinorcalciol	Bruker MetaboBASE Personal Library 2.0_in-silico	1558.5	969.25	1213.5	2183	2357.75	19652	
1149	455.38781	454.38053	18.28	C31H50O2	[M+H]+	Vitamin D3 butyrate	Bruker MetaboBASE Personal Library 2.0_in-silico	3018	2043.5	0	5208.75	5070.75	3990.5	
1150	297.24139	296.23412	18.3	C18H32O3	[M+H]+	12S,13R-EpOME	Bruker MetaboBASE Personal Library 2.0_in-silico	1849.25	0	1811.25	979	0	0	
1151	485.39722	484.38994	18.39	C32H52O3	[M+H]+	3beta-Acetoxy-19alpha-hydroxy-12-ursene	Bruker MetaboBASE Personal Library 2.0_in-silico	0	0	751.5	479.25	79457	45603.25	
1152	489.39367	488.38639	18.49	C31H52O4	[M+H]+	(22R)-1α,22,25-trihydroxy-26,27-dimethyl-24a,24b-dihomo-20-epivitamin D3	Bruker MetaboBASE Personal Library 2.0_in-silico	360.5	1226.25	13761.5	1533.75	0	1985.5	
1153	413.36283	412.35556	18.51	C25H48O4	[M+H]+	MG(22:1(13Z)/0:0/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	4117.5	750	3128	1639.5	0	0	
1154	455.33828	454.331	18.52	C26H46O6	[M+H]+	27-Norcholestanehexol	Bruker MetaboBASE Personal Library 2.0_in-silico	2117.5	3984.25	2416	0	0	0	
1155	459.38469	458.37741	18.5	C30H50O3	[M+H]+	(20S,24E)-20,26-Dihydroxy-24-dammaren-3-one	Bruker MetaboBASE Personal Library 2.0_in-silico	2706	244.5	587.75	1736.75	793.75	0	
1156	461.39864	460.39136	18.52	C30H52O3	[M+H]+	Myrrhanol A	Bruker MetaboBASE Personal Library 2.0_in-silico	0	796	0	4172	463.75	1972.25	
1157	373.27376	372.26648	18.69	C24H36O3	[M+H]+	3α-Hydroxy-5β-chola-8(14),11-dien-24-oic Acid	Bruker MetaboBASE Personal Library 2.0_in-silico	0	96	113.25	0	0	2693.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1158	501.39352	500.38624	18.75	C32H52O4	[M+H]+	3beta-Hydroxylanostane-7,11-dione acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	153.25	0	2320.5	0	3153	6321	
1159	457.3677	456.36043	18.87	C30H48O3	[M+H]+	Soyasapogenol E	Bruker MetaboBASE Personal Library 2.0_in-silico	882.5	1595.5	1494	8793	4296	3503	415
1160	441.37278	458.37615	19.08	C30H50O3	[M-H2O+H]+ [M+H]+	(3beta,24xi)-Cycloart-25-ene-3,24,27-triol	Bruker MetaboBASE Personal Library 2.0_in-silico	9151	1593.25	3879.25	2788.5	6474.25	5097.5	
1161	525.45146	524.44411	19.43	C32H60O5	[M+H]+ [M-H2O+H]+	DG(13:0/16:1(9Z)/0:0)[iso2]	Bruker MetaboBASE Personal Library 2.0_in-silico	755.25	854.25	1227.75	1930.75	2268.75	45518	
1162	469.40337	468.3961	19.93	C32H52O2	[M+H]+	Lupeol acetate	Bruker MetaboBASE Personal Library 3.0	204.25	42.5	1051	0	0	5072	63
1163	575.46733	574.46015	20.21	C36H62O5	[M+H]+ [M+Na]+	DG(15:0/18:4(6Z,9Z,12Z,15Z)/0:0)	Bruker MetaboBASE Personal Library 2.0_in-silico	2431.75	4198	2075.75	3231	1717.5	0	
1164	617.40244	616.39516	20.43	C36H56O8	[M+H]+	Phorbol myristate acetate	Bruker MetaboBASE Personal Library 2.0_in-silico	0	87.5	0	0	3140	1769.75	416
1165	537.48798	536.4807	20.45	C34H64O4	[M+H]+	9-POHSA	Bruker MetaboBASE Personal Library 2.0	1414.75	596	1328.25	1369	2211	972.75	
1166	561.48772	560.48044	20.83	C36H64O4	[M+H]+	2-methylbacteriohopane-32,33,34,35-tetrol	Bruker MetaboBASE Personal Library 2.0_in-silico	337	0	1259.5	287.25	1032.5	1229.25	
1167	133.01421	134.02149	1.23	C4H6O5	[M-H]-	L-Malic acid	Bruker HMDB Metabolite Library_2.0	3475.25	4622.75	4015	4470.25	0	1920.5	417
1168	178.02717	179.03445	7.86	C9H6FNO2	[M-H]-	5-fluoroindole-2-carboxylic acid	Bruker MetaboBASE Personal Library 3.0	0	14731	0	25141.5	1957.5	2315.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1169	179.07136	180.07863	10.88	C10H12O3	[M-H]-	2-propyl-3-hydroxyethylenepyran-4-one	Bruker MetaboBASE Personal Library 3.0	1441	569	0	119.5	0	0	
1170	191.03511	192.04238	8.82	C10H8O4	[M-H]-	4-methyldaphnetin	Bruker MetaboBASE Personal Library 3.0	143.75	251.25	373.5	719.25	2511.25	944.5	
1171	243.06221	244.06949	2.5	C9H12N2O6	[M-H]-	Uridine	Bruker MetaboBASE Personal Library 3.0	1558.75	1127	1354.25	1148.25	0	628.5	418
1172	265.14772	266.155	13.17	C15H22O4	[M-H]-	Cumanin	Bruker MetaboBASE Personal Library 3.0	37572.5	21006.25	21426.25	27339.25	34083	45784.75	
1173	313.07168	314.07896	10.93	C17H14O6	[M-H]-	Velutin	MoNA-export-GNPS_QTOF.msp	3297.25	2213.75	2685.75	1444.5	20697	8669.5	
1174	313.07179	314.07906	8.54	C17H14O6	[M-H]-	Dipteryxin	Bruker MetaboBASE Personal Library 3.0	3294	2943.75	2028.25	1868.75	1224.5	1176.75	
1175	313.23814	314.24542	12.97	C18H34O4	[M-H]-	9,10-DiHOME	Bruker MetaboBASE Personal Library 3.0	1959	1537	1109.5	1278.25	731.5	0	
1176	315.19653	316.2038	14.31	C20H28O3	[M-H]-	15-deoxy-Δ12,14-Prostaglandin A2	Bruker MetaboBASE Personal Library 2.0	0	0	0	0	790.25	2097.75	
1177	325.1841	326.19138	14.76	C18H30O3S	[M-H]-	4-Dodecylbenzenesulfonic acid	Bruker MetaboBASE Personal Library 3.0	3073.5	2127.5	2340	1784.25	1765.25	1899.5	
1178	333.20648	334.21376	12.03	C20H30O4	[M-H]-	(2E)-2-[2-(1,2,4a,5-Tetra-methyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ethyl]-2-butenedioic acid	Bruker MetaboBASE Personal Library 3.0	0	0	0	893.5	1300.25	3271.5	
1179	339.19971	340.20699	16.45	C22H28O3	[M-H]-	Norethindrone acetate	Bruker MetaboBASE Personal Library 2.0	1175.25	833.75	611.25	677	534.25	858.75	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1180	339.25385	340.26113	14.18	C20H36O4	[M-H]-	15(S)-HpEDE	Bruker MetaboBASE Personal Library 2.0	1380.5	2897.25	1501.25	1638	0	0	
1181	341.26941	342.27669	13.94	C20H38O4	[M-H]-	FAHFA 20:0; FAHFA 2:0/18:0; [M-H]-	MSDIAL-LipidDBs-VS34.msp	1836.25	2918.25	1621.25	1739	0	0	
1182	343.08209	344.08936	11.1	C18H16O7	[M-H]-	5,7-dihydroxy-3,6-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one	MoNA-export-GNPS_QTOF.msp	1680	1825.5	1423.75	1170.5	3716	1833.75	
1183	343.0824	344.08968	8.68	C18H16O7	[M-H]-	Eupatorin	Bruker MetaboBASE Personal Library 3.0	3178.75	4470.75	2318.5	1796.5	0	0	20
1184	343.11833	344.12561	9.46	C19H20O6	[M-H]-	Lasepitin	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	2979.5	1347.5	
1185	349.24182	350.24909	20.86	C18H38O4S	[M-H]-	Sulfuric acid, monooctadecyl ester	Bruker MetaboBASE Personal Library 3.0	2922.25	353.75	506.5	880.5	0	0	
1186	355.11879	356.12606	10.36	C20H20O6	[M-H]-	4,6,7-trihydroxy-5-methoxy-1,8,8,9-tetramethyl-9H-phenaleno[1,2-b]furan-3-one	MoNA-export-GNPS_QTOF.msp	739.5	420.75	805	1900.75	1923.5	3194.25	
1187	357.09781	358.10508	11.82	C19H18O7	[M-H]-	2-(2,4-Dimethoxyphenyl)-5-hydroxy-7,8-dimethoxy-4H-1-benzopyran-4-one	Bruker MetaboBASE Personal Library 3.0	1322.75	1307.25	1049	638.5	882.25	731	
1188	363.21737	364.22464	14.81	C21H32O5	[M-H]-	Acetoxy-8-gingerol	Bruker MetaboBASE Personal Library 3.0	0	0	0	0	1285.5	1779.25	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1189	371.1135	372.12077	15.82	C20H20O7	[M-H]-	[3-(4-hydroxy-3-metho-xybenzoyl)-2,3-dimethyl-oxiran-2-yl]-(4-hydroxy-3-methoxyphenyl)methanone	MoNA-export-GNPS_QTOF.msp	0	0	0	0	2696.25	1016.75	
1190	385.10817	386.11544	14.75	C22H18N4OS	[M-H]-	Axitinib	Bruker MetaboBASE Personal Library 2.0	854.25	491.5	533	198.5	0	0	
1191	417.11898	418.12626	9.1	C21H22O9	[M-H]-	2-(4-hydroxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxy-methyl)oxan-2-yl]oxy-2,3-dihydrochromen-4-one	MoNA-export-GNPS_QTOF.msp	1790	2494.75	2078	1388.25	297.75	0	
1192	417.15523	418.16251	8.85	C22H26O8	[M-H]-	2-acetyl-4-[(3-butanoyl-2,4,6-trihydroxy-5-methyl-phenyl)methyl]-3,5-dihy-droxy-6,6-dimethyl-cyclohexa-2,4-dien-1-one	MoNA-export-GNPS_QTOF.msp	616.5	279.75	1677.5	464.75	0	0	
1193	417.30028	418.30755	14.56	C26H42O4	[M-H]-	FAHFA 26:4; FAHFA 2:0/24:4; [M-H]-	MSDIAL-LipidDBs-VS34.msp	1080.25	0	0	0	2139.5	1071.75	
1194	433.2363	434.24357	15.46	C21H39O7P	[M-H]-	LPA 18:2; [M-H]-	MSDIAL-LipidDBs-VS34.msp	0	751.5	0	0	0	0	419
1195	445.11401	446.12129	9.92	C22H22O10	[M-H]-	Prunetin 5-O-glucoside	Bruker MetaboBASE Personal Library 3.0	1347.75	1097.75	1457	646.5	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude samples			
								6E	9E	12E	4E	15E	16E	
1196	447.12973	448.13701	8.42	C22H24O10	[M-H]-	2-(beta-D-Glucopyranosyloxy)benzyl (2E)-3-(3,4-dihydroxyphenyl)acrylate	MoNA-export-GNPS_QTOF.msp	1961.75	2828	3021	395.25	0	0	
1197	449.10913	450.11641	6.47	C21H22O11	[M-H]-	Eriodictyol-7-O-glucoside	Bruker MetaboBASE Personal Library 3.0	107.75	0	0	0	0	1991	
1198	461.1089	462.11618	8.37	C22H22O11	[M-H]-	5,8-Dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-4-oxo-4H-chromen-3-yl 6-deoxy-?-L-mannopyranoside	Bruker MetaboBASE Personal Library 3.0	0	103.75	305	217.25	3402	82.5	
1199	469.33245	470.33972	18.36	C30H46O4	[M-H]-	18α-glycyrrhetinic acid	Bruker MetaboBASE Personal Library 3.0	0	0	0	160.5	2092.25	970.75	
1200	471.34729	472.35457	18.39	C30H48O4	[M-H]-	Hederagenin	Bruker MetaboBASE Personal Library 2.0	0	325.25	0	1469.75	7713	2245.75	420
1201	471.34782	472.35509	17.32	$C_{30}H_{48}O_4$	$[M-H]^-$	Corosolic acid	Bruker MetaboBASE Personal Library 3.0	10310	1927.5	3670.5	429.75	6706.25	4551.5	141
1202	533.34776	488.34972	15.08	$C_{30}H_{48}O_5$	$[M+HCOOH-H]^-$, $[M-H]^-$	Olean-12-en-28-oic acid, 2,3,19-trihydroxy-, (2alpha,3beta,5xi,9xi,19alpha)-	MoNA-export-GNPS_QTOF.msp	0	0	0	1499.25	10184.5	1940	
1203	583.18312	584.1904	9.7	$C_{30}H_{32}O_{12}$	$[M-H]^-$	{(1R,2S,3R,5R,6R,8S)-3-[(6-O-Benzoyl-beta-D-glucopyranosyl)oxy]-6-hydroxy-8-methyl-9,10-dioxatetracyclo[4.3.1.0~2,5~.0~3,8~]dec-2-yl}methyl benzoate	MoNA-export-GNPS_QTOF.msp	638.75	577	1207	225.5	0	0	
1204	606.44575	607.45302	16.77	$C_{32}H_{66}NO_7P$	$[M-H]^-$	PE 27:0e; PE 18:0e/9:0; [M-H]-	MSDIAL-LipidDBs-VS34.msp	879.75	3760.25	253.25	1886.5	0	0	

No.	m/z meas.	M meas.	RT, min	Molecular formula	Ions	Compounds name	Annotation source	Sample code						Ref.
								low altitude sampels			high altitude sampels			
								6E	9E	12E	4E	15E	16E	
1205	471.15069	472.15797	6.4	$C_{21}H_{28}O_{12}$	$[M-H]^-$	beta-L-Fructofuranosyl 6-O-[(2E)-3-phenyl-2-propenoyl]-alpha-D-glucopyranoside		0	0	0	0	0	9413.25	

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Two new jatrophane diterpenes from the roots of *Euphorbia nicaeensis*

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Abstract: In the previous study fifteen jatrophane diterpenes were isolated from the *Euphorbia nicaeensis* latex. Fourteen of them have been shown to be potent P-glycoprotein (P-gp) inhibitor in two MDR cancer cells (NCI-H460/R and DLD1-TxR). The aim of this study was to determine whether and which jatrophane diterpenes can be isolated from the root of the plant, and then to examine their inhibition power on P-glycoprotein of selected cancer cell lines (NCI--H460, DLD1, U87, NCI-H460/R, DLD1-TxR and U87-TxR). Two previously undescribed jatrophane diterpenes were isolated from the root of *E. nicaeensis* collected in Deliblato Sand (Serbia). The structures of the isolated compounds were determined using 1D and 2D NMR, as well as HRESIMS data. The results obtained by MTT assay showed different antitumor potential of these two jatrophanes. Compound **1** inhibited cell growth of non-small cell lung carcinoma cell lines NCI-H460 and NCI-H460/R, as well as glioblastoma cell lines U87 and U87-TxR, while jatrophane **2** was almost completely inactive in the suppression of cancer cell growth in a given range of concentrations. The obtained results also showed that the isolated compounds have an inhibitory effect on P-glycoprotein, as well as that their inhibitory potential is similar.

Keywords: terpenoids; *Euphorbiaceae*; P-glycoprotein; MDR.

INTRODUCTION

Jatrophane diterpenes are secondary metabolites characteristic only for the family Euphorbiaceae, primarily for the plants of genus *Euphorbia*.[1] More than 350 jatrophane derivatives have been isolated up to now, of which only four have

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been isolated from the genus *Jatropha*, while the rest have been isolated from the genus *Euphorbia*.[1,2] These macrocyclic diterpenes are mostly constructed of a bicyclic [10.3.0] pentadecane system that can be highly functionalized. The great diversity in the structure of these compounds is enabled by the different oxygenation of the jatrophane skeleton, the presence of different ester groups, but also by the possibility of these compounds to appear in different conformations of the twelve-membered ring. The jatrophane diterpenes have been investigated for decades in search for new drugs that prevent multidrug resistance (MDR).[3,4] The MDR is a major medical problem because more and more tumours are becoming resistant to applicable drugs. For this reason, it is necessary to find a way to overcome MDR so that drugs can perform their function again. By the examination of the development of cell resistance to the drugs used, it was found that one of the mechanisms is the overexpression of P-glycoprotein (P-gp).[3–5] Overexpression of P-gp prevents the accumulation of the drug in a cancer cell and thus reduces the therapeutic dose of the drug and increases the resistance of tumour cells. The previous research showed that many naturally occurring jatrophane derivatives have shown higher potencies than ordinary used P-gp inhibitors cyclosporin A or verapamil; hence, they are promising candidates for further drug research.[6–8] Our previous study showed that jatrophane diterpenes isolated from *E. nicaeensis* latex have significant MDR activity, and the major mechanism of their action was inhibition of P-gp expression.[9] The aim of this study was to isolate the jatrophane diterpenes from the root of *E. nicaeensis*, and to examine their inhibitory on P-gp.

EXPERIMENTAL

Plant material

The roots of *E. nicaeensis* was collected from wild stock at Deliblato Sands (Serbia), collection site at latitude: 44°56'57" N and longitude: 21°11'13" E, in May 2018. The plant was identified by Professor Petar Marin, University of Belgrade – Faculty of Biology, Institute of Botany. Voucher specimen (No. 16,855) has been deposited at the Herbarium of Botanical Garden "Jevremovac" University of Belgrade, Belgrade (Serbia).

Isolation and purification

The roots (152 g) were dried, grounded and extracted with 96 % ethanol with heating (2 h) and then left overnight at room temperature. The obtained extract (25 g) was then subjected to the column chromatography (dry flash (SiO_2, eluent petroleum ether/acetone, gradient 10/0 → 1/9), Table S-I). Progress of separation was followed by TLC (precoated Merck silica gel 60 F_{254} plates) and ^{1}H-NMR spectra. The fraction that contained jatrophanes was eluted with 20 % acetone. That fraction was further separated using the column chromatography (dry flash (SiO_2, isocratic, petroleum ether/acetone 97/3)) to afford subfraction F1. The subfraction F1 was further purified by the preparative normal phase liquid chromatography (NP-LC), using an Agilent Technologies 1260 series liquid chromatograph equipped with diode-array detector (λ = 210 nm), autosampler and thermostated column compartment, under the following conditions: injection volume 500 µL (c ~10 mg mL^{-1}, acetone), Zorbax RX-Sil column (250 mm×9.4 mm; 5 µm), column temp. 24 °C, mobile phase 4.00 mL min^{-1},

isocratic, acetone/petroleum ether 12.5/87.5. The obtained fractions F1a and F1b were finally purified by the preparative reversed phase liquid chromatography (RP-LC) using Agilent Technologies 1100 series liquid chromatograph equipped with diode-array detector (λ 210 and 264 nm), autosampler and thermostated column compartment. For the separation of the compounds, the following LC parameters have been applied: the injection volume 1000 μL ($c \approx 10$ mg mL^{-1}, MeOH), Zorbax XDB-C18 column (250 mm×9.4 mm; 5 μm), column temp. 20 °C, mobile phase 4.00 mL min^{-1}. The mobile phase consisted of two solvents: MilliQ water (solvent A) and CH_3CN (solvent B). The following gradient was set: 40–80 % B, 0–10 min, 80–90 % B, 10–15 min. ESI-MS spectra were recorded on Agilent Technologies 6550 Funnel Q-TOF MS instrument in positive ion mode with MeOH/H_2O 1/1 with 0.2 % HCOOH as the carrying solvent solution. The samples were dissolved in MeOH (MS hypergrade purity). The selected values were as follows: capillary voltage = 3,500 V, fragmentor voltage = 175 V, nozzle voltage = 1,000 V, skimmer 1 = 65 V, octopole RF peak = 750 V, desolvatation gas (nitrogen) temperature 200 °C, desolvatation gas (nitrogen) flow 14 L min^{-1}, sheat gas (nitrogen) flow 11 L min^{-1}. From subfraction F1a (23.4 mg) the compound **1** (1.2 mg) was obtained, while from fraction F1b (2.3 mg) the compound **2** (0.8 mg) was obtained. IR spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 500 Avance III spectrometer (500.26 and 125.80 MHz, respectively) using $CDCl_3$ as a solvent and TMS as the internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hz.

Drugs

Tariquidar (TQ) was kindly provided by Dr. Sven Rottenberg from The Netherlands Cancer Institute, Amsterdam. TQ was diluted in dimethyl sulfoxide and 10 μM aliquots were kept at –20 °C. Jatrophane diterpenes (**1** and **2**) were kept as 20 mM stocks in 100 % ethanol at –20 °C. Working solutions of 200 μM were prepared in 10 % ethanol.

Cells and cell culture

The NCI-H460, DLD1, and U87 cell lines were purchased from the American Type Culture Collection, Rockville, MD, USA. NCI-H460/R cells were selected from NCI-H460 cells by their continuous culturing in a medium containing stepwise increasing concentrations of doxorubicin for three months.[10] Similarly, DLD1-TxR and U87-TxR cells were selected from DLD1 and U87 cells, respectively, by continuous exposure to the stepwise increasing concentrations of paclitaxel during six to nine months.[11] NCI-H460, NCI-H460/R, DLD1 and DLD1--TxR were grown in RPMI 1640 medium supplemented with 10 % fetal bovine serum, 1 % L-glutamine, and 1 % antibiotic–antimycotic mixture, while U87 and U87-TxR were cultivated in MEM supplemented with 10 % fetal bovine serum, 1 % L-glutamine, 1 % antibiotics, and 1 % non-essential amino acids. All cell lines were sub-cultured two-times per week using 0.25 % trypsin/EDTA and seeded into a fresh medium at the following densities: 8,000 cells cm^{-2} for NCI-H460, NCI-H460/R, DLD1 and DLD1-TxR, and 16,000 cells cm^{-2} for U87 and U87-TxR.

Cell viability assay

Cell viability was assessed by MTT assay (Sigma, St. Louis, MO, USA). MTT assay is based on the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide into formazan dye by active mitochondria of living cells. Briefly, cells were seeded in 96-well tissue culture plates (2,000 cells/well for NCI-H460, NCI-H460/R, DLD1 and DLD1-TxR; 4,000 cells/well for U87 and U87-TxR) and incubated overnight in 100 μL of appropriate medium. Afterwards, cells were treated with the increasing concentrations of jatrophane diter-

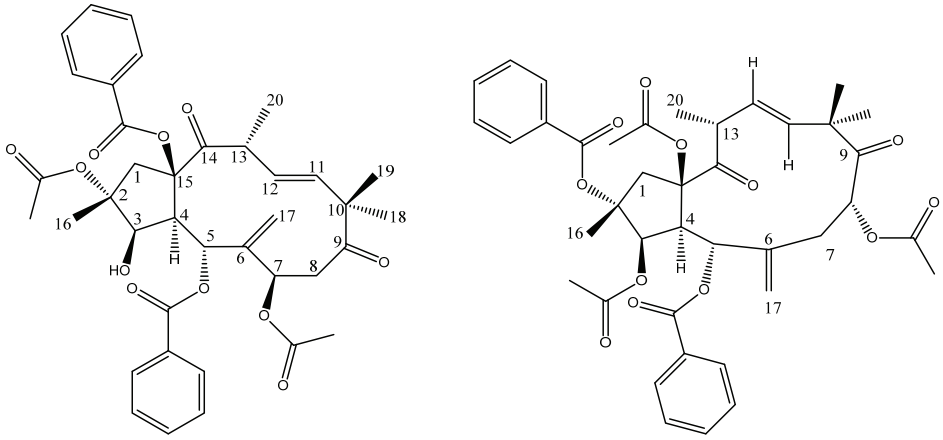
penes **1** and **2** (1, 5, 10, 20 and 50 μM). All treatments lasted 72 h. At the end of treatment period, 100 μL of MTT solution (1 mg mL^{-1}) was added to each well and plates were incubated at 37 °C for 4 h. The formazan product was dissolved in 100 μL DMSO. The absorbance of obtained dye was measured at 570 nm with reference wavelength at 690 nm using an automatic microplate reader (Multiskan Sky, Thermo Scientific, Waltham, MA, USA). IC_{50} value was defined as concentration of each drug that inhibited cell growth by 50 %. IC_{50} was calculated by non-linear regression analysis using log (inhibitor) *vs*. normalized response in GraphPad Prism 8.0.2 software.

Rhodamine 123 accumulation assay

The function of P-glycoprotein was analysed by flow cytometry exploiting the ability of its substrate rhodamine 123 to emit fluorescence. The increased level of rhodamine 123 accumulation positively correlated with the inhibited P-glycoprotein function. TQ was used as a positive control. The MDR cancer cells were suspended in 3.5 mL centrifuge tubes in a 5 μM rhodamine 123-containing medium. Then, the cells were immediately treated with jatrophanes **1** and **2** (10 μM) and TQ (50 nM) and incubated at 37 °C in 5 % CO_2 for 30 min. The samples were washed twice, suspended in 1 mL of cold phosphate-buffered saline, and analysed using a flow cytometer (Partec, Münster, Germany) and the data were analysed by Summit 4.3 (DAKO, Carpinteria, CA, USA). The fluorescence of rhodamine123 was assessed on green fluorescence channel 1 (FL1). At least 20000 events were assayed for each sample.

RESULTS AND DISCUSSION

Chemical analyses on the roots of *E. nicaeensis* afforded two unreported jatrophane diterpenes (Fig. 1). The structures of the isolated compounds and their relative configurations were established on the basis of spectroscopic analysis includeing 1D and 2D NMR (COSY, NOESY, HSQC, HMBC) and HRESIMS data.



1 **2**

Fig. 1. The structures of the isolated jatrophane diterpenes.

Compound **1**, $[\alpha]^{20}$; D = –266.3 (c 0.08, acetone), was isolated as a colourless amorphous substance with the molecular formula $C_{38}H_{42}O_{11}$, as determined by the

^{1}H- and ^{13}C-NMR data (Table S-II of the Supplementary material to this paper), as well as the (+)ESI-HRMS *m*/*z*: calculated for [$C_{38}H_{42}O_{11}$ + Na^{+}] 697.2800, observed 697.2612, and the IR (ATR): 2974*s*, 1736*s*, 1230*s*, 1121*m*, 1023*m*, cm^{-1}. Its ^{13}C-NMR spectra showed 38 carbon signals, including two keto carbonyls at 208.29 (C-9) and 204.69 (C-14), four ester carbons at 169.55 (C-1'), 163.90 (C-1''), 170.28 (C-1'''), and 165.96 (C-1^{vi}), two double bond pairs (one geminally substituted (140.78 and 116.48, C-6(17)), and one *trans* substituted (135.48 and 133.22, C-11(12))), eight aromatic carbons (129.50 (C-2''), 129.45 (C-3'') 129.24 (C-4''), 129.34 (C-5''), 130.04 (C-2^{vi}), 130.69 (C-3^{vi}) 133.85 (C-4^{vi}), and 133.94 (C-5^{vi})), five oxygenate carbons (91.30 (C-2), 77.92 (C-3), 73.94 (C-5), 76.78 (C-7) and 91.83 (C-15)), and six methyl groups (18.58 (C-16), 26.91 (C-18), 23.29 (C-19), 18.33 (C-20), 20.92 (C-2'), and 20.86 (C-2^{iv})). The ^{1}H-NMR and COSY spectra revealed the presence of four separate *J*-coupling networks (**A**–**D**): **A**) H-1α/H-1β (δ_H 3.95 *d* and 2.56 *d*); **B**) H-3/H-4/H-5 (δ_H 4.71 *brs*, 2.85 *d* and 5.55 *brs*); **C**) H-7/H-8α/H-8β (δ_H 4.97 *d*, 2.43 *dd* and 2.02 *d*); **D**) H-11/H-12/H-13/H-20 (δ_H, 5.22 *d*, 5.54 *dd*, 3.60 *dq* and 1.22 *d*), Figs. S-1 and S-3, Supplementary material). The large vicinal coupling constants of signals H-11 (δ_H 5.22, J = 16.0 Hz) and H-12 (δ_H 5.54, J = 16.0; 10 Hz) indicated *E* geometry of the double bond at C-11 (Fig. S-1 of the Supplementary material). The COSY fragments were connected using the long-range C–H correlations in the HMBC spectrum. The long-range heteronuclear couplings ($^{2\text{-}4}J_{C,H}$) of the carbons of C-15 with H-1α, H-1β, H-3, H5β and H-16 confirmed the presence of five-membered ring (ring A). The HMBC correlations between H-5 and C-6, as well as C-6 with H-7 and H-17a/17b made it possible to link the COSY fragments **A** and **B**. The long-range correlation between C-9 with H-8, H-11, H-18 and H-19 connected COSY fragments **B** and **C**. The linkage of fragment **B** with five-membered ring was enabled by the HMBC correlations between C-14 and H-1α, H-1β, H-4, H-12, H-13 and H-20 (Fig. S-4). At the end, it was concluded that the isolated compound **1** is a jatrophane diterpene with two keto groups (C-9 and C-14), and esters at positions C-2, C-5, C-7 and C-15, respectively, and with a free hydroxyl group at C-3. Benzoyl ester at C-5 was proved from HMBC correlation of the carbonyl signal (163.90) with the proton H-5, while acetate at C-7 was identified from the HMBC correlation of the carbonyl signal (170.28) with the proton H-7 (Fig. S-4). The other two ester groups were connected from NOESY correlations, because those ester groups were attached to the quaternary carbons. The proton of the second benzoate (8.10 ppm) showed coupling with the protons H-5β, H-13β and H-16β, as well as with the protons of acetate at the C-7 position (Fig. S-5).

According to the chemical shift of C-15 (91.83) and HMBC correlations of C-15 mentioned above, C-15 position of the benzoate was deduced. The protons of the methyl group (1.75 ppm) of the second acetate exhibited NOESY correl-

ation with the protons H1α, H3α, and the proton from the benzoate bound at the C-5 position, indicating that this acetate is bound at the C-2 position (Fig. S-5).

The relative configuration of **1** was deduced by the interpretation of the NOESY spectrum and coupling constants (Fig. S-5). The H-5/H-4 and H-5/H-8β NOE correlations, the absence of correlation between H-5 and H-17a/b, and a small value of $^3J_{4,5}$ (3 Hz) suggested that **1** belong to the *exo*-type conformation with exomethylene 6,17-double bond.[12] The configuration of proton H-4 was determined biogenetically as H-4α,[13] and the NOE interactions of H-4α with H-3α implied 3β-OH orientation. The correlation of H-1β with methyl group at C-16 suggested β orientation of H-16, and α-orientation of 2α-OAc. The NOE correlation H-5β/H-13β confirmed the orientation of the methyl group at C-13 (H-20α), as well as 5α-OBz and H-18α. The NOE interactions between H-4α and H-7α indicated 7β-OAc. The above evidences confirmed the structure of **1** as 2α,7β-diacetyloxy-3β-hydroxy-5α,15β-dibenzoyloxyjatropha-6(17),11*E*-diene--9,14-dione.

Compound **2**, $[\alpha]^{20}$; D = –68.8 (c = 0.08 g mL^{-1}, methanol), IR (ATR): 2976*s*, 1734*s*, 1235*s*, 1122*m*, 1021*s* cm^{-1}, was isolated as a colourless amorphous substance with the molecular formula $C_{40}H_{44}O_{12}$, as deduced by the ^{1}H- and ^{13}C-NMR data (Table S-II), as well as the (+)ESI-HRMS *m*/*z*: calculated for [$C_{40}H_{44}O_{12}$ + H^+] 717.2906, observed 717.2904. The structure of compound **2** was similar to the structure of **1**. Significant structural differences between these two compounds were in the number of ester groups, as well as in the conformation of the jatrophane skeleton. In contrast to the compound **1**, in which there were four ester groups, in compound **2** there were five (two benzoates and three acetates). The positions of ester groups in this molecule were determined on the basis of $^3J_{C,H}$ HMBC correlations of the ester carbonyls and neighbouring protons from the jatrophane skeleton (Fig. S-8). The attachment of the acetates was proved from HMBC correlations of the carbonyl signal at 169.50 with the proton H-3 (δ_H 6.01), as well as the proton H-8 (δ_H 5.13) and carbonyl 170.57. The correlation of proton H-5 (δ_H 5.77) and carbonyl signal at 165.64 enabled the binding of one benzoate to the C-5 position. The binding sites of the remaining two esters were determined on the basis of the NOE correlations and chemical shifts of carbons' positions of attachment (C-2 and C-15), as in compound **1**. The relative configuration of **2** was determined by the interpretation of the NOESY spectrum (Fig. S-9). The H-5/H-17a and H-8/H-17b NOE correlations, as well as large value of $^3J_{4,5}$ (6 Hz) suggested that **2** belonged to the *endo*-type conformation with exomethylene 6,17-double bond perpendicular to the main plane,[12] that is usually adopted in compounds lacking substituent at C-7.[14,15]

All NMR spectra and spectroscopic data for compounds **1** and **2** are given in the Supplementary material.

Jatrophane diterpenoids are well-known for their multidrug resistance (MDR) modulating potential due to the direct interaction and the inhibition of P-glycoprotein.[9,16,17] The effect of **1** and **2** on the cell growth of six human cancer cell lines (three pairs of sensitive and corresponding MDR cell lines) were examined. The results obtained by MTT assay showed different antitumor potential of these two jatrophanes. Specifically, **1** inhibited cell growth of non-small cell lung carcinoma cell lines NCI-H460 and NCI-H460/R, as well as glioblastoma cell lines U87 and U87-TxR with IC_{50} values between 10 μM and 20 μM (Table I). Both colorectal carcinoma cell lines, DLD1 and DLD1-TxR, were resistant to **1** with IC_{50} values over 50 μM (Table I, Fig. 2). However, MDR cell lines (NCI-H460/R and U87-TxR) were not resistant to **1**, meaning that the IC_{50} values of MDR cell lines were not significantly increased in comparison with the IC_{50} values of the corresponding sensitive cell lines (NCI-H460 and U87, respectively). On the contrary, jatrophane **2** was almost completely inefficient in the suppression of cancer cell growth in a given range of concentrations (1–50 μM). Only glioblastoma cell line U87 responded to **2** treatment with IC_{50} value around 20 μM (Table I, Fig. 2).

TABLE I. Cancer cell growth inhibition (IC_5 / μM, average ± standard deviation) induced by jatrophanes **1** and **2**

Cell line	Compound	
	1	**2**
NCI-H460	17.63±2.08	> 50
NCI-H460/R	20.98±2.79	> 50
DLD1	> 50	> 50
DLD1-TxR	> 50	> 50
U87	10.97±1.41	20.12±1.96
U87-TxR	15.49±3.57	> 50

Our previous results demonstrated that jatrophane diterpenoids are able to selectively inhibit cancer cell growth without harming normal cells.[18] Besides, jatrophanes showed potential to reverse resistance to paclitaxel and doxorubicin in non-small cell lung carcinoma cells.[9,18] This chemosensitization effect of jatrophanes is related to their ability to inhibit P-glycoprotein function. Therefore, the P-glycoprotein interaction with **1** and **2** in three human MDR cancer cell lines (NCI-H460/R, DLD1-TxR, and U87-TxR), using functional rhodamine 123 accumulation assay, was tested. The obtained results showed that jatrophanes **1** and **2** have similar potency in the inhibition of P-glycoprotein function (Table II). The potential of the tested compounds to inhibit P-glycoprotein activity in MDR cancer cell lines is expressed as fluorescence activity ratio (*FAR*), while sensitization index (*SI*) was used to compare their effects in MDR cancer cell lines with the rhodamine 123 accumulation in untreated sensitive cancer cell lines (Table II).

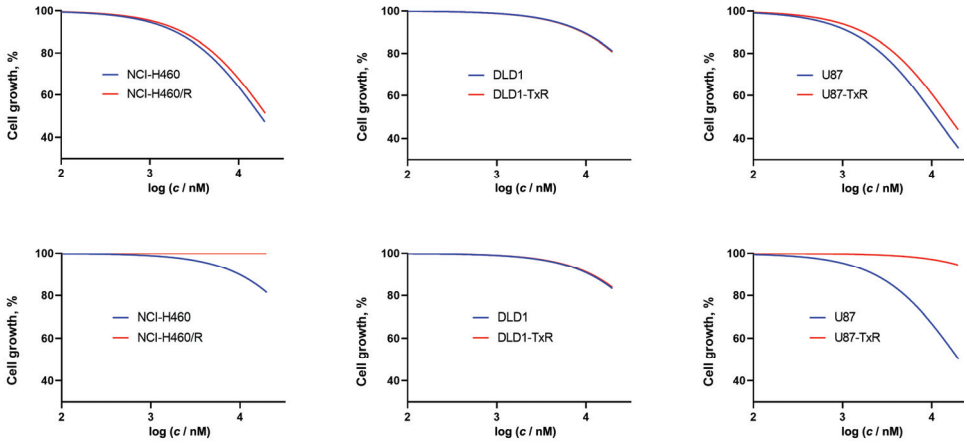


Fig. 2. Non-linear regression analysis of cell growth inhibition induced by jatrophanes **1** (above) and **2** (below). MTT results obtained with **1** and **2** treatments in NCI-H460, NCI-H460/R, DLD1, DLD1-TxR, U87, and U87-TxR cells (absorbance at 570 nm corrected with 690 nm) were transformed into percentages of cell growth normalized to untreated cells and presented in $\log_{10}$ scale of concentrations using GraphPad Prism 8.0.2 software.

TABLE II. P-glycoprotein inhibition induced by **1** and **2** in MDR cancer cell lines

Medium	NSCLC			Colorectal carcinoma			Glioblastoma		
	MFI[a]	*FAR*[b]	*SI*[c]	*MFI*	*FAR*	*SI*	*MFI*	*FAR*	*SI*
Sensitive cells[d]	100.30±2.93[e]		100.0	101.16±2.81		100.0	182.70±1.57		100.0
MDR cells	16.32±3.51		16.3	25.27±10.20		25.0	24.44±3.99		13.4
1[f]	114.62±10.92	7.0	114.3	202.93±1.34	8.0	200.6	149.80±0.98	6.1	82.0
2	92.41±2.92	5.7	92.1	212.55±1.55	8.4	210.1	144.94±1.00	5.9	79.3
TQ[g]	92.95±2.88	5.7	92.7	130.34±1.42	5.2	128.8	120.92±1.32	4.9	66.2

[a]The measured mean fluorescence intensity (*MFI*) was used for the calculation of the fluorescence activity ratio (*FAR*); [b]*via* the following equation: $FAR = MFI_{MDRtreated}/MFI_{MDRcontrol}$; [c]the sensitivity index (*SI*) was calculated on the basis of the measured mean fluorescence intensity (*MFI*) expressed *via* the following equation: $SI = (MFI_{MDRtreated} \times 100)/MFI_{sensitive\ control}$; [d]sensitive cancer cell lines and their MDR counterparts used in the study: non-small cell lung carcinoma-NSCLC (NCI-H460 and NCI-H460/R), colorectal carcinoma (DLD1 and DLD1-TxR) and glioblastoma (U87 and U87-TxR); [e]*MFI*±*SEM* (standard error of mean); [f]jatrophanes were applied at the same concentration of 10 μM; [g]*TQ* was applied at 50 nM

SI values reflect the capacity of **1** and **2** to restore the rhodamine 123 accumulation in MDR cancer cell lines close to the level of accumulation observed in sensitive cancer cell lines. On SI values scale, the strong sensitization exists when $50 << SI < 100$ and there is a complete blockade of P-glycoprotein function when $SI >> 100$ (the level of accumulated rhodamine 123 in MDR cancer cells after treatment with compounds that are P-glycoprotein inhibitors exceeds the level obtained in sensitive cancer cells). Importantly, TQ, a non-competitive inhibitor of P-glycoprotein,[19] was equally or less potent than **1** and **2** in all tested MDR cancer cell lines (Table II). The highest increases in the rhodamine 123 accumulation and

complete blockade of P-glycoprotein (*SI* > 100) were observed in non-small lung carcinoma cells (non-small cell lung cancer – NSCLC) NCI-H460/R treated with **1** and in colorectal carcinoma cells DLD1-TxR treated with both jatrophanes and TQ (Table II). Similar strong sensitization in DLD1-TxR was achieved with jatrophanes **2**, **4**–**6** and **15** that were isolated from the latex of *Euphorbia dendroides*.[17]

CONCLUSION

In conclusion, the jatrophane diterpenoides **1** and **2** completely blocked P-glycoprotein in MDR NSCLC and colorectal carcinoma cells showing even higher potential than TQ in MDR colorectal carcinoma and glioblastoma cells. Therefore, both jatrophanes could be valuable as sensitizing agents capable to decrease the effective concentrations of drugs which are P-glycoprotein substrates. Importantly, jatrophane **1** exerted cell growth inhibitory effect in NSCLC and glioblastoma cells, indicating that this compound could also have considerable anticancer properties. Generally, these jatrophane diterpenoides can be used as lead compounds for drug development and the improvement of chemotherapy.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11038, or from the corresponding author on request.

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ИЗВОД

ДВА НОВА ЈАТРОФАНСКА ДИТЕРПЕНА ИЗ КОРЕНА *Euphorbia nicaeensis*

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У претходном истраживању, петнаест дитерпена јатрофанског типа изоловано је из латекса *Euphorbia nicaeensis*. Њих четрнаест показала су се као снажни инхибитори P-гликопротеина (P-gp) у две MDR ћелијске линије рака (NCI-H460/R и DLD1-TxR). Циљ ове студије био је да се утврди да ли је и које јатрофанске дитерпене могуће изоловати из корена биљке, а затим испитивање њихове инхибиторне моћи на P-гликопротеину одабраних ћелијских линија рака (NCI-H460, DLD1, U87, NCI-H460/R, DLD1-TxR и U87-TxR). Два претходно непозната јатрофана изолована су из корена *E. nicaeensis* прикупљеног у Делиблатској пешчари. Структуре изолованих једињења одређене су применом 1D и 2D NMR метода, као и HRESIMS експеримента. Резултати добијени MTT тестом показали су различит антиканцерогени потенцијал ова два јатрофана. Једињење **1** је инхибирало раст ћелија ћелијских линија неситноћелијског карцинома

плућа NCI-H460 и NCI-H460/R, као и ћелијских линија глиобластома U87 и U87-TxR, док је јатрофан **2** био готово потпуно неефикасан у сузбијању раста ћелија карцинома у датом концентрационом опсегу. Добијени резултати су такође показали да **1** и **2** имају инхибиторно дејство на P-гликопротеин, као и да је њихов инхибиторни потенцијал сличан.

(Примљено 6. августа, ревидирано 14. септембра, прихваћено 26. октобра 2021)

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SUPPLEMENTARY MATERIAL TO

Two new jatrophane diterpenes from the roots of *Euphorbia nicaeensis*

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TABLE S-I. Elution mode for the dry flash column chromatography, eluent volume 250 mL

Faction number	Eluent composition petroleum ether/acetone (V/V)
1	100/0
2	90/10
3	85/15
4	80/20
5	70/30
6	60/40
7	50/50
8	40/60
9	20/80
10	0/100

TABLE S-II. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data for compounds **1**–**2** ($CDCl_3$, TMS)

Position	**1**		**2**	
	δ / ppm, (J / Hz)			
	^{1}H	^{13}C	^{1}H	^{13}C
1α	3.95 d (17.0)	46.03	3.97 d (17.7)	46.83
1β	2.56 d (17.0)		2.57 d (17.7)	
2	-	91.30	-	89.70
3	4.71 brs	77.92	6.01 d (4.0)	77.5[b]
4	2.85 d (3.0)	47.79	3.26 t (6.0)	47.94
5	5.55 brs	73.94	5.77 d (6.0)	73.02
6	-	140.78	-	138.68

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Position	**1**		**2**	
	δ / ppm, (J / Hz)			
	^{1}H	^{13}C	^{1}H	^{13}C
7	4.97 d (11.0)	76.78	2.32 d (7.0)	33.71
8α	2.43 dd (15.0; 11.0)	32.21	-	75.58
8β	2.02 d (15.0)		5.13 t (7.0)	
9	-	208.29	-	208.41
10	-	47,98	-	47.94[c]
11	5.22 d (16.0)	135.48	5.38 d (16.0)	135.85
12	5.54 dd (16.0; 10.0)	133.22	5.49 dd (16.0; 10.0)	133.44[c]
13	3.60 dq (10.0; 7.0)	43.93	3.58 m	43.45
14	-	204.69	-	203.74
15	-	91.83	-	91.09
16	1.88 s	18.58	1.72 s	19.06
17a	5.33 brs	116.48	5.26 brs	121.04
17b	5.41 brs		5.38 brs	
18	0.65 s	26.91	0.88 s	27.28
19	1.10 s	23.29	1.10 s	23.34
20	1.22 d (7.0)	18.33	1.20 d (7.0)	18.30
2-OR$_1$				
1'	-	169.55	-	165.20
2'	1.75 s	20.92	-	130.29[a]
3'			8.12 d (7.4)	129.91
4'			7.56 m	129.31
5'			7.62 m	134.05
3-OR$_2$				
1''			-	169.50
2''			2.07 s	21.32
5-OR$_3$				
1'''	-	163.90	-	165.64
2'''	-	129.45[a]	-	130.36[a]
3'''	8.02 d (7.0)	129.50	7.98 d (7.4)	129.79
4'''	7.52 m	129.24[a]	7.45 m	128.69
5'''	7.62 m	129.34[a]	7.57 m	133.55
7-OR$_4$				
1^{iv}	-	170.28		
2^{iv}	2.18 s	20.86		
8-OR$_5$				
1^{v}			-	170.57
2^{v}			1.72 s	20.30
15-OR$_6$				
1^{vi}	-	165.96	-	169.77
2^{vi}	-	130.04[a]	1.99 s	21.14
3^{vi}	8.10 d (7.0)	130.69		
4^{vi}	7.56 m	133.85[a]		
5^{vi}	7.62 m	133.94[a]		

[a]Uncertain assignment; [b]data obtained from the HSQC spectrum; [c]data obtained from the HMBC spectrum

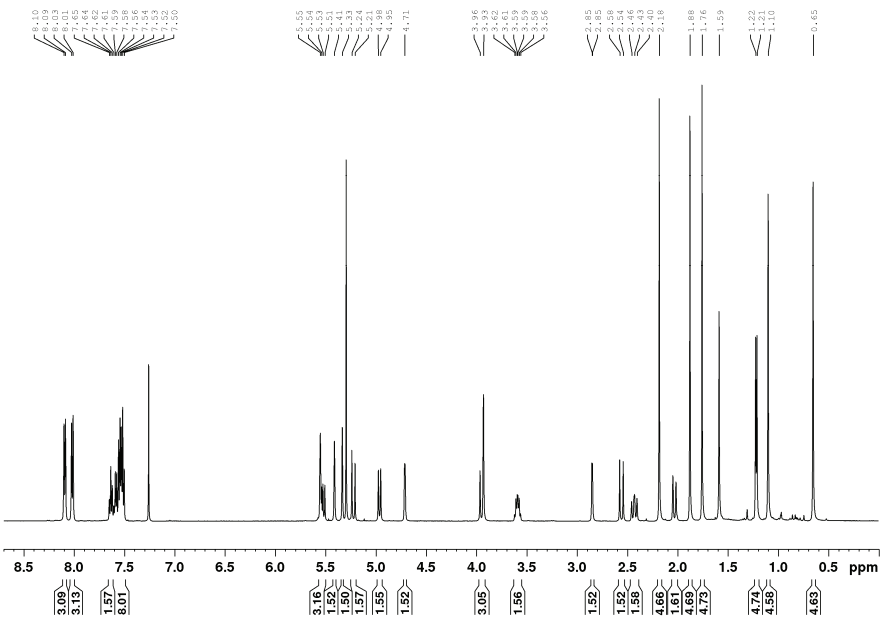

Fig. S-1. ^{1}H NMR spectrum of compound **1**

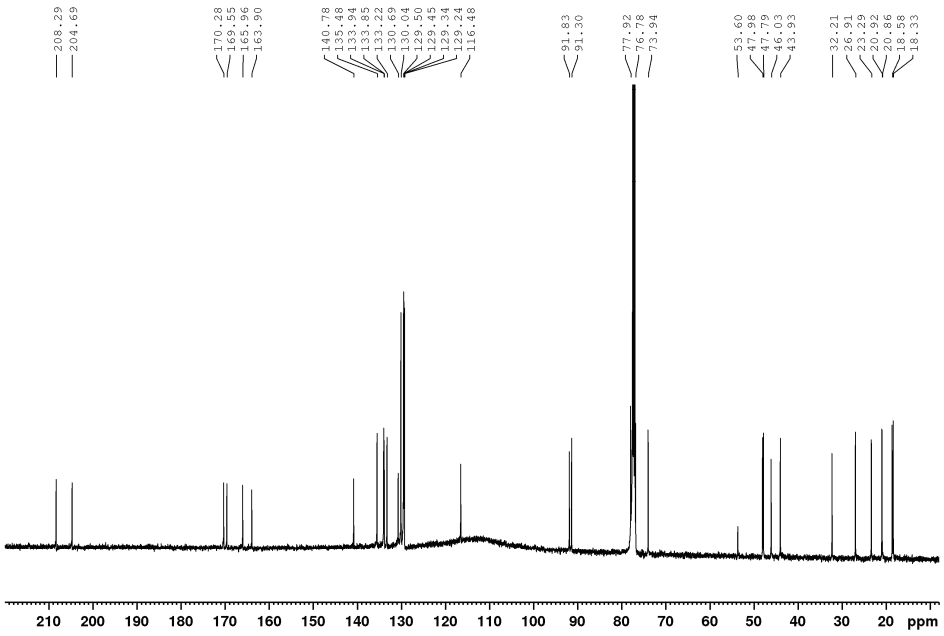

Fig. S-2. ^{13}C NMR spectrum of compound **1**

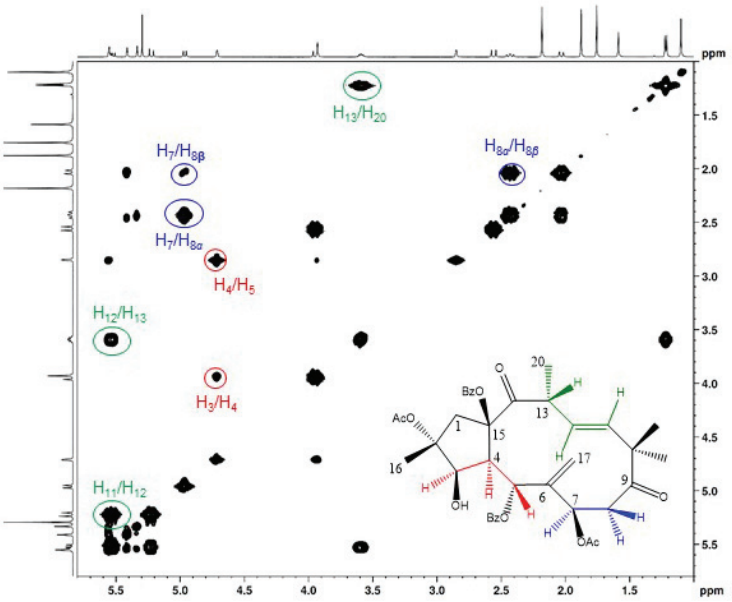

Fig. S-3. COSY spectrum of compound **1**

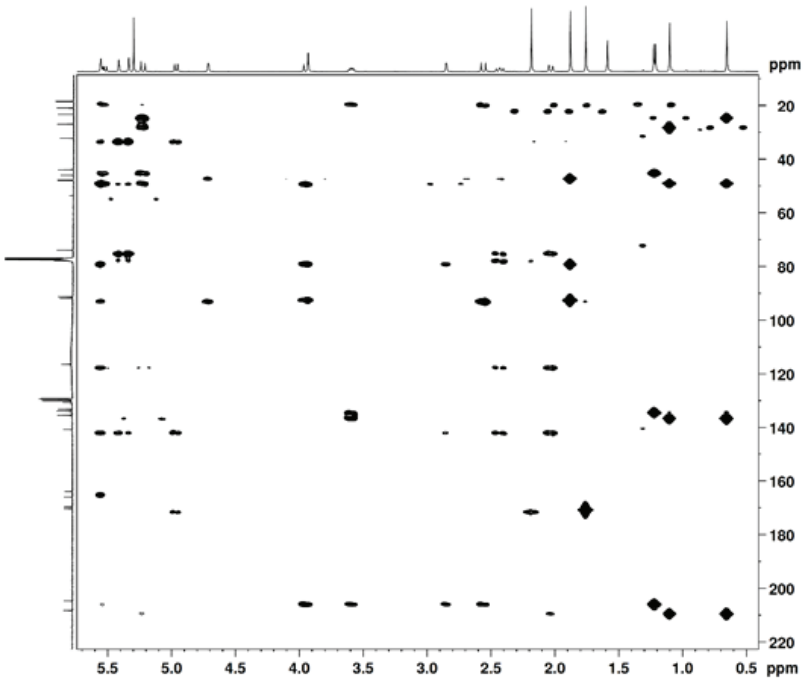

Fig. S-4. HMBC spectrum of compound **1**

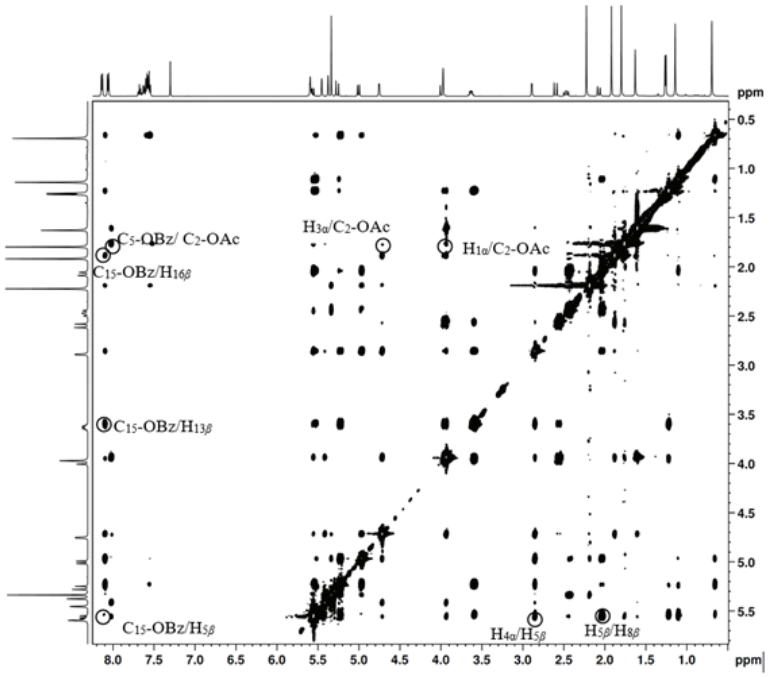

Fig. S-5. NOESY spectrum of compound **1**

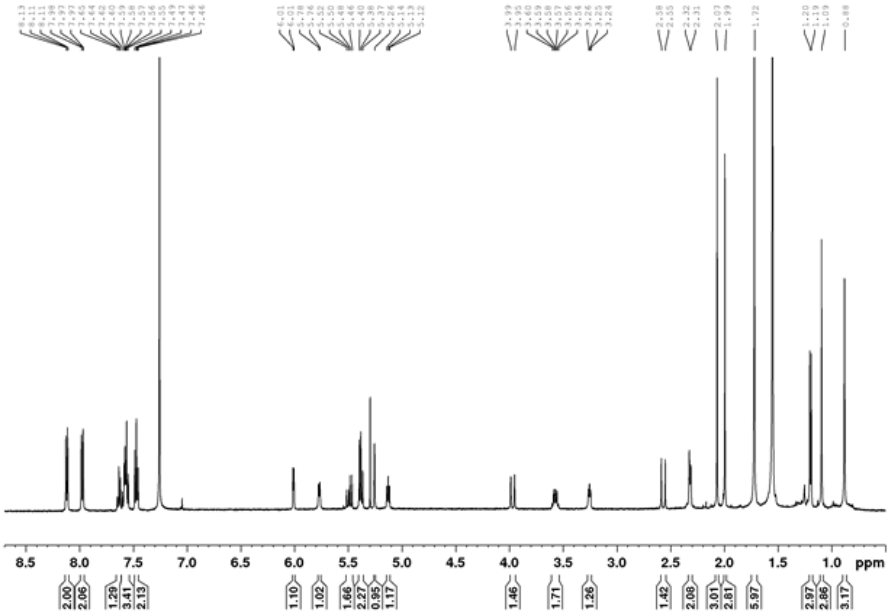

Fig. S-6. ^{1}H NMR spectrum of compound **2**

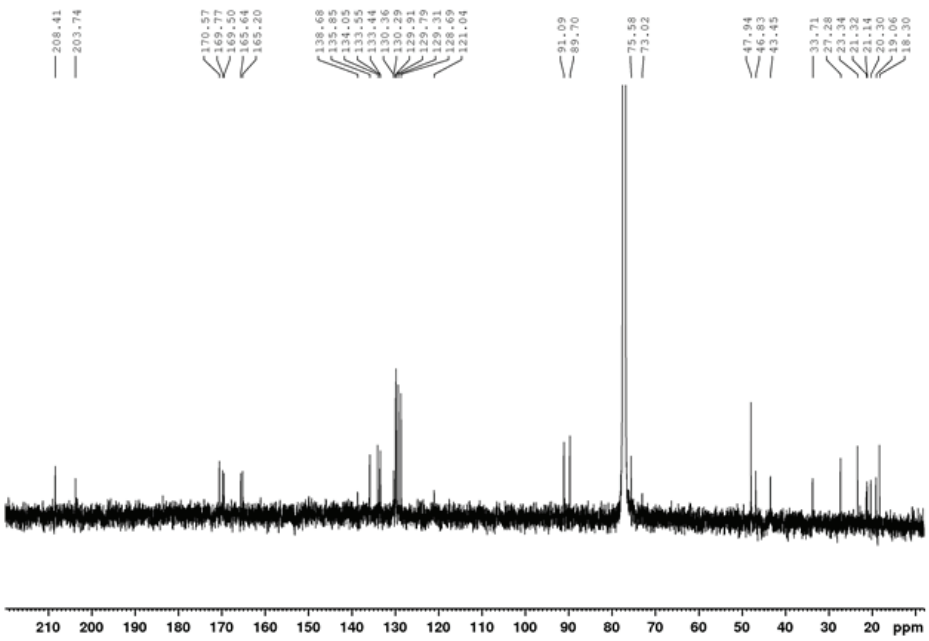

Fig. S-7. ^{13}C NMR spectrum of compound **2**

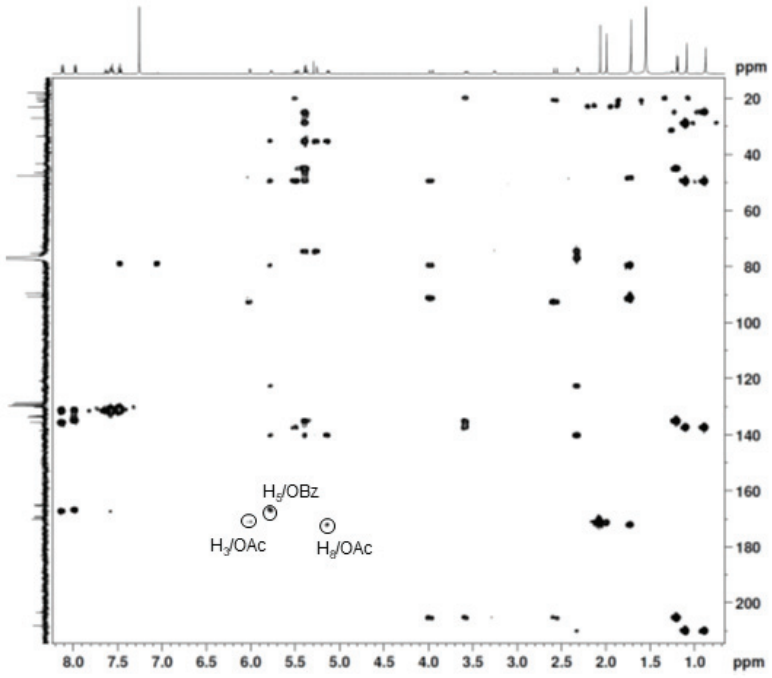

Fig. S-8. HMBC spectrum of compound **2**

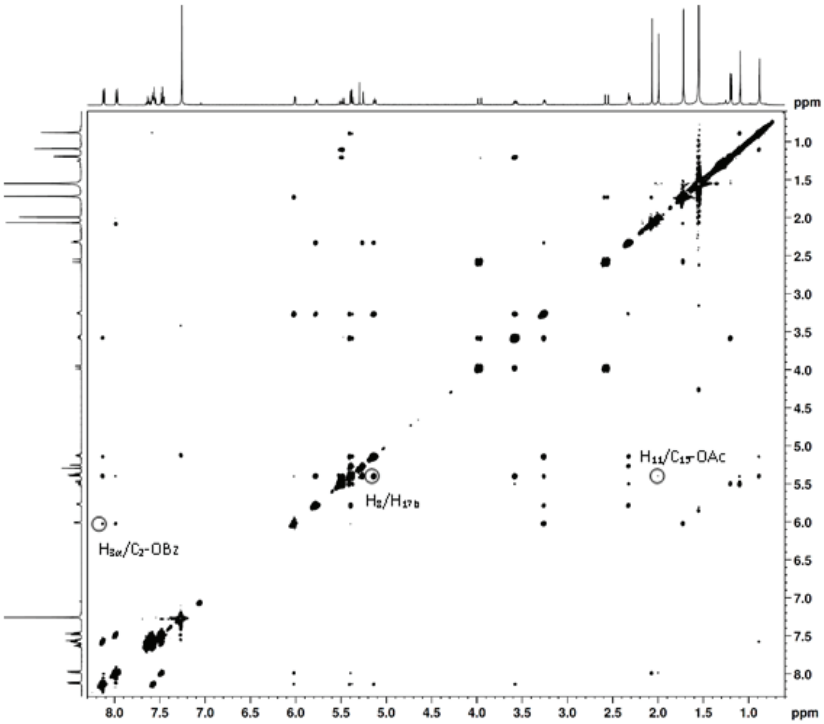

Fig. S-9. NOESY spectrum of compound **2**.

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Terpenoids in four *Inula* species from Bulgaria

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Abstract: Phytochemical study of the chloroform extract of the aerial parts of *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. and *I. salicina* L. led to the identification of 33 terpenoids. *β*- and *α*-amyrin, lupeol, taraxasterol, *ψ*-taraxasterol and their 3-*O*-acetates and 3-*O*-palmitates were identified by GC/MS. In addition, the structures of 3-*O*-palmitates of mainaladiol, arnidiol, faradiol and 16-hydroxylupeol were confirmed by NMR. *ent*-Kaur-16-en-19--oic acid and its 15*α*-(3-methylpentanoyloxy) and 15*α*-(3-methylbutanoyloxy) derivatives were isolated from *I. conyza*. Ten closely related sesquiterpene lactones (germacranolides and melampolides) were found in *I. germanica* and their structural identification was performed by spectral analyses. *I. ensifolia* and *I. salicina* were free of sesquiterpene lactones and diterpenoids. All triterpenoids and diterpenoids, grazielia acid, desacetylovatifolin and 8-(2-methylbutanoyloxy)-1(10),4,11(13)-germacrutrien-6,12-olide-14-oic acid are described for the first time in the studied species. The principal component analysis was used to find a relationship between the investigated up to now *Inula* species, growing in Bulgaria.

Keywords: *Inula*; triterpenoids; diterpenoids; sesquiterpene lactones; PCA.

INTRODUCTION

Genus *Inula* (Asteraceae) includes more than 100 species distributed mainly in Africa, Asia and Europe. The genus is paraphyletic and heterogeneous concerning several diverse characters, which makes determining taxa difficult.[1,2] *Inula* species are an inexhaustible source of new chemical diversity and approximately 500 secondary metabolites have been identified to date, some of which with relevant pharmacological activity, such as antiproliferative, antiviral, antibacterial, antifungal, anti-inflammatory, antitumour, cytotoxic, antiprotozoal,

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etc.[3–6] Undoubtedly, the most important and representative compound classes of secondary metabolites in this genus are sesquiterpene lactones. However, further important metabolites were also found, *e.g.*, thymol and chlorogenic acid derivatives, sterols and flavonoids. Sesquiterpene lactones possess a common biosynthetic origin, and are useful chemical characters for differentiating subtribes within several major tribes, or to study infra- and intraspecific variability in certain genera of Asteraceae family.[7]

So far, the phytochemical studies of *Inula* species, growing in Bulgaria, namely *I. oculus-christi*,[8,9] *I. britannica*,[10] *I. aschersoniana*[11] and *I. bifrons*[12] revealed the presence of various terpenoids, among which the most distinctive was the group of sesquiterpene lactones. Continuing our research on *Inula* species, herein we describe the terpene constituents of four *Inula* species – *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. and *I. salicina* L. The species selected for the study are the representatives of two groups according to the classification of Anderberg.[2] *Inula salicina*, *I. germanica* and *I. ensifolia* belong to the *Inula salicina* group, diagnosed by xeromorphic leaves and with ligulate peripheral flowers, 1–1.5 as long as the involucre. *Inula conyza* is a part of *Inula decurrens* group. While *I. ensifolia* and *I. conyza* are clearly distinct morphologically, the identification of *I. germanica* and *I. salicina* possesses more difficulties. *Inula ensifolia* is characterized by lanceolate and acute leaves with the parallel venation, and *I. conyza* – by much wider leaves and very short (up to 1 mm) ligulae of the ray flowers. *I. germanica* can be distinguished from *I. salicina* by the shorter ligulae, equal to or slightly exeding the involucrum, and by forming many flower heads (capitulae) usually, while *I. salicina* is characterized by ligulae clearly longer than the involucrum, and by the smaller number of the flower head (one to few). Therefore, the comparison of their chemical content could bring additional insights into their relationships.

EXPERIMENTAL

General

Column chromatography was carried out on Silica gel 60 (230–400 mesh, Merck). Thin-layer chromatography (TLC) on silica gel 60 F_{254} plates (Merck) was used for monitoring the separation of the extracts and for preparative TLC. The spots were visualised by spraying with concentrated H_2SO_4 followed by heating at 120 °C. IR spectra were obtained on a Shimadzu FTIR IR Spirit spectrometer with ATR. The 1D and 2D NMR (^{1}H- and ^{13}C-NMR, COSY, HSQC and HMBC, NOESY) spectra were recorded on a Bruker Avance II+ 600 NMR spectrometer, with the operating frequency 600 MHz (^{1}H) and 150 MHz (^{13}C), using the residual solvent signal (δ 7.26 in ^{1}H- and 77.00 ppm in ^{13}C-NMR for $CDCl_3$) as a reference.

Plant material

Plant material was collected in full flowering stage from native populations in Bulgaria during 2017. The aerial parts were air-dried and kept in a dark and cool place until extraction. *Inula germanica* L. (GPS 42°0'27.94"N 23°37'47.72"E, SOM176698) aerial parts were collected from Rila Mts, *I. ensifolia* L. (41°29'30.34"N, 23°26'59.71"E, SOM176700) – from

Chereshnitza village, Struma River Valley, *I. salicina* (41°53'18.76"N 23°22'13.18"E, SOM 176701) – from Rila Mts., and *I. conyza* (Griess.) DC. (41°45'16.25"N 24°23'57.13"E, SOM1387) – from Western Rhodopes Mts. Plant species were identified by Dr. Ina Aneva (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences). Voucher specimens (SOM) have been deposited with the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Extraction

Air-dried and powdered aerial parts (50 g) from *I. ensifolia* (IE), *I. salicina* (IS), *I. conyza* (IC) and *I. germanica* (IG) were extracted with chloroform (3×500 mL) at room temperature to give the corresponding chloroform extracts (1.4, 1.5, 1.6 and 2.1 g in total, respectively).

Fractionation of the chloroform extracts and isolation of individual compounds

A portion of the chloroform extract (0.5–1.0 g) was dissolved in $CHCl_3$ and subjected to CC on silica gel, using *n*-hexane/ethyl acetate (EtOAc) mixtures with increasing polarity (from 10:1 to 0:1). Fractions were monitored by TLC (silica gel, *n*-hexane/Et_2O, 2:1) and compared with standards *β*-amyrin palmitate, *β*-amyrin acetate, *β*-amyrin, faradiol-3-*O*-palmitate and *β*-sitosterol. Fractions, containing triterpene acetates (R_f 0.8–0.9) and free triterpene alcohols (R_f 0.5–0.6) were directly analysed using GC/MS, whereas fractions containing triterpene fatty acid esters (R_f 0.9–1.0) and 16-hydroxy triterpene fatty acid esters (R_f 0.6–0.8) were subjected to alkaline hydrolysis.

CC of *I. ensifolia* (IE) chloroform extract (1.0 g) afforded 8 fractions: IE-1 (triterpene fatty acid esters, 335 mg), IE-2 (triterpene acetates, 50 mg), IE-3 (free triterpene alcohols, 230 mg), IE-4 (16 mg), IE-5 (16-hydorxy triterpene fatty acid esters, 110 mg), IE-6 (25 mg), IE-7 (50 mg) and IE-8 (98 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IE-4 (16 mg) afforded manil-adiol-*O*-palmitate (**16**, 1.7 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IE-6 (25 mg) afforded 16*β*-hydroxylupeol 3-*O*-palmitate (**17**, 1.1 mg) and faradiol-3-*O*-palmitate (**19**, 1.5 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IE-7 (10 mg) afforded β-sitosterol (**20**, 3 mg).

CC of *I. salicina* (IS) chloroform extract (0.5 g) afforded 7 fractions: IS-1 (triterpene fatty acid esters, 202 mg), IS-2 (triterpene acetates, 21 mg), IS-3 (free triterpene alcohols, 45 mg), IS-4 (16-hydorxy triterpene fatty acid esters, 12 mg), IS-5 (5.6 mg), IS-6 (54 mg) and IS-7 (149 mg). Prep. TLC ($CHCl_3$/Et_2O, 50:1) of IS-5 (5.6 mg) afforded 16-hydroxylupeol-*O*-palmitate (**17**, 1.1 mg), arnidiol-*O*-palmitate (**18**, 1.3 mg) and faradiol-*O*-palmitate (**19**, 1.2 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IS-6 (10 mg) afforded β-sitosterol (**20**, 2.5 mg).

CC of *I. conyza* (IC) chloroform extract (0.5 g) afforded 10 fractions: IC-1 (138 mg), IC-2 (8.5 mg) and IC-3 (21 mg) contained triterpene fatty acid esters, triterpene acetates and free triterpene alcohols, respectively. Prep. TLC (*n*-hexane/Et_2O, 5:1, x2) of IC-4 (21.3 mg) gave 10.2 mg of diterpene acid **21**. Prep. TLC (*n*-hexane/Et_2O, 5:1, x3) of IC-6 (11.8 mg) afforded mixture of diterpene acids **22** and **23** (3.3 mg). Prep. TLC (*n*-hexane/Et_2O, 5:1, x2) of IC-7 (20 mg) gave additional amount **22** and **23** (5.9 mg). Prep. TLC (*n*-hexane/Et_2O, 1:1) of a portion of IC-9 (15 mg) yielded *β*-sitosterol (**20**, 6.5 mg).

CC of *I. germanica* (IG) chloroform extract (1.0 g) afforded 11 fractions of which IG-1 (265 mg) and IG-2 (118 mg) contained triterpene fatty acid esters and triterpene acetates, respectively. CC ($CHCl_3$/Et_2O, 50:1) of IG-4 (58 mg) gave **16** (6.2 mg) and a mixture of triterpenes **17**–**19** (16.8 mg). CC ($CHCl_3$/Et_2O, 50:1) of IG-5 (93 mg) afforded **18** (28 mg), 4.5 mg of **20** and a mixture of free triterpene alcohols (12.8 mg), which were further analyzed by GC/MS. Prep. TLC (*n*-hexane/EtOAc, 3:1, x2) of IG-8 (30 mg) afforded grazielia acid (**24**, 1.1 mg), 8-(2-methylbutanoyloxy)-1(10),4,11(13)-germacratrien-6,12-olide-14-oic acid (**25**,

1.0 mg), 14-hydroxy-8*β*-angeloyloxymelampolide (**32**, 0.8 mg), 2*α*-acetoxy-desacetyl-laurenobiolide (**33**, 1.8 mg), ovatifolin (**27**, 5.6 mg) and a mixture of germanin A and B (**28** and **29**, 4.4 mg). Prep. TLC ($CHCl_3$/acetone, 3:1, x2) of IG-9 (35 mg) afforded desacetylovatifolin (**26**, 1.3 mg) and mixture of 2α,14-dihydroxy-8*β*-angeloyloxymelampolide and 2*α*,14-dihydroxy-8*β*-[2-methylbutyryloxy]-melampolide (**30** and **31**, 12.2 mg).

Hydrolysis of fatty acid esters and methylation of free fatty acids

Hydrolysis of the fatty acid esters of the mixtures of triterpenes (each 10 mg) was performed with 1 M KOH in MeOH (2 mL) at 50 °C for 12 h. After cooling, the reaction mixture was diluted with water (5 mL) and extracted with Et_2O (3 times, 5 mL each). The combined Et_2O extracts were washed with water (5 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The free triterpene alcohols mixture was analysed with GC/MS.

The aqueous layer was further acidified with 1 M HCl and re-extracted with Et_2O (3 times, 5 mL each). The combined Et_2O extracts were washed with water (5 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give free fatty acid fraction. The latter was further methylated with methanolic 1 % H_2SO_4 (2 mL) at 50 °C for 2 h. After cooling, 5 % aq. NaCl (5 mL) were added to the reaction mixture and extracted with *n*-hexane (3 times, 5 mL each). The combined *n*-hexane extracts were washed with water, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The resulting methyl esters of fatty acids were analysed by GC/MS.

Gas chromatography/mass spectrometry

Analyses were carried out with an Agilent 7890B (Agilent, USA) gas chromatograph equipped with a flame ionization detector (FID) and mass selective detector (MSD) Agilent 5977A. A HP-5MS capillary column (5 %-phenyl)-methylpolysiloxane, 30 m×0.25 mm; 0.25 μm film thickness, Agilent) was used with Helium as carrier gas at a flow rate of 1.7 mL/min. The GC oven temperature gradient started at 60 °C, followed by a ramp of 5 °C/min to 300 °C, and then held for 20 min. The samples were analysed with a split ratio of 5:1 and injection volume of 1 μL. The injector temperature was 280 °C. The temperatures of the MSD and the source were 150 and 230 °C, respectively. Mass spectra were taken at 70 eV and the mass range was from *m*/*z* 45 to 800. The identification of triterpene alcohols, triterpene acetates and fatty acid methyl esters was based on comparison of their EI-mass spectra with the NIST14 and home-made databases.

Statistical analysis

Principal component analysis (PCA) was performed using the PAST 4.0 software to determine the chemical variation and relationship between the species.

RESULTS AND DISCUSSION

The chloroform extracts obtained by extraction of the aerial parts of *I. germanica* L. (IG), *I. ensifolia* L. (IE), *I. conyza* (Griess.) DC. (IC) and *I. salicina* L. (IS) were submitted to a column chromatography using *n*-hexane/EtOAc mixtures with increasing polarity. A preliminary study of the non-polar fractions by TLC revealed the presence of triterpene compounds – alcohols and their esters (acetates and long-chain aliphatic esters). The fractions containing fatty acid esters were hydrolysed in an alkaline medium. GC/MS analysis of the obtained triterpene alcohols and fatty acid methyl esters, as well as of the fractions containing free triterpene alcohols and their acetates, led to the identification of 19 triterpenoids of

β- and *α*-amyrin, lupeol, taraxasterol and *ψ*-taraxasterol type (Table S-I of the Supplementary material to this paper and Fig. 1). Thus, *β*- and *α*-amyrin (**1** and **2**) were characterized by a base peak at *m*/*z* 218, but differed in the relative intensities of the peaks at *m*/*z* 189 and 203: *β*-amyrin (**1**) had *m*/*z* 203 peak around twice the intensity of the *m*/*z* 189 peak, while *α*-amyrin (**2**) spectrum showed both peaks with similar intensities.[13] The mass spectrum of lupeol (**3**) exhibited a base peak at *m*/*z* 189, which is characteristic for the fragmentation of triterpenoids with a lupane skeleton bearing a hydroxyl group in position 3. Other abundant fragment ions were at *m*/*z* 203 and 207. The first can be related to the retention of an additional methylene group from the C ring with respect to the fragment ion at *m*/*z* 189, and the second represents the fragment obtained from the fragmentation of the C ring before the dehydration reaction led to the formation of the ion at *m*/*z* 189.

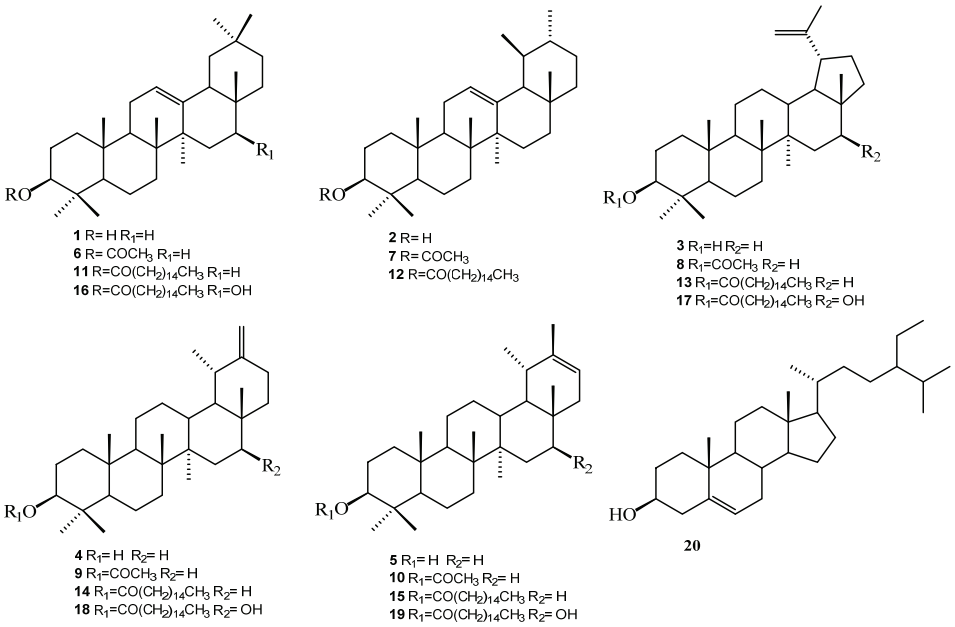


Fig. 1. Triterpenoids and sterols in *Inula* species.

The mass spectra of taraxasterol (**4**) and *ψ*-taraxasterol (**5**) revealed very intense ions at *m*/*z* 207 (80 and 85 %, respectively) and 189 (100 %), whose formation can be assumed by the cleavage of ring C, with a transfer of a hydrogen atom, followed by the loss of water.[13] The intensity of ions at *m*/*z* 218 and 203 was significantly lower than in *β*- and *α*-amyrin. The acetates **6**–**10** exhibited a molecular ion at *m*/*z* 468, an ion at *m*/*z* 408 corresponding to the loss of AcOH $[M-60]^+$ and the characteristic ions for the corresponding triterpene skeleton types. The structures of 16-hydroxy derivatives **16**–**19** were additionally confirmed by

NMR[14,15] after their isolation and purification by prep. TLC. *β*-Sitosterol (**20**) was also detected in all studied extracts by TLC comparison with authentic standard and confirmed by GC/MS of the fractions containing this compound.

The obtained results showed both similarity and difference in the studied samples (Table II).

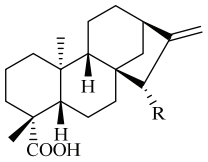
TABLE II. Distribution of triterpenoids in *Inula* species

Triterpenoid	IS	IG	IC	IE
Alcohols				
β-Amyrin (**1**)	+	+	+	+
α-Amyrin (**2**)	+	+	+	+
Lupeol (**3**)				+
Taraxasterol (**4**)	+	+	+	+
ψ-Taraxasterol (**5**)	+	+	+	+
Acetates				
β-Amyrin acetate (**6**)	+	+	+	+
α-Amyrin acetate (**7**)		+	+	
Lupeol acetate (**8**)	+		+	+
Taraxasterol acetate (**9**)	+	+	+	+
ψ-Taraxasteol acetate (**10**)	+	+	+	+
Palmitates				
β-Amyrin palmitate(**11**)	+	+	+	+
α-Amyrin palmitate (**12**)	+	+	+	+
Lupeol palmitate (**13**)				+
Taraxasterol palmitate (**14**)	+	+	+	+
ψ-Taraxasterol palmitate (**15**)	+	+	+	+
Maniladiol palmitate (**16**)		+		+
16*β*-Hydroxylupeol-3-*O*-palmitate (**17**)	+	+		+
Arnidiol palmitate (**18**)	+	+		+
Faradiol palmitate (**19**)	+	+		+

As can be seen, *I. conyza* was the only sample, which did not contain 16-hydroxy derivatives. All identified compounds are described for the first time in the here presented study of species. Fatty acid esters of triterpene alcohols have been previously found in *I. britannica*,[10,16] *I. oculus-christi*[8] and *I. bifrons*.[12] The literature data on triterpene compounds in *Inula* species are scarce and so far, there are few reports of triterpene alcohols and their esters in *I. japonica, I. helenium* and *I. cappa* only.[4] Triterpenoids of *β*- and *α*-amyrin, lupeol, taraxasterol and *ψ*-taraxasterol type are frequently found in many genera of Asteraceae family such as *Achillea*, *Chrysanthemum*, *Jurinea*, *Calendula*, *Taraxacum*, *etc.*[14,15,17–19] and therefore, could not be used as chemotaxonomic markers.

From the chloroform extract of *I. conyza* were isolated 3 compounds with very similar NMR spectral characteristics, indicating that they were diterpenoids with *ent*-kaurane skeleton and identified as *ent*-kaur-16-en-19-oic acid **(21)**,[20] *ent*-15*α*-(3-methylpentanoyloxy)-kaur-16-en-19-oic acid (**22**)[12] and *ent*-15*α*-(3-

-methylbutanoyloxy)-kaur-16-en-19-oic acid (**23**),[21] Fig. 2. The diterpene acids **22** and **23** have been recently found in *I. bifrons*[12] of Bulgarian origin, while *ent*--kaur-16-en-19-oic acid **(21**) is reporting for the first time in *Inula* species.

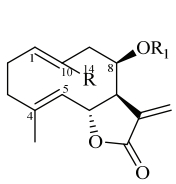


21 R = H
22 R = $OCOCH_2CH(CH_3)CH_2CH_3$
23 R = $OCOCH(CH_3)CH_2CH_3$

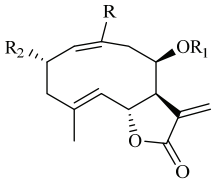
Fig. 2. Diterpene acids in *I. conyza.*

The literature survey showed only four reports on the content of diterpenoids in five species of genus *Inula – I. nervosa*, *I. cappa*, *I. japonica*, *I. britannica* and *I. bifrons.*[12,22–24] With the exception of *I. nervosa* and *I. cappa*, all other species contained diterpenoids with *ent*-kaurene skeleton, but differing in the nature and position of the substituents. According to the taxonomic classification of Anderberg,[2] *I. conyza*, *I. bifrons*, *I. britannica* and *I. japonica* belong to the section *Enula*, while *I. nervosa* and *I. cappa* are representatives of the section Duhaldea. Therefore, the presence of *ent*-kaurene diterpenoids in the section *Enula* could be of chemotaxonomic importance. Further investigations are needed to confirm this suggestion.

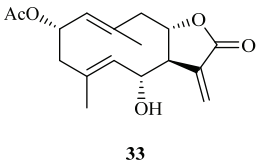
The preliminary study of the chloroform extracts of *I. germanica*, *I. ensifolia*, *I. conyza* and *I. salicina* by IR spectroscopy have shown the presence of sesquiterpene lactones in *I. germanica* only (characteristic absorption band at 1750–1765 cm^{-1}). Further separation of this extract by CC led to isolation of 10 closely related compounds with cyclodecadiene carbon skeleton and *trans*-*α*-methylene-*γ*-lactone ring at C-6 (**24**–**32**) or C-8 (**33**), Fig. 3. The lactones were isomers differing in the configuration of the double bonds: germacranolides with *trans*-$\Delta^{1,10}$ and *trans*-$\Delta^{4,5}$ (**24**–**27** and **33**) and melampolides with *cis*-$\Delta^{1,10}$ and *trans*-$\Delta^{4,5}$ (**28**–**32**) as well as in the nature of the substituents at C-2, C-8 and C-14.



24 R=COOH R_1=Ang
25 R=COOH R_1=2MeBu
26 R=CH_2OH R_1= H
27 R=CH_2OAc R_1= H



28 R=COOH R_1=Ang R_2= H
29 R=COOH R_1=2MeBu R_2= H
30 R=CH_2OH R_1=Ang R_2= OH
31 R=CH_2OH R_1=2MeBu R_2= OH
32 R=CH_2OH R_1=Ang R_2= H



33

Fig. 3**.** Sesquiterpene lactones from *I. germanica.*

The comparison of their spectral data with the literature data allowed the identification of grazielia acid (**24**),[25] 8-(2-methylbutanoyloxy)-1(10),4,11(13)--germacrutrien-6,12-olide-14-oic acid (**25**),[26] desacetylovatifolin (**26**),[27] ovatifolin (**27**),[28] germanin A (**28**),[29] germanin B (**29**),[30] 2*α*,14-dihydroxy-8*β*-angeloyloxymelampolide (**30**),[31] 2*α*,14-dihydroxy-8*β*-[2-methylbutyryloxy]-melampolide (**31**),[31] 14-hydroxy-8*β*-angeloyloxymelampolide (**32**)[31] and 2*α*-acetoxy--desacetyllaurenobiolide (**33**).[31] To the best of our knowledge, there are two reports on the content of sesquiterpenoids in *I. germanica* only. Germanin A and B (**28** and **29**) were isolated from *I. germanica* of Russian origin,[29] while lactones **27**, **30**–**33** were found in the sample cultivated in the Botanical garden of Berlin.[31] It is worth to mention that the lactones **24**–**26** are described now for the first time in *Inula* species.

In this study, no sesquiterpene lactones were detected in *I. conyza*, *I. salicina* and *I. ensifolia.* These results did not correlate with a previous investigation of *I. salicina*,[32] which reported the presence of alantolactone and isoalantolactone in this plant. Probably, the studied taxon is a new chemotype.

The obtained up to now data for the content of terpenoids in 8 *Inula* species growing in Bulgaria (*I. oculus-christi*,[8,9] *I. britannica*,[10] *I. aschersoniana*,[11] *I. bifrons*,[12] *I. germanica*, *I. conyza*, *I. salicina* and *I. ensifolia*) were analysed by the principal component aanalysis (PCA) to demonstrate their relationship. PCA performed on the different skeletal types of sesquiterpene lactones, di- and triterpenoids showed that the first two principal components accounted 80.74 % of the total variations (Fig. 4). Considering the contributions to the variances, PC 1 (61.02 % of the total variations) accounted for the positive contributions of germacranolides (GeSL), melampolides (MeSL) and triterpenoids with oleane (OleT), ursane (UrsT), taraxane (TarT) and *ψ*-taraxane (psi-TarT) carbon skeleton and negative contributions of sesquiterpene lactones with guaiane (GuSL) and eudesmane (EuSL) framework. The second component PC 2 (19.72 % of the total variations) was positively related to all types of sesquiterpene lactones (GeSL, MeSL, EuSL, GuSL, PsGuSL and SeGuSL), and negatively related to diterpenoids (DiT) and also all skeletal types of triterpenoids (OleT, UrsT, LupT, TarT and psi-TarT).

As depicted from the biplot (Fig. 4), the samples could be grouped as follows: *I. germanica* and *I. ascherosniana* constituted the first group (**A**), which was characterized by the presence of germacranolides. The other skeletal types of sesquiterpene lactones, namely melampolides in *I. germanica* and seco-guaianolides in *I. ascherosniana* are probably responsible for their placement on different sides of PC2. The second group (**B**) combined *I. oculus-christi*, *I. britannica* and *I. bifrons*, which contained eudesmanolides, guaianolides and pseudoguaianolides. The presence of diterpenoids places *I. bifrons* on the negative side of PC2. The species included in the third group (**C**) *I. conyza*, *I. ensifolia* and *I. salicina* were

characterized with the presence of triterpenoids and the lack of sesquiterpene lactones and diterpenoids. It is worth to mention that the lactone ring was 12,8-fused in all lactones found in *I. britannica*, *I. oculus-christi* and *I. bifrons* (group B), while *I. germanica* and *I. aschersoniana* (group A) produced predominantly 12,6--olides. The obtained results did not correlate well with the classification of Anderberg[2] based mainly on the morphological data. Thus, *I. germanica* differed from the species in *Inula salicina* group by the presence of sesquiterpene lactones. These compounds were absent from the plant material of *I. salicina* and *I. ensifolia*. *I. conyza* produced diterpenoids similarly to *I. bifrons*[12] from the same *Inula decurens* group. The lack of sesquiterpene lactones places *I. conyza* closer to the representatives of the *Inula salicina* group.

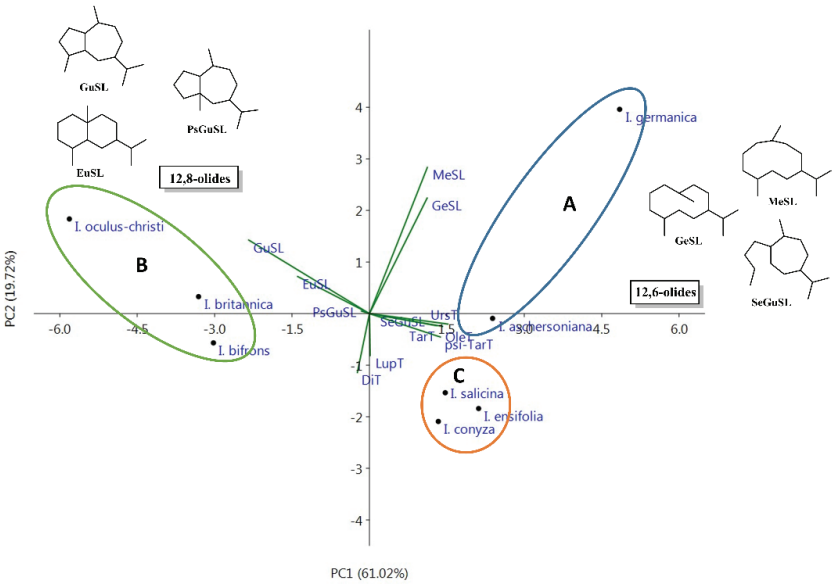


Fig. 4. Biplot (PCA) performed on the skeletal types of sesquiterpene lactones, di- and triterpenoids in *Inula* species growing in Bulgaria; germacranolides (GeSL), eudesmanolides (EuSL), guaianoilides (GuSL), pseudoguaianolides (PsGuSL), secoguaianolides (SeGuSL), diterpenoids (DiT), triterpenoids with oleane (OleT), ursane (UrsT), lupane (LupT), taraxane (TarT) and psi-taraxane (psi-TarT) skeleton.

CONCLUSION

The phytochemical studies of *I. germanica*, *I. ensifolia*, *I. conyza* and *I. salicinai,* growing in Bulgaria led to the identification of various classes of terpenoids: sesquiterpene lactones, diterpenoids, and triterpenoids. The most significant differences were observed with respect to sesquiterpene lactones and diterpenoids. They could serve as chemotaxonomic markers to clarify taxonomic problems in the genus *Inula*, which has been based mainly on morphological features so far.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11011, or from the corresponding author on request.

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ИЗВОД

ТЕРПЕНОИДИ У ЧЕТИРИ *Inula* ВРСТЕ ИЗ БУГАРСКЕ

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Фитохемијска студија хлороформског екстракта надземних делова *Inula germanica* L., *I. ensifolia* L., *I. conyza* (Griess.) DC. и *I. salicina* L. довела је до идентификације 33 терпеноида. *β*- и *α*-амирин, лупеол, тараксастерол, *ψ*-тараксастерол и њихови 3-*O*-ацетати и 3-*O*-палмитати су идентификовани помоћу GC/MS. Поред тога, NMR потврђује структуре 3-*O*-палмитата маиналадиола, арнидиола, фарадиола и 16-хидроксилупеола. *ент*-Каур-16-ен-19-оична киселина и њени 15*α*-(3-метилпентаноилокси) и 15*α*-(3-метилбутаноилокси) деривати изоловани су из *I. conyza*. У *I. germanica* је пронађено десет блиско повезаних сесквитерпенских лактона (гермакранолида и меламполида) и њихова структурна идентификација је извршена спектралним анализама. *I. ensifolia* и *I. salicina* су биле без сесквитерпенских лактона и дитерпеноида. Сви тритерпеноиди и дитерпеноиди, гразиелиа киселина, десацетиловатифолин и 8-(2-метилбутаноилокси)-1(10),4,11(13)-гермакрутриен-6,12-олид-14-оична киселина су први пут описани у проучаваној врсте. Анализа главних компоненти (PCA) је коришћена за проналажење везе између до сада истраживаних врста *Inula* које расту у Бугарској.

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SUPPLEMENTARY MATERIAL TO

Terpenoids in four *Inula* species from Bulgaria

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TABLE S-I. Triterpenoids in *Inula* species identified by GC/MS

Triterpenoid	MS data, *m/z* (relative abundance)
β-Amyrin (**1**)	426 (M^+, 5), 218 (100), 203 (58), 189 (25)
α-Amyrin (**2**)	426 (M^+, 4), 218 (100), 203 (26), 189 (25)
Lupeol (**3**)	426 (M^+, 29), 393 (14), 218 (74), 207 (87), 203 (52), 189 (100)
Taraxasterol (**4**)	426 (M^+, 32), 218 (20), 207 (80), 203 (16), 189 (100)
ψ-Taraxasterol (**5**)	426 (M^+, 23), 357 (13), 315 (8), 218 (16) 207 (85), 203 (24), 189 (100)
β-Amyrin acetate (**6**)	468 (M^+, 13), 453 (62), 408 (8), 393 (60), 218 (100), 203 (53), 189 (28)
α-Amyrin acetate (**7**)	468 (M^+, 5), 408 (2), 218 (100), 203 (212), 189 (26)
Lupeol acetate (**8**)	468 (M^+, 27), 453 (12), 408 (9), 218 (42), 204 (44), 189 (100)
Taraxasterol acetate (**9**)	468 (M^+, 15), 408 (10), 204 (17), 189 (100)
ψ-Taraxasteol acetate (**10**)	468 (M^+, 16), 408 (10), 249 (15), 204 (25), 189 (100)
Maniladiol[a]	442 (M^+, 20), 424 (3), 234 (100), 216 (40), 207 (50), 203 (73), 190 (29)
16*β*-Hydroxylupeol[a]	442 (M^+, 5), 424 (3), 234 (100), 216 (28), 207 (25), 201 (28), 190 (22)
Arnidiol[a]	442 (M^+, 27), 424 (25), 409 (10), 207 (100), 189 (89)
Faradiol[a]	442 (M^+, 5), 424 (11), 409 (4), 360 (13), 207 (46), 189 (48), 108 (100)

[a]After alkaline hydrolysis of **16**–**19**

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Phytochemical investigation of *Pimpinella serbica*

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Abstract: The plant species *Pimpinella serbica* (Vis.) Drude, endemic to West Balkans, belongs to the genus *Pimpinella* L. (Apiaceae) according to newer botanical classification. Initially, the plant was described as *Pancicia serbica* Vis. and long considered as a monotypic genus. Previous phytochemical investigations of this plant were limited to the essential oil analysis by the GC–MS method. This is first study that includes LC-DAD–MS screening of the extracts of the roots and aerial parts, followed by isolation of secondary metabolites using chromatographic techniques. Five compounds belonging to phenylpropanoids and polyacetylenes, well known for their bioactivities, were isolated and structurally determined by combined spectroscopic methods (UV, NMR, MS). Dillapiole (**1**), nothoapiole (**2**) and oplopantriol A 18-acetate (**4**) were found in both extracts, while falcarindiol (**3**) was isolated from the roots and dendrotrifidol (**5**) from the aerial parts only. The phytochemical profile of *P. serbica* L. supports its position in the *Pimpinella* L. genus.

Keywords: Apiaceae (Umbelliferae); phenylpropanoids; polyacetylenes.

INTRODUCTION

The Apiaceae (formerly Umbelliferae) family contains around 420 genera with 3100 species, found throughout most of the world, but mainly in northern temperate regions. This large family contains many important food plants, *e.g.*, *Daucus* (carrot), *Pastinaca* (parsnip), and *Apium* (celery), while others are very poisonous, *e.g. Oenanthe* (water dropwort), or are used medicinally.[1] *Pimpinella* L. is one of the largest genera of the Apiaceae family and comprises 170–180

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species.[2] This genus is well known for a number of medicinally and pharmaceutically important species, *Pimpinella anisum* (anise) being the most notable one.[3] *Pimpinella serbica* (Vis.) Drude is a species endemic to the West Balkans, limited to Serbia, Bosnia and Herzegovina, Montenegro, North Macedonia, and Albania.[4] The plant has been described as *Pancicia serbica* Vis. and has long been considered as monotypic genus named after Serbian botanist Josif Pančić.[5] In light of modern nomenclature *P. serbica* is included in the genus *Pimpinella*.[4]

It is a glabrous perennial herb up to 50 cm tall; the rhizome is vertical, thick; stems are straight, striate, rounded, branched at the top. The leaves are alternate, markedly heteromorphic: the lower ones are long petiolate, simple, cordate, serrate, the middle ones are shortly petiolate, deeply pinnately lobed, the upper ones are very short petiolate, palmate, with setaceous lobes. The umbel consists of 10––15 rays; bracts 5–8 linear, scarbid bracteoles 5, the flowers are white, rarely light pink, small, 5-numerous, with obcordate petals. The fruit is glabrous, ovoid, 3–4 mm long, with 5 narrowly winged ridges, slightly laterally compressed.[5,6]

Previous phytochemical investigations of *P. serbica* were limited to GC–MS analysis of the essential oils obtained from the fruits and aerial parts of the plant.[7,8] Here for the first time, an investigation of chemical constituents of *P. serbica* extracts, obtained from the roots and aerial parts of the plant, is reported.

EXPERIMENTAL

General

The NMR spectra were acquired on a Bruker Avance DRX 500 MHz instrument with a 5 mm inverse detection probe, in $CDCl_3$ as the solvent, at 298 K. The spectra were referenced to tetramethylsilane (TMS), chemical shifts are given in ppm (δ / ppm), and coupling constants are reported in Hz (J / Hz).

The GC and GC–MS analyses were performed on an Agilent 7890A GC equipped with a 5975C inert XL EI/CI mass selective detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology two-way splitter with make-up gas. An HP-5MSI capillary column (30 m×0.25 mm×0.25 μm) was used. The temperature of the GC oven was programmed from 60 to 300 °C at 3 °C min^{-1} and held for 10 min. Helium was used as the carrier gas at 20.343 psi (constant pressure mode). The injection volume was 1 μL with split ratio 10:1. The FID temperature was 300 °C. The MS data was acquired in the EI mode, with a scan range 30–550 m/z; the source temperature was 230 °C and the quadrupole temperature was 150 °C. The solvent delay was 3 min.

The mass spectra were obtained on an Agilent Technologies 6210 time-of-flight LC–MS system with electrospray (ESI) ion source. The separation was performed with an LC apparatus (1200 Series Agilent Technologies) comprising an on-line degasser, binary pump, auto injector, column oven and diode array (DAD) detector, equipped with analytical Zorbax Eclipse Plus C18 column (150 mm×4.6 mm, 1.8 μm ID) maintained at 40 °C at a constant flow-rate of 1.4 mL min^{-1}. The mobile phase was a mixture of solvent A (5 mM ammonium formate in H_2O) and solvent B (acetonitrile) according to a combination of gradient and isocratic modes: 95 % A, 0–1.5 min; 95–5 % A, 1.5–26 min; 5 % A, 26–35 min; 5–95 % A, 35––36 min, 95 % A, 36–41 min. The UV spectra were recorded in the 190–450 nm range, and ESI MS spectra in m/z 100–2000 Da range. ESI-TOF-MS conditions: drying gas (N_2) flow 12

L min^{-1}, nebulizer pressure = 310 kPa, drying gas temperature = 350 °C, capillary voltage = 4000 V, fragmentor voltage = 140 V, skimmer = 60 V, Oct RF voltage = 250 V, positive mode, 10,000 transients/scan. A personal computer system running MassHunter Workstation software was used for data acquisition and processing.

Optical rotation measurements were performed on a Rudolph Research Analytical Autopol IV automatic polarimeter.

UV spectra were recorded on a GBC Cintra 40 UV–Vis spectrometer and analytical LC--DAD system Agilent Technologies 6210.

Silica gel (0.063–0.200 mm) was used for column chromatography (CC). Silica gel G and silica gel F-254 were used for analytical (0.25 mm) and preparative (0.75 mm) thin layer chromatography (TLC).

Plant material

The plant material was collected during the flowering season in June 2011 at Mokra Planina near Čakor (Montenegro). The plant was identified by one of the authors (D. Stešević), and voucher specimen was deposited at the herbarium collection of the Faculty of Sciences (TGU), University of Montenegro (Voucher Code 1737603).

Extraction and isolation

Herbal material was air-dried in shade to yield 8 g of the roots and 80 g of aerial parts. Powdered roots and aerial parts were extracted with dichloromethane. The solvent was removed by vacuum evaporation; the yields the extracts were 100 mg of the roots and 960 mg of the aerial parts.

The extracts were first analyzed by LC-DAD–MS chromatography. Four major compounds were detected in the root extract (**1**–**4**), and four in the aerial parts extract (**1**, **2**, **4** and **5**). These compounds were then isolated using CC and preparative TLC.

The root extract was subjected to CC on silica gel, starting the elution with *n*-hexane/ /acetone 9/1 and increasing the polarity by adding acetone to 50 %. Fractions **4** and **5** were combined and purified by preperative TLC (*n*-hexane/acetone 8/2) to yield 3 mg of dillapiole (**1**) and 6 mg of nothoapiole (**2**). Fraction 12 contained 6 mg of falcarindiol (**3**), and fractions 17–19 gave 3 mg of oplopantriol A 18-acetate (**4**).

The extract of the aerial parts was fractionated by CC on silica with petroleum ether/ /Et_2O/ MeOH with increasing polarity to yield 50 fractions. Fraction 8, purified by preperative TLC (petroleum ether/acetone 8/2), gave 18 mg of dillapiole (**1**). Fractions 42–47 were combined and re-chromatographed by CC using dichloro methane/methanol with increasing polarity to collect 38 subfractions. Subfractions 13–15 contained 3.6 mg of dendrotrifidiol (**5**).

Analytical and spectral data are given in Supplementary material to this paper.

RESULTS

The roots and aerial parts extracts of *P. serbica* were analysed by LC-DAD– –MS chromatography prior to purification and isolation of the components. In this way, four major peaks were identified in the root extract (compounds **1**–**4**, Figs. S-1 and S-2 of the Supplementary material). In the LC-DAD chromatogram of the extract of the aerial parts, three peaks were observed (Fig. S-3). The first two peaks were identical to those detected in the root extract (compounds **1** and **4**). Careful analysis of the MS data revealed that the third peak consisted of two

unresolved components (Fig. S-4). The first was identical to compound **2** in the root extract, and the other one was an additional compound **5**.

Compound **1** exhibited the molecular ion [M+H$^+$] at *m*/*z* 223.0963 (Fig. S-2), compatible with a molecular formula $C_{12}H_{14}O_4$. The ^{1}H-NMR spectrum (Fig. S-5) suggested the phenylpropanoid structure. Specifically, the signals at δ 5.91 *ddt*, 5.05 *ddq*, 5.04 *ddq* and 3.30 ppm *dt* (each 1H) corresponded to the allyl group, and the singlet at δ 5.88 ppm (2H) indicates the presence of a methylenedioxy group. In addition, the signals at δ 4.01 (3H, *s*) and 3.75 ppm (3H, *s*) are attributed to methoxy groups. These data are compatible with the structures of apiole and dillapiole, which were identified as the major components in previous investigations of the essential oils of the plant.[7,8] By comparing the ^{1}H-NMR spectrum of compound **1** to the spectrum of apiole isolated in a study of *Malabaila aurea* essential oils,[9] it is clear that they are different compounds (Fig. S-8). At the same time, the NMR data of compound **1** matched well with published data for dillapiole.[10] GC–MS data of compound **1**, including the EI-MS spectrum and Kovats index (Fig. S-9) are also in accordance with the structure of dillapiole. While the MS spectra of dillapiole and apiole are similar, they can be distinguished by different retention times and Kovats indices on a DB-5 column (Figs. S-9 and S-10). Thus, compound **1** was unequivocally identified as dillapiole (1-allyl-2,3-dimethoxy-4,5-(methylenedioxy)benzene).

The molecular formula of compound **2** can be deduced as $C_{13}H_{16}O_5$, based on the [M+H$^+$] ion at *m*/*z* 253.1082. Its ^{1}H and ^{13}C spectra (Figs. S-11 and S-12) were similar to those of **1**, except that in spectra of compound **3**, there are three signals corresponding to methoxy groups, and the aromatic proton at C-6 is missing. Thus, compound **3** was identified as nothoapiole (1-allyl-2,3,6-trimethoxy--4,5-(methylenedioxy)bezene). Both the NMR and GC–MS data of compound **2** are in good accordance with literature values.[10]

The molecular formula of compound **3** was determined to be $C_{17}H_{24}O_2$ from the ions [M+H$^+$-H_2O] at *m*/*z* 243.1742 and [M+H$^+$-2H_2O] at *m*/*z* 225.1634. The UV spectrum (Fig. S-14) showed an absorption pattern typical for polyacetylenes: one large and intense band with a maximum around 200 nm, and three smaller bands with maxima at 230, 245 and 260 nm.[11] The ^{13}C-NMR spectrum (Fig. S-16) is in accordance with a C-17 polyacetylene structure, showing 4 sp, 4 sp^2 and 7 sp^3 hybridized carbons, with two of them oxygenated. Based on this evidence, compound **3** was identified as falcarindiol. This was confirmed by comparison to literature data.[12] Since its spectral data, including optical rotation, were identical to falcarinol isolated in a previous study of *Seseli annuum* roots,[13] its absolute configuration was assigned as (3*R*,8*S*).[12,14]

The ESI MS spectrum of compound **4** showed multiple ions and they all point to the molecular formula $C_{20}H_{28}O_4$. The UV spectrum (Fig. S-17) was compatible with a polyacetylene structure. By comparing its ^{1}H- and ^{13}C-NMR data (Figs.

S-18 and S-19) to those of falcarindiol, it could be deduced that **4** is trioxygenated C-18 polyacetylene with the acetyl group at the terminal carbon. This corresponds with the structure of oplopantriol A 18-acetate, which is supported by literature data.[15] Due to the oxygenation at the terminal position, the numbering of this compound should start from the opposite side comparing to falcarindiol. This makes comparison of NMR data to falcarindiol difficult. For this reason, and assumed common biosynthetic origin, it was decided to use the same numbering system for these compounds. The absolute configuration of **4** was accordingly assigned as (3*R*,8*S*).

From the ions at *m*/*z* 257.1900, *m*/*z* 292.2271 and *m*/*z* 297.1822, the molecular formula of compound **5** was established as $C_{18}H_{26}O_2$. The ^{1}H- and ^{13}C--NMR spectra (Figs. S-18 and S-19) suggested dioxigenated C-18 polyacetylene. The signals at δ 4.91 (1H, *br d*) 3.64 (2H, *t*) and 3.03 ppm (2H, *br d*) showed that the positions of oxigenation were 3 and 18. This led to the structure of dendrotrifidol, which is confirmed by comparison to literature data.[16] The numbering and absolute configuration were aligned to those of falcarinol.

The structures of isolated compounds are presented in Fig. 1, and their NMR data are summarized in Tables S-I and S-II of the Supplementary material.

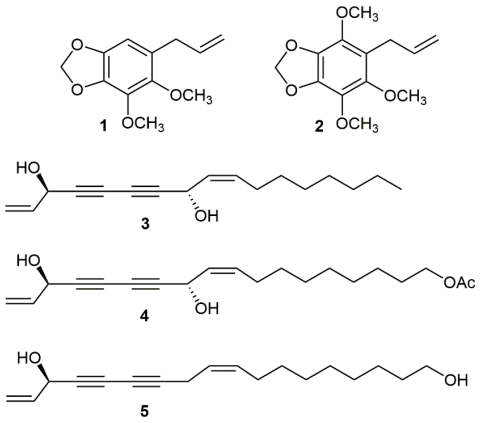

Fig. 1. The structures of the isolated compounds.

DISCUSSION

In a previous study of the essential oil obtained from the fruits of *P. serbica*, dillapiole (35.1 %) and nothoapiole (9.5 %) were among the most abundant components.[7] This is in line with the present findings, even though the plant material was collected at different localities (Kopaonik, Kodža Balkan and Zlatar). On the other hand, in the study of the essential oil from the aerial parts of the plant, apiole was major component (76.8 %); dillapiole was a minor component (0.8 %) and nothoapiole was not reported.[8] This was puzzling given that plant material was collected at one of the localities mentioned in the first study (Kopaonik). Further investigation is required to determine whether this difference can be attributed to

the existence of different chemotypes and adaptation to particular habitats. In general, the phytochemical profile of *P. serbica* is dominated by phenylpropanoids, which aligns well with the other *Pimpinella* species[17] and therefore supports its botanical classification to this genus.

Compounds **1**–**5** are known for their bioactivities. Specifically, dillapiole showed significant anti-inflammatory[18] and gastroprotective activity.[19] Nothoapiole exhibited antibacterial and antifungal activity.[20] The bioactivity of polyacetylenes (falcarinol, falcarindiol and related compounds) is also well documented, including but not limited to antifungal activity, allergenicity and cytotoxicity.[11] This makes *P. serbica* an important natural source of biologically relevant chemicals. Due to its protected status, bringing *P. serbica* into cultivation is a reasonable way to harness its potential as medicinal plant.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11073, or from the corresponding author on request.

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И З В О Д

ФИТОХЕМИЈСКО ИСПИТИВАЊЕ ВРСТЕ *Pimpinella serbica*

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Ендемска врста Западног Балкана *Pimpinella serbica* (Vis.) Drude према последњој ботаничкој класификацији припада роду *Pimpinella* L. (Apiaceae), мада је најпре била описана као *Pancicia serbica* Vis. и дуго је сматрана једином припадајућом врстом овог рода. Претходна фитохемијска испитивања ове врсте обухватају само анализу етарског уља GC–MS техником, тако да је ово прво детаљно испитивање биљке које укључује LC-DAD–MS анализу праћену изоловањем секундарних метаболита биљке применом хроматографских техника. Пет једињења која припадају групи фенилпропаноида и полиацетилена, добро познатих по својим биолошким активностима, изоловано је из екстраката надземног дела и корена биљке. Њихова структура је одређена применом спектроскопских метода (UV, NMR, MS). Дилапиол (**1**), нотоапиол (**2**) и оплопантриол A 18-ацетат (**4**) нађени су у оба екстракта, док је фалкариндиол (**3**) изолован само из екстракта корена, а дендротрифидол (**5**) само из екстракта надземног дела биљке. Фитохемијски профил биљке *P. serbica* подржава њену припадност роду *Pimpinella* L.

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SUPPLEMENTARY MATERIAL TO

Phytochemical investigation of *Pimpinella serbica*

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ANALYTICAL AND SPECTRAL DATA

Dillapiole (1-allyl-2,3-dimethoxy-4,5-(methylenedioxy)benzene) (**1**): ^{1}H-NMR and ^{13}C-NMR data are given in Table S-I. (+)ESI-HRMS *m/z*: calculated for [$C_{12}H_{14}O_4$ + H^+] 223.0965, observed 223.0963. EI MS 222 $[M]^+$ (100) 207 (15) 177 (30) 149 (17) 121 (12) 106 (13) 91 (9) 77 (15).

Nothoapiole (1-allyl-2,3,6-trimethoxy-4,5-(methylenedioxy)bezene) (**2**): ^{1}H-NMR and ^{13}C-NMR data are given in Table S-I. ((+)ESI-HRMS *m/z*: calculated for [$C_{13}H_{16}O_5$ + H^+] 253.1071, observed 253.1082. EI MS 252 $[M]^+$ (100) 237 (19) 225 (9) 221 (6) 207 (22) 191 (6) 179 (14) 164 (5) 151 (10) 121 (5) 77 (9).

Falcarindiol ((3*R*,8*S*,*Z*)-heptadeca-1,9-dien-4,6-diyne-3,8-diol) (**3**): $[\alpha]^{20}$; D = +228° (*c* 0.2, CH_2Cl_2). ^{1}H-NMR and ^{13}C-NMR data are given in Table S-II. (+)ESI-HRMS *m/z*: calculated for [$C_{17}H_{24}O_2$ + H^+ - H_2O] and [$C_{17}H_{24}O_2$ + H^+ - 2H_2O] 243.1743 and 225.1638, observed 243.1742 and 225.1634, respectively.

Oplopantriol A 18-acetate ((11*S*,16*R*,*Z*)-11,16-dihydroxyoctadeca-9,17-dien-12,14-diyn-1-yl acetate) (**4**): ^{1}H NMR and ^{13}C NMR data in Table S-II. (+)ESI-HRMS *m/z*: calculated for [$C_{20}H_{28}O_4$ + H^+ - 2H_2O], [$C_{20}H_{28}O_4$ + H^+ - H_2O], [$C_{20}H_{28}O_4$ + NH_4^+ - H_2O], [$C_{20}H_{28}O_4$ + NH_4^+] and [$C_{20}H_{28}O_4$ + Na^+] 297.1849, 315.1955, 332.2220, 350.2326 and 355.1880, observed 297.1863, 315.1948, 332.2213, 350.2319 and 355.1875, respectively.

Dentrotrifidol ((*R*,*Z*)-octadeca-9,17-dien-12,14-diyne-1,16-diol) (**5**): ^{1}H-NMR and ^{13}C-NMR data are given in Table S-II. (+)ESI-HRMS *m/z*: calculated for [$C_{18}H_{26}O_2$ + H^+ - H_2O], [$C_{18}H_{26}O_2$ + NH_4^+] and [$C_{18}H_{26}O_2$ + Na^+] 257.1900, 292.2271 and 297.1825, observed 257.1900, 292.2271 and 297.1822, respectively.

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TABLE S-I. The NMR data of phenylpropanoids from *P. serbica*

#	Dillapiole (1)		Nothoapiole (2)	
	^{1}H *m* (*J* / Hz)	^{13}C	^{1}H *m* (*J* / Hz)	^{13}C
1	-	126.0	-	118.7
2	-	144.6	-	136.6
3	-	137.6	-	133.3
4	-	135.9	-	137.7
5	-	144.3	-	134.5
6	6.35 s	102.7	-	145.0
7	5.88 *s*	101.1	5.90 *s*	101.2
8	3.30 *dt* (6.6; 1.5)	33.9	3.33 *dt* (6.1; 1.5)	28.3
9	5.91 *ddt* (16.8; 10.3; 6.6)	137.4	5.94 *ddt* (15.5; 11.5; 6.1)	137.7
10	5.04 *dq* (10.3; 1.5)	115.5	4.97 *dq* (11.5; 1.5)	114.4
-	5.05 *dq* (16.8; 1.5)	-	4.98 *dq* (15.5; 1.5)	-
2-OCH_3	3.75 *s*	61.2	3.89 *s*	61.5
3-OCH_3	4.01 *s*	59.9	3.94 *s*	60.4
6-OCH_3	-	-	3.77 *s*	60.0

TABLE S-II. The NMR data of polyacetylenes from *P. serbica*

#	Falcarindiol (3)		Aplopantriol A 18-acetate (4)		Dendrotrifidiol (5)	
	^{1}H *m* (J / Hz)	^{13}C	^{1}H *m* (*J* / Hz)	^{13}C	^{1}H *m* (*J* / Hz)	^{13}C
1	5.47 *dt* (17.2; 1.2) 5.26 *dt* (10; 1.2)	117.3	5.48 *dt* (17.2; 1.2) 5.26 *dt* (10; 1.2)	117.3	5.46 *dt* (17; 1.2) 5.23 *dt* (10; 1.2)	116.9
2	5.94 *ddd* (17.2; 10; 5.4)	135.8	5.94 *ddd* (17.2; 10; 5.2)	135.8	5.94 *ddd* (17; 10; 5.2)	136.2
3	4.94 *br t*	63.4	4.94 *br t* (4.8)	63.4	4.91 *br d* (5.2)	63.4
4	-	78.2	-	78.4	-	74.4
5	-	70.3	-	70.1	-	71.1
6	-	68.7	-	68.7	-	64
7	-	79.8	-	79.7	-	80.1
8	5.21 *br d* (8.1)	58.6	5.21 *br dd* (8.2; 3.0)	58.6	3.03 *br d* (7.0)	17.7
9	5.52 *m*	127.6	5.52 *m*	127.8	5.39 *m*	122
10	5.61 *m*	134.7	5.61 *m*	134.5	5.51 *m*	133
11	2.11*dq* (7.6; 1.2)	27.6	2.12 *dq* (7.5; 1.2)	29.1	2.04 *br q* (7.2)	27
12	1.39 *m*	29.3	1.39 *m*	29.2	1.38 *m*	28.9
13	1.28 *m*	29.15	1.29 *m*	29	1.29 *m*	29.4
14	1.27 *m*	29.1	1.29 *m*	28.9	1.29 *m*	29.3
15	1.27 *m*	31.8	1.29 *m*	28.6	1.29 *m*	29
16	1.27 *m*	22.6	1.29 *m*	27.6	1.29 *m*	25.6
17	0.88 t (7.1)	14.1	1.60 *m*	25.8	1.57 *m*	32.7
18	-	-	4.06 t (6.7)	64.7	3.64 t (6.7)	63.1
19	-	-	-	171.4	-	-
20	-	-	2.05 *s*	21	-	-
3-OH	2.01 *bd d*	-	2.20 br d (6.4)	-	1.62 br s	-
8-OH	1.91 *bd s*	-	1.94 *bd d* (4.2)	-	-	-
18-OH	-	-	-	-	1.62 br s	-

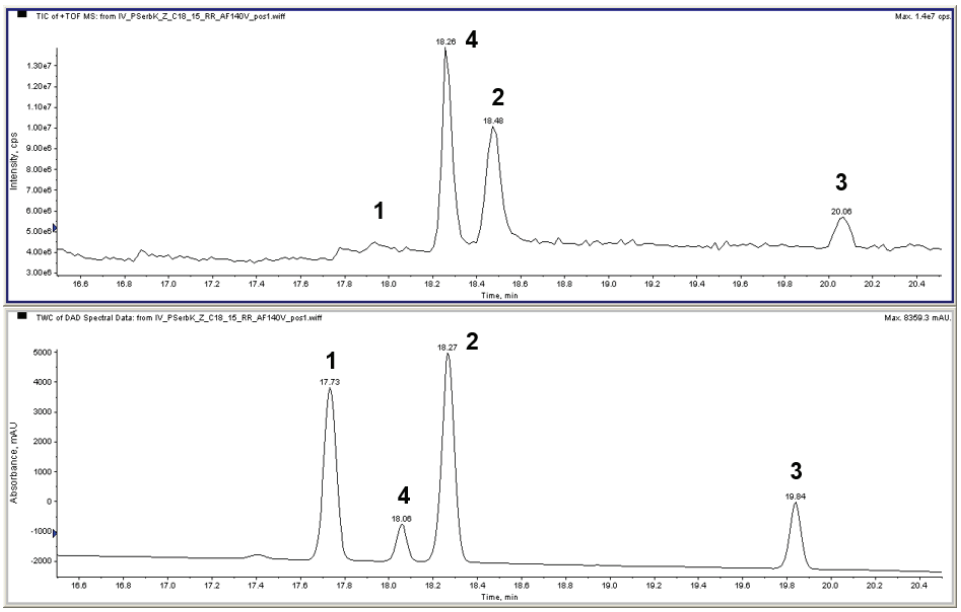


Fig. S-1. LC-MS (top) and LC-DAD (bottom) chromatogram of *P. serbica* root extract.

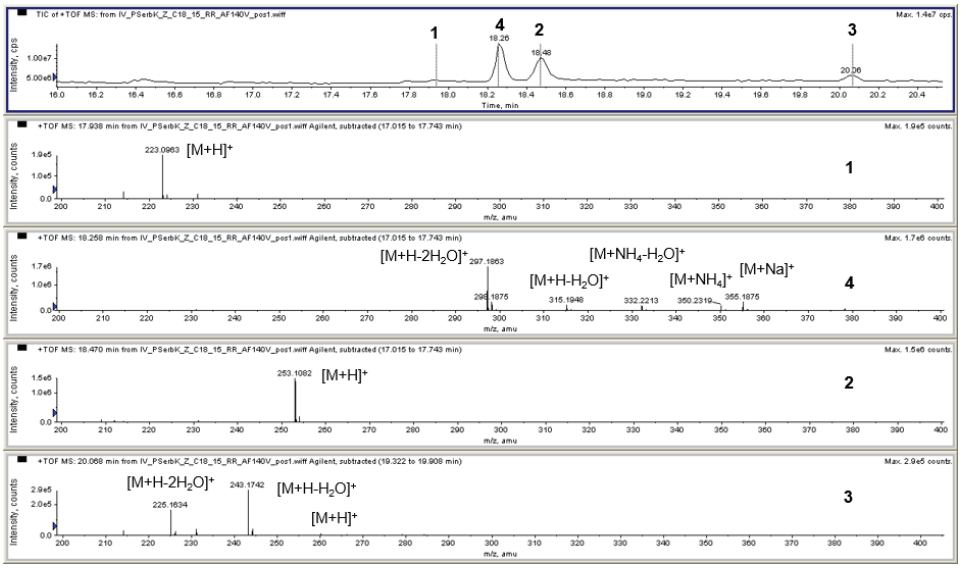


Fig. S-2. LC-MS chromatogram of *P. serbica* root extract and (+)ESI-HRMS spectra of compounds **1**–**4**.

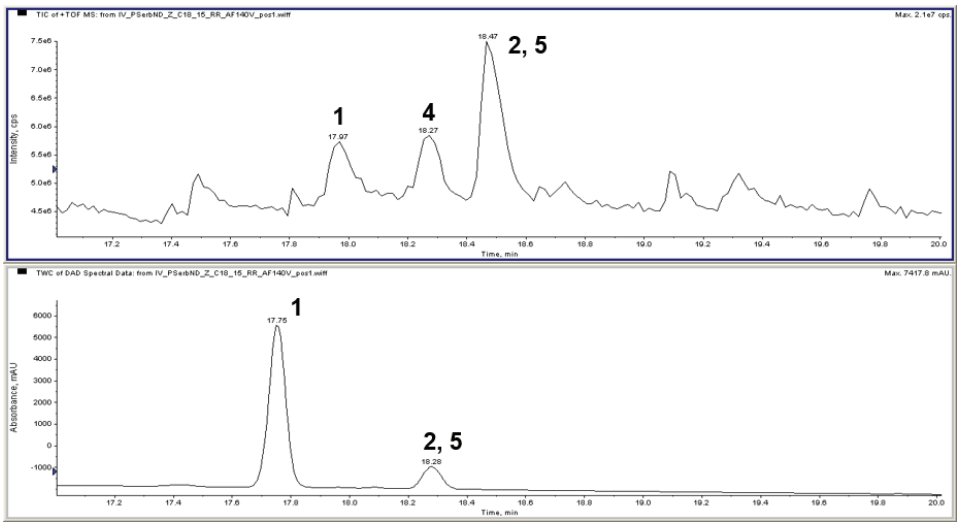


Fig. S-3. LC-MS (top) and LC-DAD (bottom) chromatogram of *P. serbica* aerial parts extract.

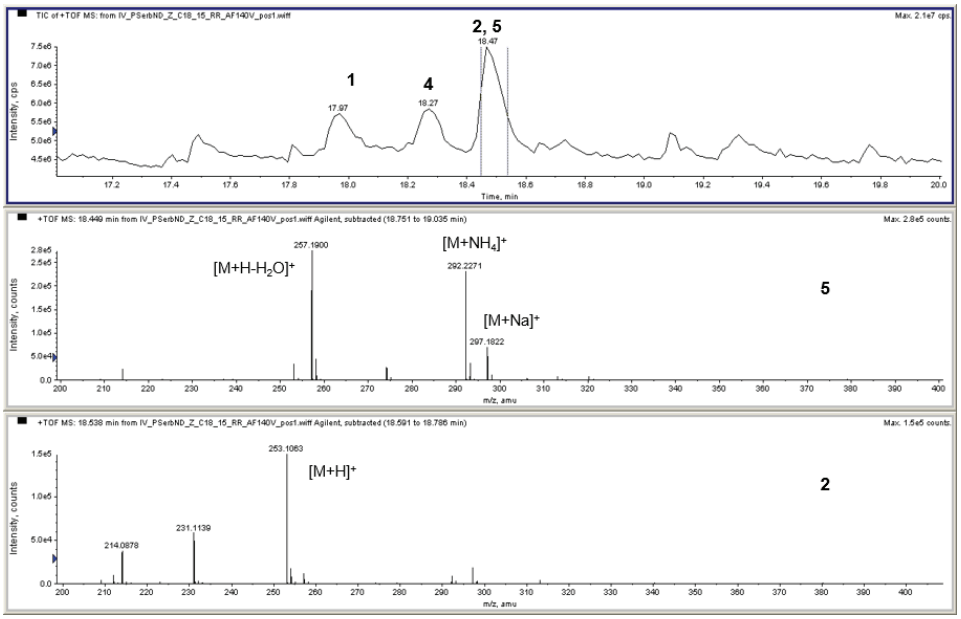


Fig. S-4. LC-MS chromatogram of *P. serbica* aerial parts extract and (+)ESI-HRMS spectra of compounds **2** and **5**.

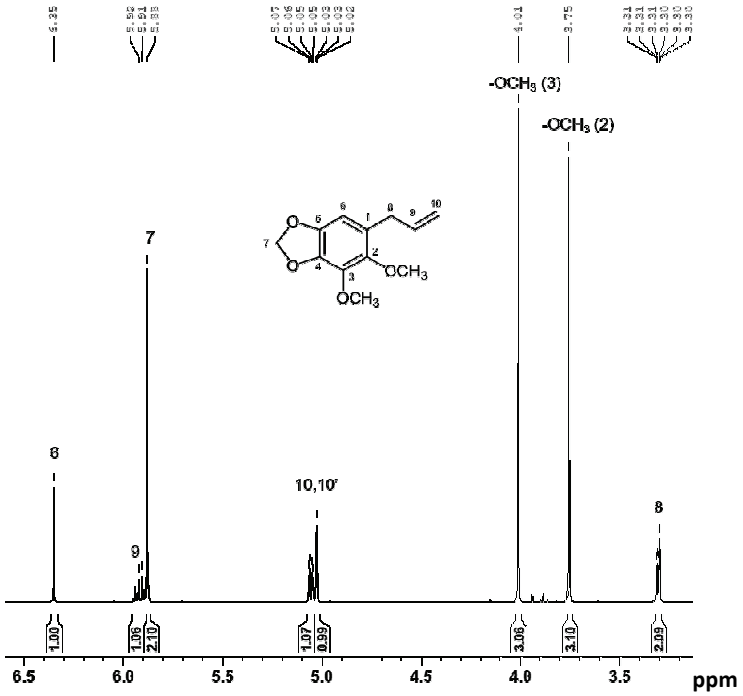


Fig. S-5. ^{1}H-NMR spectrum of compound **1** – dillapiole (500 MHz, $CDCl_3$).

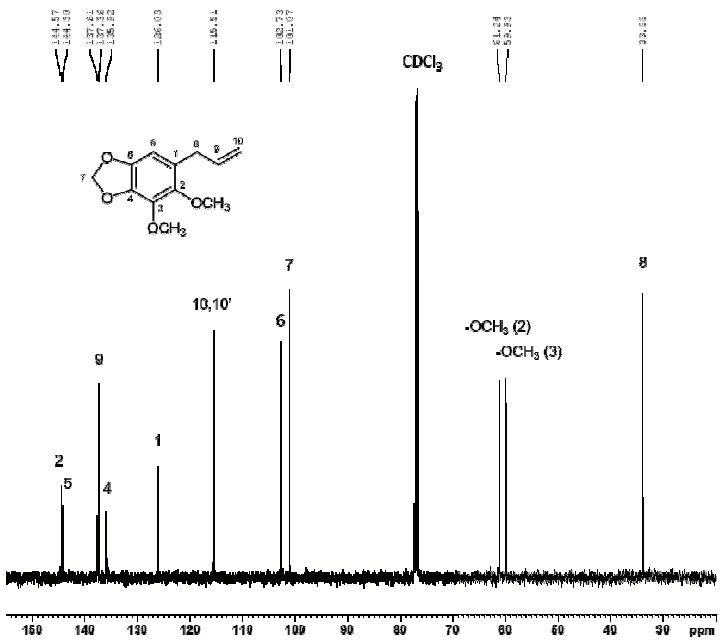


Fig. S-6. ^{13}C-NMR spectrum of compound **1** – dillapiole (125 MHz, $CDCl_3$).

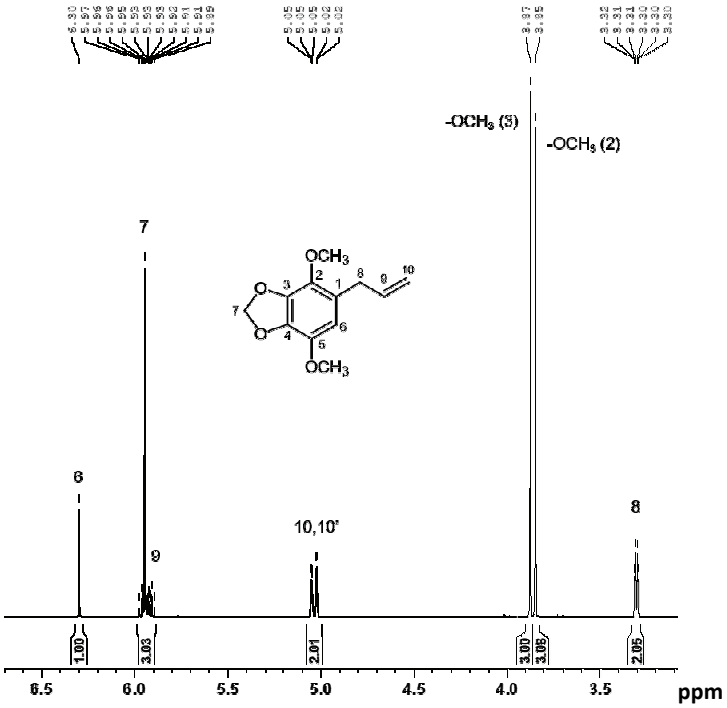


Fig. S-7. ^{1}H- NMR spectrum of apiole isolated from *Malabaila aurea* (500 MHz, $CDCl_3$).

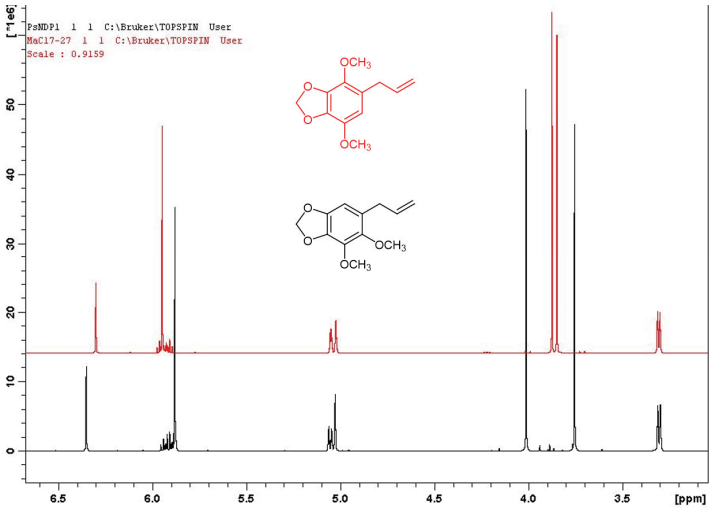


Fig. S-8. A comparison between the ^{1}H-NMR spectra of dillapiole from *Pimpinella serbica* and apiole from *Malabaila aurea*.

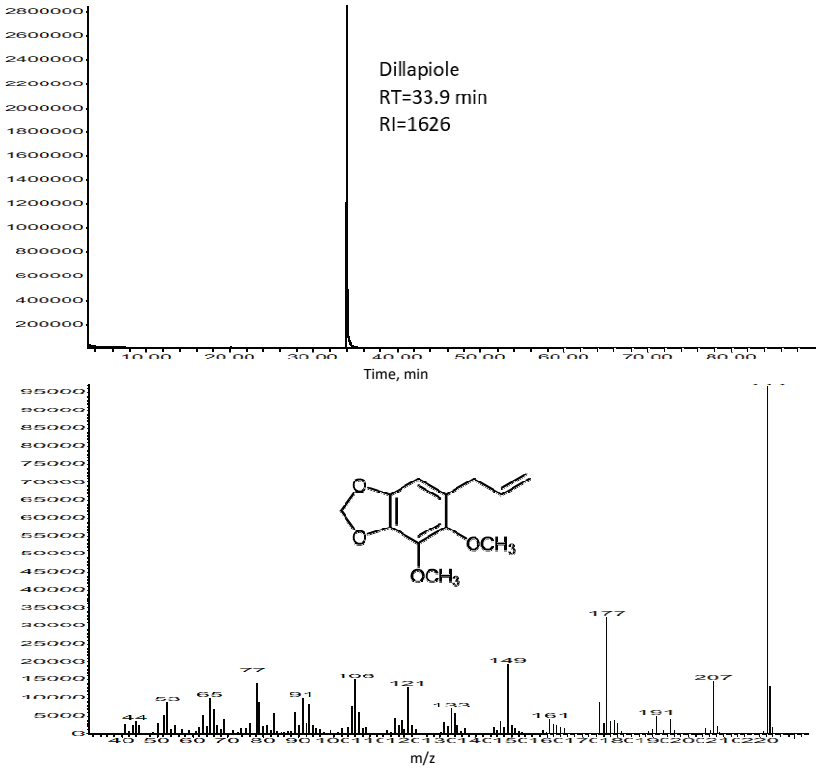


Fig. S-9. GC-MS chromatogram (top) and EI MS spectrum (bottom) of compound **1** - dillapiole.

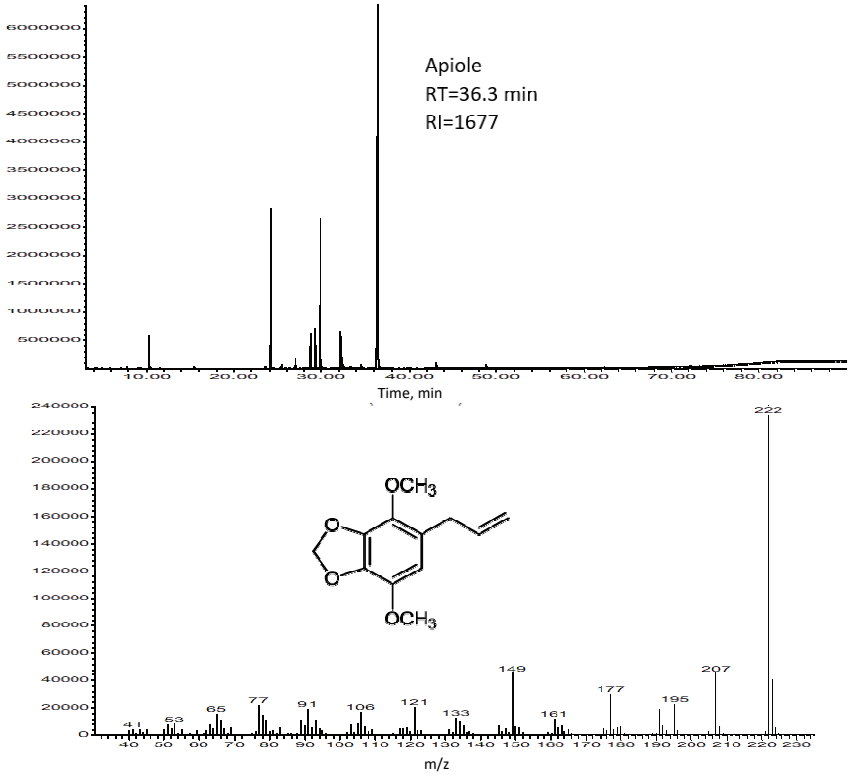


Fig. S-10. GC-MS chromatogram of *Malabaila aurea* essential oil (top) and EI MS spectrum of its major component apiole (bottom).

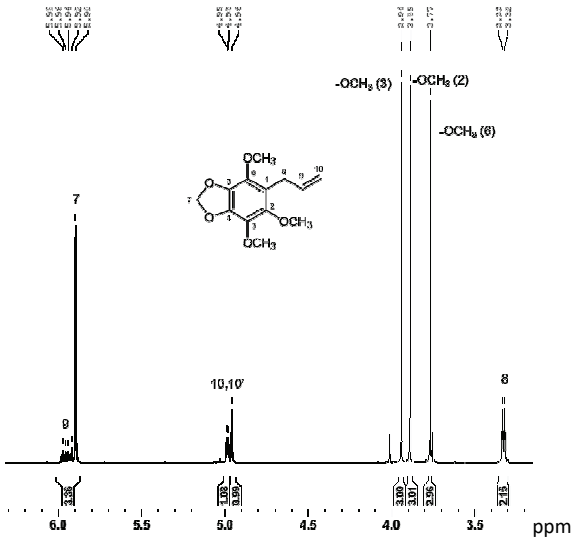
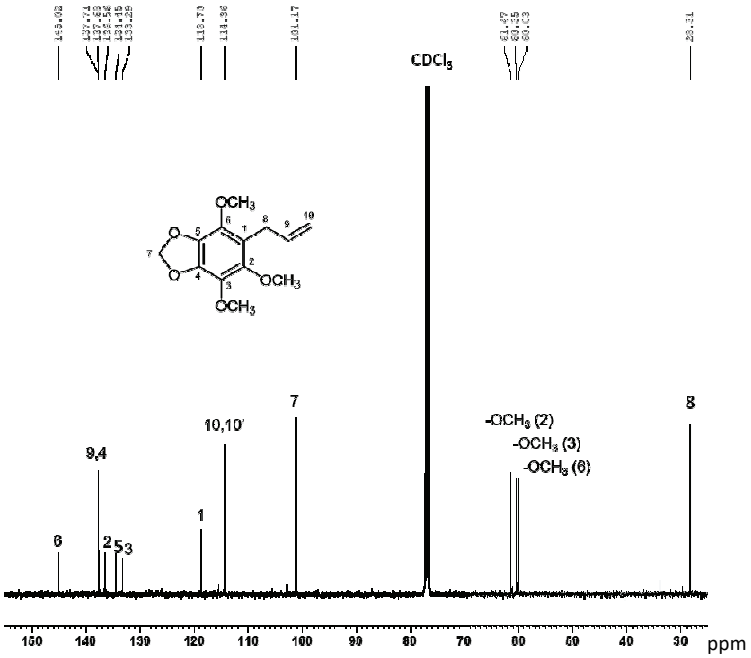


Fig. S-11. ^{1}H-NMR spectrum of compound **2** – nothoapiole (500 MHz, $CDCl_3$).



Fig' S-12. ^{13}C-NMR spectrum of compound **2** – nothoapiole (125 MHz, $CDCl_3$).

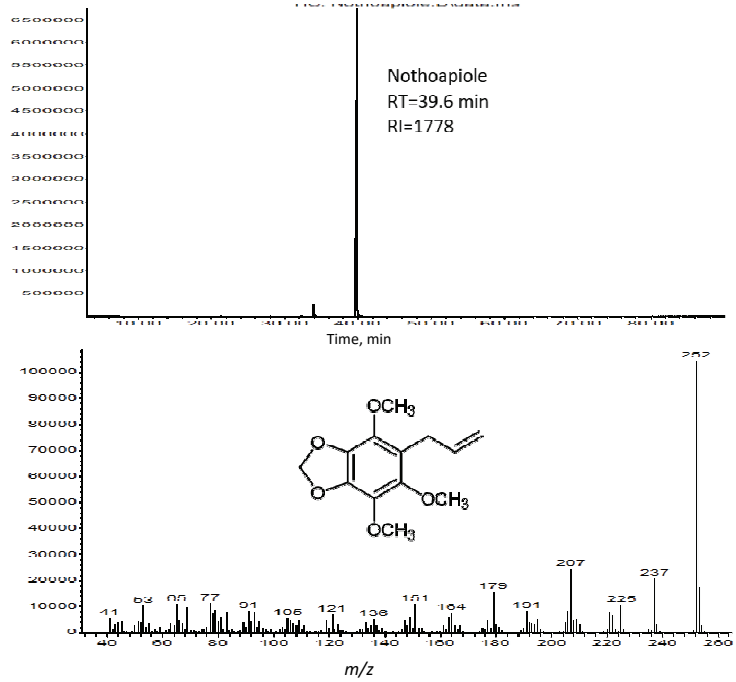


Fig. S 13. GC-MS chromatogram (top) and EI MS spectrum (bottom) of compound **2** (nothoapiole).

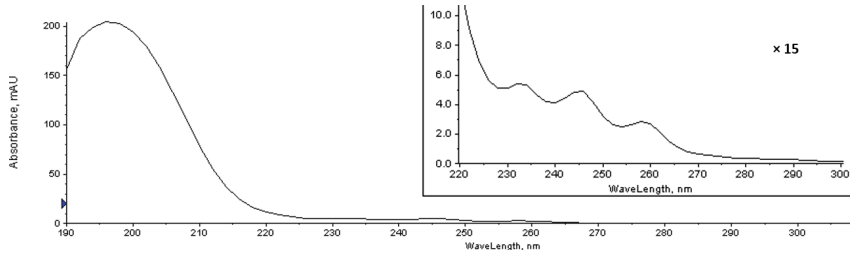


Fig. S 14. UV spectrum of compound 3 – falcarindiol.

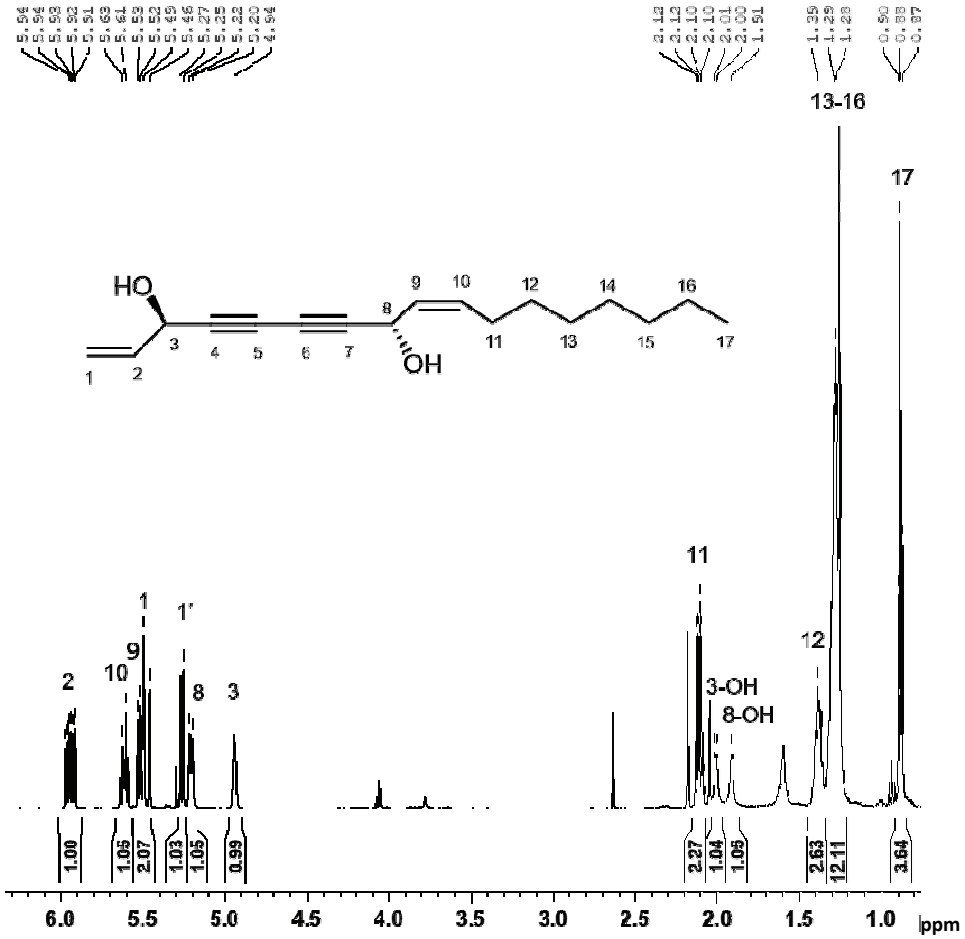


Fig. S-15. ^{1}H-NMR spectrum of compound **3** – falcarindiol (500 MHz, $CDCl_3$).

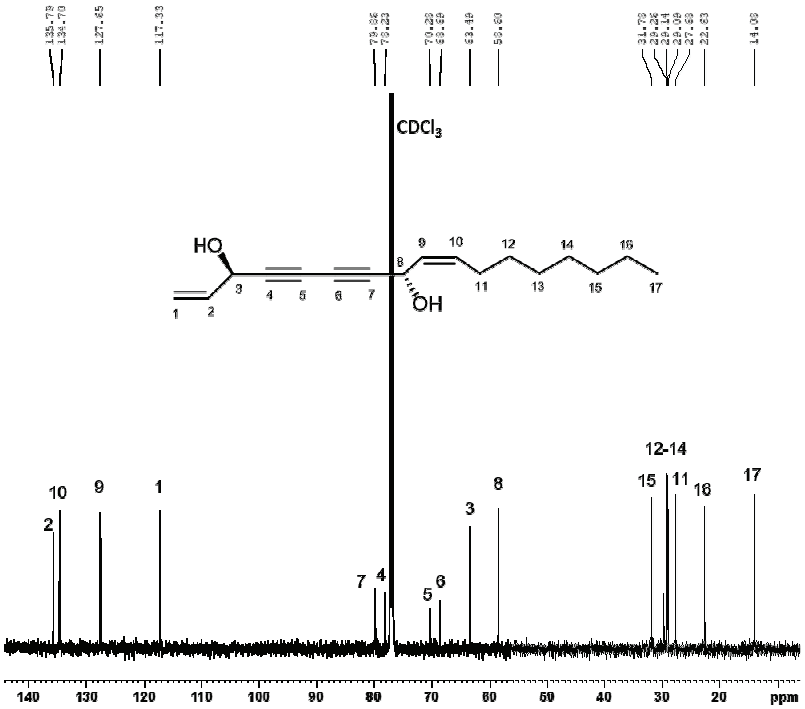


Fig. S-16. ^{13}C-NMR spectrum of compound **3** – falcarindiol (125 MHz, $CDCl_3$).

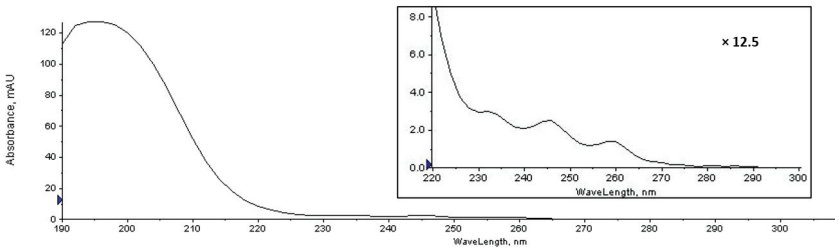


Fig. S-17. UV spectrum of compound **4** – oplopantriol A 18-acetate.

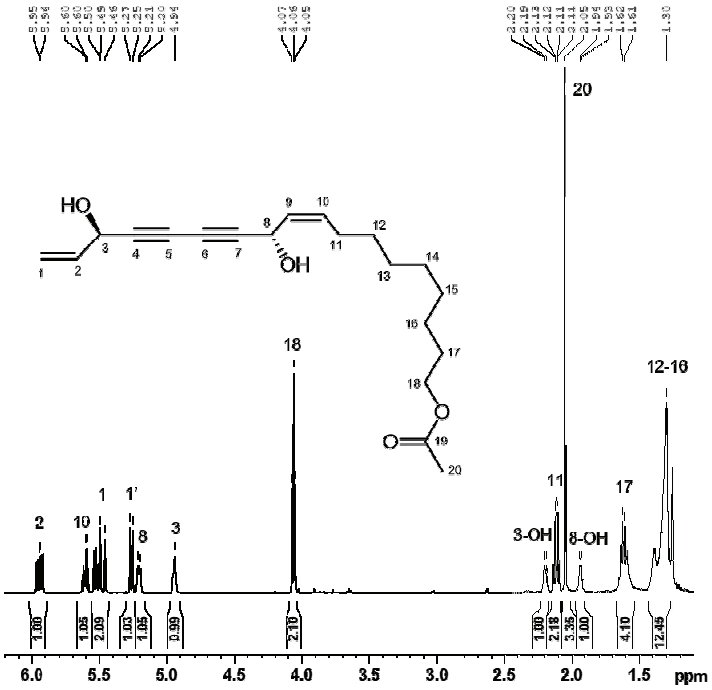


Fig. S-18. ^{1}H-NMR spectrum of compound **4** – oplopantriol A 18-acetate (500 MHz, $CDCl_3$).

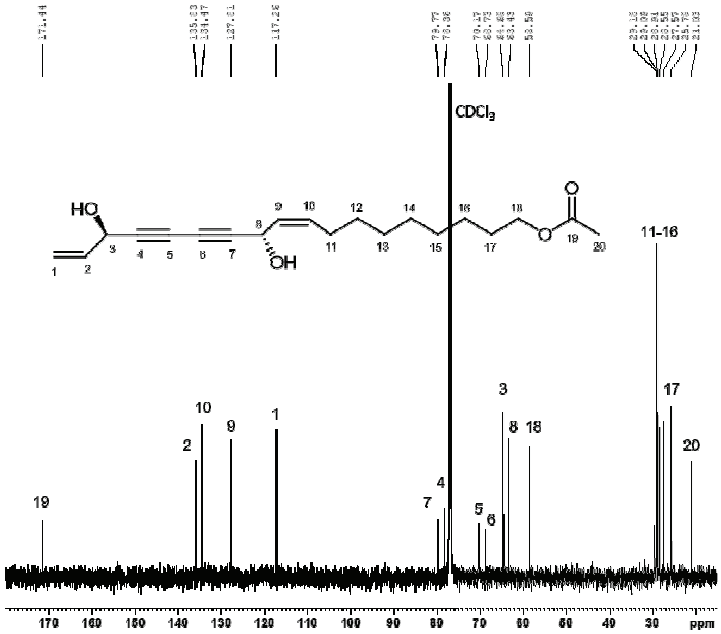
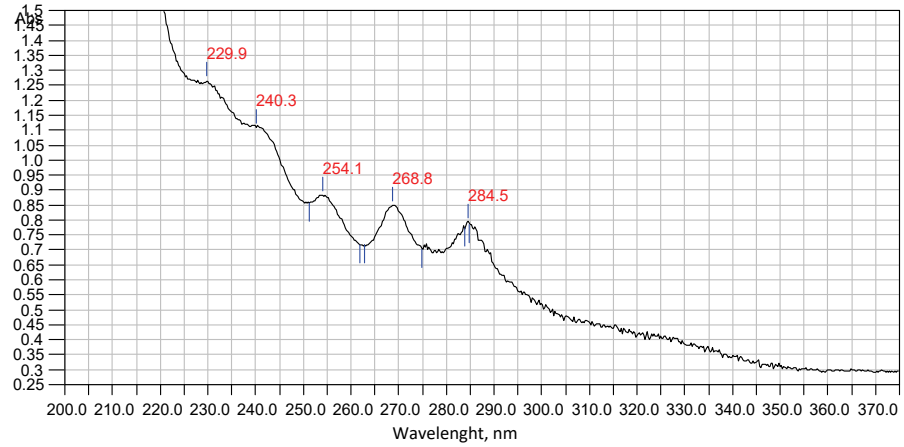


Fig.S-19. ^{13}C-NMR spectrum of compound **4** – oplopantriol A 18-acetate (125 MHz, $CDCl_3$).



Fig; S-20. UV spectrum of compound **5** – dendrotrifidiol.

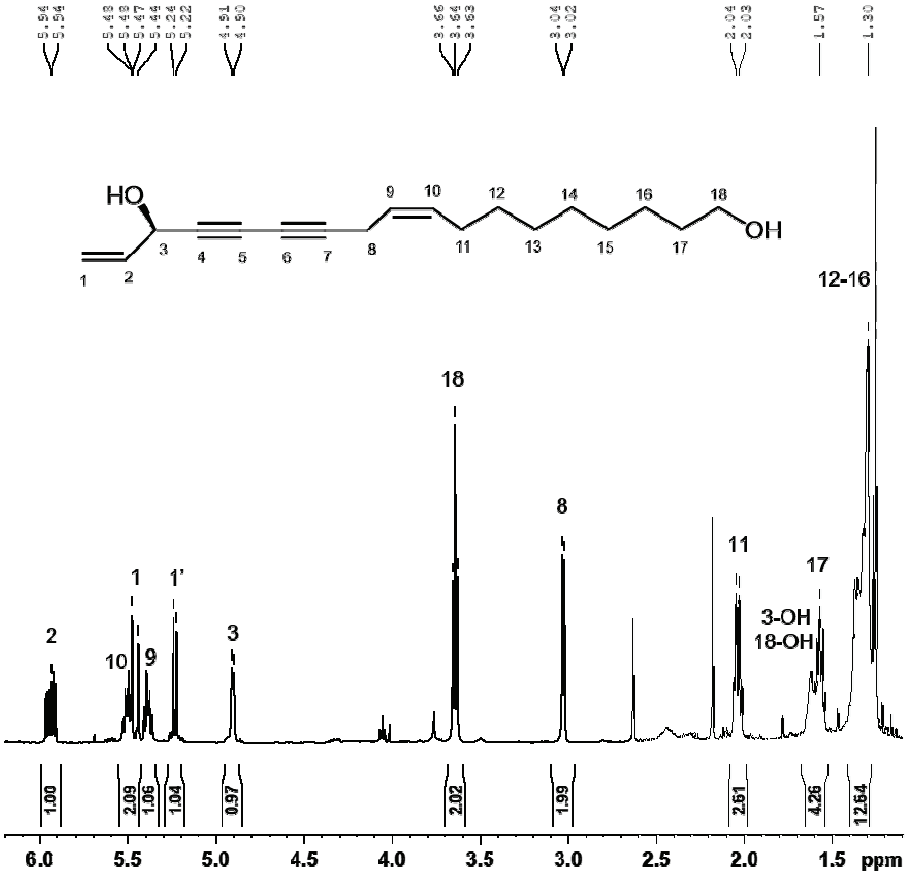


Fig. S-21. ^{1}H-NMR spectrum of compound 5 – dendrotrifidiol (500 MHz, $CDCl_3$).

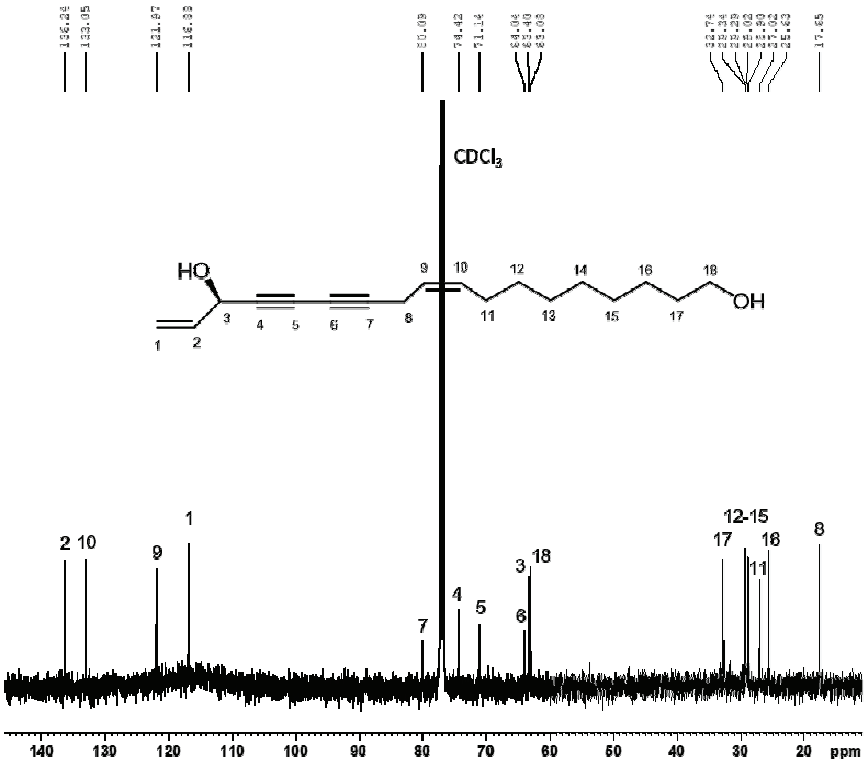


Fig. S-22. ^{13}C-NMR spectrum of compound **5** – dendrotrifidiol (125 MHz, $CDCl_3$).

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Antibacterial properties of thalloid liverworts *Marchantia polymorpha* L., *Conocephalum conicum* (L.) Dum. and *Pellia endiviifolia* (Dicks.) Dumort

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Abstract: The antimicrobial activity of methanol extracts of three thalloid liverworts, *Marchantia polymorpha*, *Conocephalum conicum* and *Pellia endiviifolia* and bis-bibenzyl marchantin A, the most dominant compound in the methanol extract of *M. polymorpha*, have been investigated in this research. ^{1}H-NMR spectroscopy revealed that the *M. polymorpha* and *P. endiviifolia* extracts of liverwort contain terpenes, oils, sugars and bis-bibenzyls, while these specific macrocyclic compounds were absent in the *C. conicum* extract. The antimicrobial potential was tested on eight bacterial strains. Antimicrobial effects of extracts and marchantin A were observed against Gram-positive bacteria, while they showed no effect against Gram-negative bacteria in both methods used – well diffusion and broth microdilution.

Keywords: bis-bibenzyls; marchantin A; extracts; antimicrobial activity; ^{1}H-NMR.

INTRODUCTION

Liverworts are small, slow-growing, terrestrial plants with cosmopolitan distribution and are mostly found in high-humidity habitats. Liverworts can be divided into two types – leafy and thalloid.

With a few exceptions among the vascular plants, liverworts are the only plants that contain specific cellular organelles, oil bodies.[1] Since the oil bodies vary in size, shape, color, number, and distribution, they are important biological markers.[2] These organelles are the sites of the synthesis of different lipophilic mono-, di- and sesquiterpenes and aromatic compounds (bibenzyls, bis-benzyls, benzoates, cinna-

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mates, naphthalenes, isocoumarins). Some liverworts emit a specific smell and have an intense bitter taste with the role in repelling herbivores and insects. They are resistant to bacteria and micromycetes, and show plant protection against UV radiation.[3]

Secondary metabolites derived from liverworts, especially terpenoids and aromatic compounds, showed diverse biological activities – antibacterial, antifungal and antioxidant, cytotoxic and anti-HIV-1, and they are known as enzyme inhibitors, as well as cardiotonic and vasopressin antagonists.[3]

Resistance of microorganisms to synthetic antibiotics is increasing. This problem has led to increased interest in natural products of plant origin as an alternative in the fight against bacterial infections. Research in the last two decades has led to the discovery of a large number of natural products showing a wide range of activities against various pathogens.[4]

The main types of chemical compounds and the antimicrobial activity of three thalloid liverwort extracts, *Marchantia polymorpha*, *Conocephalum conicum* and *Pellia endiviifolia*, were examined in this study.

EXPERIMENTAL

Plant materials

Marchantia polymorpha L. was collected at the Kopaonik Mountain, *Conocephalum conicum* (L.) Dum. at Petnica cave, *Pellia endiviifolia* (Dicks.) Dumort. at Bajina Bašta, all localities in Serbia, and identified by M. V., Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade, Serbia. Voucher specimens of these species, No.17504, No.17762 and No.17503, respectively, are deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade (BEOU). The collected material was air-dried, then packed in paper bags and stored in a dry and dark place at room temperature until further use. Marchantin A was kindly obtained from Yoshinori Asakawa who worked on *M. polymorpha* for almost 50 years and collected a large amount of this compound[5] and its purity was checked by ^{1}H-NMR.

Bacterial strains and growth conditions

Examination of the antibacterial activity of liverworts' extracts was conducted on four Gram-positive, *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19111), *Bacillus subtilis* (ATCC 6633) and *Clavibacter michiganensis* (plant tissue isolate), and four Gram-negative strains, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Pseudomonas syringae* (CFBP 2473) and *Xanthomonas arboricola* (plant tissue isolate). The bacterial strains were cultured in MHB and MHA (Mueller–Hinton broth and agar, HiMedia, Mumbai, India), except for *L. monocytogenes* that was cultured in BHI broth (Brain−Heart Infusion, Biomedics, Madrid, Spain). Incubation lasted 24 h at a temperature of 37 °C, except for *P. syringae*, *X. arboricola* and *C. michiganensis* that were grown at 30 °C. Bacterial suspensions were adjusted to McFarland standard turbidity (0.5) (BioMérieux, Marcy-l'Étoile, France), which corresponds to 10^7–10^8 CFU mL^{-1}.

Preparation of the methanol extract

For chemical analysis, dried and ground plant material (5 g) was extracted in 50 mL of methanol (MeOH). The extraction took place in the dark for 24 h. Extraction in the first and

last hour of the scheduled time was performed in an ultrasonic bath. After filtration and washing with MeOH, the extracts were evaporated using a rotary vacuum evaporator (Laborota 4001, Heidolph) at 40 °C. The yields of the obtained extracts were: *M. polymorpha* – 0.080 g (8.0 %), *C. conicum* – 0.066 g (6.6 %) and *P. endiviifolia* – 0.036 g (3.6 %). The extracts were packed in vials and stored at 4 °C until use.

Chemical analysis

Chemical analysis of MeOH extract was performed using nuclear magnetic resonance at the Faculty of Chemistry, University of Belgrade. The ^{1}H-NMR spectra of the MeOH extracts were recorded on a Bruker Avance III 500 spectrometer at 500.26 MHz in CD_3OD as the solvent, and TMS (tetramethylsilane) as the reference compound.

Determination of the antimicrobial activity

Well diffusion method. The well diffusion method was performed according to Dimkic *et al.*,[6] and used for the screening of the antibacterial activity of liverwort extracts. Sterile molds for the wells (5 mm in diameter) were placed with sterile tweezers on the MHA which was used as the solid medium. The MHA/BHI soft agar was melted, cooled to 47 °C, inoculated with 100 µL of bacterial suspension and poured onto MHA plates. After solidification, the well molds were pulled out with sterile tweezers thus creating wells. Extracts of *M. polymorpha*, *C. conicum*, *P. endiviifolia*, and marchantin A were dissolved in DMSO solvent and 10 µL of each solution was added to the wells. The antibiotic streptomycin was used as the positive control. The Petri dishes were incubated for 24 h at 37 °C, except for *P. syringae*, *X. arboricola* and *C. michiganensis* that were incubated at 30 °C. The results were analyzed by measuring the diameter of the bacterial growth inhibition zone (mm).

MIC assay. The minimum inhibitory concentrations (*MIC*) and minimum bactericidal concentrations (*MBC*) of MeOH extracts of *M. polymorpha*, *C. conicum*, *P. endiviifolia*, and marchantin A dissolved in DMSO solvent were tested using the broth microdilution method.[7] Two-fold serial dilutions with MHB medium in 96-well microtiter plates were performed, apart from *L. monocytogenes*, for which BHI medium was used. The final concentrations of all three tested extracts were 5 mg mL^{-1} (range of tested concentrations were 5–0.039 mg mL^{-1}), while for marchantin A, the concentration was 1 mg mL^{-1} (range of tested concentrations were 1–0.008 mg mL^{-1}). The final concentration of DMSO as the solvent was 10 %. The antibiotic streptomycin (Sigma–Aldrich, Carlsbad, CA, USA) was tested as the positive control in a final concentration 0.2 mg mL^{-1}. All dilutions were performed in duplicate. Each well, except for the sterility control, was inoculated with 20 µL of bacterial culture (approx. 10^8 CFU mL^{-1}), reaching a final volume of 200 µL. Finally, 22 µL of resazurin (0.675 mg mL^{-1}) was added to each well. The plates were incubated for 24 h at 37 °C for all strains except for *P. syringae*, *X. arboricola* and *C. mitchiganensis* that were incubated at 30 °C. The lowest concentration that showed no change in the resazurin color was defined as the *MIC*. The MBC was determined by sub-culturing the test dilutions from each well without color change on agar plates and incubating for 18–24 h, and the lowest concentration that did not show any bacterial growth was defined as the *MBC* value.

RESULTS AND DISCUSSION

Chemical characterization

Based on the ^{1}H-NMR analyses, it was concluded that the MeOH extract of *M. polymorpha* contained terpenes, oils, sugars (free or bound as glycosides), and

bis-bibenzyls (marchantin A as one of the most dominant) (Fig. 1) as the main groups of chemical compounds.

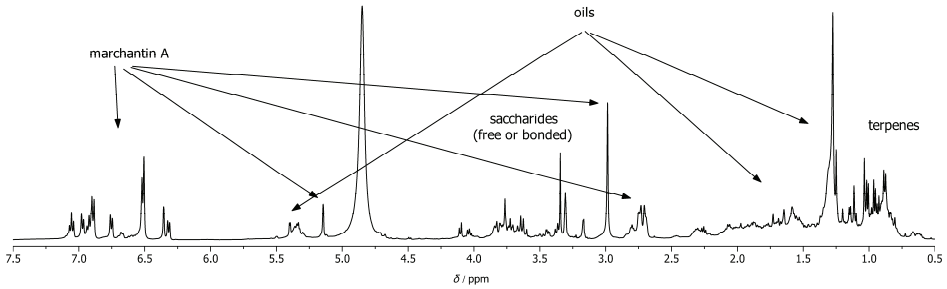

Fig. 1. ^{1}H-NMR spectrum of the *M. polymorpha* MeOH extract.

Marchantin A (Figs. 2 and 3) is a cyclic bis-bibenzyl previously isolated from different *Marchantia* species, *Plagiochasma appendiculatum* and *Wiesnerella denudata.* This is the most common bis-bibenzyl in *M. polymorpha*.[5] The content of marchantin A can be up to 60 g kg^{-1} of plant material.[5] The ^{1}H-NMR spectrum of the present methanol extract of *M. polymorpha* showed almost only marchantin A in the aromatic part, suggesting a high amount of this bibenzyl (Fig. 2).

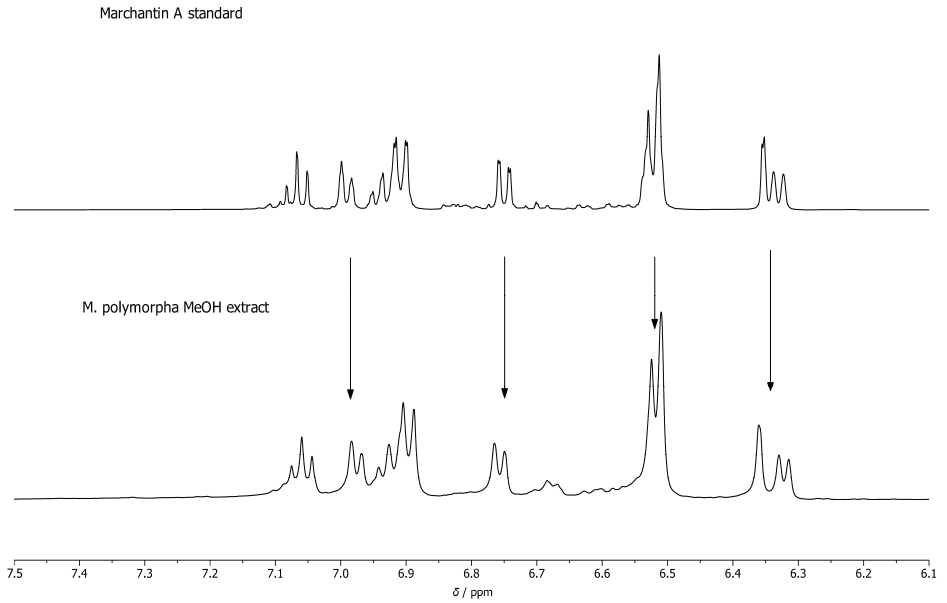

Fig. 2. Matched aromatic parts of the ^{1}H-NMR spectra of MeOH extracts *M. polymorpha* and standard marchantin A.

Based on the ^{1}H-NMR spectrum, it could be concluded that the MeOH extract of *C. conicum* contained terpenes, oils, and sugars (free or bound as glycosides, Fig. 4). According the literature, mainly monoterpenic esters,[8] sesquiterpene lactones[9] and phenethyl glycosides[10] were isolated from this liverwort, but not macrocyclic bis-bibenzyls. On the other hand, species from the same genus, *C. japonicum*, synthesized macrocyclic bis-bibenzyls, perrottetin E, isoriccardin C, marchantin A, marchantin E, marchantin C and isomarchantin C.[11]

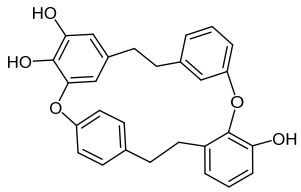

Fig. 3. Chemical structure of marchantin A.

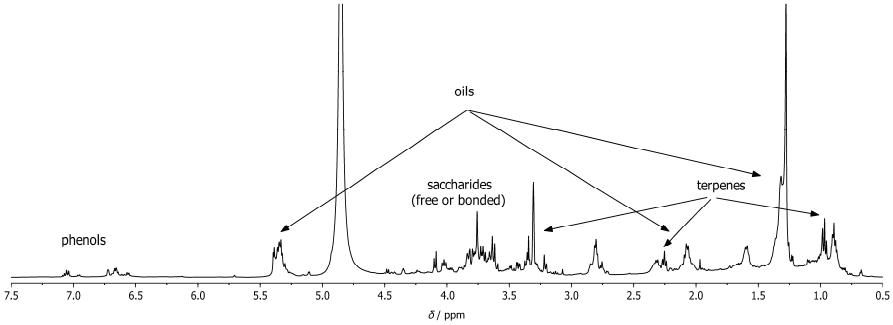

Fig. 4. ^{1}H-NMR spectrum of a MeOH extract of *C. conicum*.

According to the ^{1}H-NMR analyses, it could be proposed that the MeOH extract of *P. endiviifolia* contained terpenes, oils, sugars (free or bound as glycosides), and bis-bibenzyls as the main groups of chemical compounds (Fig. 5). Previously, few macrocyclic bis-bibenzyls, mainly perrotetins, have been isolated from the dichloromethane/methanol extract of *P. endiviifolia.*[12,13]

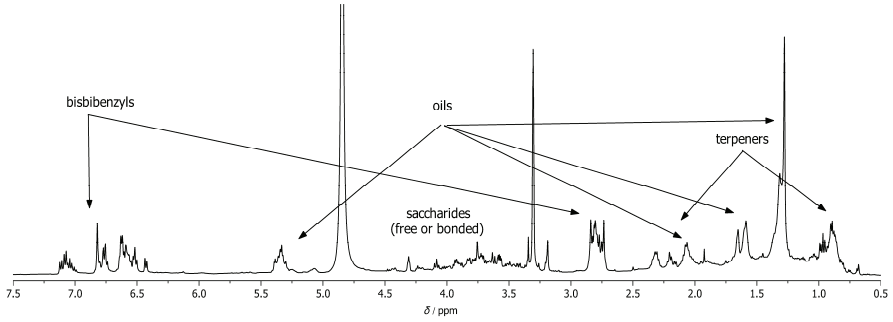

Fig 5. ^{1}H-NMR spectrum of a MeOH extract of *P. endiviifolia*.

Antimicrobial activity of the liverwort extracts

The well diffusion test was used for the initial screening of the antibacterial activity of the liverworts extracts and one active compound marchantin A. Based on the obtained results (Table I), Gram-positive bacteria, except *C. michigenesis*, were sensitive to the tested extracts and marchantin A, while Gram-negative bacteria were resistant to all the tested extracts and the compound. The diameter of the inhibition zone for *B. subtilis*, *S. aureus*, and *L. monocytogenes* ranged from 10–14 mm. The most effective antimicrobial activity was shown by the *M. polymorpha* extract against *B. subtilis* (inhibition zone 14 mm) (Table I and Fig. 6).

TABLE I. The effect of *M. polymorpha*, *C. conicum*, *P. endiviifolia* extracts and marchantin A on the tested bacteria (well diffusion method); NA – no activity

Bacterial culture	Diameter of the inhibition zone, mm
Bacillus subtilis (ATCC 6633)	–
Marchantia polymorpha	14
Conocephalum conicum	NA
Pellia endiviifolia	13
Marchantin A	13
Staphylococus aureus (ATCC 25923)	–
Marchantia polymorpha	11
Conocephalum conicum	NA
Pellia endiviifolia	10
Marchantin A	11
Listeria monocytogenes (ATCC 19111)	–
Marchantia polymorpha	13
Conocephalum conicum	NA
Pellia endiviifolia	12
Marchantin A	12

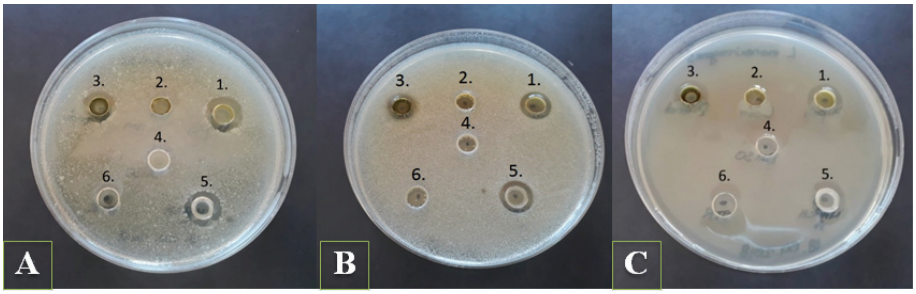

Fig. 6. Inhibition zones: A) *Bacillus subtillis*; B) *Staphylococcus aureus*; C) *Listeria monocytogenes*; 1. *Marchantia polymorpha* MeOH extract; 2. *Conocephalum conicum* MeOH extract; 3. *Pellia endiviifolia* MeOH extract; 4. DMSO; 5. marchantin A; 6. streptomycin.

The MeOH extracts of *M. polymorpha*, *C. conicum*, *P. endiviifolia* and marchantin A used in the *MIC* assay had no antimicrobial effect against Gram-negative bacteria strains. On the other hand, antimicrobial effects were determined against

Gram-positive bacteria (Table II), with the minimum inhibitory concentration (*MIC*) ranging from 0.062 to 5 mg mL^{-1}, and the minimum bactericidal concentration (*MBC*) from 1 to 5 mg mL^{-1}. The most promising antibacterial activity was shown towards *S. aureus* by marchantin A (*MIC* 0.062 mg mL^{-1}). Marchantin A also showed antibacterial effects against *L. monocytogenes* and *B. subtilis.* Both *M. polymorpha* and *P. endiviifolia* extracts showed antibacterial effects against *S. aureus* and *L. monocytogenes.* The antibacterial activity of the methanol extract of *M. polymorpha* cannot be fully ascribed to marchantin A, although it is highly abundant (Fig. 2), since terpenes can contribute to the antibacterial activity.[3] The scientific literature reports various studies in which saccharides have beneficial effects on bacterial control.[14,15] Synergistic effects among the components may also be responsible for the activity.

However, the *C. conicum* extract did not show antibacterial activity against the microorganisms tested. It may be explained by absence of bis-bibenzyls, proved in literature as good antimicrobial agents. Minimum inhibitory concentrations ranging from 0.00625–0.025 mg mL^{-1} and MBC ranging from 0.001562 to 0.025 mg mL^{-1}, proved that tested Gram-positive bacteria were more sensitive to the antibiotic streptomycin than liverwort's extracts and marchantin A (Table II).

TABLE II. Antimicrobial activity of *M. polymorpha, C. conicum, P. endiviifolia* MeOH extracts, marchantin A, and streptomycin on different strains of Gram-positive bacteria

Activity parameter	*S. aureus* (ATCC 25923)	*C. michiganensis* (plant tissue isolate)	*L. monocytogenes* (ATCC 19111)	*B. subtilis* (ATCC 6633)
Marchantia polymorpha				
MIC / mg mL^{-1}	0.156	–	2.50	–
MBC / mg mL^{-1}	–	–	–	–
Conocephalum conicum				
MIC / mg mL^{-1}	–	–	–	–
MBC / mg mL^{-1}	–	–	–	–
Pellia endiviifolia				
MIC / mg mL^{-1}	0.315	–	5.00	–
MBC / mg mL^{-1}	5.00	–	–	–
Marchantin A				
MIC / mg mL^{-1}	0.062	–	0.5–1.00[a]	1.00
MBC / mg mL^{-1}	–	–	1.00	–
Streptomycin				
MIC / mg mL^{-1}	0.025	0.00625	0.0125–0.025[a]	–
MBC / mg mL^{-1}	–	0.025	0.025	0.001562

[a]*MIC* value is between two tested concentrations

In previous investigations bibenzyls from *Radula obconica* showed an antibacterial effect against *B. subtilis* with clear zone, 2.0 cm in disc diffusion assay.[16] Extracts of *Plagiochasma japonica* also show antibacterial activity[17] as

well as *Plagiochila stephensoniana.*[18] Bibenzyl lunularin isolated from *Dumortiera hirsuta* inhibits the growth of *P. aeruginosa* (MIC 64 μg mL^{-1}).[19] Antibacterial effect exhibited extracts of *Cylindrocolea recurvifolia* and *Pleurozia subinflata.*[20] Marchantin A isolated from *M. polymorpha*, *M. tosana*, *M. plicata* and *M. chenopoda* displayed antibacterial activity against *B. cereus* (*MIC* 12.5 μg mL^{-1}), *B. subtilis* (25 μg mL^{-1}), *B. megaterium* (25 μg mL^{-1}), *Staphylococcus aureus* (3.13 – 25 μg mL^{-1}), *Enterobacter cloacae*, *Proteus mirabilis*, *E. coli*, *Salmonella typhimurium* (100 μg mL^{-1}), *Alcaligenes faecalis* (100 μg mL^{-1}), *Acinetobacter calcoaceticus* (6.25 μg mL^{-1}), *Cryptococcus neoformans* (12.5 μg mL^{-1}).[21] Methanol extract of *M. polymorpha* was screened against *E. coli*, *Proteus mirabilis* and *S. aureus* and showed the highest activity against *S. aureus*.[22] Extract of *C. conicum* showed activity against *Pseudomonas aeruginosa*,[23] *P. mirabilis* and *Salmonella sp*.[24] Methanol extract of *P. endiviifolia* inhibited the growth of *B. subtilis* (*MIC* 0.01–0.19 mg mL^{-1}),[23] as well as *S. aureus* (*MIC* 1.0 to 1.25 mg mL^{-1}).[26] Nikolajeva *et al.*[27] investigated the aqueous extract of *M. polymorpha* and concluded no influence on the growth of *S. aureus*, although in literature data can be found about the antibacterial influence of this liverwort species on Gram–positive bacteria among others.[25] This is contrary to investigation of Kamory *et al*.[28] where is highlighted effectiveness of marchantin A on Gram–negative strains *Pseudomonas aeruginosa* 170006 (*MIC* = 85.4 nM), and *Pasteurella multocida* 96101 (*MIC* = 4.5 nM). This research also confirms effectiveness of marchantin A on Gram–positive strains (*Streptococcus viridans*, *S. pyogenes*, *S. faecalis* and *S. aureus*),[28] which is in accordance with results obtained in our study. The methanol and flavonoid extracts of *M. polymorpha* showed the highest antimicrobial activity against *S. aureus* (inhibition zones 20.6 and 19.6 mm, *MIC* 0.281 and 0.312 mg mL^{-1}, *MBC* 1.125 and 0.312 mg mL^{-1}, respectively), among three bacterial and four fungal strains tested.[22]

CONCLUSION

^{1}H-NMR analysis indicate the presence of terpenes, oils, sugars, and bis-bibenzyls in the methanol extracts of *M. polymorpha* and *P. endiviifolia* and absence of bis-bibenzyls in *C. conicum* extract. Methanol extracts of *M. polymorpha* and *P. endiviifolia* and marchantin A exhibited antimicrobial activity against Gram-positive bacteria which was confirmed by well diffusion and microdilution methods. The most promising antimicrobial effect was shown by marchantin A against *Staphylococcus aureus.* Methanol extract of *C. conicum* did not show antimicrobial activity against the tested bacterial strains. Marchantin A – the dominant compound in *M. polymorpha*, fulfilled literature data that it possesses significant antibacterial activity. In addition, Gram-positive bacteria showed more sensitivity to the liverwort extracts tested than Gram-negative ones.

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ИЗВОД

АНТИБАКТЕРИЈСКА СВОЈСТВА ТАЛУСНИХ ЈЕТРЕЊАЧА *Marchantia polymorpha* L., *Conocephalum conicum* (L.) DUM. И *Pellia endiviifolia* (DICKS.) DUMORT.

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У овом раду испитиван је хемијски састав и антибактеријска активност метанолних екстраката три јетрењаче, *Marchantia polymorpha*, *Conocephalum conicum* и *Pellia endiviifolia* и бис-бибензила маршанцина А, доминантне компоненте у метанолном екстракту *M. polymorpha*. ^{1}H-NMR спектроскопија је показала присуство терпена, уља, шећера и бис-бибензила у екстрактима *M. polymorpha* и *P. endiviifolia*, док екстракт *C. conicum* не садржи специфична макроциклична једињења – бис-бибензиле. Антимикробни потенцијал је тестиран на осам бактеријских сојева. Антимикробни ефекат маршанцина А уочен је на све грам позитивне сојеве, док је ефекат изостао код грам негативних сојева у обе тестиране методе – дифузионе методе у бунарима и микродилуционе методе у хранљивом бујону.

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Variations in the composition of essential oils of selected *Artemisia* species as a function of soil type

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Abstract: Five *Artemisia* species (seven *A. alba* Turra samples and twelve samples of each four remaining species: *A. absinthium* L., *A. annua* L., *A. vulgaris* L. and *A. scoparia* Waldst. & Kit.) from Serbia were studied from the aspect of essential oil chemical composition, and potential correlations between essential oil composition with soil type determined using World Reference Base for Soil Resources (WRB). A great variety in essential oil composition was observed for *A. alba*, *A. absinthium* and *A. vulgaris* samples, while in the case of *A. annua*, as well as *A. scoparia*, the composition of the examined essential oils was more uniform. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) showed that there is no significant effect of soil type on the *Artemisia* essential oil composition while Mantel test showed that there is a correlation between samples within *A. vulgaris*, as well as *A. scoparia* and the geographical distances of the localities from which these samples were collected.

Keywords: *Artemisia alba* Turra; *Artemisia annua* L.; *Artemisia absinthium* L.; *Artemisia vulgaris* L.; *Artemisia scoparia* Waldst. *et* Kit.; gas chromatography––mass spectrometry.

INTRODUCTION

The worldwide known genus *Artemisia* L., from the tribe Anthemideae, family Asteraceae, encompasses plants significant to the medicine, cosmetic, food and beverage industry, as well as to ethnopharmacology. Many representatives of this genus have found their application in traditional medicine in treatment of diseases such as malaria, hepatitis, cancer, inflammation and infect-

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ions by fungi, bacteria, and viruses.[1] Two perhaps the most renowned members of the genus *A. annua* and *A. absinthium* are traditionally used in many cultures. *A. annua* is used as the source of antimalarial drug, treatment for fever, tuberculosis, dysentery,[2,3] while *A. absinthium* found application against gastrointestinal diseases, as anthelmintic.[4,5] *A. vulgaris*, for example, has been used for treating gynecological complications, antibacterial and antifungal infections.[6] Pharmacological studies revealed that *A. scoparia* manifests activity against inflammation, antitumor, analgesic, and protective effects on the liver.[7] *A. alba* has been deemed to heal burns and contusions.[8]

The essential oils of these plants show various healing and health-beneficial properties, biological activities and represent a source of a huge number of active components. For example, some of the proven activities would be the antimicrobial activity of *A. alba* essential oil,[9] the antipathogenic activity of *A. annua* essential oil,[10] the acaricidal activity of the essential oil from *A. absinthium*,[11] antifungal and antibacterial activity of *A. vulgaris* essential oil,[12] and the pesticidal activity of *A. scoparia* essential oil.[13]

Five above-mentioned species, members of this genus that are autochthonous to the Serbia, were collected (seven samples of *A. alba*, and twelve samples of each of the four remaining species: *A. absinthium*, *A. annua*, *A. vulgaris*, and *A. scoparia*), and studied from the aspect of essential oil chemical composition, and furthermore, statistically for potential correlations between essential oil composition and soil type determined using World Reference Base for Soil Resources (WRB). We deemed it necessary to contribute to a better and deeper understanding of correlations between the essential oil (EOs) composition and percentage of components with soil for such widely used species, for the purpose of assisting the manufacturers of drugs. Since the primary goal was to determine the dependence of the composition of the essential oils and the type of soil, no comparison was made with the published results on the composition of the essential oils of the examined species. Although there are papers about influence of various exogenous factors on the chemical composition of EOs such as altitude and soil characteristics[14], humidity[15] as well as geographical origin,[16] to the best of our knowledge, these are the first presented results on the correlation of the composition of *Artemisia* species EOs and soil type according to WRB classification.

EXPERIMENTAL

Plant material

Five selected *Artemisia* species were harvested at the blooming stage (seven specimens of *A. alba* Turra and twelve specimens of each *A. absinthium* L., *A. annua* L., *A. vulgaris* L. and *A. scoparia* Waldst. et Kit.). Voucher specimens have been deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Serbia. The labels of the samples, voucher specimen numbers, as well as habitat

specifications (locality, longitude, latitude, and soil type), are given in Table S-I of the Supplementary material to this paper.

Essential oil isolation

The aboveground part (buds, flowers, leaves and stems) of dry plant samples were hydrodistilled in a Clevenger-type apparatus for 2.5 h. The obtained essential oils were dried over anhydrous magnesium sulfate and analyzed by GC/MS. The essential oil yields were calculated using Eq. (1) and presented in Table S-I. In all experiments, the initial weight of the plant material was 500 g, so the yields are given as the mass (g) of the obtained essential oil per 500 g of plant material:

$$\text{Yield} = 100\frac{m\,(\text{essential oil})}{m\,(\text{dry plant material})} \quad (1)$$

GC/MS analysis

The essential oil samples were analyzed in triplicate by a 7890/7000B GC/MS/MS triple quadrupole system (Agilent Technologies, USA). The fused silica capillary column HP-5 MS (5 % phenylmethyl siloxane, 30 m×0.25 mm, film thickness 0.25 μm) was used. The injector and interface operated at 230 and 300 °C, respectively. Temperature program: from 45 to 290 °C at a heating rate of 4 °C/min. The carrier gas was helium with a flow of 1.0 mL/min. The injection volume of sample solutions was 1 μL and the split ratio was adjusted at 40:1. Post-run: back flash for 1.89 min, at 280 °C, with helium pressure of 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 40–440 Da, scan time 0.32 s. The percentage composition of the samples was computed from the total ion chromatogram peak areas without any corrections.

Identification of volatile compounds

Components of the essential oils were identified by comparison of their linear retention indices (relative to C8–C40 *n*-alkanes on the HP-5MS column) with literature values and their MS with those from Wiley 6, NIST11 and Agilent Mass Hunter Workstation B.06.00 software by the application of the Automated Mass Spectral Deconvolution and Identification System (AMDIS software), ver. 2.1 (DTRA/NIST, 2011).

Statistical analysis

For explaining the complex correlations between essential oil components and the WRB soil types (Pellic Vertisol – pv, Rendzic Leptosol – rl, Calcaric Fluvisol – cf; Haplic Leptosol – hlp, Dystric Cambisol – dc, Calcaric Phaeozem – cp, Eutric Cambisol – ec, Haplic Luvisol – hl), three statistical methods were used in XLSTAT 2021 (Addinsoft, 2021): principal component analysis (PCA), agglomerative hierarchical clustering (AHC) and Mantel test.

Principal component analysis. PCA enables the grouping of samples using reduced variables, obtained after mathematical transformations. Those transformations are performed to provide better insight into groupings and correlations between analyzed samples. Components with Eigenvalues are selected for the interpretation of results according to Kaisers' rule.[17] The distribution was determined by the Kolmogorov Smirnov test, with the significance level $\alpha = 0.05$. The original datasets were subjected to Grubbs' test for outliers before the application of PCA,[18] and the outliers were discarded from the used datasets.

Agglomerative hierarchical clustering. Agglomerative hierarchical clustering of the standardized variables was performed using the Ward method. The squared Euclidean distance was observed as a measure of the proximity between the samples.

Mantel test. Mantel test is used for overcoming the problem often encountered in an attempt to explain the relationships between species and environment, useful for testing the linear correlation between two proximity matrices (dissimilarity or similarity).

RESULTS AND DISCUSSION

Chemical composition

The results of the GC/MS analysis of the essential oil compositions are given in Tables S-II, S-IV, S-VI, S-VIII and S-X, while the quantities of different classes of the compounds are presented in Tables S-III, S-V, S-VII, S-IX and S-XI, all of the Supplementary material.

In the case of *A. alba* 220 components were identified in total, 81 of them were present only in one sample, while only 4 components: camphene (tr – 3.3 %), 1,8-cineole (1.9–19.7 %), camphor (1.6–51.6 %), and germacrene D (2.1––21.3 %,) were found in all seven samples. Worthy of mention is the presence of triquinane sesquiterpenes in AA2 (56.1 %) including silphiperfol-5-en-3-one A (35.0 %) as the main component. Sesquiterpenoids were the major class of compounds in two samples of *A. alba* while in the remaining five samples monoterpenoids were predominant.

A total of 234 components were identified in *A. absinthium* samples. Only 5 compounds were present in all samples with a percentage representation ≥1 %, sabinene (5.7–18.9 %), *o*-cymene (2.1–8.8 %), linalool (2.1–29.8 %), terpinen-4-ol (3.0–10.3 %) and lavandulyl isovalerate (1.2–5.7 %). In contrast, *trans*-thujone was present in traces in 5 samples while in 3 samples it was the most represented constituent (20.6–48.2 %). The situation is similar with (*Z*)-epoxy-ocimene, it was the component with the highest representation in 3 samples while in 3 samples it was not detected. In all *A. absinthium* samples, monoterpenoids were a highly dominant class of compounds, particularly oxygenated monoterpenes.

In total 153 components were identified in *A. annua* samples, 24 of them were present only in one sample (all present in traces), even 58 in all samples. The first or second represented in all samples were artemisia ketone (6.9–49.8 %), *α*-pinene (3.8–23.3 %) and camphor (2.5–18.8 %). The component present in the amount of over 5 % in all samples was 1,8-cineole. Artemisia alcohol, *trans*-pinocarveol and pinocarvone were also present in all samples but in the amount of over 1 %. As in *A. absinthium*, in all *A. annua* samples monoterpenoids were the dominant class of compounds, especially oxygenated monoterpenes.

In all samples of *A. vulgaris* a total of 315 components were found, 78 of them were present only in one sample, while 40 were present in all samples. In contrast to the relatively uniform composition of *A. annua*, in terms of the most represented compounds, composition of *A. vulgaris* essential oils was very diverse. An extreme example is the content of *cis*-chrysanthenyl acetate which was the most representative component in 4 samples (79.4–24.8 %), while in 3 samples it was present in traces, and in 3 samples it was not detected at all.

Davanon showed a similar distribution, three samples contained davanone in the highest amount (59.8–27.4 %) while in 6 samples it was not detected at all. In eight samples monoterpenoids, were the major class of compounds, while sesquiterpenoids were the major class in four samples.

A total of 156 components were identified in *A. scoparia* samples, 30 of them were present only in one sample, 43 in all samples among them *β*-pinene (4.7–14,8 %), limonene (2.0–4,2 %), (*Z*)-*β*-ocimene (4.6–8.5 %), and *γ*-terpinene (1.4 %–4.0 %), were present in percentage higher than 1 %. In all analysed samples the main component was capillene, followed by 2,4-pentadiynyl-benzene. The major class of compounds were phenyldiacetylenes with a representation of 57 % and more.

Statistical analysis regarding compound percentage in examined EOs

All statistical analysis were done with the same sets of data as for PCA, after normalization and with omitted outliers.

Principal component analysis (PCA). Kolmogorov–Smirnov test was used to check the normal distribution of the original dataset related to the percentages of compounds determined using GC/MS for each *Artemisia* species separately. None of the original datasets, except for *A. scoparia*, were normally distributed, so different mathematical functions were used in an attempt to normalize the data. The Sin function gave satisfying results, most data showed normal distribution, so transformed data were used for further work. Grubbs' test showed outliers for all datasets. The outliers were omitted from the data matrix and PCA analysis for each *Artemisia* species was performed. Due to unsatisfactory values according to Kaiser's criterion, each correlation matrix was subjected to the Varimax rotation with Kaiser normalization. Supplementary Material contains plots for samples from each investigated *Artemisia* species (Figs. S-1–S-5 of the Supplementary material), as well as tables with factor loadings after Varimax rotation (Tables S--XII–S-XVI).

In *A. alba* case, the first five factors explain more than 95 % of variability, with terpinolene, benzene acetaldehyde, *trans*-piperitol, davanone, bicyclogermacrene, *α*-eudesmol, terpinen-4-ol, *cis*-pinocamphone, *γ*-terpinene, chrysanthenone and *cis*-sabinene hydrate contributing the most.

A. alba samples from dc, cf and hlp soil type were grouped on the negative side of the plot, primarily on the basis of percentage of filifolone and *trans*-thujone. For samples from rl soil type the regularity in grouping was not observed.

For *A. absinthium* eight factors gave more than 95 % of the cumulative variability. The major contributors were 1-octen-3-ol, geraniol, *α*-fenchene, *n*-nonanal, *trans*-thujone, fragranol, neral, *n*-hexanol, *α*-terpinene, methyl salicylate and (*E*)-2--hexenal. In the case of *A. absinthium* samples, a good grouping of samples from cp soil could be noticed. What most influenced such grouping were monoterpenes

and oxygenated monoterpenes, primarily *α* and *β*-phellandrene, nerol, and neral, as well as terpinene and its derivatives. A solid grouping of samples from pv soil is also noticeable. The quadrant, in which the *x*-axis is positive and the *y*-axis negative, is predominantly determined by *α*-thujene and *trans*-thujone, *o*-cymene and ocimene derivatives.

For *A. annua* seven factors gave more than 95 % of the cumulative variability. Caryophyllene oxide, *trans*-sabinene hydrate, bicyclogermacrene, (*Z*)-jasmone, *α*-thujene, hexyl 2-methyl butyrate, tricyclene, *α*-campholenal, myrtenol, l pinocarvone, *trans*-pinocarveol, propyl 2-methyl butyrate, and *α*-humulene contributed mostly to those factors. In the case of *A. annua* all samples from pv soil were in the part of the plot where the *x*-axis is negative. Samples from the ec soil were located on opposite sides of the plot, and the component which influenced it the most was camphene. Almost all carbonic acid derivatives were located in the plot's quadrant where the *x*-axis was negative and the *y*-axis positive. There was no regularity among the samples from cf soil.

In the case of *A. vulgaris* eight factors gave more than 95 % of the cumulative variability, with terpinen-4-ol, *α*-pinene, 1,8-cineole, eugenol, *cis*-chrysanthenyl acetate, *trans*-thujone, torilenol, germacrene D, iso-3-thujanol, and *trans*--pinocarveol contributed mostly. *Artemisia vulgaris* samples from the same type of soil were on opposite sides of the plot (*e.g.*, specimens from dc, rl or cf). But, even in these cases, some regularity can be noticed. For example, in the case of two samples from cf soil: on the plot, from the opposite side to one sample from cf, it can be seen that the two grouped samples are partially determined by the percentage of *cis*-thujone, while the lone sample is determined by the percentage of *trans*-thujone and iso-3-thujanol. Also, two samples from cf soil were grouped on the basis of germacrene and its derivatives, which are responsible for grouping in the quadrant where the *x*-axis is negative, and the *y*-axis is positive. Furthermore, the position of the dc sample in the negative quadrant is defined by the percentage of chrysanthenone, while in contrast, the sample from dc soil is determined by the percentage of *cis*-chrysanthenol and *cis*-chrysanthenyl acetate. The positive quadrant is determined by both *trans*-and *cis*-sabinene hydrate.

For *A. scoparia* six factors gave more than 95% of the cumulative variability. The main contributors were *β*-pinene, *α*-pinene, sabinene, 2,4-pentadiynyl-benzene, limonene, capillene, 2,6-dimethyl-naphthalene, as well as (*Z*)-*β*-ocimene, *allo*-ocimene, butanoic acid, 2-methyl-2-methoxy-4-(2-propenyl)phenyl ester, *p*-cymene, γ-terpinene, *β*-eudesmol, *α*-humulene, and 1,8-cineole. The samples from cp soil are in opposite quadrants of the plot, and what makes that difference are the percentages of spathulenol, (*E*)-caryophyllene and *α*-humulene. The sample from pv soil, as well as four other samples from cf soils, were isolated in the negative quadrant of the plot due to the presence of butanoic acid, 2-methyl-2-methoxy-

-4-2-propenyl)phenyl ester, which was not identified in the remaining seven samples.

Agglomerative hierarchical clustering (AHC). Agglomerative hierarchical clustering (AHC) of the investigated *Artemisia* species showed three main clusters on all five dendrograms for all species based on the percentages of compounds determined by GC/MS. The same sets of data as for PCA were used (normalized, without outliers). Supplementary material contains dendrograms for samples from each investigated *Artemisia* species (Figs. S-6–S-10). Dendrogram for *A. alba* showed major irregularity in sample distribution. All samples from rl soils are located in separate clusters, yet there are some matches with PCA plots. Dendrogram for *A. absinthium* showed substantially the same as the PCA plot. At the dissimilarity level of 1, a cluster with two samples from pv soil (AB7 and AB12) was isolated, while the remaining sample from the same soil type (AB10) was in a separate cluster. Agglomerative hierarchical clustering analysis results for *A. annua* samples showed excellent grouping of samples from cf soil and interesting separation of four samples from pv soils in two clusters (each cluster contains two samples). Dendrogram of the analysed *A. vulgaris* samples revealed good correlations between cf soil type and EOs composition; three out of four samples are in one cluster. For other samples, no regularity in grouping was observed. In the case of *A. scoparia*, it can be seen that the samples from cp soil are in different clusters, as well as that one of them is in the same cluster with the sample from pv soil.

Mantel test. Mantel test was used to determine if the chemical composition distance between populations of *Artemisia* collected from different soil types is related to the geographical distance. The chemical composition distance is measured as a difference in individual component frequencies among various *Artemisia* samples. The geographical distance is calculated from the distance in longitude and latitude between the sites of interest. Proximity matrices were formed using Euclidean distance, while the Pearson's correlation was used for Mantel test, with significance level 5 %.

Mantel test revealed that the matrices for *A. vulgaris*, as well as for *A. scoparia* correlated as the computed two-tailed *p*-values (0.0273 and 0.0295) are lower than the significance level $\alpha = 0.05$. This means that there is a correlation between different samples within *A. vulgaris*, as well as *A. scoparia* and the geographical distances of the localities from which these samples were collected.

Statistics concerning the percentage of classes of compounds in the tested essential oils

All statistical investigations were done with the same sets of data as for PCA, without transformation (Sin function was done only for *A. scoparia* dataset) and with omitted outliers. Seven classes of compounds were used for statistical analysis

of both *A. alba* and *A. annua* (monoterpene hydrocarbons – M, oxygenated monoterpenes – MO, sesquiterpene hydrocarbons – S, oxygenated sesquiterpenes – SO, phenylpropanoids – PP, carbonic acid derivatives – CD and others – O), while for *A. scoparia* matrix, in addition to the mentioned seven classes of compounds, another class was used (phenyldiacetylenes – P). For *A. absinthium* six classes of compounds took place in the dataset (M, MO, S, SO, CD and O), while for *A. vulgaris* five classes were considered (M, MO, S, SO and PP).

Principal component analysis (PCA). Kolmogorov–Smirnov test was used to check the normal distribution of the original dataset related to the percentages of classes of compounds for each *Artemisia* species separately. All of the original datasets, except for *A. scoparia*, were normally distributed, so original matrixes were used for further tests. The Sin function with *A. scoparia* data showed normal distribution, so the transformed *A. scoparia* matrix was used in the statistical analyses. Grubbs' test showed outliers for all datasets. The outliers were omitted from the data matrix and PCA analysis for each *Artemisia* species, separately, was performed. Supplementary Material contains plots for the samples of each investigated *Artemisia* species (Figs. S-11–S-15).

For *A. alba* three factors gave more than 95 % of the cumulative variability, with S (0.9929), SO (0.9711), MO (–0.9932), M (–0.9382), O (0.9872), PP (0.9749) and CD (0.9479) as contributors to the factors. Biplot showed that samples from rl soil are in three different quadrants. The negative quadrant was determined by S and SO, and on the other hand, the positive one was determined by M.

For *A. absinthium* five factors gave more than 95 % of the cumulative variability. The major contributors to the factors were M (0.9286), MO (–0.6451), CD (0.9318), O (0.9278), SO (0.8387) and S (0.8705). *Artemisia absinthium* biplot differs samples from cp soil from other samples, the same was observed for samples from ec. Samples from cp well separated predominantly because of the percentages of S and SO.

For *A. annua* three factors gave more than 95 % of the cumulative variability. All classes (SO-0.9783, O-0.9242, S-0.7904, M-0.9294, CD-0.8973, MO-0.7793, PP-0.6926) contributed to the factors. Poorly grouped samples from the same soil type could be seen on the biplot. Two samples out of four from cf soil are on the side of the biplot where the *x*-axis is negative and the *y*-axis is positive, opposite to them is one sample from cf, and on the positive quadrant of the plot is the fourth sample. It is noticeable that two samples with cf soil were grouped by the percentage of S. Also, it could be seen that the samples from rl soil are very statistically close, and that proximity is determined by the percentage of PP.

For *A. vulgaris* three factors gave more than 95 % of the cumulative variability. The major contributors were SO (0.9067), MO (–0.8701, PP (0.9920), S (0.7415) and M (0.9213). Pearson's correlation matrix for *A. vulgaris* showed the highest correlation between MO and SO (–0.8592). Poorly grouped samples from

the same soil type could be seen on the biplot. Two samples out of four from cf soil are on the side of the biplot where the *x*-axis is negative and the *y*-axis is positive, opposite to them is one sample from cf, and on the positive quadrant of the plot is the fourth sample. It is noticeable that two samples with cf soil were grouped by the percentage of S. Also, it could be seen that the samples from rl soil are very statistically close, and that proximity is determined by the percentage of PP.

For *A. scoparia* four factors gave more than 95 % of the cumulative variability. The main contributors were MO (0.9632), M (0.7929), S (–0.7614), P (0.9131), O (0.9469), PP (–0.7706), and SO (0.9644). Pearson's correlation matrix for *A. scoparia* showed a very poor correlation between all percentages of classes of compounds. The scattering of samples from cf soil to all quadrants is observed on the biplot, while both samples from cp soil are very close as determined by percentages of M and MO.

Agglomerative hierarchical clustering (AHC). Dendrograms for samples of each investigated *Artemisia* species are given as Figs. S-16–S-20. Agglomerative hierarchical clustering (AHC), of the investigated *Artemisia* species, showed three main clusters for *A. alba*, *A. vulgaris* and *A. scoparia* each based on the percentages of classes of compounds, while for *A. absinthium* and *A annua* AHC grouped four main clusters. AHC analyses for classes of compounds in *A. alba* samples revealed major irregularity in sample distribution, as well as AHC based on the percentages supporting the results obtained by PCA analysis. All samples from rl soils are located in separate clusters. Dendrogram for *A. absinthium* showed a good grouping of samples. Samples from ec and rl soils are grouped in separate clusters, as well as two out of three samples from both pv and cp. AHC analysis for *A. annua* samples showed weak clustering of all samples, four very heterogenic clusters are formed. It is noticeable that the sample from dc soil was separated from all other samples. Dendrogram of the analyzed *A. vulgaris* samples revealed some correlations between cf soil type and percentages of classes of compounds; two out of four samples are in one cluster. Similar obsedvations were made for samples from rl soil. No regularity in clustering was observed for other samples. A similar grouping is on the PCA plot for this plant species. In the case of *A. scoparia*, the samples from cp soil are in the same cluster at a level of dissimilarity 1, contrary to the clustering with individual components as variables. The sample from pv soil was not separated from the samples from cf soil.

Mantel test. Mantel test was used to determine if the classes of compounds distance between populations of *Artemisia* collected from different soil types is related to the geographical distance. Proximity matrices were formed using Euclidean distance, while the Pearson's correlation was used for Mantel test, with significance level 5 %. Mantel test results showed that the classes of compounds distances and geographic distances are not correlated.

CONCLUSION

Gas chromatography/mass spectrometry analysis of fifty-five samples in total (seven samples of the essential oils of *A. alba* and twelve samples of the essential oils of each *A. annua* L., *A. absinthium* L., *A vulgaris* L. and *A. scoparia* Waldst. et Kit.) was done. Plant samples were collected from different soil types, which were determined using World Reference Base for Soil Resources (WRB). A great variety in composition was observed for *A. alba*, *A. absinthium* and *A. vulgaris* samples, while in the case of *A. annua*, as well as *A. scoparia*, the composition of the examined essential oils was more uniform. Principal component analysis and AHC, were used for a testing correlation between EO composition and soil type according to WRB. Both analyses showed that there is no significant effect of soil type on the *Artemisia* essential oil composition. According to Mantel test there is a correlation between samples within *A. vulgaris*, as well as *A. scoparia* and the geographical distances of the localities from which these samples were collected, while harvesting sites' geographic distances are not correlated to the classes of compounds of all examined *Artemisia* essential oils.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11025, or from the corresponding author on request.

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ИЗВОД

ВАРИЈАЦИЈЕ У САСТАВУ ЕТАРСКИХ УЉА ОДАБРАНИХ ВРСТА *Artemisia* У ЗАВИСНОСТИ ОД ТИПА ЗЕМЉИШТА

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Пет врста *Artemisia* (седам узорака *A. alba* Turra и по дванаест узорака сваке од четири преостале врсте: *A. absinthium* L., *A. annua* L., *A. vulgaris* L. и *A. scoparia* Waldst. & Kit.) са територије Србије проучавано је са аспекта хемијског састава етарског уља и потенцијалних корелација између састава етарског уља и типа земљишта, одређеним на основу Светске референтне базе за земљишне ресурсе (WRB). Код узорака *A. alba*, *A. absinthium* и *A. vulgaris* примећена је велика разноликост у погледу састава, док је у случају *A. annua*, као и *A. scoparia*, састав испитиваних етарских уља био униформнији. Анализа главних компоненти (PCA) и агломеративно хијерархијско груписање (AHC) показале су да нема значајног утицаја типа земљишта на састав испитиваних *Artemisia* етраских уља, док је Мантелов тест показао да постоји корелација између узорака унутар *A. vulgaris*, као и *A. scoparia* и географске удаљености локалитета са којих су ови узорци прикупљени.

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SUPPLEMENTARY MATERIAL TO

Variations in the composition of essential oils of selected *Artemisia* species as a function of soil type

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CHEMICAL COMPOSITION

Table S-I. The sample label, voucher specimen number, locality, longitude, latitude, and soil type according to WRB, and the yield of the essential oils

Plant specie	Sample	Voucher	Locality	Longitude, °	Latitude, °	WRB soil type	Yield, %
Artemisia alba Turra	AA1	13296	Kozarica, Dimitrovgrad	22.81958333	43.02902778	Pellic Vertisol (pv)	0.01
	AA2	13312	Niševac, Svrljig	22.1005	43.46488889	Rendzic Leptosol (rl)	0.05
	AA3	13313	Radov Dol, Sićevačka klisura	22.17586111	43.30177778	Calcaric Fluvisol (cf)	0.07
	AA4	13307	NULL, Kopaonik	20.82686111	43.26786111	Haplic Leptosol (hl)	0.11
	AA5	13311	Sudimlja, Kopaonik	20.95252778	43.25325	Dystric Cambisol (dc)	0.04
	AA6	14323	Put Vlkovija, Mojinci	22.90833333	43.08344444	Rendzic Leptosol (rl)	0.01
	AA7	14324	Rosomač	22.84122222	43.1535	Rendzic Leptosol (rl)	0.03
Artemisia absinthium L.	AB1	14311	Gornja Vrežina	22.01083333	43.319	Calcaric Phaeozem (cp)	0.15
	AB2	14313	Velepolje	21.84733333	43.44763889	Eutric Cambisol (ec)	0.07
	AB3	14314	Prosek	22.05511111	43.31727778	Rendzic Leptosol (rl)	0.06
	AB4	14321	Vrelo	22.04763889	43.38380556	Haplic Luvisol (hl)	0.07
	AB5	14315	Kopajkošara	21.98141667	43.44852778	Rendzic Leptosol (rl)	0.06
	AB6	14318	Lalinske pojate	21.76155556	43.35136111	Calcaric Fluvisol (cf)	0.07
	AB7	14312	Jovanovac	21.69152778	43.35183333	Pellic Vertisol (pv)	0.07
	AB8	14322	Paligrace	21.86908333	43.48055556	Eutric Cambisol (ec)	0.05
	AB9	14317	Brenica	21.92344444	43.37441667	Calcaric Phaeozem (cp)	0.06

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Plant specie	Sample	Voucher	Locality	Longitude, °	Latitude, °	WRB soil type	Yield, %
	AB10	14320	Subotinac	21.69241667	43.62577778	Pellic Vertisol (pv)	0.05
	AB11	14319	Donja Vrežina	21.97019444	43.32533333	Calcaric Phaeozem (cp)	0.07
	AB12	14316	Oblačinsko jezero	21.68008333	43.30286111	Pellic Vertisol (pv)	0.05
Artemisia annua L.	AN1	14334	Paljina	21.83569444	43.41491667	Eutric Cambisol (ec)	0.63
	AN2	14336	Stanci	21.80233333	43.53313889	Eutric Cambisol (ec)	0.51
	AN3	14335	Mozgovo	21.76130556	43.65319444	Pellic Vertisol (pv)	0.60
	AN4	14329	Lipovac	21.83516667	43.56119444	Dystric Cambisol (dc)	0.43
	AN5	14325	Tešica	21.74797222	43.45091667	Calcaric Fluvisol (cf)	0.92
	AN6	14327	Balajnac	21.79683333	43.26852778	Pellic Vertisol (pv)	1.09
	AN7	14326	Azbresnica	21.69172222	43.35211111	Pellic Vertisol (pv)	0.63
	AN8	14328	Selo Bovan	21.71675	43.63241667	Pellic Vertisol (pv)	1.56
	AN9	14333	Supovac	21.76605556	43.39669444	Calcaric Fluvisol (cf)	0.47
	AN10	14330	Naselje Broj Šest	21.98652778	43.30963889	Calcaric Phaeozem	0.86
	AN11	14331	Lalinac	21.78288889	43.34111111	Calcaric Fluvisol (cf)	0.45
	AN12	14332	Gornja Studena	22.09775	43.25388889	Rendzic Leptosol (rl)	0.75
Artemisia vulgaris L.	AV1	14359	Donji Krivodol	22.93530556	43.10255556	Rendzic Leptosol (rl)	0.12
	AV2	14353	Crnoklište	22.46238889	43.22963889	Calcaric Fluvisol (cf)	0.04
	AV3	14352	Visočka Ržana	22.67063889	43.17891667	Rendzic Leptosol (rl)	0.07
	AV4	14350	Miranovačka kula	22.35519444	43.35883333	Dystric Cambisol	0.11
	AV5	14351	Inovo	22.43183333	43.41002778	Calcaric Fluvisol (cf)	0.02
	AV6	14356	Kalna	22.41886111	43.38761111	Dystric Cambisol (dc)	0.16
	AV7	14355	Česma kod Gramade	22.05541667	43.39344444	Haplic Luvisol (hl)	0.08
	AV8	14354	Dimitrovgrad	22.81958333	43.02894444	Pellic Vertisol (pv)	0.10
	AV9	14357	Moklište	22.26555556	43.24369444	Calcaric Fluvisol (cf)	0.06
	AV10	14358	Slavinja	22.85625	43.13877778	Rendzic Leptosol (rl)	0.12
	AV11	14349	Velepolje	21.84716667	43.44777778	Eutric Cambisol (ec)	0.06
	AV12	14360	Gabrovačka reka, Ćele kula	21.92441667	43.31125	Calcaric Fluvisol (cf)	0.05
Artemisia scoparia Waldst. et Kit.	AS1	14345	Broj Šest	21.98569444	43.31013889	Calcaric Phaeozem (cp)	0.11
	AS2	14342	Čokot	21.81969444	43.30013889	Calcaric Fluvisol (cf)	0.12
	AS3	14343	Batušinac	21.82125	43.26411111	Calcaric Fluvisol (cf)	0.19
	AS4	14337	Draževac	21.79697222	43.44791667	Calcaric Fluvisol (cf)	0.17
	AS5	14344	Vinik	21.92972222	43.35038889	Calcaric Phaeozem (cp)	0.30
	AS6	14348	Trupale	21.80194444	43.35575	Calcaric Fluvisol (cf)	0.25
	AS7	14338	Supovac	21.76327778	43.37752778	Calcaric Fluvisol (cf)	0.22
	AS8	14340	Zaplanjska Toponica	21.90658333	43.15491667	Calcaric Fluvisol (cf)	0.31
	AS9	14339	Aleksandrovo	21.75272222	43.29711111	Pellic Vertisol (pv)	0.31
	AS10	14347	Aleksinac	21.70980556	43.52766667	Calcaric Fluvisol (cf)	0.25
	AS11	14346	Lalinac	21.78138889	43.34088889	Calcaric Fluvisol (cf)	0.33
	AS12	14341	Trnava	21.78633333	43.42166667	Calcaric Fluvisol (cf)	0.29

WRB World Reference Base for Soil Resources

Table S-II. Chemical composition of seven *Artemisia alba* samples collected from different soil types according to the WRB

Class	Compound	RI	RIa	Content, % AA1	AA2	AA3	AA4	AA5	AA6	AA7
O	(*Z*)-2-Penten-1-ol	775	765	/	/	/	/	/	/	tr
O	3-methyl-2-Buten-1-ol	775	765	/	/	/	/	/	tr	tr
O	3-methyl-2-Butenal	788	778	tr	tr	/	/	/	0.2	tr
O	1-Octene	793	788	/	/	/	/	/	tr	/
O	Hexanal	801	801	tr	/	/	/	/	tr	tr
O	Furfural	833	828	tr	/	/	/	/	tr	tr
O	4-Hydroxy-4-methyl-2-pentanone	841	831	/	tr	0.3	/	/	/	/
CD	Ethyl 2-methylbutyrate	848	842*	/	/	/	/	/	tr	/
O	(*E*)-2-Hexenal	850	846	/	/	/	/	/	0.2	tr
O	(*Z*)-3-Hexenol	852	850	tr	tr	/	/	/	/	tr
O	*n*-Hexanol	867	863	tr	tr	/	/	/	tr	tr
CD	Isopentyl acetate	876	869	/	/	/	/	/	tr	/
CD	2-Methyl butyl acetate	879	875	/	/	/	/	/	tr	/
A	*n*-Nonane	900	900	/	/	/	/	/	tr	/
O	Heptanal	903	901	/	/	/	/	/	tr	/
M	Santolina Triene	909	906	tr	/	/	/	/	tr	/
M	Tricyclene	924	921	/	/	/	/	/	tr	tr
M	Artemisiatriene	928	922*	/	/	/	/	/	0.2	/
M	α-Thujene	928	924	tr	tr	/	/	/	/	/
M	α-Pinene	935	932	tr	tr	/	0.5	/	1.0	tr
M	Camphene	950	946	tr	tr	0.9	3.3	0.4	1.5	0.2
M	Thuja-2,4(10)-diene	956	953	/	tr	/	/	/	tr	/
O	(*E*)-2-Heptenal	957	947	/	/	/	/	/	/	tr
O	Benzaldehyde	962	952	tr	tr	/	/	/	tr	tr
O	*n*-Heptanol	969	959	tr	tr	/	/	/	tr	tr
M	Sabinene	975	969	tr	tr	/	0.6	/	tr	tr
MO	Artemiseole	977	971	tr	/	/	/	/	tr	tr
M	β-Pinene	979	974	tr	tr	/	3.8	/	1.2	0.4
O	1-Octen-3-ol	980	974	tr	/	/	/	/	tr	/
O	6-Methyl-5-Hepten-2-one	988	981	/	/	/	/	/	/	tr
O	2-Pentyl furan	993	984	/	/	/	/	/	0.4	tr
O	Mesitylene	996	994	/	tr	/	/	/	tr	/
MO	Yomogi alcohol	1001	999	tr	/	/	/	/	7.3	tr
O	*n*-Octanal	1004	998	tr	tr	/	/	/	tr	tr
M	α-Phellandrene	1006	1002	tr	tr	/	/	/	tr	tr
O	(*E*,*E*)-2,4-Heptadienal	1012	1005	/	/	/	/	/	tr	tr
CD	Hexyl acetate	1015	1007	/	/	/	/	/	tr	/
M	α-Terpinene	1019	1014	tr	tr	tr	/	/	0.4	tr
M	*o*-Cymene	1027	1022	tr	tr	/	0.1	/	0.9	0.3
M	β-Phellandrene	1032	1025	/	tr	/	/	/	/	tr
MO	1,8-Cineole	1033	1026	5.8	1.9	15.3	16.4	19.7	9.8	2.1
MO	Santolina alcohol	1037	1034	tr	/	/	/	/	tr	/
M	(*Z*)-β-Ocimene	1039	1032	/	tr	/	/	/	/	tr
MO	Lavender Lactone	1042	1034*	tr	tr	/	/	/	tr	tr
O	Benzene acetaldehyde	1046	1036	0.6	tr	/	/	/	0.2	0.4

M	(*E*)-*β*-Ocimene	1050	1044	tr	tr	/	/	/	tr	tr
M	*γ*-Terpinene	1061	1054	0.3	tr	0.6	/	/	0.5	0.2
MO	Artemisia ketone	1063	1056	/	/	/	8.3	/	14.9	0.9
MO	*cis*-Sabinene hydrate	1069	1065	0.3	0.2	/	0.7	/	0.5	0.4
MO	*cis*-Linalool oxide	1074	1067	0.3	tr	/	/	/	tr	tr
O	*m*-Cresol	1080	1072	/	/	/	/	/	/	tr
MO	Artemisia alcohol	1085	1080	tr	/	/	/	/	6.6	tr
MO	*trans*-Linalool oxide	1090	1084	0.3	/	/	/	/	/	tr
M	Terpinolene	1091	1086	0.2	tr	/	/	/	0.2	0.2
CD	Isobutyl tiglate	1094	1088	/	/	/	/	/	/	tr
MO	*trans*-Sabinene hydrate	1100	1098	/	0.4	0.5	/	/	0.6	0.9
MO	Linalool	1103	1098	0.3	0.2	/	/	/	/	/
O	*n*-Nonanal	1105	1100	tr	tr	/	/	/	tr	tr
MO	Filifolone	1106	1103*	/	/	/	0.5	1.5	/	/
O	6-Methyl-(E)-3,5-heptadien-2-one	1107	*	/	/	/	/	/	0.5	0.5
MO	*cis*-Thujone	1108	1101	/	/	/	/	3.9	/	/
O	6-Ethenyldihydro-2,2,6-trimethyl-2H-Pyran-3(4H)-one	1110	1109*	/	/	/	/	/	0.3	/
O	Phenyl ethyl alcohol	1116	1106	/	/	/	/	/	/	tr
MO	*trans*-Thujone	1119	1112	/	/	/	1.5	2.6	0.2	tr
MO	*trans*-Chrysanthenol	1120	1114	/	/	28.3	/	/	/	/
MO	*trans*-*p*-Mentha-2,8-dien-1-ol	1121	1119	8.6	2.9	/	/	/	/	/
MO	*cis*-*p*-Menth-2-en-1-ol	1124	1118	0.3	0.6	/	/	/	0.3	0.9
MO	Chrysanthenone	1128	1124	0.2	tr	/	1.7	4.5	0.6	/
MO	*α*-Campholenal	1129	1122	tr	/	/	/	/	tr	0.2
MO	*cis*-*p*-Mentha-2,8-dien-1-ol	1135	1133	0.2	tr	/	/	/	/	/
O	Nopinone	1140	1135	/	tr	/	0.2	/	0.2	1.9
MO	*trans*-Pinocarveol	1141	1135	0.7	/	/	/	/	1.1	/
MO	*trans*-*p*-Menth-2-en-1-ol	1143	1136	/	tr	/	/	/	/	1.7
MO	*trans*-Verbenol	1147	1140	/	tr	0.3	/	/	/	/
MO	Camphor	1148	1141	3.0	1.6	22.7	27.9	51.6	8.9	14.1
MO	(*Z*)-Tagetone	1153	1148	tr	/	/	/	/	/	/
MO	Sabina ketone	1161	1154	/	tr	/	/	/	tr	tr
MO	*trans*-Pinocamphone	1165	1158	/	/	/	/	/	tr	tr
MO	Nerol oxide	1164	1154	tr	/	/	/	/	/	/
MO	Pinocarvone	1167	1160	0.8	0.2	/	0.5	/	1.4	1.0
MO	Borneol	1174	1165	/	1.6	1.5	2.7	/	3.2	3.7
MO	Artemisyl acetate	1175	1169	/	/	/	/	/	3.6	/
MO	Santalone	1177	1177	8.1	/	/	/	/	/	/
MO	cis-Pinocamphone	1178	1172	/	0.4	1.0	25.1	/	8.6	13.5
MO	Terpinen-4-ol	1181	1174	1.2	0.8	0.7	1.0	/	2.5	tr
MO	*p*-Cymen-8-ol	1188	1179	tr	0.2	0.3	/	/	0.2	tr
CD	(*Z*)-3-Hexenyl butanoate	1190	1184	0.2	/	/	/	/	0.3	/
MO	*trans*-*p*-Mentha-1(7),8-dien-2-ol	1191	1187	/	/	/	/	/	/	0.2
MO	*α*-Terpineol	1194	1186	0.2	tr	tr	/	/	0.2	0.3
CD	Methyl salicylate	1196	1190	/	/	/	/	/	tr	/
MO	Myrtenol	1197	1194	tr	tr	/	/	/	0.8	3.1
MO	*cis*-Piperitol	1199	1195	/	tr	/	/	/	/	tr
MO	Myrtenal	1200	1195	/	0.5	/	1.4	/	/	/
PP	Methyl chavicol	1202	1195	tr	/	/	/	/	tr	/
O	*n*-Decanal	1207	1201	tr	tr	/	/	/	/	/

MO	*trans*-Piperitol	1210	1207	0.2	0.4	/	/	/	tr	0.4
MO	Verbenone	1213	1204	0.1	tr	/	/	/	tr	tr
MO	*trans*-Carveol	1222	1215	0.2	tr	/	/	/	0.2	0.5
O	*m*-Cumenol	1230	1224	tr	tr	/	/	/	tr	/
MO	*cis*-*p*-Mentha-1(7),8-dien-2-ol	1231	1227	/	/	/	/	/	/	0.3
MO	*cis*-Carveol	1234	1226	0.1	tr	/	/	/	tr	0.3
MO	*trans*-Chrysanthenyl acetate	1238	1235	0.2	tr	0.7	/	/	/	/
CD	Hexyl-2-methyl butyrate	1239	1233	/	/	/	/	/	/	tr
O	Cumin aldehyde	1244	1238	tr	tr	/	/	/	tr	tr
MO	Carvone	1248	1239	tr	tr	/	/	/	tr	tr
CD	Isoamyl hexanoate	1253	1246	tr	/	/	/	/	/	/
MO	*trans*-2-hydroxy-Pinocamphone	1254	1247	/	/	/	/	/	tr	0.1
MO	Geraniol	1257	1249	/	0.2	/	/	/	/	2.4
MO	Piperitone	1258	1249	tr	0.2	/	/	/	2.3	tr
O	(*E*)-2-Decenal	1263	1260	tr	tr	/	/	/	/	/
MO	*trans*-Myrtanol	1264	1258	tr	/	/	/	/	/	tr
MO	*cis*-Chrysanthenyl acetate	1265	1261	0.1	/	/	/	/	1.2	/
MO	Geranial	1273	1264	tr	tr	/	/	/	/	tr
MO	Perilla aldehyde	1279	1269	/	/	/	/	/	/	tr
MO	*cis*-Verbenyl acetate	1281	1280	5.3	/	/	/	/	/	/
MO	*trans*-*α*-Necrodol acetate	1287	1282	tr	/	/	/	/	/	/
MO	Bornyl acetate	1290	1287	/	tr	/	/	/	0.5	0.3
MO	*trans*-Linalool oxide acetate (pyranoid)	1291	1287	tr	/	/	/	/	/	/
MO	*p*-Cymen-7-ol	1291	1289	tr	/	/	/	/	/	/
O	(*E*,*Z*)-2,4-Decadienal	1296	1292	/	tr	/	/	/	/	/
MO	Thymol	1298	1289	/	tr	tr	/	/	tr	tr
MO	Perilla alcohol	1302	1294	0.2	/	/	/	/	/	0.3
MO	Terpinen-4-ol acetate	1303	1299	tr	/	/	/	/	0.2	/
MO	Carvacrol	1304	1298	tr	tr	/	/	/	/	0.2
O	Undecanal	1309	1305	tr	/	/	/	/	tr	/
MO	6-Hydroxy-Carvotanacetone	1315	1309	/	tr	/	/	/	/	tr
O	*p*-vinyl-Guaiacol	1318	1309	tr	/	/	/	/	/	/
O	(*E*,*E*)-2,4-Decadienal	1319	1315	tr	tr	/	/	/	tr	tr
MO	*δ*-Terpinyl acetate	1321	1316	tr	/	/	/	/	/	/
MO	Myrtenyl acetate	1330	1324	tr	/	/	/	/	tr	tr
MO	*p*-Mentha-1,4-dien-7-ol	1331	1325	tr	/	/	/	/	/	tr
S	Silphiperfol-5-ene	1332	1326	/	0.6	/	/	/	tr	/
CD	Hexyl tiglate	1333	1330	/	tr	/	/	/	/	/
S	Presilphiperfol-7-ene	1338	1334	/	tr	/	/	/	/	/
S	*δ*-Elemene	1343	1335	1.5	1.0	0.8	/	/	0.3	tr
S	7-epi-Silphiperfol-5-ene	1350	1345	/	0.2	/	/	/	tr	/
MO	*α*-Terpinyl acetate	1354	1346	0.2	tr	/	/	/	/	tr
S	*α*-Cubebene	1355	1345	tr	/	/	/	/	/	/
PP	Eugenol	1362	1356	0.2	tr	/	/	/	tr	0.2
S	Silphiperfol-4,7(14)-diene	1364	1358	/	0.2	/	/	/	/	/
MO	*cis*-Carvyl acetate	1367	1365	/	/	/	/	/	/	tr
S	*α*-Copaene	1380	1374	0.2	tr	/	/	/	tr	tr
S	Silphiperfol-6-ene	1382	1377	/	0.5	/	/	/	/	/
MO	Geranyl acetate	1386	1379	/	/	/	/	/	/	tr
S	*β*-Bourbonene	1392	1387	tr	tr	/	/	/	/	tr
S	*β*-Cubebene	1396	1387	0.2	/	/	/	/	/	/

S	*β*-Elemene	1398	1389	0.6	0.2	/	/	/	0.4	0.8
O	(*Z*)-Jasmone	1401	1392	tr	/	/	/	/	/	tr
S	*iso*-Italicene	1405	1401	0.4	/	/	/	/	/	/
PP	Methyl eugenol	1407	1403	tr	tr	/	/	/	0.3	0.3
S	(*E*)-Caryophyllene	1427	1417	0.7	0.7	/	/	/	tr	0.5
S	*β*-Copaene	1437	1430	tr	tr	/	/	/	/	/
MO	Geranyl acetone	1456	1453	/	/	/	/	/	/	tr
S	(*E*)-*β*-Farnesene	1460	1454	/	/	/	/	/	tr	tr
S	*α*-Humulene	1462	1452	0.5	0.3	/	/	/	tr	tr
S	*allo*-Aromadendrene	1467	1458	tr	/	/	/	/	/	/
S	9-*epi*-(*E*)-Caryophyllene	1469	1464	tr	/	/	/	/	tr	/
S	*cis*-Muurola-4(14),5-diene	1471	1465	tr	/	/	/	/	/	/
S	*γ*-Himachalene	1487	1481	/	/	/	/	/	/	tr
S	Germacrene D	1490	1484	21.3	9.5	4.2	2.7	3.4	2.1	5.3
S	*β*-Selinene	1495	1489	/	/	/	/	/	tr	tr
S	*α*-Zingiberene	1498	1493	0.4	tr	/	/	/	/	/
SO	Indipone	1502	1496	/	/	/	/	/	0.3	/
S	Bicyclogermacrene	1505	1500	2.6	1.2	/	/	/	0.4	1.4
SO	*β*-dihydro Agarofuran	1510	1503	/	/	/	/	/	0.3	tr
S	Germacrene A	1514	1508	0.3	/	/	/	/	/	0.7
SO	Silphiperfolan-6-*α*-ol	1514	1507	/	1.9	/	/	/	0.2	/
S	*γ*-Cadinene	1522	1513	tr	/	/	/	/	/	/
S	*δ*-Cadinene	1531	1522	0.5	4.8	/	/	/	/	0.2
S	*trans*-Cadina-1,4-diene	1540	1533	tr	/	/	/	/	/	/
S	Presilphiperfolane-9-*α*-ol	1530	*	/	10.9	/	/	/	/	/
S	Silphiperfol-5-en-3-ol B	1543	1534	/	2.5	/	/	/	/	/
S	*α*-Calacorene	1551	1544	tr	/	/	/	/	tr	tr
SO	Elemol	1556	1548	5.2	/	/	/	/	0.4	0.4
SO	Silphiperfol-5-en-3-one B	1557	1550	/	2.9	/	/	/	0.3	/
SO	(*E*)-Nerolidol	1566	1561	tr	/	/	/	/	tr	0.2
SO	Silphiperfol-5-en-3-ol A	1567	1557	/	1.1	/	/	/	/	/
SO	*β*-Calacorene	1574	1564	/	/	/	/	/	tr	tr
SO	Zierone	1579	1574	0.2	/	/	/	/	/	/
SO	Silphiperfol-5-en-3-one A	1582	1574	/	35.0	2.9	/	/	0.9	/
SO	Spathulenol	1587	1577	tr	/	/	/	/	0.3	1.0
SO	Davanone	1591	1587	/	/	/	/	1.4	0.7	1.8
SO	Caryophyllene oxide	1592	1582	0.4	/	/	/	/	/	/
SO	Viridiflorol	1599	1592	tr	/	/	/	/	tr	0.2
SO	Humulene epoxide I	1603	1601*	/	0.7	/	/	/	/	/
SO	Ledol	1612	1602	/	/	/	/	/	/	tr
SO	Humulene epoxide II	1618	1608	/	/	/	/	/	tr	/
SO	10-*epi*-*γ*-Eudesmol	1631	1622	0.1	/	/	/	/	/	/
SO	Silphiperfol-6-en-5-one	1634	1624	/	0.3	/	/	/	/	/
SO	*γ*-Eudesmol	1640	1630	1.8	/	/	/	/	/	/
SO	Caryophylla-4(12),8(13)-dien-5-*α*-ol	1646	1639	/	0.3	/	/	/	/	0.4
SO	Caryophylla-4(12),8(13)-dien-5-*β*-ol	1649	1639	/	1.4	/	/	/	/	/
SO	*epi*-*α*-Murrolol	1650	1640	/	/	/	/	/	/	tr
SO	*β*-Eudesmol	1659	1649	1.3	/	/	/	/	tr	11.6
SO	*α*-Eudesmol	1662	1652	1.4	2.3	/	/	/	0.4	5.2
SO	14-hydroxy-(*Z*)-Caryophyllene	1672	1666	0.1	/	/	/	/	/	/
SO	Cadalene	1684	1675	/	/	/	/	/	tr	/

SO	Elemol acetate	1686	1680	1.8	/	/	/	/	/	/
SO	α-Bisabolol	1691	1685	0.5	/	1.1	/	/	0.9	1.7
SO	Germacra-4(15),5,10(14)-trien-1-α-ol	1695	1685	/	/	/	/	/	/	0.2
O	Pentadecanal	1718	1715*	0.2	tr	/	/	/	/	/
SO	Cyclocolorenone	1761	1759	/	/	/	/	/	tr	tr
CD	Benzyl benzoate	1769	1759	/	tr	/	/	/	/	/
SO	14-oxy-α-Muurolene	1777	1767	/	/	/	/	/	/	0.2
SO	Hexahydrofarnesyl acetone	1848	1847	/	tr	/	/	/	tr	0.2
SO	(*E*,*E*)-5,9-Farnesylacetone	1923	1913	/	tr	/	/	/	/	/
SO	(*E*,*E*)-Geranyl linalool	2033	2026	/	tr	/	/	/	/	/
A	Heneicosane	2100	2100	/	tr	/	/	/	/	/
O	Phytol	2116	2114*	/	0.2	/	/	/	/	/
O	Eicosanal	2226	2224*	/	tr	/	/	/	/	/
O	1-Eicosanol	2287	2281*	/	tr	/	/	/	/	/
A	Tricosane	2300	2300	/	tr	/	/	/	/	/
A	Tetracosane	2400	2400	/	tr	/	/	/	/	/
O	Docosanal	2430	2426*	/	tr	/	/	/	/	/
O	1-Docosanol	2461	2456*	/	tr	/	/	/	/	/
A	Pentacosane	2500	2500	/	tr	/	/	/	/	/
O	Tetracosanal	2634	2632*	/	tr	/	/	/	/	/
O	1-Tetracosanol	2678	-	/	tr	/	/	/	/	/
A	Heptacosane	2700	2700	/	tr	/	/	/	/	/
A	Nonacosane	2900	2900	/	tr	/	/	/	/	/

RI-Experimental linear retention indices relative to C8-C40 alkanes. Ria-Literature indices-Adams' retention indices and * according to NIST data base. Tr- trace<0.05 % and not detected compounds are marked as (/). M-Hydrocarbon Monoterpenoids, MO-Oxygenated Monoterpenoids, S-Hydrocarbon Sesquiterpenoids, SO-Oxygenated Sesquiterpenoids, PP-Phenylpropanoids, CD-carboxylic acid derivatives, A-Alkanes, O-Other.

Table S-III. The number of identified components per sample of *A. alba*, the percentage of each class of compounds, and the percentage of total identified components

Sample	AA1	AA2	AA3	AA4	AA5	AA6	AA7
Contribution in total peaks area of ion chromatogram, %	81.0	90.8	82.1	98.7	89.1	91.7	83.8
Number of components	117	116	20	19	9	117	121
				Content, %			
Total monoterpenoids	37.6	12.3	72.8	95.8	84.2	81.4	48.9
Monoterpene hydrocarbons (M)	0.5	/	1.5	8.3	0.4	5.8	1.2
Oxygenated monoterpenes (MO)	37.1	12.3	71.2	87.5	83.8	75.6	47.6
Total sesquiterpenoids	42.1	78.2	9.1	2.7	4.9	7.8	31.6
Sesquiterpene hydrocarbons (S)	29.3	32.4	5.0	2.7	3.4	3.1	8.8
Oxygenated Sesquiterpene (SO)	12.8	45.8	4.1	/	1.4	4.7	22.9
Phenylpropanoids (PP)	0.2	/	/	/	/	0.3	0.5
Carboxylic acid derivatives (CD)	0.2	/	/	/	/	0.3	tr
n-Alkanes (A)	/	/	/	/	/	tr	/
Other (O)	0.9	0.2	0.3	0.2	/	1.9	2.8

Table S-IV. Chemical composition of twelve *Artemisia absinthium* samples collected from different soil types according to the WRB

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
O	3-Methyl-2-buten-1-ol	775	765	/	/	/	/	/	/	/	tr	/	tr	/	tr
O	Methyl 2-methyl-butyrate	784	780*	tr	tr	/	/	/	/	tr	tr	/	/	/	/
O	3-Methyl-2-butenal	788	778	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	1-Octene	793	788	tr	tr	tr	/	/	tr	tr	tr	tr	tr	tr	tr
O	Hexanal	801	801	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	2-Methyl-1-pentanol	831	824*	/	/	/	/	/	/	/	/	/	tr	/	tr
O	4-Methyl-pentanol	834	830	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Methyl angelate	843	843	tr	/	/	/	/	/	/	/	/	/	/	/
CD	Ethyl-2-methylbutyrate	848	842*	/	/	tr	/	/	/	/	/	/	/	/	/
O	(*E*)-2-Hexenal	850	846	tr	0.7	0.7	0.2	0.2	0.7	tr	0.3	0.2	0.2	tr	0.3
O	(*Z*)-3-Hexenol	852	850	tr	0.3	0.2	0.2	0.2	0.4	tr	0.4	tr	0.2	/	0.2
O	7-methyl-1-Octene	855	852*	/	/	/	/	/	/	/	/	/	/	0.2	/
O	4-methyl-3-Pentenol	858	868*	/	/	/	/	/	/	/	tr	/	/	/	/
O	(*E*)-Salvene	861	858	/	/	/	/	/	/	tr	/	/	/	/	/
O	(*Z*)-2-Hexenol	865	859	/	tr	tr	tr	tr	0.3	tr	tr	tr	tr	tr	tr
O	*n*-Hexanol	867	863	tr	0.3	0.3	0.2	0.3	0.5	tr	0.4	tr	0.2	tr	0.2
A	*n*-Nonane	900	900	tr	tr	tr	/	/	/	tr	tr	/	/	/	/
O	Heptanal	903	901	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	(*E,E*)-2,4-Hexadienal	912	907	/	/	tr	tr	/	tr	tr	tr	tr	tr	/	/
CD	Isobutyl isobutyrate	915	908	tr	tr	tr	/	/	/	/	/	/	/	/	/
M	Tricyclene	924	921	/	tr	tr	tr	tr	tr	/	tr	tr	/	tr	/
M	α-Thujene	928	924	0.2	tr	0.2	tr	tr	0.2	tr	0.3	0.2	0.2	0.3	0.2
M	α-Pinene	935	932	0.3	0.2	0.2	tr	0.1	0.3	0.3	0.4	0.2	0.3	0.2	0.2
CD	Ethyl tiglate	939	929	tr	/	/	/	/	/	tr	/	/	/	/	tr
M	α-Fenchene	949	945	tr	tr	0.3	tr	tr	tr	tr	0.3	0.2	0.2	0.2	tr
M	Camphene	950	946	/	/	/	/	/	/	/	tr	/	/	/	/
CD	Butyl isobutyrate	953	952*	/	/	tr	/	/	/	/	/	/	/	/	/
O	(*E*)-2-Heptenal	957	947	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	Benzaldehyde	962	952	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	(*Z*)-4-Heptenol	966	959	tr	tr	/	/	/	/	/	/	/	/	/	/
O	*n*-Heptanol	969	959	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Isoamyl propionate	970	960	/	/	/	/	/	tr	/	/	/	/	/	/
CD	2-methylbutyl propanoate	973	968*	/	/	tr	/	/	/	/	/	/	/	/	/
M	Sabinene	975	969	18.9	17.6	16.1	5.7	13.9	15.3	9.5	18.5	17.0	16.4	18.2	12.1
O	1-Octen-3-ol	980	974	0.7	0.8	0.6	0.3	0.4	0.7	0.6	0.7	0.7	0.5	0.5	0.4
O	3-Octanone	988	979	/	/	/	/	/	/	tr	/	/	/	/	/
O	6-methyl-5-hepten-2-one	988	981	/	/	tr	tr	tr	tr	/	tr	tr	tr	tr	tr
M	Myrcene	992	988	2.9	14.8	6.7	2.4	2.7	4.0	0.8	4.5	2.2	1.2	4.7	1.0
M	*cis*-*m*-Mentha-2,8-diene	993	983	/	/	/	/	/	/	/	/	/	tr	/	tr
O	3-Octanol	995	988	tr	/	/	tr	tr	/	/	/	/	/	/	/
MO	Yomogi alcohol	1001	999	/	/	/	/	/	tr	/	/	/	/	/	/
O	*n*-Octanal	1004	998	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	α-Phellandrene	1006	1002	1.5	0.2	tr	0.3	0.7	2.2	1.5	2.5	2.4	0.2	0.8	0.6

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
O	(*E*,*E*)-2,4-Heptadienal	1012	1005	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Isoamyl isobutyrate	1014	1007	tr	tr	tr	tr	/	tr	tr	tr	/	/	/	/
CD	2-methylbutyl Isobutyrate	1017	1017*	/	/	/	/	/	/	/	/	/	tr	tr	/
M	*α*-Terpinene	1019	1014	0.6	tr	0.4	tr	0.3	0.4	0.3	0.6	0.5	0.3	0.8	0.4
M	*o*-Cymene	1027	1022	5.1	4.9	2.1	2.1	2.5	8.8	3.9	6.2	8.4	2.1	2.1	3.2
M	Limonene	1031	1024	/	tr	/	/	/	/	/	/	/	/	/	/
M	*β*-Phellandrene	1032	1025	0.4	/	0.3	tr	0.2	0.3	0.3	tr	0.3	0.2	0.3	0.2
MO	1,8-Cineole	1033	1026	0.4	4.5	0.3	0.2	0.3	1.5	0.6	6.9	1.3	0.3	0.2	0.3
O	Benzyl alcohol	1036	1026	/	/	/	tr	tr	tr	/	/	/	tr	/	tr
M	(*Z*)-*β*-Ocimene	1039	1032	tr	tr	tr	0.6	0.2	0.2	tr	0.4	0.2	2.1	0.6	0.5
O	Benzene acetaldehyde	1046	1036	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr
M	(*E*)-*β*-Ocimene	1050	1044	tr	tr	tr	tr	0.1	tr	tr	tr	0.2	0.2	tr	tr
CD	Prenyl isobutyrate	1055	1048	tr	/	/	/	/	/	tr	/	tr	tr	tr	tr
M	*γ*-Terpinene	1061	1054	1.5	0.5	1.1	0.4	0.9	1.0	0.7	1.4	1.3	0.7	1.6	0.9
MO	Artemisia ketone	1063	1056	tr	tr	/	/	/	0.3	/	/	/	/	/	/
MO	*cis*-Sabinene hydrate	1069	1065	tr	tr	tr	tr	0.5	tr	0.3	0.4	0.7	0.5	0.4	0.3
O	*n*-Octanol	1071	1063	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*cis*-Linalool oxide	1074	1067	tr	tr	0.3	0.1	tr	0.2	tr	0.3	tr	tr	tr	0.2
O	1-Nonen-3-ol	1082	1078*	0.4	tr	tr	tr	/	tr	tr	tr	tr	/	/	/
M	Terpinolene	1091	1086	0.3	0.2	0.5	0.2	0.3	0.4	tr	0.4	0.4	0.2	0.4	0.2
CD	Isobutyl tiglate	1094	1088	/	/	/	/	/	/	tr	/	/	/	/	/
MO	6,7-Epoxymyrcene	1095	1090	/	/	/	/	tr	tr	/	tr	/	tr	tr	tr
CD	Methyl benzoate	1098	1088	tr	tr	tr	tr	/	/	tr	tr	/	/	/	/
MO	*trans*-Sabinene hydrate	1100	1098	/	/	/	/	/	/	tr	/	/	/	/	/
MO	Linalool	1103	1098	2.1	15.1	29.8	12.5	21.6	28.9	6.0	13.2	12.8	4.3	6.6	5.3
O	*n*-Nonanal	1105	1100	0.3	0.4	0.4	tr	0.3	0.4	tr	0.3	0.4	0.4	0.4	0.2
MO	*cis*-Thujone	1108	1101	0.4	/	/	0.5	tr	/	1.1	/	/	/	/	0.5
CD	2-Methyl butyl isovalerate	1109	1103	/	tr	tr	/	/	tr	/	tr	tr	tr	tr	/
MO	*cis*-Rose oxide	1113	1106	tr	/	/	/	tr	/	/	tr	0.2	0.2	/	tr
CD	3-Methyl-3-butenyl 3-methyl butyrate	1115	1112	/	tr	tr	tr	/	/	/	/	/	/	/	/
MO	*trans*-Thujone	1119	1112	20.6	tr	tr	26.2	5.5	0.3	48.2	tr	0.3	tr	tr	21.7
MO	*cis*-*p*-Menth-2-en-1-ol	1124	1118	0.4	0.4	0.5	0.2	0.4	0.3	tr	0.6	0.5	0.3	0.4	0.2
MO	*trans*-Rose oxide	1128	1122	/	/	/	/	/	/	tr	tr	tr	tr	/	tr
MO	*α*-Campholenal	1129	1122	tr	tr	tr	tr	tr	/	/	/	/	/	/	/
MO	*cis*-Limonene oxide	1131	1132	/	/	/	/	/	tr	/	/	/	/	/	/
MO	(*Z*)-epoxy-Ocimene	1134	1128	/	/	/	5.9	9.6	0.9	5.3	7.6	2.3	25.6	27.2	29.5
MO	*iso*-3-Thujanol	1137	1134	tr	/	/	0.2	tr	tr	2.1	tr	tr	tr	tr	0.4
CD	Pentyl 2-methylbutyrate	1140	1136*	/	/	tr	/	/	/	/	/	/	/	/	/
MO	*trans*-Pinocarveol	1141	1135	/	/	/	0.5	/	/	/	0.9	/	/	/	/
MO	*trans*-*p*-Menth-2-en-1-ol	1143	1136	/	0.8	0.7	tr	tr	/	/	/	0.6	/	/	/
MO	*trans*-Sabinol	1144	1137	0.5	/	/	/	0.8	0.5	0.8	/	/	/	/	/

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
MO	(*E*)-epoxy-Ocimene	1145	1137	/	/	/	/	/	/	/	/	/	1.2	2.5	1.9
CD	(*Z*)-3-Hexenyl isobutyrate	1146	1142	tr	tr	tr	tr	tr	tr	tr	tr	tr	/	/	/
MO	*trans*-Verbenol	1147	1140	/	/	/	tr	/	/	/	/	/	/	/	/
MO	Camphor	1148	1141	tr	tr	tr	/	tr	tr	tr	/	/	tr	/	/
CD	Hexyl isobutyrate	1150	1147	tr	tr	0.3	tr	tr	tr	tr	tr	tr	tr	/	tr
MO	Nerol oxide	1157	1154	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Sabina ketone	1161	1154	tr	0.3	0.3	0.2	0.3	0.2	tr	0.2	0.4	0.3	0.5	0.4
MO	Isoborneol	1161	1155	tr	/	/	/	/	/	/	/	/	/	/	/
MO	Pinocarvone	1167	1160	tr	tr	tr	tr	tr	tr	tr	/	/	/	/	/
MO	Lavandulol	1172	1165	1.1	0.8	1.5	0.4	2.0	1.1	0.9	1.4	1.1	1.9	0.7	1.7
O	*n*-Nonanol	1173	1165	/	/	/	/	/	tr	/	/	/	/	/	/
MO	Borneol	1174	1165	tr	tr	tr	/	tr	tr	tr	tr	tr	/	/	/
MO	*cis*-Linalool oxide	1175	1170	tr	tr	tr	tr	/	/	/	/	/	/	/	/
MO	Rosefuran epoxide	1178	1173	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3	0.2	0.3
MO	Terpinen-4-ol	1181	1174	6.6	10.2	10.3	3.0	4.9	6.0	3.6	7.1	6.0	4.7	6.6	5.1
MO	*p*-Cymen-8-ol	1188	1179	/	0.3	/	/	/	/	/	/	/	/	/	/
MO	Thuj-3-en-10-al	1189	1181	tr	/	0.2	0.6	/	0.3	/	/	0.4	/	/	/
CD	(*Z*)-3-Hexenyl butyrate	1190	1184	/	tr	tr	/	tr	/	/	/	/	/	/	1.2
MO	α-Terpineol	1194	1186	0.5	1.4	0.6	0.3	0.4	0.6	0.4	1.4	0.4	0.4	0.4	0.3
CD	Methyl salicylate	1196	1190	tr	0.5	0.3	0.3	0.3	/	tr	0.3	0.4	0.3	tr	0.3
MO	Myrtenol	1197	1194	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*cis*-Piperitol	1199	1195	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr
CD	4-Methylpentyl 2-methylbutyrate	1201	1197	tr	tr	tr	tr	/	tr	tr	tr	tr	tr	tr	tr
MO	Sabinyl acetate	1205	1298*	/	/	/	tr	tr	0.2	tr	tr	tr	/	/	/
O	*n*-Decanal	1207	1201	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*trans*-Piperitol	1210	1207	tr	0.3	0.3	tr	0.2	0.2	tr	0.3	0.2	0.2	tr	tr
MO	Fragranol	1217	1214	0.3	0.3	0.2	0.2	0.1	0.2	0.3	tr	0.2	0.3	0.2	0.2
MO	γ-Isogeraniol	1221	1222*	/	1.0	0.5	0.5	0.2	0.3	0.5	0.4	0.3	0.3	0.4	0.2
MO	β-Cyclocitral	1224	1217	/	/	/	/	/	/	tr	/	tr	/	/	/
MO	Nerol	1231	1227	0.9	1.2	0.5	0.5	0.8	0.7	1.4	1.5	2.5	2.3	1.1	1.0
CD	(*Z*)-3-Hexenyl 2-methyl butyrate	1234	1229	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Hexyl 2-methyl butyrate	1239	1233	tr	tr	0.2	tr	0.1	tr	tr	tr	tr	0.2	0.1	tr
MO	Neral	1244	1235	0.5	/	/	0.2	/	/	0.3	0.3	0.7	0.4	0.3	0.2
O	Cumin aldehyde	1244	1238	/	0.6	0.5	/	0.4	0.4	/	/	/	/	/	/
MO	*cis*-Myrtanol	1247	1250	/	/	/	/	/	/	/	tr	tr	tr	tr	tr
CD	Heptyl isobutyrate	1248	1246	tr	/	/	/	/	/	/	/	/	/	/	/
MO	Carvotanacetone	1251	1244	tr	tr	/	tr	/	/	tr	/	/	/	/	tr
MO	Geraniol	1257	1249	0.4	0.5	0.2	0.2	0.2	0.5	0.4	0.6	0.7	0.4	0.4	0.3
CD	Benzyl propanoate	1261	1257	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Carvenone	1262	1255	/	/	/	tr	/	/	/	/	/	/	/	/
MO	*cis*-Chrysanthenyl acetate	1265	1261	/	/	/	/	/	1.6	/	/	/	/	/	/
MO	*iso*-3-Thujanol acetate	1269	1267	tr	/	/	/	/	/	tr	/	/	/	/	/
MO	Geranial	1273	1264	0.5	0.7	0.5	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.2
MO	Perilla aldehyde	1279	1269	tr	0.3	tr	tr	0.1	tr	tr	tr	tr	0.2	0.2	tr
MO	α-Terpinen-7-al	1288	1283	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Bornyl acetate	1290	1287	/	/	0.2	/	/	/	/	tr	0.2	/	/	/
MO	Lavandulyl acetate	1292	1288	tr	tr	0.9	0.2	0.6	0.3	tr	0.4	0.9	0.4	0.1	tr

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
MO	*p*-Cymen-7-ol	1293	1289	tr	0.3	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	6-Undecanol	1294	1284	/	/	/	/	/	/	/	tr	tr	tr	tr	tr
O	(*E*,*Z*)-2,4-Decadienal	1296	1292	/	/	/	tr	tr	/	/	/	/	/	/	/
CD	Benzyl isobutyrate	1300	1297	tr	0.3	tr	tr	tr	tr	0.3	tr	tr	tr	tr	tr
MO	Perilla alcohol	1302	1294	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Carvacrol	1304	1298	tr	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	4-Methylhexyl 2-methylbutyrate	1308	1304	/	/	/	/	/	/	/	/	/	tr	/	/
CD	(*E*)-3-Hexenyl tiglate	1316	1315	tr	tr	tr	/	tr	/	tr	tr	tr	tr	tr	tr
O	(*E*,*E*)-2,4-Decadienal	1319	1315	/	/	/	tr	tr	/	tr	tr	tr	tr	tr	tr
MO	*cis*-2,3-Pinanediol	1320	1318	tr	0.2	/	/	/	/	/	/	tr	/	/	/
CD	(*Z*)-3-Hexenyl tiglate	1327	1319	tr	0.2	tr	/	0.2	tr	tr	tr	tr	0.2	tr	tr
MO	*p*-Mentha-1,4-dien-7-ol	1331	1325	tr	/	/	/	/	tr	/	/	tr	/	tr	/
CD	Hexyl tiglate	1333	1330	/	tr	tr	tr	0.2	tr	tr	tr	tr	0.2	tr	tr
CD	Heptyl 2-methyl-butyrate	1337	1336*	tr	tr	tr	/	tr	/	/	/	/	/	/	/
O	Fragranyl acetate	1348	1345*	tr	tr	tr	tr	tr	tr	tr	/	tr	0.1	tr	tr
MO	Citronellyl acetate	1355	1350	/	/	/	/	tr	0.2	tr	tr	tr	tr	tr	tr
CD	2-phenyl ethyl Propanoate	1355	1351	0.2	tr	tr	tr	/	tr	/	/	/	/	/	/
PP	Eugenol	1362	1356	0.3	tr	0.2	tr	0.2	tr	tr	tr	tr	tr	0.2	tr
MO	Neryl acetate	1366	1359	0.4	0.2	0.2	0.1	0.2	0.4	tr	tr	0.4	0.3	tr	tr
O	Methyl *p*-anisate	1379	1371	tr	/	/	/	/	/	/	/	/	/	/	/
MO	Myrtanyl acetate	1382	1381*	/	/	/	/	0.1	tr	tr	tr	tr	/	/	/
MO	Geranyl acetate	1386	1379	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Benzyl 2-methylbutyrate	1391	1392*	tr	0.3	tr	0.1	tr	0.2	tr	tr	0.2	0.1	0.2	tr
O	(*E*)-Jasmone	1395	1390	/	/	/	/	tr	/	/	/	/	/	/	/
S	7-*epi*-Sesquithujene	1395	1390	/	/	tr	tr	/	tr	/	/	/	tr	/	/
S	*β*-Cubebene	1396	1387	/	/	/	/	/	/	/	/	tr	/	/	/
S	*β*-Elemene	1398	1389	tr	0.6	0.6	tr	0.1	0.2	/	tr	0.2	tr	/	/
CD	Benzyl valerate	1398	1396*	/	/	/	/	/	/	tr	/	/	/	tr	tr
CD	Phenyl ethyl isobutyrate	1399	1393	/	/	/	tr	/	/	/	/	/	/	/	/
O	(*Z*)-Jasmone	1403	1392	tr	0.2	0.2	0.2	0.3	tr	tr	tr	tr	tr	tr	tr
S	Italicene	1411	1405	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	/	tr
MO	Linalool butyrate	1425	1421	1.9	0.7	2.0	0.7	1.4	0.8	/	/	/	/	/	/
MO	Lavandulyl isobutyrate	1426	1421	/	/	/	/	/	/	0.8	1.9	2.2	1.4	1.0	0.6
S	(*E*)-Caryophyllene	1427	1417	0.9	0.9	tr	1.4	0.9	1.3	0.4	tr	1.0	0.8	0.9	0.6
S	*β*-Copaene	1437	1430	/	/	/	/	/	/	tr	/	tr	/	/	/
CD	2-Phenyl ethyl butyrate	1445	1439	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Citronellyl propanoate	1445	1444	tr	/	/	/	tr	tr	/	/	tr	tr	tr	/
S	(*Z*)-*β*-Farnesene	1448	1440	tr	/	0.2	0.2	tr	tr	tr	tr	tr	0.1	/	tr
MO	Neryl propanoate	1456	1452	0.3	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
S	*α*-Humulene	1462	1452	tr	tr	tr	0.2	0.1	tr	tr	tr	tr	tr	tr	tr
O	*γ*-Decalactone	1472	1465	tr	/	/	/	/	/	/	/	/	/	/	/

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
SO	dehydro-Sesquicineole	1474	1469	/	/	0.3	0.2	0.1	tr	/	tr	0.2	tr	/	/
MO	Geranyl propanoate	1476	1476	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
S	*γ*-Selinene	1483	1479*	/	/	/	/	/	/	/	/	/	tr	tr	/
S	*γ*-Curcumene	1485	1481	0.4	/	0.3	0.5	0.1	0.3	0.3	0.3	0.5	0.2	/	tr
MO	Citronellol isobutyrate	1486	1482	/	tr	tr	/	/	/	/	/	/	/	/	/
S	*ar*-Curcumene	1489	1479	0.4	tr	0.9	1.0	0.2	0.6	0.6	0.3	1.1	0.7	/	tr
MO	Neryl isobutyrate	1493	1490	2.6	1.5	1.0	0.5	1.0	1.0	0.6	0.7	1.6	1.4	tr	0.3
S	*β*-selinene	1495	1489	0.7	1.2	1.6	1.2	0.4	0.6	0.3	1.1	1.5	1.0	4.4	0.7
CD	Benzyl tiglate	1503	1497	/	tr	/	tr	tr	/	tr	tr	tr	/	/	/
S	*α*-Selinene	1503	1498	/	/	tr	/	/	/	/	/	/	/	tr	/
S	Bicyclogermacrene	1505	1500	tr	/	/	/	/	tr	tr	/	/	/	/	tr
SO	Lavandulyl isovalerate	1513	1509	5.7	2.0	1.2	2.5	2.5	2.4	2.1	3.8	5.4	4.9	3.2	1.7
SO	Lavandulyl 2-methyl butyrate	1514	1511	tr	tr	0.6	tr	2.2	/	/	/	tr	tr	tr	tr
MO	Geranyl isobutyrate	1516	1514	tr	0.2	tr	tr	tr	tr	/	/	tr	/	/	tr
S	*α*-dehydro-*ar*-Himachalene	1521	1516	0.4	/	/	tr	0.2	tr	tr	0.3	0.2	tr	0.4	tr
SO	10-*epi*-Italicene ether	1523	1515	/	/	tr	tr	/	/	/	/	/	/	/	/
S	7-*epi*-*α*-Selinene	1527	1520	/	tr	/	/	/	tr	/	/	/	/	/	/
S	*β*-Sesqui-phellandrene	1530	1521	/	/	/	/	/	/	/	/	/	tr	/	/
S	*δ*-Cadinene	1531	1522	tr	tr	tr	tr	tr	tr	tr	tr	tr	/	/	/
SO	Italicene ether	1543	1536	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	/	/
SO	Silphiperfo lan-6-*β*-ol	1547	1546	tr	/	/	/	/	/	/	/	/	/	/	/
SO	*cis*-Sesquisabinene hydrate	1550	1542	/	/	tr	tr	/	tr	tr	/	/	tr	/	/
SO	(*E*)-Nerolidol	1566	1561	0.3	0.4	0.2	0.3	0.5	0.2	0.3	0.4	0.4	0.2	0.2	0.2
SO	Citronellyl isovalerate	1573	1563	tr	/	tr	tr	0.1	tr	tr	tr	0.3	0.3	tr	tr
SO	*trans*-Sesquisa-binene hydrate	1586	1577	4.4	2.0	0.9	1.4	2.0	1.4	1.5	2.0	3.0	3.9	1.3	0.7
SO	Neryl isovalerate	1587	1582	2.4	0.9	0.9	1.2	1.6	1.1	0.9	1.1	2.2	2.3	0.7	0.4
SO	Caryophyllene oxide	1592	1582	0.6	1.2	0.5	0.9	1.0	1.3	0.3	0.5	1.5	0.8	0.9	0.4
SO	10-*epi*-Junenol	1593	1590*	/	0.2	/	0.2	0.2	0.2	tr	/	0.3	/	/	/
SO	Humulene epoxide I	1603	1601*	/	/	/	tr	tr	tr	tr	tr	/	/	tr	/
SO	Geranyl 2-methyl butyrate	1604	1601	0.6	tr	0.3	tr	0.2	tr	tr	0.2	0.3	0.3	0.2	tr
SO	Khusimone	1614	1604	tr	/	/	/	/	/	tr	tr	0.2	tr	tr	tr
SO	Geranyl isovalerate	1616	1606	0.8	0.5	0.5	0.3	0.4	0.4	tr	0.4	0.7	0.5	0.4	tr
SO	Humulene epoxide II	1618	1608	/	tr	tr	/	/	/	/	/	/	/	/	/
SO	Junenol	1628	1618	/	/	/	/	/	/	/	/	0.4	/	/	/
SO	Caryophylla-4(12), 8(13)-dien-5-*α*-ol	1646	1639	/	/	/	/	/	tr	/	/	tr	tr	tr	/
SO	*allo*-Aroma-dendrene epoxide	1647	1639	/	/	/	/	0.3	0.3	tr	/	/	/	/	/
SO	*β*-Eudesmol	1659	1649	/	/	0.6	/	/	/	/	/	tr	/	/	/
SO	*α*-Eudesmol	1662	1652	/	/	tr	tr	/	/	/	/	/	/	/	/
SO	*α*-Bisabolol oxide B	1663	1656	/	/	/	tr	tr	/	/	/	tr	0.2	/	/

Class	Compound	RI	Ria	Content, %											
				AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
SO	Intermedeol	1670	1665	0.4	tr	/	/	tr	0.3	tr	tr	0.4	0.2	tr	tr
SO	*epi*-*β*-Bisabolol	1678	1670	/	/	/	/	/	tr	tr	tr	tr	tr	/	/
SO	*β*-Bisabolol	1678	1674	/	/	0.2	0.1	0.3	/	/	/	/	/	/	/
SO	Elemol acetate	1686	1680	/	/	/	/	/	/	/	/	/	/	tr	/
SO	*epi*-*α*-Bisabolol	1690	1683	/	/	/	0.6	/	/	/	/	/	/	/	/
SO	*α*-Bisabolol	1691	1685	tr	/	1.3	0.4	0.5	0.2	tr	0.5	0.9	0.4	/	tr
SO	Germacrone	1694	1693	/	tr	/	/	/	/	/	/	/	/	tr	tr
SO	Shyobunol	1695	1687	/	/	/	/	/	/	/	/	0.3	0.2	/	/
SO	*β*-Sinensal	1709	1699	/	/	tr	/	/	/	/	/	/	/	/	/
O	Pentadecanal	1718	1715*	/	/	/	tr	/	/	/	/	/	/	/	/
O	Chamazulene	1740	1730	0.4	tr	tr	0.2	0.4	0.2	tr	0.3	0.3	0.2	0.2	tr
SO	Eudesma-2,4(15),11-triene	1751	*	/	/	tr	/	/	/	/	/	/	/	/	/
SO	*γ*-Costol	1755	1745	/	tr	tr	tr	/	tr	tr	tr	tr	tr	/	/
O	Eupatorio-chromene	1771	1761	tr	tr	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr
SO	*β*-Costol	1775	1765	tr	tr	tr	0.4	tr	tr	tr	tr	0.2	0.2	0.2	tr
O	Phenanthrene	1786	1784*	/	/	/	/	tr	/	/	/	/	/	/	/
SO	Bisabola-1(6),10-dien-*trans*-2,3-diol	1789	*	/	/	/	0.2	/	/	/	/	/	/	/	/
SO	Hexahydrofarnesyl acetone	1848	1846*	/	tr	tr	0.3	tr	tr	tr	tr	tr	0.1	tr	tr
SO	(*Z*)-Lanceol acetate	1861	1854	/	/	/	tr	tr	/	/	/	/	/	/	/
MO	Linalyl phenylacetate	1913	*	/	/	/	/	/	tr	/	/	/	/	/	/
O	(*E*)-3-Cembrene A	1957	1947	/	/	/	/	tr	/	/	/	/	/	/	/
O	Geranyl-*α*-terpinene	1962	1962	/	/	/	/	/	/	/	tr	tr	0.4	tr	tr
O	Geranyl-*p*-cymene	2003	1993*	/	/	1.0	1.8	0.8	0.7	tr	0.5	0.8	1.8	0.3	0.2

RI-Experimental linear retention indices relative to C8-C40 alkanes. Ria-Literature indices-Adams' retention indices and *according to NIST data base. Tr- trace<0.05 % and not detected compounds are marked as (/). M-Hydrocarbon Monoterpenoids, MO-Oxygenated Monoterpenoids, S-Hydrocarbon Sesquiterpenoids, SO-Oxygenated Sesquiterpenoids, PP-Phenylpropanoids, CD-carboxylic acid derivatives, A-Alkanes, O-Other

Table S-V. The number of identified components per sample of *A. absinthium*, the percentage of each class of compounds, and the percentage of total identified components

Sample	AB 1	AB 2	AB3	AB 4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12
Contribution in total peaks area of ion chromatogram, %	92.0	94.8	94.6	82.9	91.5	95.6	98.6	95.5	95.1	93.7	94.3	98.1
Number of components	134	131	142	143	138	142	141	136	144	137	119	127
	Content, %											
Total monoterpenoids	71.8	79.8	78.5	65.8	73.4	80.5	90.9	81.7	70.7	71.8	79.6	90.5
Monoterpene hydrocarbons (M)	31.7	38.4	27.9	11.6	21.8	33.0	17.1	35.4	33.6	24.1	29.9	19.4
Oxygenated monoterpenes (MO)	40.1	41.4	50.7	54.2	51.6	47.6	73.8	46.4	37.1	47.7	49.7	71.1
Total sesquiterpenoids	17.9	9.9	11.2	13.4	13.9	10.7	6.8	10.7	21.3	17.1	12.7	4.5
Sesquiterpene hydrocarbons (S)	2.5	2.7	3.6	4.4	2.1	3.1	1.6	1.9	4.6	2.8	5.7	1.3
Oxygenated Sesquiterpene (SO)	15.5	7.2	7.6	9.0	11.8	7.6	5.1	8.8	16.8	14.3	7.0	3.3
Phenylpropanoids (PP)	0.3	tr	0.2	tr	0.2	tr	tr	tr	tr	tr	0.2	tr
Carboxylic acid derivatives (CD)	0.2	1.6	0.7	0.5	0.8	0.2	0.3	0.3	0.6	0.9	0.3	1.5
n-Alkanes (A)	tr	tr	tr	/	/	/	tr	tr	/	/	/	/
Other (O)	1.8	3.4	4.0	3.2	3.1	4.2	0.6	2.8	2.4	4.0	1.5	1.6

Table S-VI. Chemical composition of twelve *Artemisia annua* samples collected from different soil types according to the WRB

Class	Compound	RI	RIa	Content, %											
				AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
O	3-Methyl-2-buten-1-ol	775	765	/	tr	/	/	/	tr	tr	/	tr	/	tr	/
CD	Methyl 2-methylbutyrate	784	780*	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	3-Methyl-2-butenal	788	778	/	tr	/	/	tr	tr	tr	tr	tr	tr	/	/
O	Hexanal	801	801	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Ethyl 2-methylbutyrate	848	842*	0.4	0.6	0.6	0.5	0.6	0.4	0.4	0.7	0.5	0.7	0.5	0.3
O	(*E*)-2-Hexenal	850	846	/	/	/	/	/	/	0.1	/	tr	/	/	tr
CD	Ethyl 3-methylbutyrate	851	849	tr	tr	tr	tr	0.1	/	/	0.1	/	tr	tr	/
O	(*Z*)-3-Hexenol	852	850	/	tr	/	/	/	/	tr	/	tr	/	tr	/
CD	Propyl isobutyrate	854	856*	/	/	0.1	0.1	tr	tr	/	0.1	/	tr	/	tr
O	(*E*)-2-Hexenol	864	854	/	/	/	/	/	tr	/	/	/	/	/	tr
O	(*Z*)-2-Hexenol	865	859	/	/	/	/	tr	/	tr	/	tr	/	/	/
O	*n*-Hexanol	867	863	tr	tr	tr	tr	0.1	0.1	0.1	tr	tr	tr	tr	tr
M	Santolina triene	909	906	0.4	0.2	tr	0.2	0.3	0.3	0.3	0.3	0.8	0.5	0.4	0.3
M	Tricyclene	924	921	0.1	tr	0.1	0.1	tr	0.1	tr	0.2	0.1	tr	0.1	0.1
M	α-Thujene	928	924	0.1	tr	0.1	tr	0.1	0.1	tr	0.1	0.1	0.1	0.1	tr
M	α-Pinene	935	932	12.8	12.2	20.3	3.8	14.9	10.9	13.4	10.1	8.6	16.4	23.3	9.0
CD	Ethyl tiglate	939	929	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	/	tr
CD	Propyl 2-methylbutyrate	946	944*	0.2	0.4	0.5	0.5	0.3	tr	0.2	0.4	0.3	0.4	0.3	0.1
M	Camphene	950	946	1.2	0.5	3.3	2.7	1.1	1.6	0.7	3.7	1.9	1.0	1.7	1.6
CD	Butyl isobutyrate	953	952*	/	/	tr	/	/	/	/	/	/	/	/	/
M	Thuja-2,4(10)-diene	956	953	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	Benzaldehyde	962	952	tr	/	tr	/	/	tr	tr	/	tr	tr	tr	tr
M	Sabinene	975	969	1.1	0.7	1.8	1.5	1.1	0.8	0.6	1.5	0.7	0.8	1.8	1.0
M	β-Pinene	979	974	1.2	1.1	2.4	0.7	1.3	1.0	1.1	1.4	1.0	1.3	2.1	0.9
O	6-Methyl-5-hepten-2-one	988	981	/	tr	/	/	/	/	/	/	/	/	/	/
M	Myrcene	992	988	0.9	0.4	2.9	0.5	1.5	1.0	0.7	4.3	0.1	0.7	0.6	0.7
MO	Yomogi alcohol	1001	999	0.6	1.2	0.2	0.9	1.1	1.1	1.0	0.9	1.0	1.0	0.4	0.6
CD	Isobutyl isovalerate	1005	1004*	/	/	/	/	/	/	/	/	tr	/	/	/
M	α-Phellandrene	1006	1002	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	δ-3-Carene	1013	1008	/	/	tr	/	/	/	/	/	/	/	/	/
M	α-Terpinene	1019	1014	0.1	0.2	0.3	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.1
M	*o*-Cymene	1027	1022	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr
M	Limonene	1031	1024	tr	tr	/	tr	tr	tr	tr	0.2	tr	tr	tr	tr
MO	1,8-Cineole	1033	1026	5.8	5.4	11.0	11.9	7.6	6.0	4.9	9.7	5.4	5.0	8.4	6.6
MO	Santolina alcohol	1037	1034	0.2	0.2	tr	0.1	0.2	0.2	0.2	0.1	0.3	0.3	0.1	0.2
M	(*Z*)-β-Ocimene	1039	1032	/	/	tr	/	/	/	/	/	/	/	/	/
CD	Butyl 2-methylbutyrate	1043	1041*	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	Benzene acetaldehyde	1046	1036	/	tr	tr	/	tr	tr	/	tr	tr	tr	0.1	tr
M	(*E*)-β-Ocimene	1050	1044	tr	/	tr	/	tr	tr	tr	tr	/	tr	tr	tr
CD	Prenyl isobutyrate	1055	1048	/	/	tr	/	/	/	/	tr	/	/	/	/
M	γ-Terpinene	1061	1054	tr	tr	0.4	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Artemisia ketone	1063	1056	38.1	49.8	6.9	40.8	43.9	45.7	49.7	32.8	48.8	46.5	20.7	36.0
MO	*cis*-Sabinene hydrate	1069	1065	0.9	0.6	0.5	0.4	0.4	0.5	0.5	0.2	0.3	0.5	0.8	0.7
MO	*cis*-Linalool oxide	1074	1067	/	/	tr	/	/	/	/	/	/	/	/	/
MO	Artemisia alcohol	1085	1080	5.8	4.7	1.2	5.2	4.2	6.4	4.4	4.6	5.2	6.2	3.3	6.6
M	Terpinolene	1091	1086	tr	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr
MO	*trans*-Sabinene hydrate	1100	1098	0.4	0.2	0.3	0.2	0.2	tr	0.2	0.1	0.2	0.2	0.4	0.3
MO	α-Pinene oxide	1102	1099	/	tr	0.1	/	/	/	tr	/	/	tr	0.1	tr
CD	2-Methyl butyl 2-Methyl butyrate	1104	1100	tr	/	/	/	/	tr	tr	/	tr	tr	tr	tr
O	*n*-Nonanal	1105	1100	/	tr	/	tr	tr	/	/	/	/	/	/	/
CD	3-Methyl-3-butenyl	1115	1112	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1

Class	Compound	RI	RIa	Content, %											
				AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
	3-Methyl butyrate														
MO	*exo*-Fenchol	1117	1114	tr	/	/	/	tr	/	tr	tr	tr	tr	tr	tr
MO	*trans*-Thujone	1119	1112	tr	/	/	/	/	/	/	/	/	/	/	/
MO	*trans*-*p*-Mentha-2, 8-dien-1-ol	1121	1119	/	0.1	tr	/	0.1	tr	tr	tr	/	/	/	/
MO	*cis*-*p*-Menth-2-en-1-ol	1124	1118	0.1	/	0.2	/	/	tr	/	0.1	0.1	/	0.1	tr
MO	*trans*-Pinene hydrate	1125	1119	/	/	/	/	/	/	0.1	/	/	0.1	/	0.1
MO	*α*-Campholenal	1129	1122	0.1	0.5	0.5	0.2	0.1	0.3	0.4	0.2	0.3	0.3	0.4	0.3
MO	*cis*-*p*-Mentha-2, 8-dien-1-ol	1135	1133	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Butyl 2-methylbutyrate	1140	1141*	/	/	/	/	/	tr	/	/	/	/	/	/
MO	*trans*-Pinocarveol	1141	1135	2.8	4.3	5.4	1.7	3.3	2.4	4.1	1.3	1.8	2.6	3.4	2.3
MO	Camphor	1148	1141	7.8	2.5	18.2	18.8	5.6	10.6	3.6	17.8	10.3	4.0	8.6	12.8
MO	*β*-Pinene oxide	1159	1154	tr	0.3	1.4	0.1	0.1	0.1	0.1	0.2	/	0.1	/	0.1
MO	Isoborneol	1161	1155	/	/	/	0.1	/	/	/	/	/	/	/	/
MO	*cis*-chrysanthenol	1166	1160	0.1	tr	/	/	0.1	0.1	tr	0.1	0.2	tr	0.1	0.1
MO	Pinocarvone	1167	1160	1.9	3.9	4.2	1.7	3.0	2.1	3.6	1.2	1.5	2.2	2.8	2.4
MO	*δ*-Terpineol	1171	1162	/	/	tr	/	/	/	/	/	/	/	/	/
MO	Lavandulol	1172	1165	1.1	tr	/	tr	0.8	0.7	0.6	0.6	tr	tr	tr	tr
MO	Borneol	1174	1165	tr	0.4	0.6	0.3	tr	tr	tr	tr	0.9	0.9	1.0	0.8
MO	Artemisyl acetate	1175	1169	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*cis*-Pinocamphone	1178	1172	0.4	tr	tr	tr	tr	tr	0.1	tr	0.1	0.1	0.3	tr
MO	Terpinen-4-ol	1181	1174	0.3	0.6	1.4	0.6	0.7	0.7	0.6	0.6	0.5	0.5	0.8	0.4
MO	*p*-Cymen-8-ol	1188	1179	tr	tr	/	/	/	/	/	/	tr	tr	0.1	/
MO	Thuj-3-en-10-al	1189	1181	/	/	0.1	tr	tr	tr	tr	tr	/	/	/	tr
MO	*trans*-*p*-Mentha-1(7), 8-dien-2-ol	1191	1187	/	/	tr	0.1	/	/	/	/	/	/	/	/
MO	*α*-Terpineol	1194	1186	0.5	0.2	0.6	0.3	0.5	0.4	0.2	0.4	0.3	0.4	0.9	0.5
MO	*p*-Mentha-1,5-dien-7-ol	1196	1191	/	/	0.1	0.1	/	/	tr	tr	tr	tr	/	/
MO	Myrtenol	1197	1194	tr	0.7	1.0	0.5	0.5	0.4	0.6	0.3	0.4	0.4	0.6	0.5
MO	Myrtenal	1200	1195	0.4	/	/	/	/	/	/	/	/	/	/	/
MO	*trans*-Piperitol	1210	1207	/	tr	tr	tr	tr	tr	tr	/	tr	tr	tr	/
MO	Verbenone	1213	1204	tr	tr	tr	/	tr	/	tr	/	tr	tr	tr	tr
MO	*trans*-Carveol	1222	1215	0.1	0.1	0.4	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.4	/
MO	*cis*-*p*-Mentha-1(7),8-dien-2-ol	1231	1227	/	tr	tr	tr	/	tr	tr	/	tr	tr	tr	tr
CD	(*Z*)-3-Hexenyl 2--methyl butyrate	1234	1229	0.1	0.2	0.3	0.1	/	/	0.1	/	0.2	0.1	0.3	0.2
CD	(*Z*)-3-Hexenyl 3-methyl butyrate	1238	1232	0.1	/	0.2	/	0.1	0.2	/	0.2	/	/	/	/
CD	Hexyl 2-methyl butyrate	1239	1233	/	0.2	/	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.1
O	Cumin aldehyde	1244	1238	/	/	/	tr	/	/	/	/	/	/	/	/
CD	Hexyl 3-methylbutyrate	1245	1241	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Carvone	1248	1239	tr	tr	0.1	tr	tr	0.1	tr	tr	tr	tr	0.1	0.1
MO	Carvotanacetone	1251	1244	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Geraniol	1257	1249	0.1	/	tr	tr	0.1	tr	tr	tr	tr	tr	0.1	0.1
MO	*cis*-Chrysanthenyl acetate	1265	1261	tr	tr	tr	tr	/	/	/	/	/	/	/	/
MO	Geranial	1273	1264	tr	/	/	/	tr	/	/	/	/	/	tr	/
MO	*cis*-Verbenyl acetate	1281	1280	/	/	0.1	/	/	/	/	/	/	/	/	/
MO	Isobornyl acetate	1289	1283	/	/	tr	0.1	/	/	/	/	/	/	/	/
MO	Bornyl acetate	1290	1287	tr	tr	tr	0.1	/	tr	tr	/	tr	/	tr	tr
O	1-Tridecene	1293	1290	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
CD	Benzyl isobutyrate	1300	1297	tr	/	tr	tr	tr	tr	/	/	/	/	/	/
MO	*trans*-Pinocarvyl acetate	1304	1298	0.1	0.2	0.4	0.2	0.1	0.1	0.2	/	0.1	tr	0.1	0.1

Class	Compound	RI	RIa	Content, %											
				AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
CD	(*Z*)-3-Hexenyl tiglate	1327	1319	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Myrtenyl acetate	1329	1324	/	/	tr	tr	/	/	/	/	/	/	/	/
CD	Hexyl tiglate	1333	1330	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*trans*-Carvyl acetate	1341	1339	/	/	tr	/	/	/	/	/	/	/	/	/
S	*α*-Cubebene	1355	1345	tr	/	tr	/	/	/	/	/	/	/	/	/
PP	Eugenol	1362	1356	0.4	0.2	0.3	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.4
S	*α*-Copaene	1380	1374	0.3	0.3	0.3	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.4	0.3
CD	Benzyl 2-methylbutyrate	1391	1392*	/	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1
S	*β*-Cubebene	1396	1387	/	0.1	0.1	tr	tr	tr	0.1	tr	tr	tr	0.1	0.1
S	*β*-Elemene	1398	1389	0.2	tr	tr	tr	tr	tr	tr	tr	0.1	tr	0.1	0.1
O	(*Z*)-Jasmone	1401	1392	0.1	tr	0.1	tr	tr	tr	tr	tr	0.1	0.1	0.1	0.1
S	*α*-Cedrene	1420	1410	/	/	tr	/	/	/	/	/	/	/	/	/
S	(*E*)-Caryophyllene	1427	1417	2.1	0.9	0.9	0.5	0.9	0.7	1.0	0.5	1.6	1.0	2.3	1.7
S	*β*-Copaene	1437	1430	tr	/	/	/	/	/	tr	/	/	tr	tr	tr
S	Amorpha-4,11-diene	1458	1449	/	/	/	/	/	/	/	/	/	0.4	0.3	/
S	(*E*)-*β*-Farnesene	1460	1454	0.5	/	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.5	0.5
S	*α*-Humulene	1462	1452	tr	0.1	0.1	0.1	0.3	0.4	0.1	tr	0.1	tr	tr	0.6
S	*cis*-Muurola-4(14), 5-diene	1471	1465	tr	tr	0.1	/	/	/	/	/	/	/	/	/
S	*γ*-Selinene	1483	1479*	0.5	tr	0.1	tr	0.3	0.2	0.2	0.1	0.5	0.4	1.1	0.6
S	Germacrene D	1490	1484	3.4	1.5	1.3	1.1	1.3	1.0	1.7	0.8	1.4	1.5	2.8	2.7
S	*β*-Selinene	1495	1489	2.2	2.1	3.6	0.5	0.5	0.7	0.2	2.1	0.3	0.6	0.4	0.5
SO	Indipone	1502	1496	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
S	Bicyclogermacrene	1505	1500	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2
S	(*E*,*E*)-*α*-Farnesene	1512	1505	/	tr	/	/	/	/	/	/	/	/	/	/
S	*β*-Bisabolene	1513	1505	/	/	/	/	tr	/	tr	/	/	/	/	/
S	Germacrene A	1514	1508	0.1	tr	tr	/	/	tr	/	/	tr	tr	0.1	0.1
SO	Cubenol	1522	1514	/	tr	/	/	tr	/	tr	tr	/	tr	0.1	tr
SO	Isobornyl isovalerate	1523	1521	tr	/	tr	tr	/	/	/	/	/	/	/	/
S	*δ*-Cadinene	1531	1522	0.1	tr	0.1	tr	tr	tr	tr	tr	tr	tr	0.1	0.1
S	*γ*-Cuprenene	1542	1532	/	/	/	/	/	/	/	/	tr	/	/	/
S	*α*-Calacorene	1551	1544	/	/	tr	/	/	/	/	/	/	/	/	/
SO	Bornyl angelate	1569	1564	tr	tr	tr	tr	tr	tr	/	tr	tr	tr	tr	tr
CD	(*Z*)-3-Hexenyl benzoate	1575	1565	tr	/	0.1	/	/	/	tr	/	/	/	/	/
SO	Germacrene D-4-ol	1584	1574	tr	/	/	/	/	/	/	/	/	/	/	0.2
SO	Spathulenol	1587	1577	/	/	tr	tr	/	/	/	tr	/	tr	tr	tr
SO	Thujopsan-2-*α*-ol	1590	1586	/	/	0.1	/	/	/	/	/	/	/	/	/
SO	Caryophyllene oxide	1592	1582	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.3	0.2
SO	*β*-Copaen-4-*α*-ol	1598	1590	/	tr	0.1	/	tr	/	/	/	/	/	/	/
SO	1,10-di-*epi*-Cubenol	1624	1618	/	/	/	/	/	/	/	/	/	/	tr	/
SO	*epi*-Cedrol	1626	1618	/	0.1	0.1	tr	/	/	/	/	/	/	/	/
SO	Muurola-4,10(14)-dien-1-*β*-ol	1637	1630	/	/	0.1	0.2	/	/	/	/	/	/	/	/
SO	Longifolenaldehyde	1635	1631*	/	/	/	/	/	/	0.2	/	0.3	0.2	0.5	0.5
SO	*cis*-Cadin-4-en-7-ol	1640	1635	0.1	0.1	0.1	tr	tr	0.1	tr	0.1	0.1	0.1	0.2	0.2
SO	Selina-3,11-dien-6-*α*-ol	1642	1642	/	0.2	0.1	0.3	0.2	0.2	0.4	tr	0.4	0.3	0.7	0.7
SO	Caryophylla-4(12),8(13)-dien-5-α-ol	1646	1639	0.1	0.1	0.1	0.1	/	tr	tr	tr	0.1	0.1	0.1	0.2
SO	*allo*-Aromadendrene epoxide	1647	1639	0.1	/	/	/	tr	/	/	/	/	/	/	/
SO	Pogostol	1655	1651	/	/	/	/	/	/	/	/	/	0.1	/	/
SO	Selin-11-en-4-*α*-ol	1665	1658	0.1	tr	0.1	0.1	0.1	tr	0.1	/	0.1	0.1	0.3	0.3
SO	Intermedeol	1670	1665	0.1	/	tr	/	/	/	/	/	/	/	/	/
SO	14-hydroxy-(*Z*)-	1672	1666	/	tr	/	/	/	/	/	/	/	/	/	/

Class	Compound	RI	RIa	Content, %											
				AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
	Caryophyllene														
SO	*epi*-Zizanone	1675	1668	tr	/	/	/	/	/	/	/	/	/	/	/
SO	(*Z*)-*α*-Santalol	1679	1674	0.1	/	/	/	tr	tr	0.1	/	0.1	0.1	0.3	/
SO	*α*-Bisabolol	1691	1685	0.1	/	/	/	/	/	/	/	/	/	0.2	0.2
SO	Germacra-4(15),5, 10(14)-trien-1-*α*-ol	1695	1685	0.4	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	tr	0.3	0.6
SO	Cedr-8(15)-en-9-yl-acetate	1746	1741	/	/	tr	/	/	/	/	/	/	/	/	/

RI-Experimental linear retention indices relative to C8-C40 alkanes. Ria-Literature indices-Adams' retention indices and * according to NIST data base. Tr- trace<0.05% and not detected compounds are marked as (/). M-Hydrocarbon Monoterpenoids, MO-Oxygenated Monoterpenoids, S-Hydrocarbon Sesquiterpenoids, SO-Oxygenated Sesquiterpenoids, PP-Phenylpropanoids, CD-carboxylic acid derivatives, A-Alkanes, O-Other

Table S-VII. The number of identified components per sample of *A. annua*, the percentage of each class of compounds, and the percentage of total identified components

Sample	AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12
Contribution in total peaks area of ion chromatogram, %	97.5	98.5	96.8	98.8	99.1	99.0	98.9	99.1	98.8	99.3	97.7	96.9
Number of components	98	97	114	94	94	94	99	87	96	99	99	97
	Content, %											
Total monoterpenoids	85.4	91.2	86.5	94.1	93.2	93.9	92.5	93.3	91.1	92.1	84.4	85.2
Monoterpene hydrocarbons (M)	17.9	15.2	32.0	9.8	20.7	16.0	17.1	22.0	13.5	21.0	30.5	13.6
Oxygenated monoterpenes (MO)	67.5	75.9	54.6	84.3	72.5	77.9	75.45	71.3	77.5	71.1	53.9	71.6
Total sesquiterpenoids	10.7	5.7	7.9	3.2	4.2	3.9	4.9	4.0	6.0	5.4	11.3	10.3
Sesquiterpene hydrocarbons (S)	9.4	5.0	6.6	2.4	3.7	3.4	3.8	3.8	4.5	4.5	8.4	7.2
Oxygenated Sesquiterpene (SO)	1.2	0.7	1.3	0.8	0.5	0.4	1.1	0.2	1.6	0.9	2.9	3.1
Phenylpropanoids (PP)	0.4	0.2	0.3	0.2	0.2	0.3	0.2	0.1	0.2	0.2	0.3	0.4
Carboxylic acid derivatives (CD)	1.0	1.5	2.0	1.4	1.3	1.0	1.1	1.7	1.4	1.6	1.6	0.9
Other (O)	0.1	tr	0.1	tr	0.1	0.1	0.1	tr	0.1	0.1	0.2	0.1

Table S-VIII. Chemical composition of twelve *Artemisia vulgaris* samples collected from different soil types according to the WRB

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
O	(*Z*)-2-Penten-1-ol	775	765	/	/	/	/	/	/	/	/	/	/	0.2	/
O	3-Methyl-2-butenal	788	778	/	/	/	/	/	/	/	/	/	tr	tr	tr
O	1-Octene	793	788	/	/	/	/	/	/	tr	tr	tr	tr	/	tr
O	Hexanal	801	801	/	/	tr	/	tr	tr	tr	tr	tr	tr	tr	tr
O	Furfural	833	828	/	/	/	/	tr	/	/	/	/	/	/	/
O	4-Methyl-pentanol	834	830	/	tr	/	/	/	/	/	/	/	/	/	/
CD	Methyl 3-methyl-2-butyrate	843	842*	/	/	/	/	/	/	/	/	/	tr	tr	/
O	(*E*)-2-Hexenal	850	846	/	/	/	/	/	/	/	tr	tr	tr	0.8	/
O	(*E*)-3-Hexenal	851	846	tr	/	tr	/	/	/	/	/	/	/	/	/
O	(*Z*)-3-Hexenol	852	850	/	tr	/	/	tr	/	/	/	/	/	0.4	tr
O	7-Methyl-1-octene	853	852*	/	/	/	/	/	/	tr	tr	tr	tr	/	/
O	(*E*)-2-Hexenol	864	854	/	/	/	/	/	tr	/	/	/	/	/	/
O	(*Z*)-2-Hexenol	865	859	/	/	tr	/	tr	/	/	/	/	/	/	/
O	Cyclohexanol	865	869*	/	/	/	/	/	/	tr	tr	tr	tr	tr	tr
O	*n*-Hexanol	867	863	tr	tr	tr	/	tr	tr	tr	tr	tr	tr	0.4	tr
CD	Isopentyl acetate	876	869	/	/	/	/	/	/	/	tr	/	/	/	/
O	1-Hepten-3-ol	879	870*	/	/	/	/	/	/	/	/	/	/	/	tr
O	1-Nonene	892	892*	/	/	/	/	/	tr	tr	tr	tr	tr	/	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
O	(*Z*)-4-Heptenal	895	893	/	/	/	/	/	/	/	/	/	/	tr	tr
A	*n*-Nonane	900	900	/	/	/	/	/	/	/	/	/	/	/	tr
M	Santolina Triene	909	906	4.2	tr	1.5	0.4	/	3.4	tr	2.3	6.1	3.2	tr	/
O	(*E*,*E*)-2,4-Hexadienal	912	907	/	/	/	/	tr	/	/	/	/	/	tr	/
CD	Isobutyl isobutyrate	915	908	/	/	/	/	/	/	/	/	/	tr	/	/
O	2,5-diethenyl-2-methyl-Tetrahydrofuran	917	912	/	/	/	/	/	/	/	/	/	tr	/	/
M	Tricyclene	924	921	/	/	tr	tr	/	tr	tr	tr	tr	tr	/	tr
M	*α*-Thujene	928	924	tr	tr	tr	tr	tr	0.2	0.4	0.1	tr	0.2	tr	tr
M	*α*-Pinene	935	932	1.3	tr	0.4	tr	tr	1.5	4.5	1.0	1.6	2.3	tr	tr
M	Camphene	950	946	0.1	tr	0.2	tr	/	0.3	0.8	tr	0.4	0.2	tr	tr
M	Thuja-2,4(10)-diene	956	953	/	/	/	/	/	/	tr	tr	tr	tr	/	tr
O	(*E*)-2-Heptenal	957	947	/	/	/	/	tr	/	/	/	/	/	tr	tr
O	Benzaldehyde	962	952	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	Isoamyl propionate	970	960	/	tr	tr	/	/	/	/	/	/	tr	/	/
M	Sabinene	975	969	2.5	tr	0.7	tr	tr	6.0	15.4	3.0	3.3	2.4	0.8	0.5
M	*β*-Pinene	979	974	0.9	tr	0.5	tr	tr	1.5	1.3	1.0	1.4	0.8	tr	0.4
O	1-Octen-3-ol	980	974	tr	0.2	tr	tr	0.2	tr	/	tr	/	tr	0.5	tr
O	6-methyl-5-Hepten-2-one	988	981	/	tr	tr	/	tr	tr	tr	tr	tr	tr	tr	tr
M	Myrcene	992	988	3.6	tr	1.0	1.6	0.7	2.6	4.9	0.7	1.4	3.3	0.6	2.0
O	3-Octanol	995	988	/	/	/	/	tr	tr	/	tr	tr	tr	tr	tr
O	Mesitylene	996	994	/	/	/	/	/	/	tr	/	/	/	/	tr
MO	Yomogi alcohol	1001	999	/	tr	/	/	/	/	tr	tr	tr	/	0.6	/
O	*n*-Octanal	1004	998	/	/	/	/	tr	/	/	/	/	/	tr	tr
CD	Isobutyl 2-methylbutanoate	1005	1004*	tr	tr	tr	tr	/	/	/	/	/	tr	/	/
M	*α*-Phellandrene	1006	1002	tr	tr	0.6	/	/	tr	tr	tr	tr	0.4	tr	tr
CD	(*Z*)-3-Hexenyl acetate	1008	1004	/	/	/	/	/	/	/	/	/	/	/	tr
O	(*E*,*E*)-2,4-Heptadienal	1012	1005	/	/	/	/	tr	/	/	/	/	/	tr	/
M	*δ*-3-Carene	1013	1008	/	/	/	/	tr	/	/	/	tr	/	/	/
CD	Isoamyl isobutyrate	1014	1007	/	/	tr	/	/	/	/	/	/	tr	/	/
CD	2-Methylbutyl isobutyrate	1017	1017*	tr	tr	tr	tr	/	/	/	/	/	tr	/	/
M	*α*-Terpinene	1019	1014	0.2	tr	tr	tr	tr	0.4	0.5	0.3	0.3	0.4	tr	tr
M	*o*-Cymene	1027	1022	0.5	tr	0.9	tr	0.1	0.8	0.3	0.5	0.7	1.4	0.2	tr
O	2-Ethylhexanol	1030	1027*	/	tr	/	/	tr	/	/	/	/	/	/	/
M	Limonene	1031	1024	tr	tr	tr	tr	tr	/	tr	/	/	/	tr	tr
MO	1,8-Cineole	1033	1026	16.2	2.6	6.9	0.9	1.0	31.5	8.3	16.1	23.1	23.8	1.4	3.5
MO	Santolina alcohol	1037	1034	/	/	/	/	/	/	/	/	/	/	tr	/
M	(*Z*)-*β*-Ocimene	1039	1032	tr	tr	tr	tr	tr	0.3	tr	tr	tr	0.2	tr	tr
MO	Lavender Lactone	1042	1034*	/	/	tr	tr	/	/	/	/	/	/	/	/
CD	Butyl 2-methylbutyrate	1043	1041*	/	tr	/	/	/	/	/	/	/	/	/	/
O	*β*-Isophorone	1043	1043*	/	/	/	/	/	/	/	/	/	/	/	tr
O	Benzene acetaldehyde	1046	1036	tr	tr	tr	tr	0.2	tr	/	tr	tr	tr	0.5	tr
M	(*E*)-*β*-Ocimene	1050	1044	0.5	1.2	0.3	tr	tr	1.9	0.3	0.3	0.8	1.8	tr	tr
MO	Santolina epoxide	1055	-	0.2	/	0.2	tr	/	tr	/	tr	0.2	tr	tr	/
MO	Bergamal	1055	1051	/	tr	/	/	/	/	/	/	/	/	/	/
M	*γ*-Terpinene	1061	1054	0.8	tr	0.2	tr	tr	1.2	1.2	0.8	1.0	1.1	tr	tr
MO	Artemisia ketone	1063	1056	tr	tr	/	/	/	/	/	/	/	/	16.3	tr
MO	*cis*-Sabinene hydrate	1069	1065	1.0	0.2	0.3	tr	0.2	0.8	1.5	0.3	0.4	0.3	1.0	tr

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
O	*n*-Octanol	1071	1063	/	tr	/	/	/	/	/	/	/	/	/	tr
MO	*trans*-Arbusculone	1072	1066	/	/	/	tr	/	/	/	/	/	/	/	/
MO	*cis*-Linalool oxide	1074	1067	/	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr
O	1-Nonen-3-ol	1082	1078*	/	/	/	/	tr	tr	tr	/	/	tr	tr	tr
MO	Artemisia alcohol	1085	1080	tr	/	tr	/	/	/	/	/	/	tr	2.1	tr
MO	*trans*-Linalool oxide	1090	1084	/	tr	tr	/	tr	/	/	/	/	/	/	/
M	Terpinolene	1091	1086	0.2	/	/	tr	/	tr	0.3	0.2	tr	0.2	tr	tr
MO	6,7-Epoxymyrcene	1095	1090	0.2	/	tr	tr	tr	tr	0.1	tr	tr	0.2	tr	tr
MO	*trans*-Sabinene hydrate	1100	1098	0.7	tr	tr	tr	0.1	0.9	1.1	0.3	0.5	0.5	0.9	tr
MO	Linalool	1103	1098	/	0.4	1.0	tr	tr	/	/	/	/	/	/	0.4
CD	2-Methyl butyl-2-methyl butyrate	1104	1100	/	0.4	tr	tr	/	/	/	/	tr	tr	/	/
O	*n*-Nonanal	1105	1100	/	/	/	/	0.3	tr	tr	0.1	/	/	2.0	0.5
MO	Filifolone	1106	1103*	1.8	/	/	/	/	/	/	/	/	1.1	/	/
MO	*cis*-Thujone	1108	1101	5.9	/	3.3	0.4	1.2	0.2	tr	1.1	tr	/	/	/
CD	2-Methyl butyl isovalerate	1109	1103	/	tr	/	/	/	tr	/	/	tr	tr	/	/
O	1-Octen-3-yl acetate	1113	1110	/	0.8	/	/	/	/	tr	tr	tr	/	tr	0.6
O	Benzeneethanol	1116	1116*	/	tr	/	/	/	tr	tr	/	/	tr	/	/
MO	*trans*-Thujone	1119	1112	1.7	tr	1.5	10.3	22.5	4.5	0.9	23.3	2.8	/	0.3	tr
MO	*cis*-*p*-Menth-2-en-1-ol	1124	1118	0.1	tr	tr	tr	tr	tr	0.3	0.1	tr	tr	tr	tr
MO	Chrysanthenone	1128	1124	16.5	tr	tr	1.3	tr	/	tr	/	tr	3.0	/	1.5
MO	*α*-Campholenal	1129	1122	/	/	/	/	/	/	tr	tr	tr	/	tr	/
MO	(*Z*)-Epoxy-ocimene	1134	1128	/	/	/	/	/	/	/	/	/	/	tr	tr
MO	*trans*-*p*-Mentha-2,8-dien-1-ol	1134	1128*	/	/	/	/	/	/	/	/	tr	0.2	/	/
MO	*cis*-*p*-Mentha-2,8-dien-1-ol	1135	1133	/	/	0.4	/	/	tr	/	/	/	/	/	/
MO	1-Terpineol	1137	1130	/	/	/	/	/	/	/	/	/	tr	tr	/
MO	*iso*-3-Thujanol	1137	1134	0.1	/	0.2	2.7	10.0	0.9	0.3	3.7	0.3	/	/	/
CD	Pentyl 2-methylbutyrate	1140	1136*	/	tr	/	/	/	/	/	/	/	/	/	/
MO	*trans*-Pinocarveol	1141	1135	0.2	tr	tr	/	0.2	0.2	0.4	/	tr	0.2	0.5	tr
MO	*trans*-Sabinol	1144	1137	/	/	/	tr	/	/	/	0.7	/	/	/	/
MO	*cis*-Verbenol	1145	1137	/	/	/	/	tr	/	tr	/	/	/	/	tr
MO	(*E*)-Myroxide	1146	1140	/	/	/	/	/	tr	/	/	/	/	/	/
MO	*trans*-Verbenol	1147	1140	tr	tr	/	/	0.3	/	1.0	/	/	/	/	0.4
MO	Camphor	1148	1141	0.9	0.5	4.9	1.0	/	1.5	/	0.8	2.0	0.4	2.4	/
CD	3-Methyl-2-butenyl 3-methyl-butyrate	1152	1147	/	tr	/	/	/	/	/	/	/	/	/	/
MO	*neo*-3-Thujanol	1152	1149	/	/	tr	tr	/	/	/	/	/	/	/	/
MO	*p*-Mentha-1,5-dien-8-ol	1152	1159	/	/	/	/	/	/	/	/	/	tr	tr	tr
O	(*E*,*Z*)-2,6-Nonadienal	1155	1150	/	/	/	/	tr	/	/	/	/	/	tr	/
O	*m*-Cresol acetate	1159	1158	/	/	/	/	/	/	tr	/	/	/	/	/
MO	Sabina ketone	1161	1154	/	/	/	/	/	/	0.2	tr	/	/	/	tr
MO	Isoborneol	1161	1155	/	/	0.3	tr	/	0.2	/	/	tr	/	/	/
O	(*E*)-2-Nonen-1-al	1161	1157	/	tr	/	/	tr	/	/	/	/	/	/	/
MO	*trans*-Chrysanthenol	1162	1163*	0.2	/	/	/	/	/	/	/	0.2	0.2	1.0	tr
MO	*cis*-Chrysanthenol	1166	1160	0.2	/	0.2	tr	/	/	6.0	0.4	2.4	0.2	1.6	4.1
MO	Pinocarvone	1167	1160	/	tr	tr	tr	tr	0.2	/	tr	/	/	/	/
MO	*δ*-Terpineol	1171	1162	/	0.2	/	/	tr	/	/	0.7	tr	tr	tr	/
O	*n*-Nonanol	1173	1165	/	/	/	/	tr	/	/	/	/	/	/	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
MO	Borneol	1174	1165	1.9	/	1.0	1.3	0.2	3.1	8.0	tr	1.9	1.9	3.1	/
MO	*cis*-Pinocamphone	1178	1172	/	/	/	/	/	/	/	/	/	/	/	tr
MO	Terpinen-4-ol	1181	1174	1.4	0.6	0.7	tr	0.4	2.0	3.0	1.6	1.2	1.7	2.3	0.6
MO	*p*-Cymen-8-ol	1188	1179	/	tr	tr	tr	tr	tr	tr	/	/	tr	/	tr
MO	Thuj-3-en-10-al	1189	1181	tr	/	/	/	/	tr	tr	tr	tr	/	tr	tr
MO	*α*-Terpineol	1194	1186	2.2	0.5	1.9	0.3	0.3	5.5	1.2	2.2	3.3	3.7	0.3	tr
MO	*p*-Mentha-1, 5-dien-7-ol	1196	1191	tr	/	/	tr	tr	/	tr	tr	tr	/	/	/
MO	Myrtenol	1197	1194	0.2	0.1	tr	tr	tr	tr	0.4	tr	tr	tr	tr	tr
MO	*cis*-Piperitol	1199	1195	/	/	/	/	/	/	tr	tr	/	/	/	/
MO	Myrtenal	1200	1195	/	/	/	/	/	/	/	/	/	/	tr	tr
CD	4-Methylpentyl 2-methylbutyrate	1201	1197	/	tr	tr	/	/	/	/	tr	/	/	/	/
MO	Safranal	1203	1196	tr	/	/	tr	tr	/	tr	/	/	/	tr	tr
MO	*α*-Campholenol	1205	1202*	/	/	/	/	/	/	/	/	/	tr	0.4	tr
O	*n*-Decanal	1207	1201	/	tr	tr	/	0.1	/	/	tr	/	tr	tr	tr
MO	*trans*-Piperitol	1210	1207	tr	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Verbenone	1213	1204	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*trans*-Carveol	1222	1215	0.1	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr
MO	*β*-Cyclocitral	1224	1217	/	tr	/	/	tr	/	/	/	/	tr	tr	/
MO	Nerol	1231	1227	/	tr	tr	/	/	tr	tr	tr	tr	tr	/	/
MO	*nor*-Davanone	1233	1228	/	/	/	tr	/	/	/	/	/	/	/	/
MO	*cis*-Carveol	1234	1226	0.1	/	tr	/	/	tr	tr	0.1	tr	0.2	/	tr
CD	(*Z*)-3-Hexenyl 2-methyl butyrate	1234	1229	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Isogeraniol	1235	1232	/	/	/	/	tr	tr	/	/	/	/	tr	/
CD	(*Z*)-3-Hexenyl 3-methyl butyrate	1238	1232	/	tr	/	/	/	/	/	/	/	/	/	/
CD	Hexyl 2-methyl butyrate	1239	1233	/	/	/	tr	/	/	/	/	/	/	/	/
MO	*trans*-Chrysanthenyl acetate	1239	1235	0.4	tr	/	/	/	/	tr	/	/	/	/	tr
MO	Neral	1244	1235	0.2	0.8	/	/	/	/	/	tr	/	/	/	/
O	Cumin aldehyde	1244	1238	/	/	/	tr	tr	0.2	tr	0.1	tr	tr	tr	tr
O	2-Methyl-3-phenyl-propanal	1244	1244	/	/	tr	/	/	/	/	/	/	/	/	/
MO	Carvone	1248	1239	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Carvotanacetone	1251	1244	/	/	/	/	tr	/	/	tr	/	/	/	/
MO	Geraniol	1257	1249	tr	1.6	/	tr	tr	tr	tr	tr	/	tr	tr	/
O	2-Phenyl ethyl acetate	1260	1254	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Carvenone	1262	1255	/	/	/	/	/	/	/	tr	/	/	/	/
MO	*cis*-Chrysanthenyl acetate	1265	1261	0.6	0.3	/	/	tr	tr	24.8	tr	29.1	/	41.5	79.4
MO	*iso*-3-Thujanol acetate	1269	1267	/	/	/	/	1.9	/	/	/	/	/	/	/
MO	Geranial	1273	1264	tr	1.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	Isopiperitenone	1276	1272*	0.2	/	/	/	/	/	/	/	/	tr	tr	tr
O	*α*-ethylidene-Benzeneacetaldehyde	1276	1274*	/	/	/	/	tr	tr	tr	/	tr	/	/	/
O	2-phenyl-2-butenal	1276	1274*	/	tr	/	/	/	/	/	/	/	/	/	/
MO	Perilla aldehyde	1279	1269	0.1	/	tr	tr	tr	0.2	tr	tr	tr	tr	tr	tr
MO	4-Thujen-2-*α*-yl acetate	1279	1275*	/	tr	/	/	/	/	/	/	/	/	/	/
MO	*cis*-Verbenyl acetate	1281	1280	/	/	/	/	/	/	tr	/	/	/	/	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
MO	*trans*-α-Necrodol acetate	1288	1282	/	/	/	/	/	/	/	tr	/	/	tr	/
MO	α-Terpinen-7-al	1288	1283	/	/	/	/	tr	tr	/	/	/	/	/	/
MO	Bornyl acetate	1290	1287	/	0.4	tr	tr	/	tr	0.3	tr	tr	tr	tr	tr
MO	Lavandulyl acetate	1292	1288	0.3	/	tr	/	/	tr	/	/	tr	tr	tr	tr
MO	*trans*-Sabinyl acetate	1295	1289	tr	tr	tr	tr	0.1	tr	tr	23.1	tr	tr	tr	tr
O	Indole	1300	1290	/	/	/	/	/	/	/	tr	/	/	tr	/
MO	Perilla alcohol	1302	1294	/	/	/	/	/	tr	/	/	/	/	/	/
MO	Carvacrol	1304	1298	/	/	tr	tr	tr	tr	tr	tr	/	tr	tr	/
O	Undecanal	1309	1305	/	tr	tr	tr	0.1	tr	/	tr	tr	tr	tr	tr
CD	(*E*)-3-Hexenyl tiglate	1316	1315	/	tr	/	/	/	/	/	/	/	/	/	/
O	(*E*,*E*)-2,4-Decadienal	1319	1315	/	tr	/	/	tr	/	/	/	/	/	tr	tr
MO	δ-Terpinyl acetate	1321	1316	/	/	0.2	/	/	/	tr	/	tr	/	/	/
MO	Myrtenyl acetate	1330	1324	/	tr	/	/	tr	/	0.2	tr	tr	/	tr	tr
MO	*p*-Mentha-1,4,-dien-7-ol	1331	1325	/	/	/	/	/	tr	tr	/	/	/	tr	/
MO	Silphiperfol-5-ene	1332	1326	/	/	/	/	/	/	/	/	/	/	tr	/
CD	Heptyl 2-methylbutyrate	1337	1336*	/	tr	/	/	/	/	/	/	/	/	/	/
MO	*trans*-Carvyl acetate	1341	1339	0.2	tr	/	/	/	/	0.5	tr	/	/	tr	tr
S	δ-Elemene	1343	1335	tr	0.2	/	tr	/	tr	tr	tr	tr	/	tr	tr
S	Bicycloelemene	1344	1336	/	/	/	/	tr	/	/	/	/	/	/	/
MO	*exo*-2-Hydroxycineole acetate	1346	1346*	/	tr	/	/	/	/	/	/	/	/	/	/
S	Silphinene	1351	1345	/	tr	/	/	/	/	/	/	/	/	/	/
MO	α-Terpinyl acetate	1354	1346	/	/	/	tr	tr	/	tr	tr	tr	/	tr	tr
S	α-Cubebene	1355	1345	tr	tr	tr	tr	tr	tr	tr	tr	/	tr	/	/
S	*cis*-Muurola-3,5-diene	1356	1348	/	/	/	/	/	/	/	tr	/	/	/	/
S	α-Longipinene	1358	1350	tr	/	/	/	/	/	/	/	/	/	/	/
PP	Eugenol	1362	1356	0.3	0.5	tr	tr	0.3	0.2	0.2	0.2	0.2	0.2	tr	tr
MO	*cis*-Carvyl acetate	1367	1365	0.1	0.6	/	/	/	/	tr	tr	tr	/	0.2	tr
S	Cyclosativene	1373	1369	/	/	/	/	tr	/	/	/	/	/	/	/
S	α-Ylangene	1378	1373	/	/	/	/	tr	/	/	/	/	tr	/	/
S	α-Copaene	1380	1374	0.4	0.1	0.5	tr	1.0	0.3	0.2	0.2	0.3	0.3	0.3	tr
MO	Geranyl acetate	1386	1379	tr	2.3	/	tr	/	/	tr	tr	tr	tr	/	tr
S	β-Bourbonene	1392	1387	0.2	tr	tr	tr	0.6	tr	tr	tr	tr	tr	tr	tr
S	α-Isocomene	1394	1387	/	tr	/	tr	tr	/	/	/	/	/	/	/
S	7-*epi*-Sesquithujene	1395	1390	/	/	/	/	/	/	/	0.2	/	/	/	/
S	β-Elemene	1398	1389	3.1	0.3	0.7	0.6	0.9	1.3	0.4	0.9	0.7	0.7	0.4	0.5
A	Tetradecane	1400	1400	/	tr	/	/	tr	/	/	/	/	/	/	/
PP	Methyl eugenol	1407	1403	/	tr	/	/	tr	tr	tr	/	/	tr	tr	/
S	Sesquithujene	1410	1405	tr	/	tr	tr	0.3	tr	tr	tr	tr	tr	tr	/
S	β-Isocomene	1415	1407	/	/	/	/	tr	/	/	/	/	/	/	/
S	(*Z*)-Caryophyllene	1415	1408	/	tr	/	/	/	/	/	/	/	/	/	/
S	α-Gurjunene	1417	1409	tr	/	/	/	/	/	tr	/	/	0.5	/	/
S	*cis*-α-Bergamotene	1421	1411	0.2	tr	0.3	tr	0.9	0.3	0.2	tr	tr	tr	0.2	tr
S	(*E*)-Caryophyllene	1427	1417	1.3	7.2	2.5	0.8	4.1	1.1	0.8	0.6	1.8	2.5	0.9	0.7
S	β-Copaene	1437	1430	tr	tr	tr	tr	0.2	tr	tr	tr	tr	tr	tr	tr
S	*trans*-α-Bergamotene	1441	1432	0.2	tr	0.2	tr	0.7	0.3	0.2	tr	tr	tr	tr	tr
S	Aromadendrene	1447	1439	tr	/	tr	tr	/	tr	/	/	/	tr	tr	tr
S	(*Z*)-β-Farnesene	1448	1440	/	/	/	/	/	tr	/	0.2	/	/	/	/
S	6,9-Guaiadiene	1452	1442	/	/	/	/	tr	/	/	/	/	/	/	/
S	*epi*-β-Santalene	1454	1445	/	/	/	/	tr	/	/	/	/	/	/	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
MO	Geranyl acetone	1456	1453	/	0.4	tr	tr	tr	tr	tr	tr	tr	tr	tr	/
S	(*E*)-*β*-Farnesene	1460	1454	tr	/	/	0.8	2.3	tr	tr	tr	tr	/	/	tr
S	*α*-Humulene	1462	1452	0.5	1.6	1.4	tr	tr	0.8	0.6	0.4	0.8	0.6	0.5	tr
S	9-*epi*-(*E*)-Caryophyllene	1469	1464	/	0.1	/	/	/	/	/	/	/	0.3	/	/
S	Dauca-5,8-diene	1471	1471	/	/	/	/	tr	/	/	/	/	/	/	/
S	10-*epi*-*β*-Acoradiene	1475	1474	/	/	/	/	/	/	/	tr	/	/	/	/
S	4,5-di-*epi*-Aristolochene	1478	1471	/	0.2	/	/	/	tr	/	tr	/	/	/	/
S	Selina-4,11-diene	1483	-	/	/	/	/	/	0.9	/	/	/	tr	tr	tr
S	*γ*-Selinene	1483	1479*	0.2	1.0	/	/	/	/	/	/	/	/	/	/
S	*γ*-Muurolene	1484	1478	/	/	/	tr	0.5	/	tr	/	tr	0.1	tr	tr
S	*γ*-Curcumene	1485	1481	/	/	/	/	/	/	/	1.1	/	/	/	/
S	*ar*-Curcumene	1489	1479	/	/	/	/	tr	/	/	/	/	/	/	/
S	Germacrene D	1490	1484	9.1	1.1	12.3	7.2	15.1	8.7	5.4	3.4	6.0	3.9	2.3	1.3
S	*δ*-Selinene	1498	1492	/	tr	/	/	/	/	/	/	/	/	/	/
S	*α*-Zingiberene	1498	1493	/	/	/	/	/	/	/	/	/	tr	/	/
S	*β*-Selinene	1495	1489	1.8	24.6	0.8	1.3	0.5	3.6	0.4	0.4	0.5	1.1	0.6	1.0
SO	10,11-epoxy-Calamenene	1498	1491	/	/	/	/	/	/	/	/	/	/	tr	/
SO	*epi*-Cubebol	1502	1493	/	tr	/	/	/	/	/	/	/	/	/	/
S	*α*-Selinene	1503	1498	/	/	/	/	/	tr	/	/	/	/	/	/
S	Bicyclogermacrene	1505	1500	1.8	0.8	2.1	1.4	2.8	1.3	0.6	1.3	1.4	1.3	0.4	tr
S	*α*-Muurolene	1507	1500	/	0.2	/	/	/	/	/	/	tr	/	/	/
S	(*E*,*E*)-*α*-Farnesene	1512	1505	/	0.5	tr	tr	0.2	0.2	tr	/	tr	tr	/	/
S	Germacrene A	1514	1508	0.4	0.2	0.8	0.6	0.8	0.6	0.3	0.2	0.3	0.7	tr	tr
SO	Davana ether isomer 1	1516	1514*	/	/	0.4	0.3	/	/	/	/	/	/	/	/
SO	Davana ether, isomer 2	1517	1517*	/	/	/	/	/	/	/	/	/	tr	/	/
S	*β*-Curcumene	1517	1514	/	/	/	/	/	/	tr	0.4	/	/	/	/
S	*γ*-Cadinene	1522	1513	tr	0.8	0.2	tr	0.5	tr	tr	tr	tr	tr	/	tr
SO	Isobornyl isovalerate	1523	1521	/	/	/	/	/	/	/	/	/	/	tr	/
S	7-*epi*-*α*-Selinene	1527	1520	/	/	/	/	/	tr	/	/	/	tr	tr	/
S	*δ*-Cadinene	1531	1522	0.3	1.1	0.4	0.5	0.9	0.3	0.4	0.3	0.2	0.2	0.3	tr
S	*trans*-Cadina-1,4-diene	1540	1533	/	tr	/	/	tr	tr	/	/	/	/	/	/
SO	Davana ether isomer 3	1536	1535*	/	/	0.2	tr	/	/	/	/	/	tr	/	/
S	(*E*)-*iso*-*γ*-Bisabolene	1538	1528	/	/	/	/	/	/	/	tr	/	/	/	/
S	*α*-Cadinene	1546	1537	/	tr	tr	/	tr	tr	/	tr	/	tr	/	/
SO	*cis*-Sesquisabinene hydrate	1550	1542	0.3	/	/	/	/	/	/	0.3	/	/	tr	/
S	*α*-Calacorene	1551	1544	/	tr	tr	tr	/	tr	tr	/	/	/	tr	tr
SO	Salviadienol	1562	1549*	0.2	0.3	0.6	/	1.0	0.4	tr	tr	tr	0.2	0.5	tr
SO	(*E*)-Nerolidol	1566	1561	tr	0.3	1.0	/	tr	tr	/	/	tr	/	0.2	/
S	*β*-Calacorene	1572	1564	/	tr	/	/	/	/	tr	/	/	/	tr	/
SO	Davanone B	1574	1564	/	/	0.2	0.8	/	/	/	/	/	0.4	/	/
SO	Palustrol	1577	1567	/	/	/	/	/	/	/	/	/	0.2	/	/
SO	Germacrene D-4-ol	1584	1574	/	0.5	tr	tr	tr	tr	/	/	/	/	/	/
SO	*trans*-Sesquisabinene hydrate	1586	1577	/	/	/	/	/	/	/	2.7	/	/	/	/
SO	Spathulenol	1587	1577	3.1	tr	2.9	1.9	3.9	1.4	0.6	tr	1.2	0.9	2.2	0.8
SO	Caryophyllene oxide	1592	1582	1.0	13.2	tr	tr	3.8	0.9	0.5	0.4	1.2	tr	3.1	0.9
SO	Davanone	1596	1587	/	/	32.1	59.8	tr	tr	/	/	/	27.4	tr	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
SO	*β*-Copaen-4-*α*-ol	1598	1590	/	0.2	/	/	/	/	tr	/	/	/	/	tr
SO	Viridiflorol	1599	1592	/	tr	/	/	/	/	/	/	/	/	/	/
SO	Fokienol	1601	1596	/	/	/	/	/	/	/	/	/	0.2	/	/
SO	Humulene epoxide I	1603	1601*	/	tr	/	/	/	/	/	/	/	/	/	/
SO	Ledol	1612	1602	/	/	/	/	/	/	/	/	/	0.3	tr	tr
SO	Salvial-4(14)-en-1-one	1613	1603*	0.1	tr	0.3	tr	0.4	tr	tr	tr	tr	tr	0.3	tr
SO	Torilenol	1614	1604	/	/	1.1	0.3	1.8	0.9	0.3	0.4	0.4	0.4	1.1	tr
SO	Geranyl isovalerate	1616	1606	/	0.3	/	/	/	/	/	/	/	/	/	/
SO	Humulene epoxide II	1618	1608	0.7	1.9	/	/	/	/	tr	tr	tr	tr	tr	/
O	Tetradecanal	1619	1611	/	tr	/	/	0.4	/	/	/	/	/	/	/
SO	1,10-di-*epi*-Cubenol	1624	1618	/	tr	tr	/	tr	/	/	/	tr	/	/	/
SO	*epi*-Cedrol	1626	1618	/	/	/	/	/	/	/	tr	/	/	tr	/
SO	1-*epi*-Cubenol	1637	1627*	/	/	/	tr	0.1	tr	/	tr	/	/	0.2	tr
SO	Guaia-6,10(14)-dien-4-*β*-ol	1638	1631*	tr	1.2	0.5	/	/	/	/	tr	/	/	/	/
SO	*cis*-Cadin-4-en-7-ol	1642	1635	/	/	/	/	/	/	/	/	/	/	/	tr
SO	*β*-Acorenol	1643	1636	/	/	/	/	/	/	/	tr	/	/	/	/
SO	*epi*-*α*-Cadinol	1648	1638	0.1	tr	/	/	/	tr	/	/	/	/	/	/
SO	Caryophylla-4(12),8(13)-dien-5-α-ol	1646	1639	tr	0.3	tr	/	tr	tr	/	/	/	tr	/	/
SO	*allo*-Aromadendrene epoxide	1647	1639	/	/	/	/	/	/	/	/	/	tr	tr	tr
SO	epi-*α*-Murrolol	1650	1640	/	/	0.5	/	0.6	tr	/	/	/	/	tr	tr
SO	Selina-3,11-dien-6-*α*-ol	1642	1642	/	/	/	/	/	tr	/	/	tr	/	/	tr
SO	*α*-Muurolol	1654	1644	0.2	0.2	/	/	/	/	/	/	/	/	/	/
SO	*β*-Eudesmol	1659	1649	tr	0.2	/	/	/	/	/	/	/	/	/	0.4
SO	*α*-Cadinol	1662	1652	4.2	1.4	/	0.5	2.0	0.8	0.2	0.4	0.4	0.3	0.6	tr
SO	Selin-11-en-4-*α*-ol	1665	1658	/	/	3.5	/	/	/	/	tr	tr	tr	tr	tr
SO	*cis*-Calamenen-10-ol	1668	1660	tr	0.2	/	/	/	/	/	/	/	/	/	/
SO	14-hydroxy-9-*epi*-(*E*)-Caryophyllene	1669	1668	0.3	1.6	/	tr	0.9	0.5	tr	tr	tr	tr	0.3	tr
SO	*trans*-Calamenen-10-ol	1677	1668	/	/	/	/	/	/	/	/	/	/	tr	tr
SO	*β*-Bisabolol	1678	1674	/	/	/	/	0.1	/	/	0.7	/	/	/	/
SO	(*Z*)-*α*-Santalol	1679	1674	/	/	/	/	/	/	/	/	/	/	0.4	/
SO	Mustakone	1686	1676	0.1	tr	/	/	/	/	/	/	/	/	/	/
SO	Longiborneol acetate	1690	1684	/	/	/	/	/	/	/	/	/	/	/	tr
SO	Eudesma-4(15),7-dien-1-*β*-ol	1691	1685	/	/	tr	tr	0.4	tr	tr	tr	tr	tr	tr	/
SO	Germacra-4(15),5,10(14)-trien-1-*α*-ol	1695	1685	0.6	0.8	1.2	0.4	2.3	0.8	0.2	0.3	0.4	0.2	0.8	tr
SO	(*Z*)-*α*-*trans*-Bergamotol	1698	1690	/	/	/	/	0.9	0.6	0.1	/	0.2	/	/	/
SO	(*Z,Z*)-2,6-Farnesol	1701	1698	/	/	/	/	/	/	/	/	/	tr	/	/
O	Pentadecanal	1718	1715*	0.2	0.5	0.3	/	1.2	/	/	/	/	/	0.3	tr
SO	(*E,Z*)-2,6-Farnesal	1721	1713	tr	1.3	tr	tr	/	tr	/	/	tr	/	/	/
SO	(*Z,E*)-2,6-Farnesal	1721	1715	/	/	/	/	/	/	/	/	/	tr	/	/
SO	*β*-Davanone-2-ol	1725	1718	/	/	0.7	0.7	/	/	/	/	/	tr	/	/
SO	(*Z,E*)-2,6-Farnesol	1728	1722	tr	4.6	/	/	/	/	/	/	/	/	/	/
SO	Longifolenaldehyde	1732	-	/	/	/	/	/	/	/	/	/	/	tr	tr
SO	(*E,E*)-2,6-Farnesal	1749	1740	tr	2.1	tr	tr	/	tr	/	/	tr	tr	/	/
SO	Mint sulfide	1750	1740	/	/	/	/	tr	/	tr	/	/	/	/	/

Class	Compound	RI	RIa	Content, %											
				AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
SO	*γ*-Costol	1755	1745	tr	2.3	tr	tr	tr	0.2	/	/	/	/	/	/
SO	Cyclocolorenone	1761	1759	/	/	/	/	/	/	/	/	/	/	tr	/
SO	*β*-Acoradienol	1765	1762	/	/	/	/	tr	/	/	/	/	/	/	/
SO	*β*-Costol	1775	1765	tr	5.4	tr	tr	0.3	0.2	/	tr	/	tr	tr	tr
SO	*α*-Costol	1782	1773	/	/	/	tr	0.1	tr	tr	tr	/	/	/	/
SO	2-*α*-hydroxy-Amorpha-4,7(11)-diene	1786	1775	/	/	/	/	tr	/	/	/	/	/	/	/
SO	14-hydroxy-*δ*-Cadinene	1810	1803	/	/	/	/	tr	/	/	/	/	/	/	/
O	Hexadecanal	1819	1818*	tr	tr	/	/	tr	/	/	/	/	/	/	/
SO	(*E*,*E*)-2,6-Farnesyl acetate	1846	1845	tr	0.9	/	/	/	/	/	/	/	/	/	/
SO	Hexahydrofarnesyl acetone	1848	1846*	tr	tr	/	/	tr	/	/	/	/	/	tr	tr
SO	(*Z*)-Lanceol acetate	1861	1854	/	/	/	tr	tr	/	/	/	/	/	/	/
A	Nonadecane	1900	1900	tr	0.2	/	/	0.4	/	/	/	/	/	tr	/
O	*m*-Camphorene	1960	1960*	/	/	tr	/	/	/	/	/	/	/	/	/
SO	(*E*,*Z*)-Geranyl linalool	1992	1987	/	/	/	/	tr	/	/	/	/	/	/	/
A	Eicosane	2000	2000	tr	tr	/	/	tr	/	/	/	/	/	/	/
A	Heneicosane	2100	2100	tr	0.8	/	/	0.5	/	/	/	/	/	/	/
O	Phytol	2116	2114*	tr	tr	tr	/	tr	/	/	/	/	/	/	/
O	(*Z*,*Z*)-9,12-octade-cadienoic acid	2156	2156*	/	/	/	/	tr	/	/	/	/	/	/	/
A	Tricosane	2300	2300	tr	0.2	/	/	tr	/	/	/	/	/	/	/
A	Pentacosane	2500	2500	tr	tr	/	/	tr	/	/	/	/	/	/	/
A	Heptacosane	2700	2700	tr	tr	/	/	tr	/	/	/	/	/	/	/
A	Nonacosane	2900	2900	/	/	/	/	tr	/	/	/	/	/	/	/

RI-Experimental linear retention indices relative to C8-C40 alkanes. Ria-Literature indices-Adams' retention indices and * according to NIST data base. Tr- trace<0.05% and not detected compounds are marked as (/). M-Hydrocarbon Monoterpenoids, MO-Oxygenated Monoterpenoids, S-Hydrocarbon Sesquiterpenoids, SO-Oxygenated Sesquiterpenoids, PP-Phenylpropanoids, CD-carboxylic acid derivatives, A-Alkanes, O-Other

Table S-IX. The number of identified components per sample of *A. vulgaris*, the percentage of each class of compounds, and the percentage of total identified components

	Content, %											
Sample	AV1	AV2	AV3	AV4	AV5	AV6	AV7	AV8	AV9	AV10	AV11	AV12
Contribution in total peaks area of ion chromatogram, %	99.1	96.3	96.8	98.0	93.6	98.5	99.8	99.7	100.0	98.4	97.8	99.5
Number of components	120	156	119	110	152	125	122	132	116	137	146	133
Total monoterpenoids	68.8	13.8	29.2	20.1	39.1	71.6	88.3	84.5	84.3	55.5	77.3	92.9
Monoterpene hydrocarbons (M)	14.7	1.2	6.3	1.9	0.8	19.9	29.9	10.1	16.8	17.8	1.6	2.9
Oxygenated monoterpenes (MO)	54.0	12.6	23.0	18.2	38.3	51.7	58.4	74.4	67.5	37.7	75.7	90.1
Total sesquiterpenoids	29.9	79.0	67.3	77.9	50.9	26.5	11.4	14.8	15.5	42.6	15.4	5.6
Sesquiterpene hydrocarbons (S)	19.1	40.0	22.1	13.2	32.4	19.7	9.4	9.7	11.8	12.2	5.8	3.5
Oxygenated Sesquiterpene (SO)	10.8	39.0	45.2	64.7	18.5	6.8	2.0	5.2	3.7	30.5	9.6	2.1
Phenylpropanoids (PP)	0.3	0.5	tr	tr	0.3	0.2	0.2	0.2	0.2	0.2	tr	tr
Carboxylic acid derivatives (CD)	tr	0.4	tr	tr	/	tr	/	tr	tr	tr	tr	tr
n-Alkanes (A)	tr	1.2	/	/	0.9	/	/	/	/	/	tr	tr
Other (O)	0.2	1.5	0.3	tr	2.5	0.2	tr	0.3	tr	/	5.1	1.1

Table S-X. Chemical composition of twelve *Artemisia scoparia* samples collected from different soil types according to the WRB

Class	Compound	RI	RIa	Content, %											
				AS1	AS2	AS3	AS4	AS5	AS6	AS7	AS8	AS9	AS10	AS11	AS12
O	3-Methyl-2-butenal	788	778	/	/	tr	0.1	tr	/	/	tr	/	tr	tr	tr
A	*n*-Octane	800	800	/	/	/	/	/	/	/	/	tr	/	/	/
O	Hexanal	801	801	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr
O	1,3-Octadiene	829	827	/	tr	tr	tr	tr	tr	/	tr	tr	tr	tr	tr
CD	2-Methyl-butanoic acid	842	832	/	/	/	tr	tr	/	/	/	/	tr	/	/
O	(*E*)-2-Hexenal	850	846	tr	tr	tr	tr	tr	tr	/	tr	tr	tr	tr	tr
O	(*Z*)-3-Hexenol	852	850	tr	tr	tr	tr	tr	/	tr	tr	tr	tr	tr	tr
O	(*Z*)-2-Hexenol	865	859	/	/	tr	/	/	/	/	/	/	tr	tr	/
O	*n*-Hexanol	867	863	/	tr	tr	tr	tr	tr	/	tr	tr	tr	tr	tr
O	(*E,Z*)-1,3,5-Octatriene	882	880	/	/	/	/	tr	/	/	/	/	/	/	/
A	*n*-Nonane	900	900	/	/	/	/	/	/	/	/	/	tr	/	/
O	Heptanal	903	901	/	/	/	/	/	tr	/	/	/	/	/	/
M	Tricyclene	924	921	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	*α*-Thujene	928	924	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	*α*-Pinene	935	932	0.9	0.9	1.3	1.4	1.3	1.0	0.7	2.0	1.0	1.2	2.5	1.9
M	*α*-Fenchene	949	945	/	/	/	/	/	/	/	/	/	/	tr	/
M	Camphene	950	946	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	(*E*)-2-Heptenal	957	947	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	Benzaldehyde	962	952	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	Sabinene	975	969	0.3	0.3	0.4	0.4	0.5	0.4	0.2	0.5	0.3	0.4	0.7	0.5
M	*β*-Pinene	979	974	7.3	4.7	7.9	8.0	11.6	8.3	5.3	10.6	8.5	9.7	16.9	14.8
O	6-Methyl-5-hepten-2-one	988	981	/	tr	/	/	/	/	/	tr	/	tr	tr	/
M	Myrcene	992	988	0.6	1.9	1.8	2.9	1.1	0.8	1.3	2.6	0.9	1.9	2.7	1.9
O	Mesitylene	996	994	/	/	/	/	/	/	/	/	/	/	tr	/
O	*n*-Octanal	1004	998	/	/	/	/	tr	/	/	/	/	/	/	/
M	*α*-Phellandrene	1006	1002	tr	tr	tr	/	/	tr	tr	tr	tr	tr	tr	tr
CD	(*E*)-3-Hexenyl acetate	1007	1001	/	/	/	tr	tr	/	/	/	/	/	/	/
CD	(*Z*)-3-Hexenyl acetate	1008	1004	tr	tr	/	/	/	/	/	/	/	tr	/	/
M	*α*-Terpinene	1019	1014	0.1	0.1	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1
M	*p*-Cymene	1027	1020	0.1	0.3	0.1	0.2	0.2	0.1	tr	0.3	0.2	0.2	0.2	0.1
M	Limonene	1031	1024	2.3	2.4	2.5	2.6	2.0	2.4	2.1	3.9	2.1	2.0	4.2	2.9
MO	1,8-Cineole	1033	1026	0.3	tr	0.1	0.3	0.4	tr	tr	0.2	0.2	0.2	0.1	0.2
M	(*Z*)-*β*-Ocimene	1039	1032	6.9	4.6	4.7	8.5	6.4	7.7	6.2	8.5	6.4	4.6	5.4	6.2
O	Benzene acetaldehyde	1046	1036	tr	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	(*E*)-*β*-Ocimene	1050	1044	0.4	0.3	0.4	0.5	0.3	0.4	0.4	0.7	0.4	0.5	0.7	0.4
M	*γ*-Terpinene	1061	1054	2.5	3.7	3.6	3.2	2.8	1.9	1.4	4.6	3.9	3.0	4.0	1.5
MO	*cis*-Sabinene hydrate	1069	1065	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	*n*-Octanol	1071	1063	/	/	/	/	/	/	/	tr	/	tr	tr	/
M	Terpinolene	1091	1086	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	/	0.1
MO	*trans*-Sabinene hydrate	1100	1098	/	/	tr	/	tr	tr	tr	/	tr	tr	/	tr
MO	Linalool	1103	1098	/	/	/	tr	/	/	/	tr	/	/	tr	tr
O	*n*-Nonanal	1105	1100	tr	tr	tr	tr	0.1	tr	tr	0.1	tr	tr	tr	tr
CD	2-Methyl butyl isovalerate	1109	1103	/	/	/	/	/	/	/	/	/	tr	/	tr
MO	*endo*-Fenchol	1116	1114	/	/	/	/	/	/	/	/	/	/	tr	tr
O	4,8-dimethyl-1,3,7-Nonatriene	1118	1114	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*cis*-*p*-Menth-2-en-1-ol	1124	1118	tr	/	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
M	*allo*-Ocimene	1131	1128	0.1	tr	tr	0.1	0.1	0.1	tr	0.1	0.1	tr	tr	0.1
MO	(*Z*)-epoxy-Ocimene	1134	1128	/	/	/	tr	/	tr	/	tr	/	/	tr	tr
MO	*trans*-Pinocarveol	1141	1135	/	/	tr	tr	tr	tr	tr	/	tr	/	/	/
MO	*trans*-*p*-Menth-2-en-1-ol	1143	1136	/	/	/	/	/	/	/	tr	/	/	/	/

MO	*trans*-Sabinol	1144	1137	/	/	/	/	/	/	/	/	/	tr	tr	tr
MO	*neo-allo*-Ocimene	1144	1140	/	/	/	/	/	/	tr	/	/	/	/	/
CD	(*Z*)-3-Hexenyl isobutyrate	1146	1142	/	tr	/	/	/	/	/	/	/	tr	tr	tr
MO	Camphor	1148	1141	tr	/	tr	/	/	/	/	/	/	/	tr	/
MO	Camphene hydrate	1152	1145	/	/	/	/	/	/	/	/	/	/	tr	tr
O	pentyl-Benzene	1159	1152	/	/	/	/	/	/	tr	/	/	/	/	/
O	(*E*)-2-Nonen-1-al	1161	1157	/	/	tr	tr	/	tr	tr	tr	tr	tr	tr	tr
MO	Pinocarvone	1167	1160	/	/	/	/	tr	/	/	/	/	/	/	/
MO	Lavandulol	1172	1165	0.6	0.1	0.2	tr	0.2	0.3	0.1	0.3	0.2	0.1	0.2	tr
O	*n*-Nonanol	1173	1165	/	/	/	/	tr	/	/	/	/	/	/	/
MO	Umbellulone	1176	1167	/	tr	tr	/	/	tr	/	tr	tr	tr	tr	tr
MO	*cis*-Pinocamphone	1178	1172	tr	/	/	tr	/	tr	/	tr	tr	tr	tr	tr
MO	Terpinen-4-ol	1181	1174	0.1	tr	0.1	0.1	0.1	tr	tr	0.1	0.1	0.1	0.1	0.1
O	Naphthalene	1187	1178	/	/	/	/	/	/	tr	/	/	tr	tr	/
CD	(*Z*)-3-Hexenyl butyrate	1190	1184	/	tr	tr	tr	/	/	/	/	/	/	/	/
MO	*α*-Terpineol	1194	1186	tr	tr	tr	0.1	0.1	tr	tr	tr	tr	tr	0.1	0.1
MO	Myrtenol	1197	1194	/	/	/	tr	/	/	/	/	/	tr	tr	tr
MO	Myrtenal	1200	1195	/	/	/	/	/	tr	/	/	/	/	/	/
O	*n*-Decanal	1207	1201	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
MO	*trans*-Piperitol	1210	1207	/	/	/	/	/	/	tr	/	/	/	tr	/
MO	Citronellol	1230	1223	/	/	/	tr	tr	tr	/	tr	tr	/	tr	tr
CD	(*Z*)-3-Hexenyl 2-methyl butyrate	1234	1229	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
CD	(*Z*)-3-Hexenyl 3-methyl butyrate	1238	1232	tr	tr	tr	0.1	tr	tr	tr	tr	tr	0.1	tr	tr
CD	Hexyl 2-methyl butyrate	1239	1233	/	/	/	/	tr	/	/	/	/	/	/	/
CD	Hexyl 3-methylbutyrate	1245	1241	/	tr	tr	tr	/	tr	tr	tr	tr	tr	tr	/
CD	(*E*)-2-Hexenyl isovalerate	1246	1243	/	/	/	/	/	/	/	/	tr	tr	/	/
MO	Geraniol	1257	1249	/	/	tr	/	tr	/	/	/	/	/	/	/
MO	Piperitone	1258	1249	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	(*E*)-2-Decenal	1263	1260	/	/	/	tr	/	tr	/	/	/	/	/	/
MO	*cis*-Chrysanthenyl acetate	1265	1261	tr	/	/	/	/	/	/	/	/	/	/	/
MO	Citronellyl formate	1277	1271	/	/	/	tr	/	0.1	tr	tr	tr	/	/	tr
O	1-Decanol	1284	1274	/	/	/	/	tr	/	/	/	/	/	tr	/
P	2,4-pentadiynyl-Benzene	1290	1286	13.5	9.8	17.2	14.8	21.1	24.5	16.3	18.7	24.7	12.5	24.0	24.1
MO	Lavandulyl acetate	1292	1288	tr	tr	/	/	/	/	/	/	/	tr	/	/
O	(*E,Z*)-2,4-Decadienal	1296	1292	/	/	/	tr	/	/	/	/	/	/	/	/
O	2-methyl-Naphthalene	1299	1299	/	/	/	/	/	/	tr	/	/	/	/	/
MO	Geranyl formate	1304	1298	/	/	tr	/	/	/	/	tr	tr	/	tr	/
O	Undecanal	1309	1305	/	/	tr	tr	/	tr	tr	tr	/	/	tr	tr
O	*p*-vinyl-Guaiacol	1318	1309	/	/	/	/	/	/	/	/	/	/	tr	tr
CD	(*E*)-3-Hexenyl tiglate	1316	1315	tr	/	/	/	/	/	/	/	/	tr	/	/
O	(*E,E*)-2,4-decadienal	1319	1315	/	/	tr	tr	tr	tr	tr	tr	/	tr	tr	/
O	1-methyl-Naphthalene	1326	1317	/	/	/	/	/	/	tr	/	/	/	/	/
CD	(*Z*)-3-Hexenyl tiglate	1327	1319	0.2	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
P	1,3-Hexadienyl-benzene	1330	-	/	tr	/	/	tr	tr	/	/	tr	tr	tr	tr
CD	Hexyl tiglate	1333	1330	tr	/	/	tr	tr	/	/	/	tr	tr	/	/
S	*δ*-Elemene	1343	1335	/	/	/	/	tr	tr	tr	/	/	/	/	/
S	Bicycloelemene	1344	1336	tr	tr	tr	tr	/	/	/	tr	tr	tr	tr	tr
S	Silphinene	1351	1345	/	/	tr	tr	/	/	/	/	/	tr	/	/
MO	*α*-Terpinyl acetate	1354	1346	/	/	/	/	/	tr	/	/	/	/	/	/
MO	Citronellyl acetate	1355	1350	/	/	/	/	/	/	tr	/	/	/	/	tr

PP	Eugenol	1362	1356	2.1	tr	1.1	tr	tr	0.4	0.4	0.3	0.7	1.1	1.6	0.7
S	Cyclosativene	1373	1369	/	/	/	/	/	/	/	/	/	tr	tr	tr
S	*α*-Ylangene	1378	1373	/	tr	/	/	/	/	tr	/	/	/	/	/
S	*α*-Copaene	1380	1374	tr	tr	tr	/	tr	tr	/	tr	tr	tr	tr	tr
CD	(*Z*)-3-Hexenyl hexenoate	1383	1378	/	/	/	tr	/	/	/	tr	/	/	/	/
S	Modheph-2-ene	1389	1382	tr	/	tr	tr	/	/	/	/	/	tr	/	/
S	*α*-Isocomene	1394	1387	tr	tr	tr	tr	/	/	/	tr	tr	tr	tr	/
S	*β*-Elemene	1398	1389	/	/	/	/	/	/	/	/	/	tr	/	/
O	2-ethyl-Naphthalene	1402	1398	tr	tr	/	/	/	/	/	/	/	/	/	/
O	2,6-dimethyl-Naphthalene	1405	1400	0.4	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.5	0.2	0.3
O	2,7-dimethyl-Naphthalene	1406	1402	/	/	/	tr	/	tr	/	tr	tr	tr	/	tr
PP	Methyl eugenol	1407	1403	tr	0.1	0.4	/	0.2	/	2.3	/	0.7	tr	tr	0.4
S	*β*-Isocomene	1415	1407	tr	/	tr	tr	/	/	/	/	/	tr	/	/
O	1,6-dimethyl-Naphthalene	1424	1419	/	/	/	/	/	/	tr	/	/	/	/	/
S	(*E*)-Caryophyllene	1427	1417	1.6	0.7	0.9	1.6	0.3	0.7	1.3	1.1	0.9	1.3	0.8	0.6
S	Aromadendrene	1447	1439	tr	tr	tr	/	/	/	/	/	/	/	/	/
O	2,3-dimethyl-Naphthalene	1444	1444	/	/	/	/	/	/	tr	/	/	/	/	/
MO	Citronellyl propanoate	1445	1444	/	/	tr	tr	/	tr	/	/	/	/	/	/
S	(*Z*)-*β*-Farnesene	1448	1440	/	/	/	tr	tr	tr	/	/	/	tr	tr	tr
S	6,9-Guaiadiene	1452	1442	/	/	/	/	/	/	/	tr	tr	/	/	/
O	1-Naphthalene carboxaldehyde	1457	1467	/	/	/	/	/	tr	/	/	/	/	/	/
S	(*E*)-*β*-Farnesene	1460	1454	/	/	/	/	/	/	tr	/	/	/	tr	/
S	*α*-Humulene	1462	1452	0.1	tr	0.1	0.2	tr	0.1	0.2	0.1	0.1	0.1	0.1	0.1
S	*γ*-Curcumene	1485	1481	0.3	0.1	0.3	0.4	0.4	0.3	0.2	0.1	0.3	0.3	0.4	0.3
S	Germacrene D	1490	1484	0.4	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.2	0.2	0.7	0.1
S	*β*-Selinene	1495	1489	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
P	Capillene	1503	1493	56.7	67.9	52.1	51.0	49.6	48.3	59.5	43.8	47.1	58.6	33.6	42.1
S	Bicyclogermacrene	1505	1500	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
SO	Lavandulyl isovalerate	1513	1509	tr	tr	/	/	/	tr	/	/	/	/	tr	/
SO	Lavandulyl 2-methyl butyrate	1514	1511	/	/	/	/	/	/	/	tr	tr	tr	tr	/
S	*β*-Curcumene	1517	1514	tr	/	/	/	/	/	/	/	tr	tr	tr	tr
SO	Shyobunone	1523	1519	/	tr	tr	/	/	/	/	tr	tr	tr	tr	tr
S	*δ*-Cadinene	1531	1522	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
O	2,3,6-trimethyl-Naphthalene	1533	1533	/	/	/	/	/	/	tr	/	/	/	/	/
O	1,3,6-trimethyl-Naphthalene	1537	MS	/	/	/	/	/	/	tr	/	/	/	/	/
SO	Italicene ether	1543	1536	/	/	tr	tr	/	/	tr	/	tr	tr	tr	tr
SO	*α*-Copaen-11-ol	1549	1539	/	/	/	/	/	/	/	/	/	/	/	tr
SO	*cis*-Sesquisabinene hydrate	1550	1542	/	/	tr	tr	/	tr	tr	/	/	/	/	/
SO	Italicene epoxide	1557	1547	/	tr	tr	tr	/	tr	tr	tr	tr	tr	tr	tr
SO	(*E*)-Nerolidol	1566	1561	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
SO	Citronellyl isovalerate	1573	1563	/	tr	tr	/	tr	tr	tr	tr	tr	tr	0.0	tr
SO	Germacrene D-4-ol	1584	1574	/	tr	tr	/	/	tr	/	/	/	/	/	/
SO	Spathulenol	1587	1577	0.3	tr	tr	0.6	0.1	tr	0.4	0.2	tr	tr	0.2	0.1
SO	Caryophyllene oxide	1592	1582	0.2	tr	tr	0.6	tr	tr	0.3	0.1	tr	tr	0.1	tr
SO	Viridiflorol	1599	1592	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr
SO	Ledol	1612	1602	/	tr	tr	0.1	/	tr	0.2	0.1	/	tr	tr	0.1
SO	Geranyl isovalerate	1616	1606	/	/	/	/	/	/	/	/	/	/	tr	/
SO	Junenol	1628	1618	0.2	/	/	/	/	/	/	/	/	/	/	/

SO	1-*epi*-Cubenol	1637	1627*	/	/	/	0.1	/	/	/	/	/	/	/
SO	(*E*)-Sesquilavandulol	1639	1631	0.1	/	tr	/	tr	/	/	/	/	/	tr	/
SO	Caryophylla-4(12),8(13)-dien-5-*β*-ol	1649	1639	/	/	/	0.3	/	/	/	/	/	/	/	/
SO	*β*-Eudesmol	1659	1649	0.4	tr	0.6	0.7	0.1	tr	0.2	0.4	0.2	0.2	0.1	0.4
SO	(*E*)-Sesquilavandulyl acetate	1743	1739	/	/	tr	/	/	/	/	/	/	/	tr	/
SO	(*S,R*)-6,7-Bisabolone	1754	1748	/	/	tr	tr	/	/	/	/	/	/	/	/
CD	Butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	1767	MS	/	tr	0.2	/	/	tr	0.1	/	0.1	0.1	0.1	/
SO	Hexahydrofarnesyl acetone	1848	1847	/	/	tr	tr	/	/	tr	/	/	/	/	/

RI-Experimental linear retention indices relative to C8-C40 alkanes. Ria-Literature indices-Adams' retention indices and * according to NIST data base. Tr- trace<0.05% and not detected compounds are marked as (/). M-Hydrocarbon Monoterpenoids, MO-Oxygenated Monoterpenoids, S-Hydrocarbon Sesquiterpenoids, SO-Oxygenated Sesquiterpenoids, PP-Phenylpropanoids, CD-carboxylic acid derivatives, A-Alkanes, O-Other

Table S-XI. The number of identified components per sample of *A. scoparia*, the percentage of each class of compounds, and the percentage of total identified components

Sample	AS1	AS2	AS3	AS4	AS5	AS6	AS7	AS8	AS9	AS10	AS11	AS12
Contribution in total peaks area of ion chromatogram, %	98.8	98.3	96.6	99.4	99.3	98.1	99.6	99.6	99.3	99.1	99.6	99.8
Number of components	69	73	86	87	74	81	78	79	79	95	98	85
						Content, %						
Total monoterpenoids	22.3	19.0	23.0	28.2	27.0	23.4	17.7	34.3	24.2	24.1	37.7	30.7
Monoterpene hydrocarbons (M)	21.4	18.9	22.7	27.7	26.2	23.1	17.6	33.7	23.8	23.7	37.3	30.3
Oxygenated monoterpenes (MO)	0.9	0.1	0.4	0.5	0.8	0.3	0.1	0.6	0.4	0.4	0.4	0.4
Total sesquiterpenoids	3.7	0.9	2.2	4.9	1.0	1.2	2.9	2.3	1.6	2.1	2.3	1.6
Sesquiterpene hydrocarbons (S)	2.4	0.9	1.5	2.4	0.9	1.2	1.8	1.5	1.4	1.9	1.9	1.0
Oxygenated Sesquiterpene (SO)	1.2	tr	0.6	2.4	0.1	tr	1.1	0.8	0.2	0.2	0.4	0.6
Phenyldiacetylenes (P)	70.3	77.7	69.3	65.8	70.7	72.8	75.8	62.4	71.7	71.1	57.6	66.2
Phenylpropanoids (PP)	2.1	0.1	1.5	tr	0.2	0.4	2.7	0.3	1.4	1.1	1.6	1.1
Carboxylic acid derivatives (CD)	0.2	0.1	0.2	0.1	tr	tr	0.1	tr	0.1	0.3	0.1	tr
Other (O)	0.4	0.5	0.4	0.5	0.4	0.3	0.4	0.3	0.3	0.5	0.2	0.3

STATISTICAL ANALYSIS

Statistics on the content of compounds determined using GC/MS of the investigated Artemisia *species*

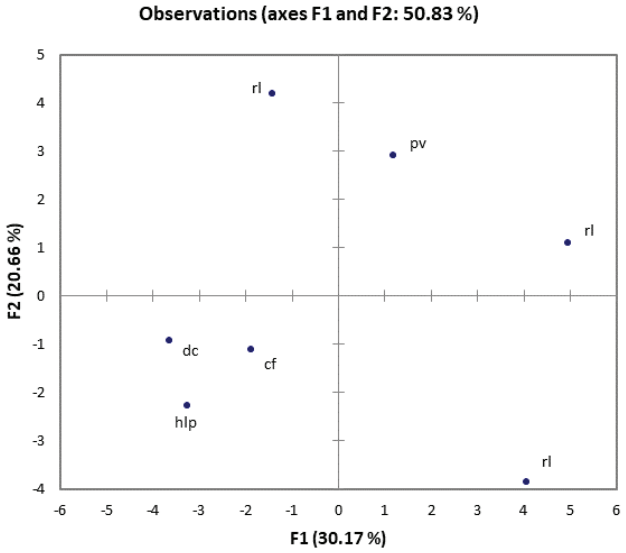

Fig. S-1. PCA plot of the analyzed *A. alba* samples showing correlations between the type of soil and percentages of components determined by GC/MS.

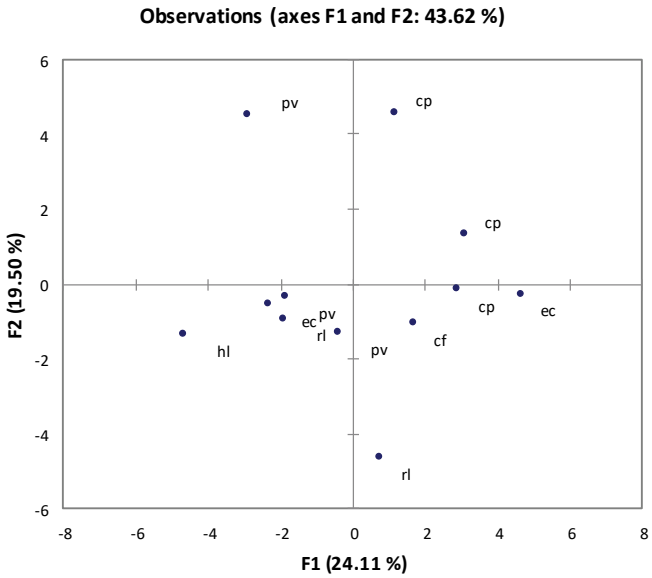

Fig. S-2. PCA plot of the analyzed *A. absinthium* samples showing correlations between the type of soil and percentages of components determined by GC/MS.

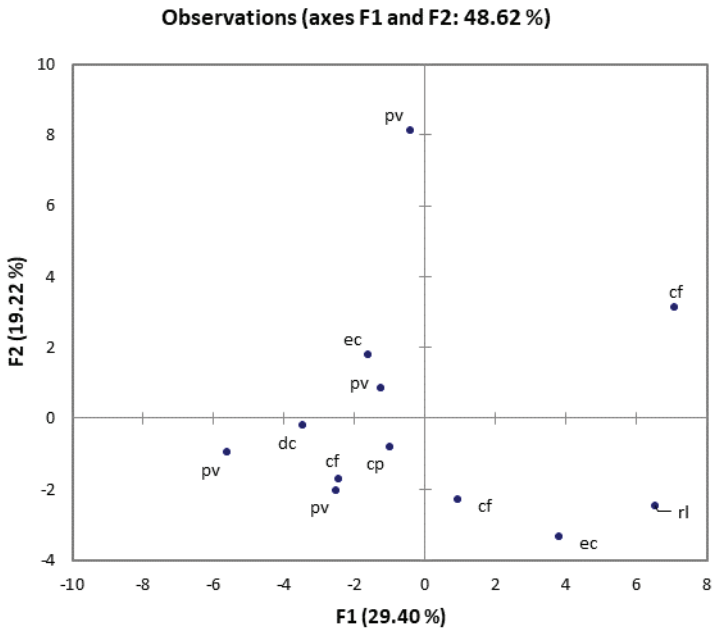

Fig. S-3. PCA plot of the analyzed *A. annua* samples showing correlations between the type of soil and percentages of components determined by GC/MS.

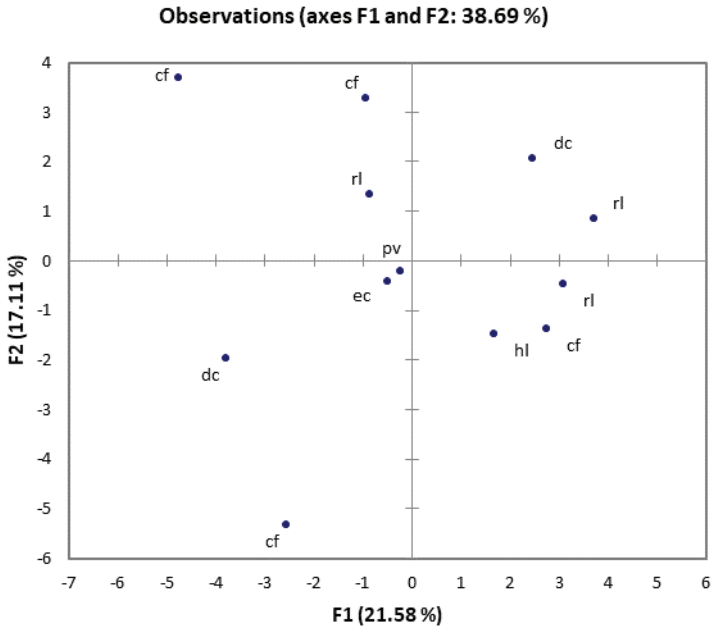

Fig. S-4. PCA plot of the analyzed *A. vulgaris* samples showing correlations between the type of soil and percentages of components determined by GC/MS.

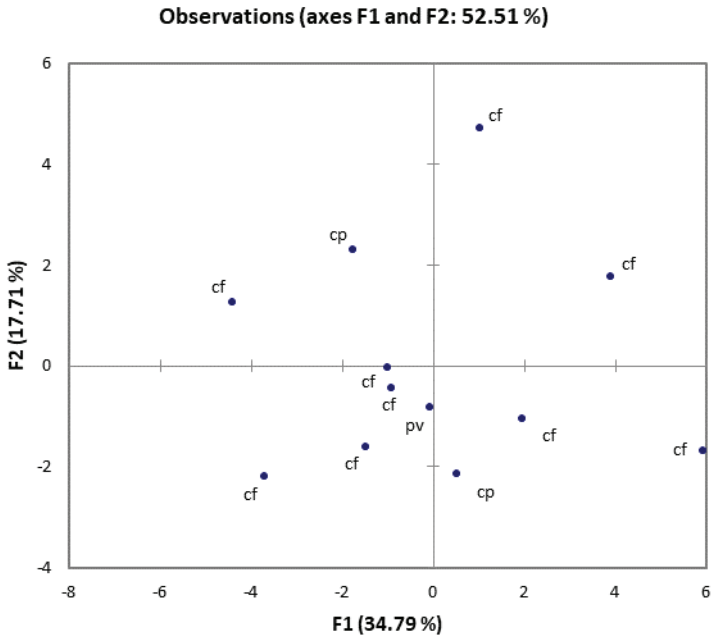

Fig. S-5. Dendrogram of the analyzed *A. scoparia* samples showing correlations between the type of soil and percentages of components determined by GC/MS.

Table S-XII. Factor loadings after Varimax rotation for *A. alba* regarding chemical composition

	D1	D2	D3	D4	D5	D6
α-Pinene	0.1938	-0.6191	0.1375	0.1642	0.6877	0.2457
Camphene	0.0856	-0.5571	0.2358	0.7642	-0.1188	0.1692
β-Pinene	0.5577	0.0453	0.3905	0.6752	-0.0508	0.2755
1,8-Cineole	-0.2921	0.5431	0.4334	0.1417	-0.6387	0.0623
Benzene acetaldehyde	0.8855	0.3982	-0.1786	0.0392	0.1083	-0.1101
γ-Terpinene	0.2631	-0.3127	-0.1656	0.8933	0.0078	-0.0866
Artemisia ketone	0.2380	-0.1518	0.5843	0.0148	0.7603	-0.0273
cis-Sabinene hydrate	0.3709	0.0493	0.1251	-0.2156	0.8932	0.0034
Terpinolene	0.9038	0.2226	0.0700	0.2592	0.2466	0.0276
trans-Sabinene hydrate	0.1481	0.4434	0.5186	0.7158	-0.0099	0.0044
Linalool	0.2593	0.4746	-0.7795	-0.2838	-0.1388	-0.0017
Filifolone	-0.2965	-0.5235	0.3726	-0.6795	-0.1747	0.0835
6-Methyl-(E)-3,5-heptadien-2-one	0.5148	0.1358	0.6550	0.4540	0.2669	0.1012
trans-Thujone	-0.2714	-0.6024	0.1955	-0.5835	0.4294	0.0160
cis-*p*-Menth-2-en-1-ol	0.3445	0.8956	0.2482	0.1256	-0.0390	0.0194
Chrysanthenone	0.1113	-0.1666	-0.3236	0.0722	0.9172	-0.0933
trans-Pinocarveol	0.7535	-0.2925	-0.3415	0.2526	0.3353	0.2320
Camphor	0.1029	0.5158	0.6124	-0.5162	-0.0756	0.2760
Pinocarvone	0.7815	0.1859	0.2017	0.1605	0.5352	0.0438
Borneol	-0.7503	-0.0901	-0.6290	0.0945	-0.1555	-0.0158

	D1	D2	D3	D4	D5	D6
cis-Pinocamphone	0.0417	0.1726	0.3639	0.9132	-0.0423	-0.0187
Terpinen-4-ol	-0.0731	-0.1085	-0.9218	-0.0785	0.3534	-0.0461
p-Cymen-8-ol	-0.4062	-0.1794	-0.3259	0.8094	-0.1466	0.1413
α-Terpineol	0.7254	0.4006	0.4403	0.2915	0.1798	-0.0456
trans-Piperitol	0.1001	0.9755	-0.0263	-0.0706	-0.1808	-0.0058
δ-Elemene	-0.0029	0.2921	-0.8987	0.1991	-0.2576	-0.0301
β-Elemene	0.7339	0.6474	0.1113	0.1457	0.0832	-0.0416
(E)-Caryophyllene	0.2902	0.8441	-0.3783	-0.1773	-0.1641	-0.0415
Germacrene D	0.3656	-0.3543	-0.5067	-0.3579	0.5389	0.2559
Bicyclogermacrene	0.3101	0.9344	0.1229	0.0934	-0.0592	0.0589
Silphiperfol-5-en-3-one A	0.3820	-0.6487	0.2230	0.5245	0.3121	0.1053
Davanone	0.3385	-0.0035	0.9124	0.0578	-0.1731	0.1401
α-Eudesmol	-0.0010	-0.0526	-0.9512	-0.2247	-0.0461	0.1994
α-Bisabolol	0.4536	0.0882	0.2659	0.8304	0.0299	-0.1594

Table S-XIII. Factor loadings after Varimax rotation for *A. absinthium* regarding chemical composition

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
(*E*)-2-Hexenal	0.0180	0.3112	-0.8737	-0.3306	0.0216	-0.0453	0.1033	-0.1073	0.0123	0.0371
n-Hexanol	-0.0183	0.2031	-0.9190	-0.1573	-0.0383	0.2734	0.0000	0.0560	0.0616	0.0776
α-Thujene	0.2340	0.5574	0.0353	0.7022	0.2697	0.1662	0.1475	0.1203	-0.0505	-0.0199
α-Pinene	0.7526	0.0056	0.1621	0.6124	-0.0471	0.0048	0.1479	-0.0115	-0.0499	-0.0003
α-Fenchene	0.0498	0.8976	0.0564	0.1387	0.0840	0.2744	0.2152	0.0905	0.0054	-0.1731
Sabinene	-0.3018	-0.3445	-0.2509	0.5433	-0.4420	0.0852	-0.2655	-0.3106	0.0833	0.2177
1-Octen-3-ol	0.8619	0.0343	-0.2070	0.1902	0.0596	-0.2479	0.0743	-0.3169	0.0686	0.0059
Myrcene	-0.3406	-0.2517	0.2302	-0.7574	-0.1050	-0.3071	0.2361	-0.1479	-0.0529	0.0780
α-Phellandrene	0.3237	-0.4365	0.4513	0.6592	-0.0737	-0.0441	-0.1229	-0.1446	0.0692	0.1109
α-Terpinene	0.1925	0.3126	0.2913	0.8729	0.0395	0.1007	0.0105	0.0139	-0.0792	-0.0045
o-Cymene	-0.6969	0.6473	-0.0025	-0.0065	-0.0038	0.1571	0.0214	0.2332	0.1178	-0.0043
β-Phellandrene	-0.2279	0.1383	0.4564	0.6699	-0.1455	-0.3842	0.1025	-0.2805	-0.0070	0.1088
(*Z*)-*β*-Ocimene	-0.4231	0.2909	0.3201	-0.1250	0.1046	0.2607	0.0629	0.7278	0.0150	-0.0048
γ-Terpinene	0.2284	0.4754	0.1596	0.8117	0.0266	0.0991	0.0107	-0.1230	-0.1040	0.0292
cis-Sabinene hydrate	-0.0753	0.3505	0.7530	0.0543	-0.4279	0.1911	0.0481	0.2619	-0.0285	0.1006
cis-Linalool oxide	-0.1509	0.3211	-0.7383	0.0912	0.1704	0.4565	0.2666	-0.0137	-0.0572	-0.0886
Terpinolene	0.0094	0.8014	-0.4191	0.3430	0.1999	0.1113	0.0087	-0.0928	-0.0504	-0.0225
Linalool	0.6809	-0.2684	0.3274	0.1679	0.1730	0.0894	-0.5091	-0.1734	-0.0277	-0.0162
n-Nonanal	0.1760	0.8896	-0.2975	0.0891	-0.0700	-0.2313	-0.0117	0.0310	-0.0415	0.1165
trans-Thujone	-0.0822	0.0074	-0.0438	0.0659	0.9905	-0.0630	-0.0180	0.0332	-0.0003	0.0203
(*Z*)-Epoxy-ocimene	0.3559	0.7525	-0.0238	0.4053	0.1514	0.1469	-0.1855	0.1946	0.1581	-0.0111
Sabina ketone	-0.2723	0.8408	-0.0568	-0.1916	-0.1719	0.0716	-0.0391	0.3137	-0.1634	0.0587
Terpinen-4-ol	0.4853	-0.0778	0.1925	0.5383	0.4695	0.3419	-0.2256	0.0592	0.0905	-0.1752
α-Terpineol	0.7907	0.1409	-0.5365	-0.1360	0.0297	0.1668	-0.0917	-0.0582	-0.0176	-0.0649
Methyl salicylate	-0.0249	0.2892	-0.2285	-0.9111	-0.0225	0.1250	0.0051	0.0461	-0.1155	0.0431
Fragranol	0.0624	-0.3642	0.3351	-0.1697	0.1741	-0.8094	0.0433	-0.1829	-0.0005	-0.0265
Nerol	0.7870	-0.4155	0.2364	0.1775	-0.2468	0.0744	-0.0623	0.1859	-0.1222	-0.0240
Neral	0.1952	-0.0187	0.8976	0.2573	0.2589	0.0023	0.1097	0.0816	0.0506	-0.0220
Geraniol	0.8572	0.2997	0.3127	0.2163	0.0341	0.0203	-0.0162	0.0674	0.1295	0.0412
Lavandulyl acetate	-0.3870	0.8565	-0.1119	-0.0707	-0.1243	0.1988	0.0680	-0.1588	0.1137	0.0309

Table S-XIV. Factor loadings after Varimax rotation for *A. annua* regarding chemical composition

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Ethyl 2-methylbutyrate	-0.6822	0.2167	0.2977	-0.1518	0.1082	-0.5376	-0.0364	-0.0291	0.2304	0.1389
Santolina triene	0.1167	-0.8033	0.0687	0.4766	-0.2473	-0.0534	0.1341	-0.1643	-0.0127	-0.0068
Tricyclene	-0.1100	0.0655	0.1645	-0.1839	0.9391	-0.1437	-0.0433	-0.1136	-0.0425	0.0580
α-Thujene	0.1324	-0.2062	0.9355	0.0220	0.1453	-0.1593	-0.1255	0.0392	0.0076	0.0084
α-Pinene	0.2372	0.2592	-0.3630	-0.2580	-0.1279	0.0846	0.8085	-0.0144	-0.0031	-0.0040
Propyl 2-methylbutyrate	-0.3447	0.4152	0.0196	-0.1729	0.3551	-0.7286	0.0289	0.0779	0.1174	-0.0248
Camphene	0.5449	-0.4097	-0.0452	0.4144	-0.4640	0.3453	0.0132	0.0258	0.0067	-0.1594
Sabinene	0.0877	-0.0477	0.3809	-0.3281	0.7779	-0.1599	-0.1714	0.2598	0.0906	-0.0390
β-Pinene	-0.1388	-0.6140	0.3733	-0.1131	-0.5995	-0.0585	-0.0142	0.1444	-0.0507	0.2520
Yomogi alcohol	-0.7349	-0.4255	-0.1606	0.1479	-0.4562	0.0826	-0.0986	0.0365	-0.0638	0.0117
o-Cymene	-0.1723	0.7559	0.4259	-0.0737	0.1055	-0.4172	0.0941	-0.1196	-0.0128	-0.0580
1,8-Cineole	0.4104	-0.3706	0.3965	0.2713	0.0099	0.4011	-0.0987	0.5398	-0.0168	0.0137
Artemisia ketone	-0.2612	0.1093	0.6918	-0.3759	0.3254	-0.1738	-0.4027	0.0033	-0.0081	-0.0004
cis-Sabinene hydrate	0.9309	0.1129	-0.0403	0.0162	-0.2464	0.1921	-0.1095	0.0854	-0.0342	-0.0245
Artemisia alcohol	0.6132	0.4421	0.1466	-0.1791	0.3744	0.3264	0.0489	-0.2450	0.2554	-0.0232
Terpinolene	-0.5486	0.6120	0.3335	-0.2125	0.3339	-0.2153	0.0395	0.0701	0.0566	-0.0215
trans-Sabinene hydrate	0.9685	-0.0174	-0.0330	0.1401	0.0894	-0.1270	0.0522	0.1076	-0.0148	-0.0414
3-methyl-3-butenyl 3-Methyl butyrate	-0.5247	-0.0009	0.1864	-0.5965	0.3505	-0.3351	0.2187	-0.1553	0.0623	0.1522
α-Campholenal	0.0697	0.9619	-0.1061	0.1722	0.0180	-0.0539	0.0091	-0.1586	-0.0165	0.0121
trans-Pinocarveol	-0.1767	-0.9075	-0.0263	0.1178	0.3132	0.0921	-0.0743	-0.0942	0.0942	-0.0278
Camphor	0.9216	-0.0085	-0.1608	0.0883	-0.1538	-0.0147	-0.1792	0.1973	-0.1308	-0.0278
cis-Chrysanthenol	0.4180	-0.7689	0.0623	0.3427	0.0568	0.1267	0.2419	-0.0919	-0.1294	0.0657
Pinocarvone	0.0014	-0.9622	0.0936	0.1387	0.1287	0.0704	-0.1346	-0.0389	0.0342	-0.0555
Lavandulol	-0.2452	-0.5192	0.3195	-0.5458	-0.3087	0.2407	-0.0155	0.1752	-0.2833	0.0041
Terpinen-4-ol	-0.2949	0.8521	0.2455	-0.1180	0.3049	-0.1201	0.0216	0.0146	0.0554	-0.0385
α-Terpineol	0.7735	0.0849	0.4850	0.0411	0.3684	0.0434	-0.0198	0.1336	0.0298	0.0040
Myrtenol	-0.1718	0.9536	-0.0586	0.1435	0.1597	-0.0212	0.0478	0.0151	0.0902	-0.0200
trans-Carveol	0.3044	0.7931	0.4249	-0.0646	0.1220	-0.2564	0.0525	-0.0764	-0.0227	-0.0598
(*Z*)-3-Hexenyl 2-methyl butyrate	0.7141	0.5404	-0.0642	0.3050	0.1329	-0.1947	0.1411	-0.1476	0.0470	-0.0329
(*Z*)-3-Hexenyl 3-methyl butyrate	-0.4488	0.1197	0.4325	-0.6765	0.3433	0.1168	0.0446	-0.0012	-0.0414	0.0532
Hexyl 2-methyl butyrate	0.0108	0.0322	0.1615	0.8980	-0.2740	0.1431	-0.2115	0.0586	-0.1272	0.0504
Eugenol	0.8710	-0.1916	0.1024	-0.1467	-0.1118	0.3945	0.0174	-0.0148	0.0143	-0.0468
α-Copaene	0.9023	0.3024	0.1193	0.1258	-0.2178	-0.0943	0.0094	0.0338	-0.0645	0.0465
Benzyl 2-methylbutyrate	-0.1817	0.8103	-0.2223	0.3123	0.2408	-0.1262	-0.0644	-0.2859	0.0165	0.0347
β-Cubebene	0.3810	0.8541	-0.2382	0.1714	-0.1385	0.0261	0.0579	0.0036	-0.0783	0.0908
(*Z*)-Jasmone	0.9428	-0.0957	0.0981	0.0172	0.0960	-0.0835	0.1676	-0.1779	0.1260	-0.0122
(*E*)-Caryophyllene	0.6997	-0.0730	-0.2332	0.2487	-0.4106	0.1570	0.4018	-0.1662	0.0726	0.0303
(*E*)-β-Farnesene	0.8382	-0.4104	0.1498	0.1085	-0.1128	0.2654	-0.0347	0.0852	0.0216	-0.0156
α-Humulene	0.1602	-0.2868	-0.2928	0.2039	-0.0826	0.8436	0.1557	0.0978	0.1046	0.0168
γ-Selinene	0.7798	-0.3364	0.1819	0.4591	-0.1374	0.1092	0.0482	0.0155	0.0127	-0.0078
Bicyclogermacrene	0.9453	-0.1467	-0.0149	0.2128	-0.1521	0.1116	0.0087	0.0444	-0.0211	-0.0085
Caryophyllene oxide	0.9642	0.0517	0.0535	0.1398	-0.1018	0.0235	0.1507	-0.0203	-0.1040	-0.0015
Longifolenaldehyde	0.6864	-0.0738	-0.1116	0.6781	-0.0776	0.1505	0.0926	-0.0366	0.0567	0.0467
cis-Cadin-4-en-7-ol	0.8472	0.0124	0.0212	0.4486	0.1290	0.1480	0.0071	-0.1297	0.0976	0.1118
Selina-3,11-dien-6-*α*-ol	0.5870	0.0771	-0.1651	0.7459	-0.0527	0.2085	0.0693	0.0358	0.0837	-0.0430
Caryophylla-4(12),8(13)-dien-5-*α*-ol	0.8430	-0.0908	-0.3709	0.1978	0.1933	0.1266	0.1444	-0.0897	0.1419	-0.0030
Selin-11-en-4-*α*-ol	0.8131	-0.1004	-0.1099	0.5097	0.0327	0.1921	0.0950	0.0558	0.0586	0.0056
Germacra-4(15),5, 10(14)-trien-1-*α*-ol	0.8950	0.0438	-0.3035	0.0059	0.1026	0.2830	0.0959	0.0426	-0.0174	0.0444

Table S-XV. Factor loadings after Varimax rotation for *A. vulgaris* regarding chemical composition

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Santolina Triene	-0.2772	-0.2757	-0.1478	0.3758	-0.6892	0.3505	-0.1951	0.1329	-0.1029	0.1144
α-Pinene	0.9612	0.0595	-0.1674	-0.0280	-0.1061	-0.0072	0.0994	0.1037	0.0959	0.0498
Sabinene	-0.0436	-0.3856	0.8492	0.0587	0.2394	0.0551	-0.2080	0.0155	0.0301	-0.0792
Myrcene	-0.0186	-0.3681	-0.4169	0.2618	-0.7107	-0.2995	-0.0367	0.1337	-0.0367	0.0691
α-Terpinene	0.5102	-0.0619	-0.1118	-0.2825	0.5888	0.4997	0.1970	-0.0895	-0.0114	-0.0142
o-Cymene	0.8156	-0.0932	0.0924	0.1412	0.2641	0.4297	0.2051	0.0157	-0.0018	-0.0249
1,8-Cineole	-0.8715	0.1752	0.0304	0.4209	-0.0064	-0.1174	-0.0475	0.0933	-0.0300	0.0703
(*E*)-*β*-Ocimene	0.6893	0.4567	-0.1082	-0.2236	0.2097	0.1996	0.3719	-0.0264	-0.1139	-0.1363
cis-Sabinene hydrate	0.1222	0.0750	0.3350	-0.0689	0.8821	0.1204	0.1896	0.1328	-0.0518	0.1162
trans-Sabinene hydrate	0.3035	-0.0918	0.1094	-0.1173	0.8881	0.1376	0.2273	0.1080	-0.0467	0.0224
cis-Thujone	-0.2486	0.0658	-0.7164	0.3175	-0.3189	0.0166	-0.4583	-0.0035	0.0805	0.0361
trans-Thujone	0.0179	-0.0034	0.9095	-0.0758	0.3539	0.1188	0.0907	0.0293	-0.0568	0.0512
Chrysanthenone	-0.4224	-0.5526	-0.3409	-0.1108	-0.5608	-0.1418	0.0775	-0.1710	0.0054	-0.1296
iso-3-Thujanol	0.1831	-0.1462	-0.0175	-0.1884	0.2474	0.0433	0.9179	-0.0539	0.0173	0.0210
trans-Pinocarveol	-0.2098	-0.1017	0.2954	0.1763	0.8981	-0.0111	-0.0068	0.0923	-0.0410	-0.0851
cis-Chrysanthenol	0.4611	-0.0025	0.1258	0.3940	0.2901	0.0221	-0.0608	0.7250	-0.0303	0.0089
Terpinen-4-ol	0.9518	0.0256	0.0510	-0.1099	0.2135	0.0012	-0.0161	0.1340	-0.1055	0.0198
α-Terpineol	-0.5379	0.2327	0.5926	0.0088	-0.0391	0.2270	-0.3608	0.1341	0.0820	0.3120
cis-Chrysanthenyl acetate	0.1599	0.8736	0.1236	0.1315	-0.0243	0.1455	-0.1052	-0.0838	0.3451	0.0658
Eugenol	0.2975	0.8894	-0.0593	-0.1656	0.2505	0.0489	-0.1027	-0.0678	-0.0077	-0.0808
β-Elemene	0.2867	-0.1361	-0.7762	0.4007	-0.3145	0.1727	0.0234	-0.0578	-0.0544	-0.0123
α-Humulene	0.4830	0.5076	0.3331	-0.0162	0.1865	0.2931	0.3812	0.2197	-0.2842	0.0478
Germacrene D	-0.4439	0.1964	-0.2378	0.0435	-0.3522	-0.7586	0.0404	-0.0049	0.0060	0.0006
β-Selinene	-0.0396	-0.7232	0.4828	0.0232	-0.1220	0.0730	-0.2724	-0.0902	0.3674	-0.0475
Bicyclogermacrene	0.6949	0.2742	-0.0998	-0.0941	0.0051	0.4853	0.2924	0.1618	0.2657	0.0732
Germacrene A	0.1517	0.2953	-0.2755	0.7033	-0.2570	0.3152	0.0248	-0.2174	0.3159	-0.0607
δ-Cadinene	-0.4660	0.7506	-0.1460	0.3307	-0.2354	-0.0743	-0.1555	0.0276	0.0271	-0.0241
Salviadienol	-0.1590	0.4052	-0.0102	0.8381	0.0086	-0.2388	-0.2186	0.0265	-0.0431	-0.0229
Spathulenol	0.2406	-0.6434	-0.0932	-0.3358	0.1619	0.0383	0.5989	0.0561	-0.0418	-0.1195
Caryophyllene oxide	0.4637	0.0069	0.0797	-0.7091	0.2506	-0.0531	0.4100	0.0041	-0.1542	0.1346
Torilenol	-0.1420	-0.1123	-0.2562	0.9159	0.0068	-0.0050	-0.1055	0.2015	-0.0865	0.0533
14-hydroxy-9-*epi*-(*E*)-Caryophyllene	-0.2365	0.8333	-0.1871	0.2260	0.0167	-0.3708	-0.0908	0.0168	-0.1193	-0.0566
Germacra-4(15),5, 10(14)-trien-1-*α*-ol	-0.1045	0.6423	0.1098	0.6767	-0.0611	-0.1659	0.0346	0.2334	-0.0484	0.1313

Table S-XVI. Factor loadings after Varimax rotation for *A. scoparia* regarding chemical composition

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
α-Pinene	0.9260	-0.0139	0.3379	-0.1174	0.0461	-0.0187	-0.0186	0.1065	0.0149	0.0066
Sabinene	0.9233	-0.0542	0.2046	-0.2702	0.1440	-0.0278	0.0407	0.0730	0.0307	0.0113
β-Pinene	0.9328	-0.0336	0.1277	-0.2650	0.1881	0.0530	0.0230	0.0542	0.0066	0.0208
Myrcene	0.6055	-0.0032	0.5988	0.2391	-0.0971	-0.3527	0.0149	0.2831	0.0446	0.0355
α-Terpinene	0.5510	0.0784	0.7614	-0.2777	0.1389	0.0586	-0.0851	-0.0580	-0.0087	0.0086
p-Cymene	0.1293	0.0194	0.9550	-0.2279	0.0790	-0.0248	0.0558	0.0902	0.0094	0.0278
Limonene	0.8744	0.0516	0.4279	-0.0097	-0.1888	0.0049	-0.0194	0.1125	0.0020	-0.0280
1,8-Cineole	0.1209	0.3771	0.0862	0.0430	0.9129	0.0152	-0.0132	0.0061	-0.0078	-0.0032
(*Z*)-*β*-Ocimene	0.2708	0.8634	-0.0592	0.3877	0.0421	-0.0401	0.0358	-0.1455	-0.0249	-0.0080
(*E*)-*β*-Ocimene	0.8296	0.1114	0.4627	0.2604	-0.0526	0.0554	0.0743	0.0176	-0.0684	0.0310
γ-Terpinene	0.2588	-0.0611	0.9380	-0.1715	-0.0472	-0.0210	-0.0885	-0.0898	-0.0081	-0.0385
allo-Ocimene	0.3035	0.8617	0.0720	0.2770	0.2228	-0.0609	-0.0918	-0.1409	0.0238	0.0278

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Terpinen-4-ol	0.8170	0.1263	0.3557	-0.0684	0.4142	0.0820	-0.0656	0.0364	-0.0332	0.0138
2,4-pentadiynyl-benzene	0.8787	0.1564	-0.1585	-0.3262	0.0652	0.0481	0.0115	-0.2546	0.0095	0.0040
Eugenol	0.3092	-0.6039	-0.0699	0.1665	0.0654	0.7082	0.0337	0.0048	0.0033	0.0047
2,6-dimethyl-Naphthalene	-0.9371	-0.2938	-0.0823	0.1159	-0.0345	-0.0671	-0.0023	0.0881	-0.0095	0.0425
(*E*)-Caryophyllene	-0.5272	0.0472	-0.0653	0.8205	-0.0661	0.1618	0.0815	0.0287	-0.0542	0.0359
α-Humulene	-0.2928	-0.0068	-0.2554	0.8929	-0.0948	0.1533	0.0868	-0.0777	-0.0667	0.0350
γ-Curcumene	0.5756	-0.1890	-0.3518	0.0096	0.5286	0.1058	0.4520	-0.0886	0.0782	0.0143
Capillene	-0.9681	-0.1159	-0.0941	0.1313	-0.0895	-0.0541	-0.0160	0.1092	-0.0137	-0.0004
Spathulenol	-0.1273	0.3686	-0.2848	0.8247	0.0018	-0.1411	0.2055	0.1235	-0.0318	-0.0894
β-Eudesmol	-0.0010	0.1352	-0.0465	0.9238	0.1670	-0.0639	-0.2841	-0.0102	0.1155	0.0078
Butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester	0.0199	-0.9682	0.0060	0.0591	-0.1394	0.0602	-0.0059	-0.1881	0.0000	0.0128

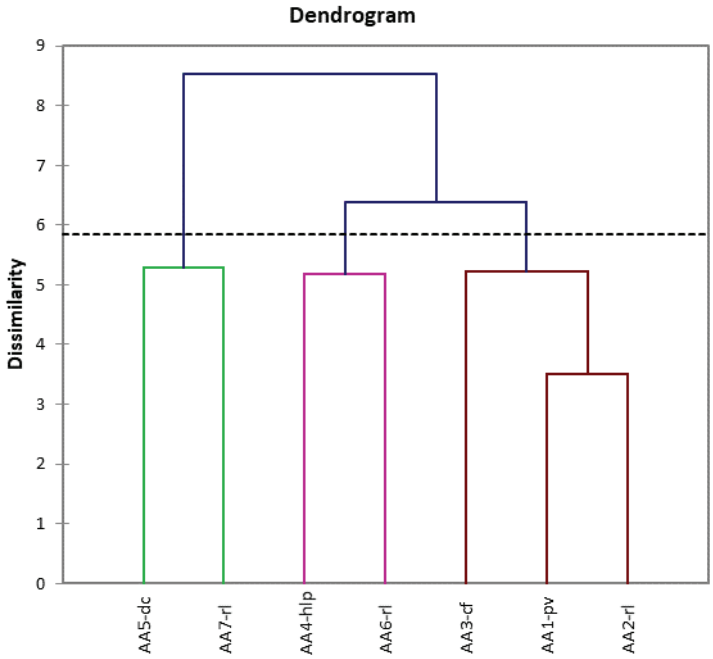

Fig. S-6. Dendrogram of the analyzed *A. alba* samples showing correlations between the type of soil and percentage of components determined by GC/MS.

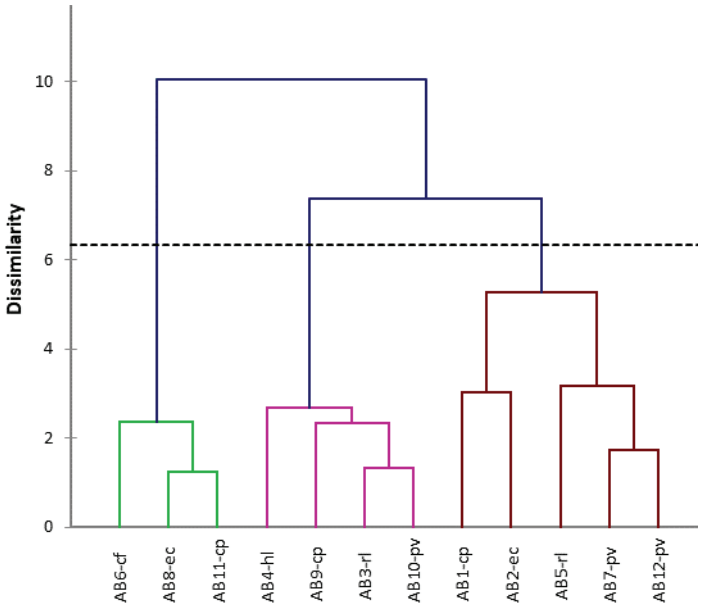

Fig. S-7. Dendrogram of the analyzed *A. absinthium* samples showing correlations between the type of soil and percentage of components determined by GC/MS.

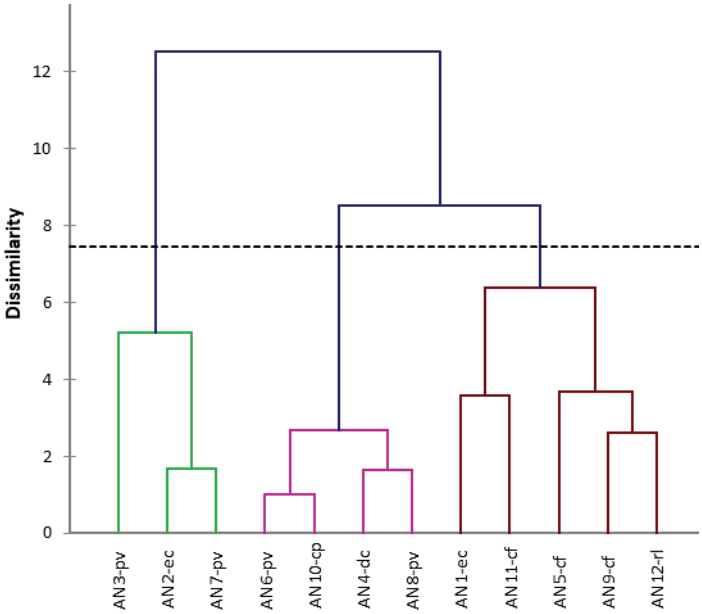

Fig. S-8. Dendrogram of the analyzed *A. annua* samples showing correlations between the type of soil and percentage of components determined by GC/MS.

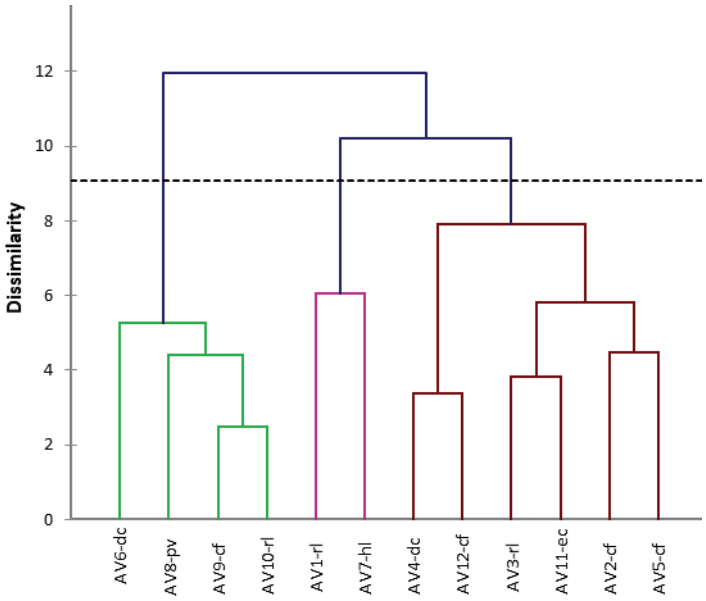

Fig. S-9. Dendrogram of the analyzed *A. vulgaris* samples showing correlations between the type of soil and components determined by GC/MS.

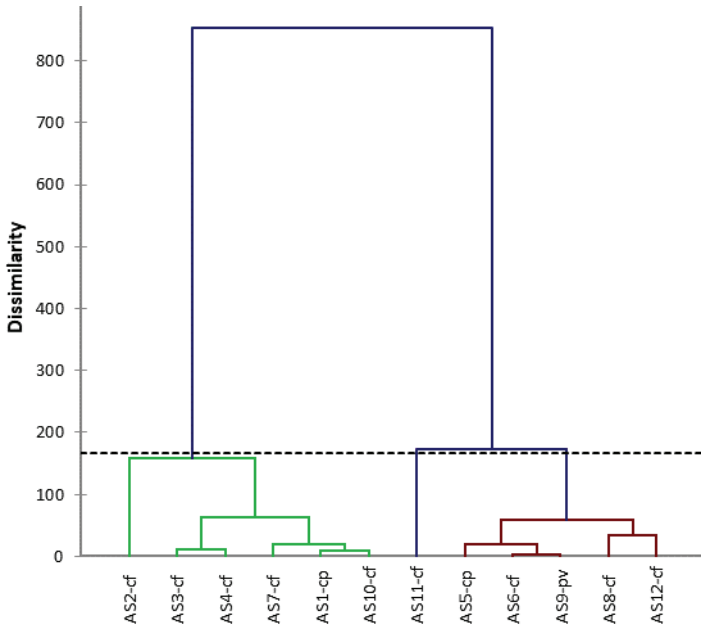

Fig. S-10. Dendrogram of the analyzed *A. scoparia* samples showing correlations between the type of soil and components.

Statistics on the percentage of classes of compounds determined using GC/MS of the investigated Artemisia *species*

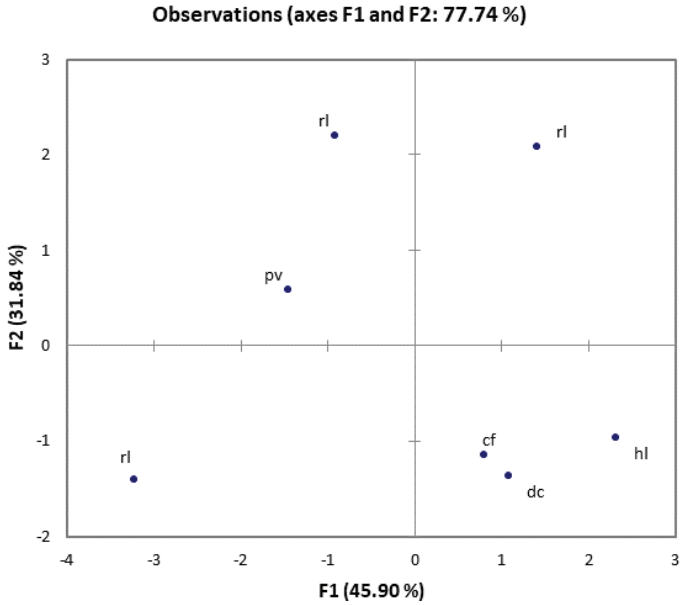

Fig. S-11. PCA plot of the analyzed *A. alba* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

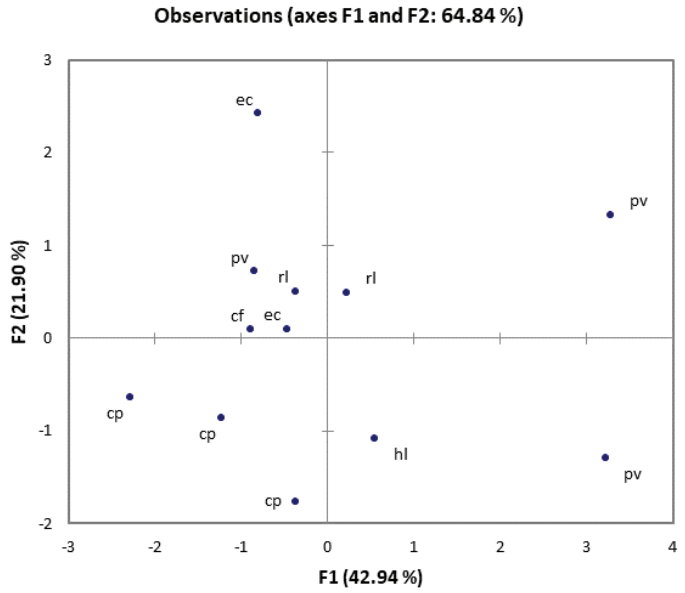

Fig. S-12. PCA plot of the analyzed *A. absinthium* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

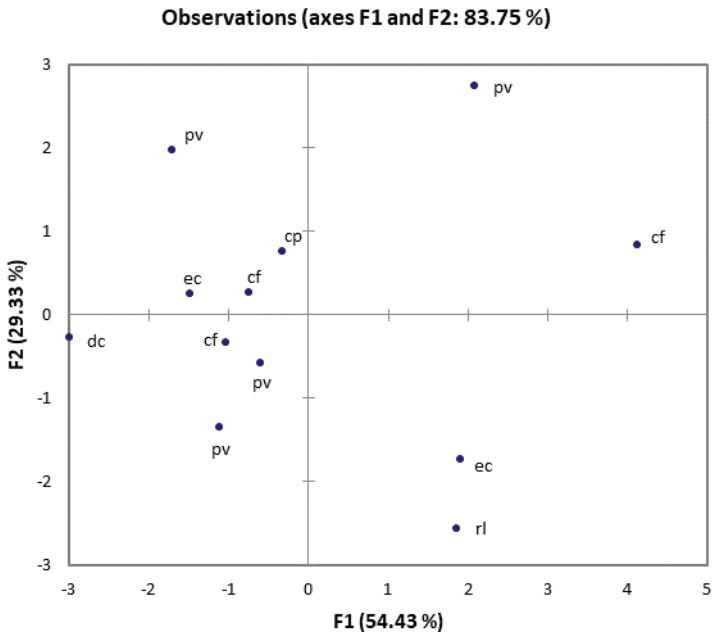

Fig. S-13. PCA plot of the analysed *A. annua* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

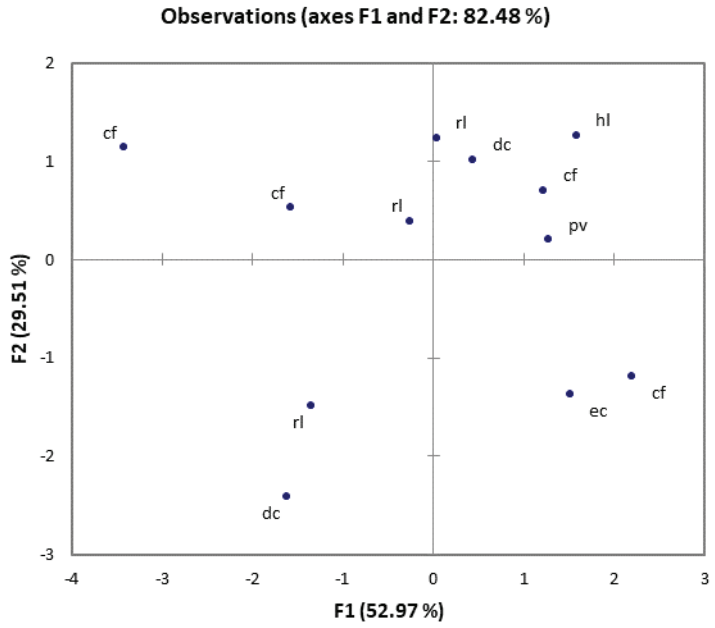

Fig. S-14. PCA plot of the analysed *A. vulgaris* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

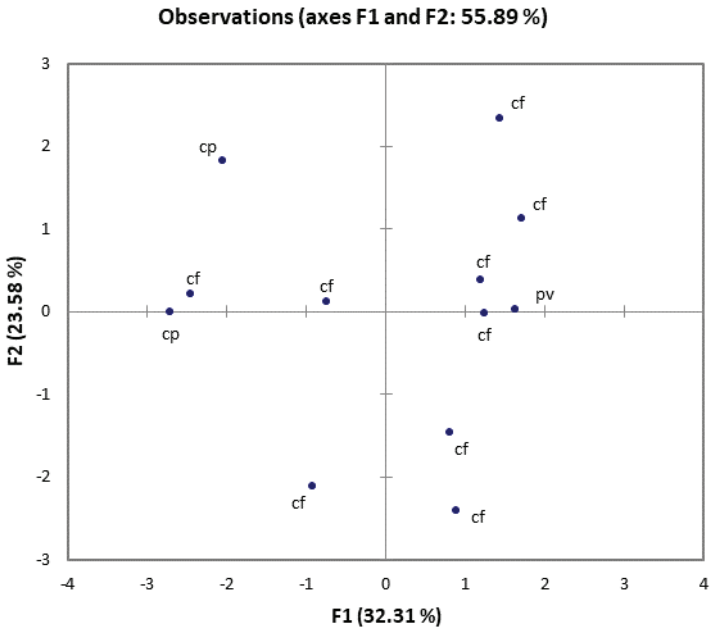

Fig. S-15. Dendrogram of the analysed *A. scoparia* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

Table S-XVII. Factor loadings after Varimax rotation for *A. alba* regarding classes of compounds

	D1	D2	D3	D4	D5
(M)	-0.9382	-0.1177	0.1259	0.2999	0.0102
(MO)	-0.9932	-0.0972	0.0506	-0.0398	-0.0049
(S)	0.9929	-0.0953	0.0436	0.0323	0.0452
(SO)	0.9711	0.0942	-0.1922	0.0960	-0.0446
(PP)	0.1271	0.9749	0.1781	-0.0336	0.0234
(CD)	-0.1331	0.2892	0.9479	0.0075	0.0019
(O)	-0.0031	0.9872	0.1561	0.0184	-0.0260

Table S-XVIII. Factor loadings after Varimax rotation for *A. absinthium* regarding classes of compounds

	D1	D2	D3	D4	D5
(M)	0.9286	-0.0407	0.2447	0.2434	0.1300
(MO)	-0.6451	0.1578	-0.3959	-0.5388	-0.3346
(S)	0.1959	-0.3743	0.1344	0.2137	0.8705
(SO)	0.3507	-0.2642	0.2421	0.8387	0.2125
(CD)	-0.0561	0.9318	0.0728	-0.1924	-0.2936
(O)	0.2801	0.0757	0.9278	0.2076	0.1088

Table S-XIX. Factor loadings after Varimax rotation for *A. annua* regarding classes of compounds

	D1	D2	D3	D4	D5
(M)	0.3124	0.9294	0.1651	0.1063	0.0082
(MO)	-0.5520	-0.7793	-0.2926	-0.0450	-0.0189
(S)	0.7904	0.2756	0.5395	-0.0531	0.0734
(SO)	0.9783	0.0548	0.1908	-0.0540	-0.0221
(PP)	0.7135	-0.0801	0.6926	0.0659	-0.0211
(CD)	-0.3038	0.8973	-0.2910	-0.1333	-0.0030
(O)	0.9242	0.1853	0.2608	0.2079	0.0184

Table S-XX. Factor loadings after Varimax rotation for *A. vulgaris* regarding classes of compounds

	D1	D2	D3	D4
(M)	-0.3868	-0.0386	0.9213	-0.0122
(MO)	-0.8701	-0.4086	0.2724	-0.0421
(S)	0.5601	0.7415	-0.3519	0.1124
(SO)	0.9067	0.0872	-0.4124	-0.0153
(PP)	0.1149	0.9920	0.0478	-0.0216

Table S-XXI. Factor loadings after Varimax rotation for *A. scoparia* regarding classes of compounds

	D1	D2	D3	D4	D5
(M)	0.7929	0.0715	0.3921	0.1277	-0.4334
(MO)	0.9632	0.0595	-0.1786	0.1187	-0.1471
(S)	-0.7614	-0.3443	-0.4708	-0.2116	0.1639
(SO)	0.1838	-0.1822	-0.0513	0.9644	-0.0055
(P)	0.2062	0.9131	0.1729	-0.2389	0.1906
(PP)	-0.3526	0.1103	-0.7706	0.0201	0.5168
(CD)	-0.5047	0.4273	0.0501	0.0012	0.7449
(O)	-0.0793	0.2607	0.9469	-0.0814	0.1430

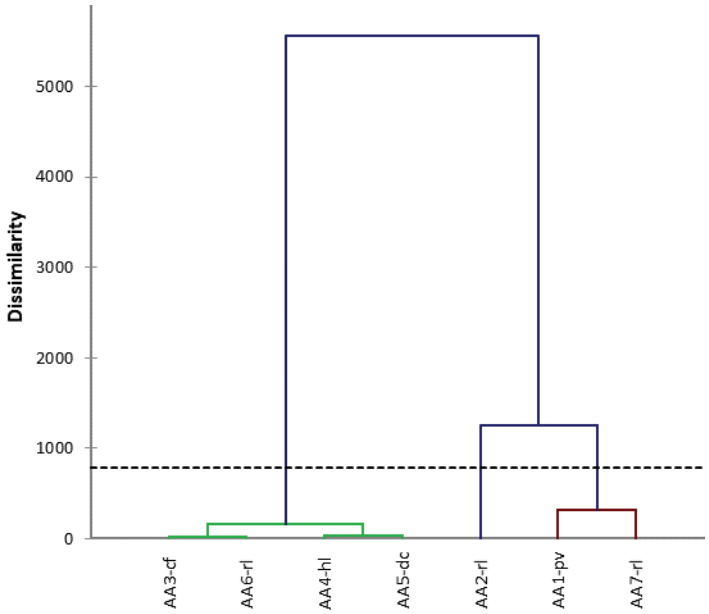

Fig. S-16. Dendrogram of the analysed *A. alba* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

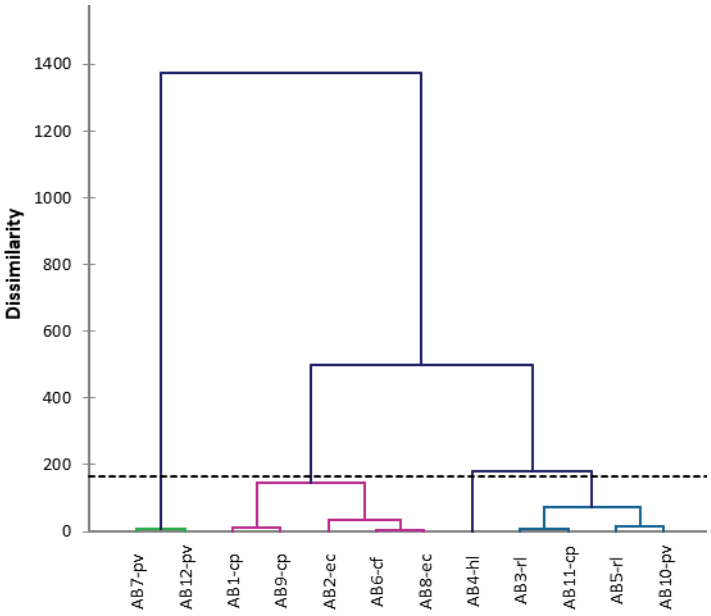

Fig. S-17. Dendrogram of the analysed *A. absinthium* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

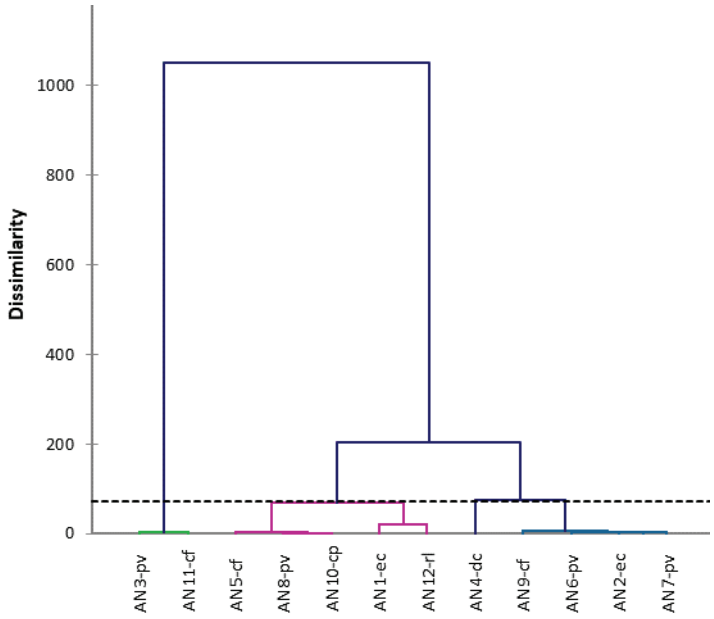

Fig. S-18. Dendrogram of the analysed *A. annua* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

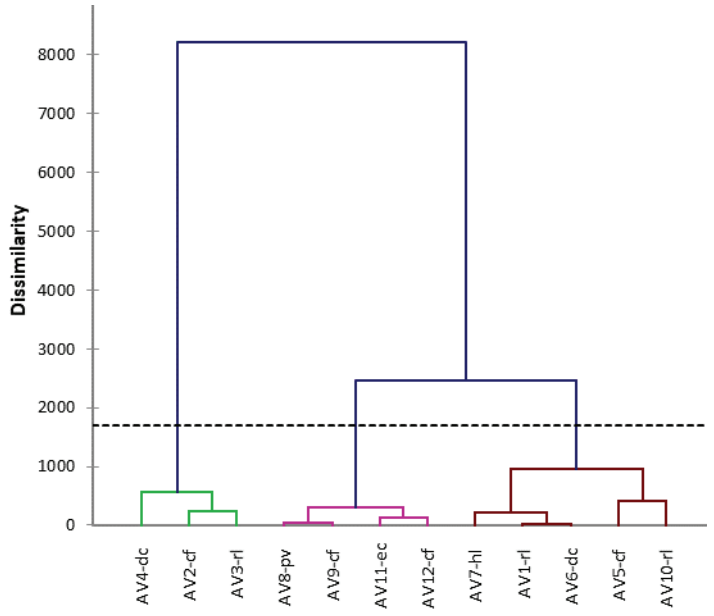

Fig. S-19. Dendrogram of the analysed *A. vulgaris* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

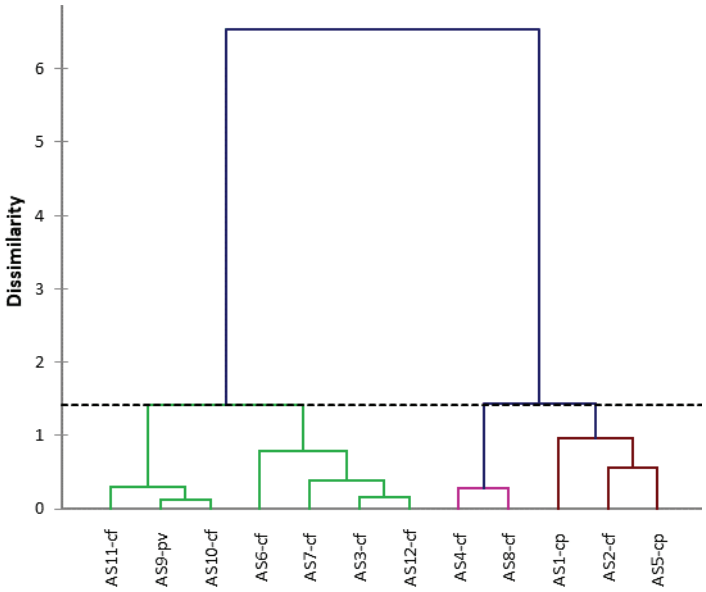

Fig. S-20. Dendrogram of the analysed *A. scoparia* samples showing correlations between the type of soil and percentage of classes of compounds determined by GC/MS.

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Comparative study on the elemental composition of different parts of cultivated *Physalis alkekengi* (Solanaceae)

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Abstract: This study aimed at analyzing and comparing the elemental composition of different parts of cultivated *Physalis alkekengi* (ljoskavac): rhizome with roots, stem with leaves, fruit, and inflated calyx. The contents of twenty-one macro- and micro-elements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, the patterns of the distributions of both macro- and micro-elements were subjected to AHC analysis which gave different grouping of samples in sub-clusters. Generally, potassium, calcium, iron, and aluminum were the most abundant elements, but with different distribution in examined parts. High contents of iron and aluminum were detected in a stem with leaves, followed with samples of rhizome with roots and calyx, while potassium dominates in samples of calyx and stem with leaves. Edible fruits did not contain potentially toxic metals in concentration higher than permissible limits, wherein the lowest contents of lead and aluminum were detected; cadmium was under limit of quantification. Arsenic, mercury, and thallium were below the method detection limit.

Keywords: ljoskavac; ICP-OES; macro- and microelements; AHC.

INTRODUCTION

Among cultivated species frequently represented in the diet of humans (chili – *Capsicum annuum*, tomato – *Lycopersicon esculentum* and potato – *Solanum tuberosum*), the Solanaceae family contains wild growing species, such as those from the fifth largest genus within the family, *Physalis* L.[1] The genus *Physalis*, a clearly defined genus within the nightshade family, comprises about 70–95 species famous for their attractive appearance, application as foods and natural remedies, which justifies worldwide cultivation. The *Physalis* species are low to

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large annual or perennial herbaceous plants (up to 1 m tall), or shrubs with attractive papery husk calyx (lantern-like) wrapping the globose yellow to orange, smooth-skinned berry (fruit) small (4-7 mm) or large (10-20 mm) with a juicy or dry pericarp and with many small seeds. Name *physalis*, derived from the Greek word *phusa*, means bladder and corresponds to the inflated calyx which completely covers the fruit during its growing and ripening periods and protects it against conditions of biotic and abiotic stress. Members of the genus are mostly native to the Americas, with the centers of distribution in Mexico (over 70 species) with pronounced endemism; then United States and Central America, and finally South America with the least species. A few species are registered in Asia and Europe, and six in China.[2,3] Surely, the most attractive in a diet and ethnomedicine are fruits of various species, such as *P. peruviana*, *P. pubescens*, *P. alkekengi*, *P. angulata*, *etc*. Depending on the region of origin and national cuisine, the fruits are consumed raw, or in sauces, compotes, pies, jams, or relishes. According to an ethnobotanical survey of Arenas & Kamienkowski, the leaves of the species *P. angulata* are also edible and used in salads.[3] *Physalis* species are natural sources of diverse compounds, thus many ethnopharmacological properties are attributed to phytochemicals as potential bioactive principles: minerals, vitamins, carotenoids, sterols, phenols, phenolic acids, flavonoids, glycosides, tannins, alkaloids, *etc*. Due to edible fruits, numerous taxa from *Physalis*, cultivated or wild growing, present economically useful crops. Less than *P. peruviana*, but also *P. philadelphica*, *P. ixocarpa*, *P. pubescens* and *P. alkekengi* are cultivated or collected from native populations for their edible fruits and nutritional value.[4] The most famous representative of the genus is *P. peruviana*, colloquially known as golden berry or Cape gooseberry, is frequently used in the food industry due to its nutritional value, pleasant sensory characteristics and as a great source of vitamins C, A, E, B3, B6; and the elements iron, magnesium, potassium, phosphorus, and calcium.[1,3,5–10] *P. alkekengi* is also recognized and described in modern and traditional medicine and also cuisine. Mature fruit of the plant (*Fructus alkekengi*) is a strong diuretic and laxative and could be locally applied (balm) as a healing agent in gout, rheumatism, erysipelas, syphilis therapy, as an accelerator in wound healing, *etc*. Juicy, sweet fruit with a certain bitter-sour note, contains sugars, organic acids, a trace of alkaloids, and high content of vitamins and carotenoids. Unripe fruit can be mildly poisonous, so it is necessary to collect mature fruit in August and September and subsequently discard the poisonous calyx. Whether used as a food or in medicine, the fruits of *P. alkekengi* can be consumed raw or dried, as salad, aqueous extracts, syrup, compote, or jam. An adult should not eat more than 30 raw berries a day. The edibleness of the fruits is widely known in the countries of Europe and Japan. Except for mature fruit, other parts of the *P. alkekengi* are not edible.[8,11–13] According to the literature, there is no data on the elemental composition of *P.*

alkekengi, except the study on the rhizome by Xu *et al.*[14] Aware of the many benefits from the *Physalis* taxa, and the importance of novel research, this study aimed at determining and comparing the contents on macro- and micro-elements in different parts of cultivated *Physalis alkekengi* – rhizome with roots (hereafter rhizome-roots), stem with leaves (hereafter stem-leaves), mature fruits and inflated calyx (hereafter calyx). Furthermore, these would be the first results (comparative study) on the elemental composition of different parts of *P. alkekengi*.

EXPERIMENTAL

Plant material

The plant material was planted in March and collected in September 2020. Cultivation of *P. alkekengi* individuals was performed from seeds in sets of plastic containers of uniform diameter under the same light conditions depending on the seasonal variation of the photoperiod, on the standardized substrate Floradur® (mixed with sand in the mass ratio of 4:1), and watering twice a week with an equal amount of water. Voucher specimens were deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš (HMN; Voucher No. 14543). All the examined plant parts of cultivated *P. alkekengi* are summarized in Fig. 1.

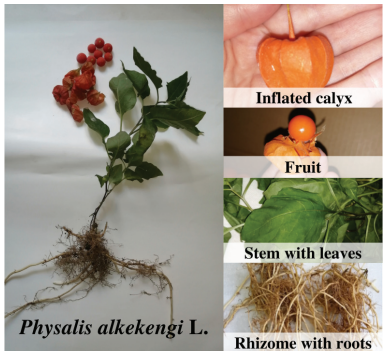

Fig 1. Cultivated *P. alkekengi* L.; plant material used to determine the elemental composition.

Sample preparation and parameters of ICP-OES analysis

For the experiment, fifteen plant individuals were collected. The plant material was divided and organized into four plant parts: rhizome with roots, stem with leaves, fruits, and calices. Prior a digestion, the rhizomes with roots were washed with deionized water and drained. All samples were chopped into small pieces with stainless-steel scissors, dried in a drying oven (at 60 °C) to a constant mass, and finally, the samples were weighed: rhizome--roots 1.0134 g, stem-leaves 1.0515 g, fruit 1.2661 g, and calyx 1.0169 g of average dry mass, performed in triplicate, $n = 3$. Digestion of samples was realized according to slightly modified procedure of Mosetlha *et al.*[15] Each sample was mineralized in an Erlenmeyer flask with 20 mL of concentrated HNO_3. The samples were covered with a watch glass and left overnight. The following day, the digests were evaporated, and then diluted with 0.5 % HNO_3 (in ultra-pure deionized water, 0.05 μs cm^{-1}) up to the volume of 25 mL, followed by filtration (grade 589/3 blue ribbon). The analysis was performed using an iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK), which uses the Echelle optical design and a charge injection device solid state detector. The nebu-

lizer was glass concentric. iTEVA software from Thermo Scientific (Cambridge, UK) was used to collect and analyze the data.[16] Multi-element standard solution IV of the microelements Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V and Zn, standard solution III of the macroelements Ca, K, Mg and Na, as well as individual standard solutions of Si, P and Hg (TraceCERT, Fluka Analytical, Switzerland) were used for calibration. Linearity in checked intervals was satisfied with a coefficient of determination above 0.9994. All measurements were performed in triplicate. Parameters of conducted ICP-OES analysis based on a calibration curve: wavelength of selected emission lines, linearity of the calibration curves, coefficient of determination (R^2), limit of detection (*LOD*) and limit of quantification (*LOQ*) of the calibration for each element determination are given in Table S-I of the Supplementary material. The *LOD* and *LOQ* values were calculated using the 3σ and 10σ criterion, respectively.[17]

Statistical processing data

Statistical data processing was performed by Statistica 8 software (Statsoft, Inc., Tulsa, OK, USA). Two statistical matrices included the content of macro- and microelements as original variables. Agglomerative Hierarchical Clustering (AHC, using the Ward's method and Euclidean distance) was conducted to visualize how the contents of macro- and microelements affect differentiation among studied samples (plant parts).

RESULTS AND DISCUSSION

The results obtained on the elemental composition of different parts of cultivated *P. alkekengi* are presented in Table I – macroelements: Ca, K, Mg, P, Na; and Table II – microelements: Al, B, Ba, Be, Co, Cr, Cu, Cd, Fe, Mn, Ni, Pb, Se, Si, V, Zn.

TABLE I. The content ($c \pm SD^a$ / mg g^{-1}; mean values of element content (all measurements were performed in triplicate, $n = 3$); *SD* – standard deviation) on macroelements in the studied samples of cultivated *P. alkekengi*; samples: R – rhizome-roots, SL – stem-leaves, C – calyx, F – fruit

Element	Sample			
	R	SL	F	C
Ca	3.28±0.02	**14.51**±0.05	0.503±0.002	4.20±0.04
K	13.48±0.08	34.81±0.04	12.66±0.03	**38.5**±0.3
Mg	1.59±0.09	**3.11**±0.05	1.361±0.004	1.568±0.003
P	1.65±0.010	2.98±0.02	3.32±0.02	**4.15**±0.02
Na	0.0018±0.0001	0.0018±0.0001	0.0015±0.0001	**0.0114**±0.0001

Among all measured macroelements, K was the most abundant in all samples (Table I): C (calyx) > SL (stem-leaves) >> R (rhizome-roots) ≈ F (fruit). Previously, Aguilar-Carpio *et al.*[18] determined the growth dynamics and yield of Cape gooseberry cultivation by varying the concentrations of the nutrient solution under greenhouse conditions. They claimed K^+ could be absorbed to the greatest extent by plants, thus, adequate nutrition enriched with this element is associated with increases in fruit yield and quality. Right after K, Ca was the next abundantly present element in the studied samples, but with the highest content

in the SL samples. Considering other macroelements, Mg dominates in the SL samples, while P in the C samples. The conducted statistical (AHC) analysis segregates plant parts according to the content of macroelements (Fig. 2a). In the cluster, two branches are distinguished, R and F samples on one side, and SL and C on the other side. Regarding the microelements, Al and Fe are the two elements that obviously dominate in the group, with similar contents among the studied samples (Table I): SL >> R > C >> F. The AHC singled out three entities: 1) SL; 2) F; 3) R and C (Fig. 2b).

TABLE II. The content ($c \pm SD^a$ / mg g^{-1}; mean values of element content (all measurements were performed in triplicate, $n = 3$); *SD* – standard deviation)on microelements in the studied samples of cultivated *P. alkekengi*; samples: R – rhizome-roots, SL – stem-leaves, C – calyx, F – fruit; *SD* – standard deviation; ND – not detected; *LOQ* – the limit of quantification

Element	Sample			
	R	SL	F	C
Al	424±4	**899**±11	7.60±0.04	255±1
B	12.7±0.2	23.8±0.2	11.4±0.1	**32.6**±0.4
Ba	8.54±0.07	**17.8**±0.2	0.948±0.002	6.57±0.04
Be	0.0148±0.0000	**0.0357**±0.0000	ND[b]	0.0049±0.0000
Co	0.301±0.007	**0.51**±0.01	0.079±0.002	0.202±0.003
Cr	**11.12**±0.06	10.73±0.01	7.94±0.01	10.93±0.08
Cu	4.56±0.06	8.01±0.09	8.7±0.1	**19.7**±0.2
Cd	0.046±0.002	**0.058**±0.002	<*LOQ*[c]	0.027±0.000
Fe	406±3	**741**±6	10.36±0.06	240±1
Mn	21.3±0.2	**42.7**±0.3	11.54±0.03	18.2±0.1
Ni	**7.66**±0.08	6.89±0.07	5.99±0.03	6.98±0.03
Pb	**1.37**±0.02	1.31±0.05	0.99±0.02	1.19±0.04
Se	**1.72**±0.08	1.37±0.02	1.16±0.03	1.36±0.05
Si	17±1	26±2	24±1	**34**±1
V	4.82±0.05	**12.3**±0.1	3.26±0.03	4.58±0.02
Zn	19.39±0.09	20.73±0.06	17.62±0.04	**25.4**±0.1

As above mentioned, there is no data on the elemental composition of *P. alkekengi*, except for the study on rhizome by Xu *et al*, but with different aims.[14] Concerned by the fact that the continuous growth of the plant is strongly endangered by changing the soil elemental composition, leading to the succession cropping obstacle, they tried to find answers in the variation of elemental content between plant rhizome and the surrounding soil. By conducting ICP-MS analysis on the annual, biennial, and triennial *P. alkekengi* (healthy and rotten) rhizomes, they observed variation in trace elements with the time and state of rhizomes: reduction of Mg, K, Ca, and enrichment of V, Fe, Co, Se and Pb. Surely, most studies have focused on the study of the most famous species *P. peruviana*, especially studies on the fruit. Erkaya *et al.* considered Cape gooseberry as a good natural source of nutritive ingredients in ice cream production.[19]

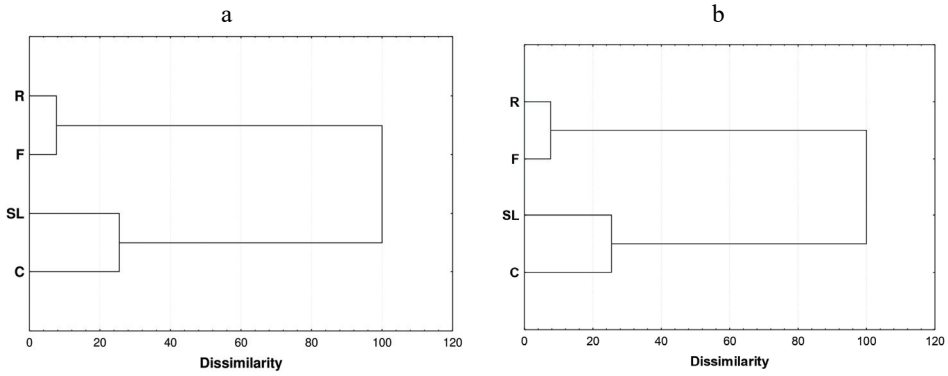

Fig 2. Results of AHC analysis conducted on two patterns: a) macroelements contents and b) microelemens contents of studied *P. alkekengi* samples (R – rhizome-roots, SL – stem-leaves, C – calyx, and F – fruit).

They determined content of selected macro- and micro-elements in the fruit Ca, K, Mg, P, S, Na, Fe, Zn, Mn, and Ni. If comparing that elemental composition of Cape gooseberry[16] with the results of this study, the fruit of *P. alkekengi* contained higher amounts of K, P, Mg, Ni, Mn, while the contents of Ca, Fe and Zn were lower than published. In the study of Karasakal different microwave acid digestion procedures were tested to determine concentrations of Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Zn, P in many tropical fruits.[20] In case of the *P. peruviana* fruit, most of tested systems for digestion gave the following order for the contents were K >> Mg > S > Na > Ca > Zn > Mn > Fe, which largely agrees with the present results obtained for the *P. alkekengi* fruit, as follows: K >> P > > Na > Mg > Ca >>> Zn > Mn > Fe. Furthermore, the results of El Sheikha *et al.*[21] indicate the highest content of K, followed by P in the fruit juice of *P. pubescens*. A Study of *P. angulata*, grown under normal conditions and treated by Al in the nutrient solution, showed different patterns of elemental composition caused by stress.[22] Obviously, the root of *P. angulata* accumulates the highest levels of Al. Moreover, the certain condition of stress (0.16 M of Al) increased the content of P in the stems and roots; K, Cu, and Mo in all parts of the plants; and reduced the content of Ca, Mg, Fe, and Zn in the tested *P. angulata* plants. Since the contents of Al are high in SL, R, and C samples, as follows, the question is whether it affects the content of other macro- and micro-elements. Surely, by varying the cultivation conditions, the correlation between Al content with other elements could be determined. Karasakal also found a high content of Al in the fruit of Cape gooseberry.[20] High doses of toxic Al could cause damage to the human nervous system and the advised limit value for Al intake is 24 μg g^{-1} 60 kg body weight.[20] If these parameters are considered, an adult person could eat approx. 3 g (dry matter) of *P. alkekengi* fruits, which suggests that concentrations of Al are a kind of acceptable level for human health. On the contrary, other parts

of the plant contain Al in a very high concentration. Overall, considering the allowed intake of dry fruit (calculated *via* the Al content), other nutritional macro- and microelements concentrations are below the recommended daily allowance levels. The most common toxic heavy metals include As, Pb, Cd and Hg. The FAO/WHO (2007)[23] prescribes limits of these toxic heavy metals in raw herbs. The permissible limit for Pb in herbs is 10 and 0.3 mg kg^{-1} for Cd. The content of Pb and Cd is quite lower, especially in the fruit sample where the content of Cd falls under the limit of quantification. In all studied samples As, Hg, and Tl were below the limit of detection.

CONCLUSIONS

According to data obtained, there are differences in the elemental composition of the studied parts of the cultivated *Physalis alkekengi*. K, Ca, Fe and Al were the most abundant elements, but with different distribution in the studied samples. The conducted statistical analysis showed different grouping of samples depending on the contents of macro- and micro-elements. Respecting similarities from AHC analysis on the macro-elements pattern, the rhizome-roots sample corresponds to the fruit sample, while the stem-leaves to the calyx. On the other hand, the distribution pattern of microelements led to segregation of the rhizome--roots sample and calyx sample. Respecting the nutritional aspect, the edible fruits did not contain potentially toxic elements (Pb, Cd, Al) in concentration higher than the permissible limits, while the content of useful elements is not negligible. Furthermore, the contents of Pb and Al were the lowest in the fruit sample, while Cd was present at a value under the *LOQ*. In all studied samples, As, Hg, and Tl were below the limit of the method detection. By improving the growing conditions, primarily to reduce the Al content, the content of the macro- and micro-elements could be influenced. Consequently, by increasing the daily intake of the fruits, concentrations of useful elements could reach up to the recommended daily allowance levels. Thus, *P. alkekengi* presents a useful crop and should be studied from multiple aspects.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/10947, or from the corresponding author on request.

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ИЗВОД

УПОРЕДНА ЕЛЕМЕНТНА АНАЛИЗА РАЗЛИЧИТИХ ДЕЛОВА ГАЈЕНЕ БИЉНЕ ВРСТЕ *Physalis alkekengi* (SOLANACEAE)

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У овом раду први пут је испитиван елементни састав различитих делова гајене биљне врсте *Physalis alkekengi* (љоскавац): ризома са кореном, стабла са лишћем, плода и каликса. Садржај двадесет и једног макро- и микроелемента одређен је методом оптичке емисионе спектрометрије са индуктивно куплованом плазмом (ICP-OES). На матрицама са садржајима испитиваних елемената спроведена је кластер анализа, на основу које је утврђено различито груписање узорака према садржају микро- и макроелемената. Генерално, K, Ca, Fe и Al јесу најзаступљенији елементи, али са различитим обрасцем дистрибуције у испитиваним узорцима. Висок садржај Fe и Al нађен је у узорку стабла са лишћем, затим у узорку ризома са кореном и, напослетку, у узоку каликса. Највећи садржај K нађен је у узорку каликса, потом у узорку стабла са лишћем. Потенцијално токсични метали нису детектовани у плоду у концентрацији већој од прописане, већ је нађен најнижи садржај Pb и Al, док је садржај Cd испод квантификационог лимита методе. У свим испитиваним узорцима садржај за As, Hg и Tl је испод лимита детекције за дату методу.

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SUPPLEMENTARY MATERIAL TO

Comparative study on the elemental composition of different parts of cultivated *Physalis alkekengi* (Solanaceae)

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TABLE S-I. ICP-OES parameters: wavelengths (λ) of the analytical lines, coefficient of determination (R^2), linearity of the calibration curves, the limit of detection and quantification (*LOD* and *LOQ*) of the calibration curve for each element

Element	λ / nm	Plasma view mode	R^2	*LOD*, μg g^{-1}	*LOQ*, μg g^{-1}	Linear range of the calibration curve, μg g^{-1}
Al	396.152	Axial	0.9995	0.0323	0.1076	0-100
As	189.042	Axial	0.99995	0.0612	0.2040	0-5
B	249.773	Axial	0.9999	0.0199	0.0663	0-25
Ba	493.409	Axial	1	0.0015	0.0049	0-25
Be	234.861	Axial	1	0.0014	0.0048	0-25
Ca	393.366	Radial	0.9999	0.0034	0.0115	0-100
Cd	226.502	Axial	1	0.0039	0.0129	0-5
Co	228.616	Axial	1	0.0063	0.0209	0-5
Cr	283.563	Axial	0.9999	0.0139	0.0463	0-5
Cu	324.754	Axial	1	0.0149	0.0489	0-50
Fe	259.940	Axial	1	0.0100	0.0331	0-50
Hg	184.950	Axial	1	0.0274	0.0913	0-5
K	766.490	Radial	0.9994	1.0622	3.5406	0-100
Mg	279.553	Radial	0.9999	0.0065	0.0217	0-100
Mn	257.610	Axial	1	0.0022	0.0075	0-50
Na	588.995	Radial	0,9998	0.0127	0.0425	0-100
Ni	221.647	Axial	1	0.0083	0.0275	0-50
P	213.618	Radial	0.9999	0.1090	0.3632	0-100
Pb	220.353	Axial	0.9999	0.0422	0.1407	0-5
Se	203.985	Axial	0.9999	0.1978	0.6595	0-50

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Si	251.611	Axial	1	0.0373	0.1243	0-100
Tl	276.787	Axial	0.9996	1.1221	3.7404	0-50
V	309.311	Axial	0.9999	0.0073	0.0243	0-25
Zn	213.856	Axial	0.9999	0.0026	0.0086	0-50

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Five wild-growing *Artemisia* (Asteraceae) species from Serbia and Montenegro: Essential oil composition and its chemophenetic significance

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Abstract: In this work, the essential oils (EOs) obtained by hydrodistillation from the aerial parts of five *Artemisia* species*: A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam., *A. vulgaris* L., originating from Serbia and *A. umbelliformis* Lam. subsp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas, originating from Montenegro were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). In total, 91 compounds were detected, and 78 were identified. Even though a high number of compounds were detected, each sample had only 18 to 35, attesting to a great diversity of compounds within these taxa. Depending on the species and the locality (geographical origin), the EO was dominated by either monoterpenes or sesquiterpenes, with artemisia ketone, 1,8-cineole (eucalyptol), fragranol, *α*-thujone, *β*-thujone and myrcene being the dominant compounds. The obtained results were coupled with extensive literature data and used in multivariate chemometric approach to assess the chemophenetic significance of the EO.

Keywords: hydrodestilation; GC/MS; chemometrics.

INTRODUCTION

Artemisia L. (Artemisiinae, Anthemidae, Asteraceae) is a large genus that contains nearly 500 mostly perennial taxa. However, there is still no universal agreement on the number of taxa within the genus.[1] *Artemisia* taxa mainly grow in different ecosystems of the northern hemisphere in Asia, Europe, and North America[2], while a broad area of Central Asia is the center of their diversity.[3]

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Artemisia taxa show large variability in morphological and phytochemical characters.[4] The systematics and nomenclature of the genus is complex, and it is a challenge for taxonomists. Although there are some conflicts between classical and molecular datasets, *Artemisia* has been traditionally divided into five subgenera: *Artemisia*, *Absinthium* (Miller) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser ex Less. and *Tridentatae* (Rydb.) McArthur[5], to which one more, *Pacifica* Hobbs & Baldwin, has been added.[6]

Literature data about the phytochemistry of different *Artemisia* species showed large structural diversity of specialized metabolites. These plants are very aromatic, with a characteristic pungent smell.[7] The specific aromatic odor is a consequence of a high quantity of volatile terpenes, primarily present in the flowers and leaves.[8] Monoterpenes and sesquiterpenes are dominant compounds in the essential oil (EO) of many species.[9–11]

Plant metabolic profile is genetically determined, thus similarity in metabolite content is applicable in assessing the phylogenetic relationship of higher plants.[12] It was shown that plant metabolites (*e.g.*, EOs) could be helpful in assessing the taxonomic relationship among some *Artemisia* taxa[8]. In this regard, continuing chemophenetic studies (description of the diversity of specialized metabolites in any given plant taxon)[13] contribute to the phenetic description of its taxa, and, in combination with other tools (*e.g.*, morphology, anatomy, molecular methods), could help in establishing natural classification of the genus *Artemisia*.

The objectives of the present study were to investigate and determine composition of the EOs of five wild-growing *Artemisia* species: four species from Serbia (*A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam. and *A. vulgaris* L.), and one species from Montenegro (*A. umbelliformis* Lam. subsp. *Eriantha* (Ten.) Vallès-Xirau & Oliva Brañas); and to evaluate their significance in chemophenetics using a chemometric multivariate approach.

EXPERIMENTAL

Plant material

Plant material (aerial parts) of four species from Serbia and one species from Montenegro was collected during the growing season (Table S-I of the Supplementary material to this paper). The collected plant material in full bloom was identified using floras of Serbia and Europe.[14,15] Voucher specimens were deposited at the Herbarium (BEOU) of the University of Belgrade – Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac." Standard herbarium acronym follows Index herbariorum.[16]

Isolation of EO

The aerial parts of each plant species were dried at room temperature and then chopped. Between 26 and 340 g of plant material was placed in a round-bottomed flask and 800–2000 mL of distilled water was added. Hydrodistillation was performed for 3 h using a Clevenger-type apparatus, according to the procedure described in Ph. Eur. 6.[16] The obtained oils were

stored at 4 °C before GC analysis. The extraction yield of oil was calculated according to an earlier described equation.[8] For GC analysis, 10 μL of crude essential oil was dissolved in 1 mL of dichloromethane.

GC-FID and GC/MS analyses

The GC-FID and GC/MS analyses were performed with an Agilent 7890 A apparatus equipped with a 5975 C mass-selective detector (MSD), a flame ionization detector (FID), and an HP-5 MSI fused-silica cap (column length 30 m, diameter 0.25 mm, film thickness 0.25 mm). The oven temperature was programmed linearly, rising from 60 to 240° at 3° min^{-1}; the injector temperature was 220°; the detector temperature was 300°, and the transfer-line temperature was 240°. The carrier gas was He (1.0 mL min^{-1} at 210°, constant pressure mode) with an injection volume of 1 μL and a split ratio of 10:1. Electron impact mass spectra (EI-MS; 70 eV) were acquired over the *m*/*z* range 40–550. Library search and mass spectral deconvolution and extraction were performed using the NIST AMDIS (automated mass spectral deconvolution and identification system) software, version 2.64.113.71, with the retention index (*RI*) calibration data analysis parameters set to the strong level and a 10 % penalty for compounds without an *RI*. The *RI*s were experimentally determined using the standard method involving retention times (t_R) of *n*-alkanes injected after the EO under the same chromatographic conditions. The search was performed against our homemade library, containing 4972 spectra. The relative contents of identified compounds were computed from the GC peak areas.

Statistical analysis

Statistical analysis was performed on 3875 numerical data. Standard statistics (mean, standard deviation, distribution) were used to study the data prior to Discriminant Analysis (DA). All statistical analyses were performed using PAST 4.06b.[17]

RESULTS AND DISCUSSION

Artemisia *EO composition and yield*

The yield and organoleptic characteristics of the EOs of the studied *Artemisia* species are given in Table I.

TABLE I. Yield and organoleptic characteristics for essential oils of the investigated *Artemisia* species

Sample	*m* / g		Yield wt. %	Organoleptic characteristics
	Dry plant material	Obtained oil		
A. alba	31.8	0.0138	0.043	Transparent yellow, mild herbaceous sweet odor
A. pontica	32.2	0.0816	0.253	Transparent yellow, strong sharp bitter odor
A. scoparia	140.0	0.3181	0.227	Transparent yellow, strong, sour greasy odor
A. umbelliformis subsp. *eriantha*	26.6	0.1493	0.561	Transparent yellow, strong herbaceous sharp odor
A. vulgaris	340.0	0.1363	0.040	Bright yellow, strong unpleasant moldy odor

The conducted GC-FID and GC/MS analyses resulted in the detection of 91 compounds, making on average 96.7% of the total oil. All compounds are listed in Table II.

TABLE II. Chemical constituents of the essential oils of investigated *Artemisia* species; *RI*, retention indices relative to *n*-alkanes on HP-5 MS; %, Relative percentage obtained from the peak area; SO, sesquiterpene oxygenated; #, tentatively identified; Ni, not identified, Ni_1, *m/z* 41, 55, 70, 83, 97, Ni_2, *m/z* 81, 43, 109, 71 53, Ni_3, *m/z* 81, 41, 69, 135, 107, Ni_4, *m/z* 95, 147, 162, Ni_5, *m/z* 68, 43, 93, 108, 121, Ni_6, *m/z* 91, 43, 93, 119, 134, Ni_7, *m/z* 71, 43, 107, 93, 79, Ni_8, *m/z* 123, 71, 107, 81, 41, Ni_9 SO, *m/z* 157, 143, 218, 129, 91, Ni_{10} SO, *m/z* 216, 178, 159, 147, 95, Ni_{11}, *m/z* 109, 148, 175, 43, 193, Ni_{12} SO, *m/z* 217, 232, 171, 91, 105, Ni_{13} SO 214, 156, 115, 55, 171

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformis* subsp. *eriantha*	*A. vulgaris*
1	865	(*Z*)-Salvene	–	–	–	0.4	–
2	903	Santolina Triene	–	–	–	–	0.3
3	922	α-Thujene	–	–	–	0.2	0.5
4	929	α-Pinene	–	–	0.7	–	0.5
5	944	Camphene	–	–	0.1	–	–
6	945	α-Fenchene	–	–	–	0.4	–
7	969	Sabinene	–	–	0.1	0.2	6.2
8	973	Artemiseole	0.3	–	–	–	–
9	973	β-Pinene	–	0.4	0.1	0.4	0.7
10	987	Myrcene	–	–	–	–	22.0
11	999	Yomogi alcohol	2.0	–	–	–	–
12	1015	α-Terpinene	–	–	–	–	0.4
13	1024	*p*-Cymene	0.8	1.3	0.9	0.2	1.3
14	1032	1,8-Cineole	12.2	58.2	57.2	0.3	6.2
15	1058	γ-Terpinene	–	0.4	0.1	0.1	–
16	1060	Artemisia ketone	45.3	–	–	–	17.6
17	1066	*cis*- Sabinene hydrate	–	0.2	–	0.2	–
18	1084	Artemisia alcohol	2.3	–	–	–	0.4
19	1094	Ni_1	–	–	–	0.2	–
20	1095	6,7-Epoxymyrcene	–	–	–	–	0.8
21	1101	*cis*-Sabinene hydrate (*cis* for IPP vs OH)	–	0.2	–	–	–
22	1107	Ni_2	0.7	–	–	–	–
23	1108	β-Thujone	–	6.8	34.5	73.7	–
24	1117	α-Thujone	–	0.7	3.8	15.8	–
25	1122	*cis*-*p*-Menth-2-en-1-ol	–	0.2	0.2	–	–
26	1130	Ni_3	7.7	–	–	–	–
27	1138	*trans*-Pinocarveol	–	0.2	0.4	–	–
28	1139	*iso*-3-Thujanol	–	–	–	0.1	–
29	1139	Monoterpenol	0.7	–	–	–	–
30	1139	*trans*-Sabinol	–	–	–	0.4	–
31	1143	*trans*-Verbenol	–	0.4	0.2	0.1	–

TABLE II. Continued

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformis* subsp. *eriantha*	*A. vulgaris*
32	1148	Camphor	3.7	–	–	–	–
33	1149	*neo*-3-Thujanol	–	–	–	0.1	–
34	1155	Sabina ketone	–	–	–	0.2	–
35	1155	Isoborneol	–	–	0.1	–	–
36	1162	Pinocarvone	–	–	0.3	0.1	–
37	1165	*δ*-Terpineol	–	0.4	–	–	–
38	1166	Borneol	0.7	–	0.2	–	–
39	1171	Artemisyl acetate	0.4	–	–	–	–
40	1175	Terpinen-4-ol	1.0	1.0	0.3	0.3	1.3
41	1182	*cis*-3-Hexenyl butyrate	0.6	–	–	–	–
42	1193	Myrtenal	–	0.3	0.2	–	–
43	1197	Myrtenol	–	–	–	0.4	–
44	1202	*γ*-Terpineol	–	–	–	0.1	–
45	1213	Fragranol	–	14.7	–	–	–
46	1277	Ni_4	–	0.4	–	–	–
47	1291	*trans*-Sabinyl acetate	–	–	–	0.1	–
48	1337	*δ*-Elemene	–	–	–	0.1	–
49	1342	Ni_5	–	4.1	–	–	–
50	1375	*α*-Copaene	–	–	–	–	0.5
51	1384	*β*-Bourbonene	–	0.7	–	–	0.7
52	1391	*β*-Elemene	–	–	–	–	2.2
53	1414	Ni_6	–	–	–	0.2	–
54	1415	*trans*-*α*-Bergamotene	–	–	–	–	0.6
55	1419	(*E*)-Caryophyllene	–	–	–	–	3.1
56	1435	*cis*-α-Bergamotene	–	–	–	–	0.4
57	1453	*α*-Humulene	–	–	–	–	1.3
58	1456	(*Z*)-β-Farnesene	–	–	–	–	0.8
59	1460	Cabreuva oxide B	–	0.5	–	–	–
60	1477	Cabreuva oxide D	–	0.3	–	–	–
61	1476	*γ*- Muurolene	–	–	–	0.1	0.6
62	1481	Germacrene D	–	–	–	–	5.0
63	1484	*β*-Selinene	–	–	–	–	9.9
64	1495	*α*-Selinene	–	0.4	–	–	1.2
65	1496	Bicyclogermacrene	–	–	–	–	1.0
66	1523	*δ*-Cadinene	–	–	–	–	0.7
67	1553	$C_{15}H_{24}$ isomer#	–	–	–	–	0.6
68	1555	Ni_7	–	0.8	–	–	–
69	1563	(*Z*)-Nerolidol	–	0.7	–	–	–
70	1576	Spathulenol	–	1.1	–	–	3.5
71	1581	Caryophyllene oxide	–	0.6	0.9	–	4.6
72	1593	Ni_8	–	0.2	–	–	–
73	1608	Humulene epoxide II	–	–	–	–	1.3
74	1610	$C_{15}H_{24}O$ isomer#	–	–	–	–	0.7
75	1623	$C_{15}H_{22}O$ isomer#	0.8	–	–	–	–

TABLE II. Continued

No.	*RI*	Compound	Content, %				
			A. alba	*A. pontica*	*A. scoparia*	*A. umbelliformi* subsp. *eriantha*	*A. vulgaris*
76	1628	1-*epi*-Cubenol	–	–	–	0.1	–
77	1654	Pogostol	–	–	–	–	1.0
78	1670	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	–	–	–	–	0.8
79	1672	Valeranone	–	–	–	0.1	–
80	1675	Cadalene	0.3	–	–	–	–
81	1684	α-Bisabolol	1.0	–	–	–	–
82	1685	Germacra-4(15),5,10(14)-trien--1-α-ol	0.8	–	–	–	0.8
83	1691	Solavetivone	7.9	–	–	–	–
84	1713	Ni_9 SO	0.5				
85	1720	(8*S*,8*aS*)-3,8-Dimethyl-4-propan--2-ylidene-1,2,6,7,8,8*a*-hexahydroazulen5-one	1.1	–	–	–	–
86	1749	Cyclocolorenone	0.9	–	–	–	–
87	1788	Ni_{10} SO	0.4	–	–	–	–
88	1847	6,10,14-trimethyl-2-pentadecanone	0.3	–	–	–	–
89	1849	Ni_{11}	0.7	–	–	–	–
90	1945	Ni_{12} SO	–	–	–	1.4	–
91	1954	Ni_{13} SO	–	–	–	0.4	–
		Total monoterpenes	**69.2**	**85.2**	**98.9**	**93.7**	**58.3**
		Monoterpene hydrocarbons	3.0	1.9	1.8	1.9	31.9
		Monoterpenes oxygenated	66.1	83.2	97.0	91.7	26.4
		Total sesquiterpenes	**13.7**	**8.3**	**0.9**	**2.1**	**41.3**
		Sesquiterpene hydrocarbons	0.0	5.1	0.0	0.1	28.6
		Sesquiterpene oxygenated	13.7	3.2	0.9	1.9	12.6
		Other	0.9	0.0	0.0	0.0	0.0
		Unknown	9.0	1.3	0.0	0.4	0.0
		Total	**92.9**	**94.9**	**99.8**	**96.2**	**99.6**
		Total (No. of identified compounds)	**25**	**26**	**18**	**29**	**35**

Depending on the species and locality, different groups of volatile terpenes were present in the EOs. The results showed that monoterpenes are the dominant constituents in the EOs of all investigated species. The EOs of *Artemisia scoparia*, *A. umbelliformis* subsp. *eriantha* and *A. pontica* were strongly dominated by oxygenated monoterpenes (97.1, 91.7, and 83.2 %, respectively). In *A. vulgaris*, however, monoterpene hydrocarbons were found in a similar amount as oxygenated monoterpenes (31.9 and 26.4%, respectively).

Additionally, *A. vulgaris* was the only analyzed species with a high abundance of sesquiterpenes, with sesquiterpene hydrocarbons representing the greatest part (28.64 %). In the other species, sesquiterpenes were moderately present in the EOs,

and oxygenated sesquiterpenes were more common: *A. alba* – 13.8 %, and *A. vulgaris* – 12.7 %, the exception being *A. scoparia,* which had less than 1 % of sesquiterpenes present. The most dominant compound of *A. alba* was artemisia ketone (45.317 %), followed by 1,8-cineole (12.174 %), while in the oil of *A. pontica*, the most prevalent was 1,8-cineole (58.2 %), followed by fragranol (14.7 %). The EO of *A. scoparia* was characterized by an extremely high percentage of 1,8-cineole (57.2 %), followed by *β*-thujone (34.5%). In contrast, the EO of *A. umbelliformis subsp. eriantha* was characterized by *β*-thujone (73.7 %) and *α*-thujone (15.8 %). Myrcene (22.0 %) and artemisia ketone (17.6 %) were the most dominant constituents in the EO of *A. vulgaris*. The present data are in concordance with literature data.[1,8] Based on the obtained results, the EOs of *Artemisia* species can be put into two groups – those highly dominated with a single compound and those with two to three co-dominant compounds. All EOs of the studied species belong to the formergroup, except for *A. vulgaris* that belongs to the latter.

Chemophenetics of Artemisia *based on EO*

To check the chemophenetic significance, discriminant analysis, including 120 literature data,[8,18–24] was performed. Only compounds present on average in mid-to-high amounts (above 0.5%) of the essential oil profile were used. Species represented with only one or two data points were not assigned to a group but marked for the discriminant linear classifier and placed on the scatter plot after discriminant analysis (marked with an asterisk), Fig. 1. Three components were

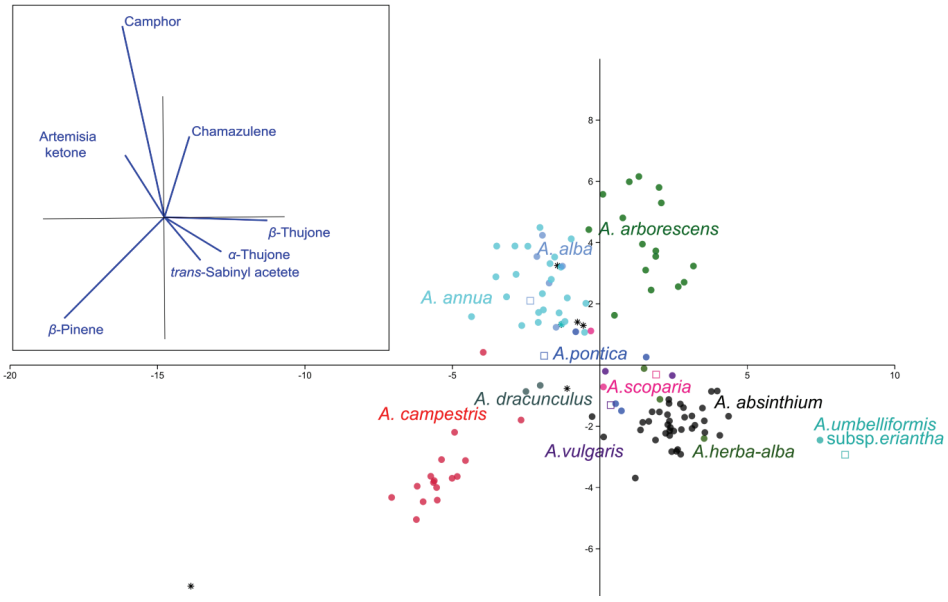

Fig. 1. Discriminant analysis (DA) scatter plot: ● – literature data, □ – present data, * –plotted after DA according to the results of discriminant linear classifier.

responsible for most of the separation species – camphor, *β*-pinene, and *β*-thujone. An additional four compounds contributed the most to the separation (artemisia ketone, chamazulene, *α*-thujone, and *trans*-sabinyl acetate). The present samples grouped according to the species and not the locality or the phenophase, with the exception of *A. pontica* that showed more similarity with *A. annua* and *A. absinthium* essential oil than with other *A. pontica* samples, though only several data points were available for this species, so that it can be an artifact caused by a low number of samples in the discriminant analysis.

CONCLUSIONS

The results are in agreement with a previous detailed chemometric analysis of *Artemisia* EO, where the variability detected in the EO composition of different taxa, attributed to both genetical and environmental factors, correspond to evolutionary trends and molecular data. The present results, although obtained from a rather limited number of investigated taxa, indicate that the EO composition could be a useful chemophenetic character within this genus.

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ИЗВОД

ПЕТ САМОНИКЛИХ ВРСТА *Artemisia* (ASTERACEAE) ИЗ СРБИЈЕ И ЦРНЕ ГОРЕ: САСТАВ ЕТАРСКОГ УЉА И ЊЕГОВ ХЕМОФЕНЕТИЧКИ ЗНАЧАЈ

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У овом раду анализирана су етарска уља добијена хидродестилацијом из надземних делова пет врста рода *Artemisia*: *A. alba* Turra, *A. pontica* L., *A. scoparia* Waldst. & Kitam., *A. vulgaris* L., из Србије и *A. umbelliformis* Lam. subsp. *eriantha* (Ten.) Vallès-Xirau & Oliva Brañas, пореклом из Црне Горе, коришћењем гасне хроматографије комбиноване са масеном спектрометријом (GC/MS). Укупно је детектовано 91 једињење, од чега је 78 идентификовано. Иако је укупно детектован велики број једињења, у сваком узорку је детектовано између 18 и 35 једињења, што сведочи о великом хемијском диверзитету етаских уља испитиваних таксона. У зависности од врсте и локалитета (географског порекла) етарским уљима су доминирали монотерпени или сесквитерпени, где су артемизија кетон, 1,8-цинеол (еукалиптол), фрагранол, *цис*-тујон, *транс*-тујон и мирцен били доминантна једињења. Добијени резултати су упарени са литературним и искоришћени у мултиваријантном хемометријском приступу како би се проценио хемофенетички значај етарског уља.

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SUPPLEMENTARY MATERIAL TO

Five wild-growing *Artemisia* (Asteraceae) species from Serbia and Montenegro: Essential oil composition and its chemophenetic significance

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TABLE S-I. Investigated *Artemisia* species from Serbia and Montenegro

Species	Country	Locality	Coordinates	Month	Year	BEOU
A. alba	Serbia	Mt. Rtanj	N 43. 44' 23.9" E 21 51' 49.6"	June	2018	17457
A. pontica	Serbia	Zaječar	N 43. 55' 24.4" E 22 17' 46.17"	June	2018	17453
A. scoparia	Serbia	Grocka	N 44° 39' 31" E 20° 43' 27"	July	2020	17760
A. umbelliformis subsp. *eriantha*	Montenegro	Mt. Durmitor (Zeleni Vir)	N 43 07' 8.06" E 19 02' 21.73"	August	2018	17458
A. vulgaris	Serbia	Opovo, Sakule	N 45° 08' 38" E 20° 28' 06"	July	2020	17761

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Chemical composition and biological properties of *Pelargonium graveolens*, *Leptospermum petersonii* and *Cymbopogon martinii* var. *motia* essential oils and of *Rosa centifolia* absolute

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Abstract: Chemical composition of the essential oils (EO) of *Pelargonium graveolens*, *Leptospermum petersonii* and *Cymbopogon martinii* var. *motia*, and the absolute of *Rosa centifolia* and their bioactivity were examined. Major compounds in *P. graveolens* EO were monoterpene alcohols citronellol, geraniol and linalool; in *L. petersonii* EO monoterpene aldehydes geranial, neral and citronellal; in *C. martiniii* var. *motia* EO monoterpene alcohol geraniol and ester geranyl acetate, while in absolute of *R. centifolia* aromatic alcohol 2-phenylethanol. The EO of *L. petersonii* showed the strongest antibacterial while the EO of *C. martinii* var. *motia* the strongest antifungal potential. The best biofilm inhibition capacity was observed with *R. centifolia* absolute. The results of scanning electron microscopy analysis indicated that the EOs of *L. petersonii* and *P. graveolens* changed the number and morphology of *C. albicans* cells. The *L. petersonii* EO was the most potent toward tumour cells and exhibited the best biological activity. This is first comparative report summarizing efficacy of studied aromatic samples against pathogenic microbes, providing deeper insight into the modes of antimicrobial action, and at the same time describing their cytotoxicity against cell lines.

Keywords: volatiles; antibacterial effect; anticandidal activity; cytotoxic; antibiofilm; SEM.

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INTRODUCTION

In addition to the fact that synthetic drugs have numerous side effects, the increase in resistance to commonly used antimicrobial agents seriously worries health professionals worldwide. Although the use of plants in treatments of various human health issues originates in the distant past,[1] the secondary metabolites they produce still attracts considerable attention of researchers in attempt to discover their safe application.[2] Aromatic plants are rich in compounds with terpene core (present in their essential oils), which exhibit a range of important biological properties, such as antibacterial, antifungal, antiviral, cytotoxic, anticancer, anti-inflammatory, *etc.*[3] A number of them appear promising for resolving some human health issues, as a vast of scientific studies already proved their strong potential against certain harmful microbes,[4–6] but also some other important activities, including antibiofilm,[7,8] antitumor,[9] antiquorum sensing[10,11] and antioxidant.[12–14] As the balance between free radicals and antioxidants is crucial for a proper physiological functioning of the human body, in addition to the fact that some commonly used synthetic antioxidants proved to be dangerous to humans, it is not surprising that the search for safe and effective natural antioxidants, particularly among the essential oils, has been in focus in the recent years.[15]

Consequently, the aim of this study was to determine chemical composition of three commercial essential oils (*Pelargonium graveolens*, *Cymbopogon martinii* var. *motia* and *Leptospermum petersonii*) and an absolute (*Rosa centifolia*), and estimate their antimicrobial, antioxidant, and to the authors' best knowledge, for the first time, their cytotoxic, antibiofilm and antiquorum sensing potential. The study was performed in an attempt to contribute to the industrial application of tested aromatic products as they could be promising for use in human health treatments.

EXPERIMENTAL

Origin of aromatic samples

Four products from the aromatic products collection of the Institute for Medicinal Plant Research "dr Josif Pančić" Belgrade, Serbia, were used in the study: the essential oil from the flowers of *Pelargonium graveolens* L'Hér., the essential oil from the leaves of *Leptospermum petersonii* F.M. Bailey, the essential oil from the aboveground plant parts of *Cymbopogon martinii* (Roxb.) Wats. var. *motia* Burk., and the absolute from the petals of *Rosa centifolia* L.

Chemical analysis of aromatic samples

The gas chromatography analysis was performed using GC Agilent Technologies 7890A apparatus equipped with the split–splitless injector and automatic liquid sampler, attached to HP-5 column, coupled with flame-ionisation detector, while the gas chromatography/mass spectrometry analysis was performed using HPG 1800 C Series II GCD analytical system equipped with HP-5MS column. Relative percentage of components in the samples was calculated from the peak areas of the area-percentage reports (as a result of standard processing of GC-FID chromatograms), without correction factors, using the normalization method. The preparation of aromatic samples, details on the used columns and the operating conditions for

both apparatuses, as well as the procedures for identification and quantification of individual constituents in studied aromatic samples are all explained in the previous study.[16]

Microorganisms

Following oral bacteria and fungi were included in this study: clinical isolates of *Streptococcus sanguis* (IBR S002 & IBR S003), *Streptococcus pyogenes* (IBR S004 & IBR S005), *Streptococcus mutans* (IBR S001), *Pseudomonas aeruginosa* (IBR P001), *Lactobacilus* sp. (IBR L002) and the *Staphylococcus aureus* ATCC 25923, as well as clinical isolates of *Candida* spp., and *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 750. The referent strains used in this study were purchased from American Type Culture Collection (ATCC), Manassas, VA, USA, while the clinical isolates were taken from the patients at the Department of Pediatric and Preventive Dentistry, Faculty of Dental Medicine, University of Belgrade, Serbia, and identified as previously reported.[17,18]

Antimicrobial activity

Minimum inhibitory concentration (*MIC*), and minimum bactericidal (*MBC*) or fungicidal concentrations (*MFC*) were determined by microdilution method as previously described.[19,20] As a positive control Hexoral® (Hemofarm, Serbia) was included.

Antibiofilm activity

The impact of selected aromatic samples on the course of biofilm formation by *S. mutans* and *C. albicans* was estimated by using crystal violet dye, as described previously.[21] The optical readings were performed in an automated Elisa reader at a wavelength of 570 nm. The results were presented as inhibition percentages. Three commercial medicaments were used as positive controls, antibiotics Streptomycin and Ampicillin and antifungal drug fluconazole (Sigma, USA).

Scanning electron microscopy (SEM) of pre-formed C. albicans *biofilm*

The SEM observations were conducted on *C. albicans* cells. The microbial cells were treated with hexamethyldisilazane (HMDS, Polyisience, Europe GmbH, Germany), placed on aluminium columns and covered with a layer of gold, and then viewed under SEM (JOEL JSM5300), as reported by Braga *et al*.[22]

Cytotoxicity against tumour and non-tumour cells

Cytotoxic activity of four aromatic products were investigated on five human tumour cell lines, as follows: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HCT-15 (colon carcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma). Sulforhodamine B assay was carried out as previously described.[23]

For hepatotoxicity evaluation in PLP2 cells, cell culture was prepared according to the previously established procedure.[24] As a positive control, Ellipticine was used, and the results were presented as GI_{50} values (sample concentration responsible for 50 % inhibition of the net cellular growth).

Statistical analysis

In all the assays, three replications of the samples were used and triplicates for each concentration reading were carried out. The results were expressed as mean values ± standard deviation (*SD*) and analysed as a one-way analysis of variance (ANOVA) using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA).

RESULTS AND DISCUSSION

Composition of aromatic samples

Comparative presentation of chemical compositions of EOs of *P. graveolens*, *L. petersonii*, *C. martinii* var. *motia* and *R. centifolia* absolute is given in Table I. In total, 43 different compounds were identified. Oxygenated monoterpenes represented the major portion in all EOs, with the highest content confirmed in *C. martinii* var. *motia* EO (98.48 %), while in *R. centifolia* absolute oxygenated monoterpenes also represented a great portion (34.7 %) but the major constituents belonged to a group of benzenoid/phenylpropenoid compounds (60.09 %).

TABLE I. Results of chemical analysis (Content, %) of commercial aromatics samples used in the current study

RI	EO constituent	Essential oils			Absolute
		Microorganism			
		P. graveolens	*L. petersonii*	*C. martini* var. *motia*	*R. centifolia*
925	α-Pinene	0.3	0.3		
984	Dehydro-1,8-Cineol		0.1		
1067	*cis*-Linalool oxide	0.2			
1094	Linalool	11.4	2.3	2.1	
1104	*cis*-Rose oxide	1.0			
1111	Phenyl ethyl alcohol				57.7
1120	*trans*-Rose oxide	0.4			
1139	*trans*-Verbenol		4.8		
1145	Menthone	3.9			
1147	Citronellal		21.1		
1156	*iso*-Menthone	2.7			
1186	α-Terpineol	0.4			
1224	Citronellol	27.0	8.5		21.6
1236	Neral	0.4	22.2	0.9	
1250	Geraniol	19.2	3.3	76.9	12.1
1266	Geranial	0.7	32.9	2.1	
1269	Citronellyl formate	8.7			0.5
1295	Geranyl formate	5.5		0.9	
1349	Citronellyl acetate	0.5			0.5
1356	Eugenol				2.2
1365	α-Copaene	0.4			
1374	β-Bourbonene	0.3			
1377	Geranyl acetate			15.7	
1382	β-Elemene	1.0	3.8		
1407	Methyl eugenol				0.2
1408	*cis*-Caryophyllene	1.2		0.8	1.4
1428	α-Guaiene	0.9			
1432	*6,9*-Guaiadiene	5.6			
1438	Aromadendrene	0.3			
1468	Geranyl propanoate	0.7			
1506	Geranyl isobutanoate	0.3			

TABLE I. Continued

RI	EO constituent	Essential oils			Absolute
		Microorganism			
		P. graveolens	*L. petersonii*	*C. martini* var. *motia*	*R. centifolia*
1513	δ-Cadinene	1.6			
1520	Citronellyl butanoate	0.6			
1554	Geranyl butanoate	0.9			
1557	*trans*-Nerolidol			0.7	
1584	*2*-Phenyl ethyl tiglate	1.6			
1594	Geranyl isovalerate	0.4			
1658	*trans*-Citronellyl tiglate	0.3			
1689	Heptadecane (C17)				0.8
1693	Geranyl tiglate	1.6			
1861	*n*-Hexadecanol				1.5
1887	Nonadecane (C19)				0.5
2076	*n*-Octadecanol				0.8
Monoterpene hydrocarbons		0.32	0.25	0.00	
Oxygenated monoterpenes		82.10	95.09	98.48	34.74
Sesquiterpene hydrocarbons		11.38	3.82	0.82	1.37
Benzoid/phenylpropanoid cmpds.					60.09
Oxygenated sesquiterpenes		6.21	0.00	0.70	
Aliphatic hydrocarbons					1.32
Oxygenated aliphatics					2.30
Total of identified constituents[a]		100.0	99.16	100.0	99.83

[a]Relative percentage of identified constituents are obtained by GC-FID peak areas

Thirty-one compounds were identified in *P. graveolens* EO, as presented in Table I; citronellol and geraniol were the major constituents followed by linalool, all of them being monoterpene alcohols (57.6 % of EO). Similar to our results, Ben ElHadj Ali *et al.*[25] showed the same major constituents in *P. graveolens* EOs from Tunisia.

In the EO of *L. petersonii*, 10 compounds were identified (Table I), among which geranial (32.9 %) was the major one, followed by neral and citronellal, all of them belonging to monoterpene aldehydes (76.2 % of EO). A huge variation in the chemical composition of *L. petersonii* EO was recently confirmed[26] in *L. petersonii* EO from Australia with the major constituent being geranyl acetate (31.4 %). In our study *L. petersonii* EO the most nearly resembles *L. petersonii* EO – type I,[27] which has a pleasant scent and is abundant in monoterpene aldehydes (geranial, neral and citronellal) that represent 83.5 % of EO. Apart to this, our EO also contained monoterpene alcohols: citronellol and geraniol (11.8 %).

In the EO of *C. martiniii* var. *motia* (syn. *C. martiniii* var. *martinii*), 8 constituents were identified (Table I). The most dominant was monoterpene alcohol geraniol (76.9 %), followed by its ester geranyl acetate; together they accounted for 92.6 % of the EO. According to Kakaraparthi *et al.*,[28] the contents of geraniol and geranyl acetate in the EO are inversely related, and depend on the harvest time.

In *R. centifolia* absolute, 12 compounds were identified, as presented in Table I, the major compound was aromatic alcohol 2-phenylethanol (57.7 %), followed by monoterpene alcohols, citronellol (21.6 %) and geraniol (12.1 %). Although not so extensively studied, chemical characterization of *Rosa centifolia* L. absolute was previously reported;[29] the most abundant constituents were 2-phenylethanol (66.5 and 64.8–73.0 %, respectively), followed by citronellol (10.1 and 8.8–12.0 %, respectively) and geraniol (5.6 and 4.9–6.4 %, respectively), which is in agreement with our findings.

Although the chemical composition of selected essential oils and an absolute were more or less recently reported, as discussed above, this is the first comparative comprehensive report on their chemistry.

Antimicrobial activity

The obtained results of antibacterial and antifungal activity are summarized in Tables II and III, respectively. The tested aromatic samples exhibited significant antimicrobial activity against all strains, and the inhibition values ranged as follows: *MIC* 0.01–0.50 mg mL^{-1}, *MBC* 0.03–1.00 mg mL^{-1}. Among the four tested aromatic samples, the EO of *C. martiniii* var. *motia* exhibited the lowest antibacterial potential (*MIC* 0.25–0.50 mg mL^{-1}, MBC 0.50–1.00 mg mL^{-1}). On the other hand, the EO of *L. petersonii* EO proved to have the strongest achievements (*MIC* 0.01–0.25 mg mL^{-1}, *MBC* 0.03–0.25 mg mL^{-1}, Table II).

TABLE II. Antibacterial activity of tested aromatic samples compared to Hexoral (*MIC* and *MBC* in mg/mL)

Bacteria	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
S. aureus	0.50	1.00	0.50	1.00	0.13	0.25	0.13	0.25	1.56	3.12
S. pyogenes	0.50	1.00	0.50	1.00	0.13	0.25	0.25	0.50	0.65	1.31
S. mutans	0.50	1.00	0.50	1.00	0.06	0.13	0.25	0.50	1.56	3.12
L. acidophilus	0.25	0.50	0.25	0.50	0.06	0.13	0.13	0.25	1.56	3.12
S. salivarius	0.50	1.00	0.25	0.50	0.06	0.13	0.13	0.25	0.78	1.56
S. sanguis	0.25	0.50	0.25	0.50	0.06	0.13	0.13	0.25	0.19	0.39
P. aeruginosa	0.50	1.00	0.13	0.25	0.25	0.25	0.25	0.50	0.78	1.56
E. faecalis	0.25	0.50	0.13	0.25	0.01	0.03	0.13	0.25	0.78	1.56

However, this is the first report on comparative evaluation of four essential oils and an absolute against the most common bacteria isolated from the human oral cavities.

The results of antifungal activity showed the strongest antifungal potential of *C. martinii* var. *motia* EO *(MIC* 0.003–0.007 mg mL^{-1}, *MFC* 0.007–0.015 mg mL^{-1}), while the lowest one was achieved with *R. centifolia* absolute (0.06–0.13 mg mL^{-1}, MFC 0.12–0.25 mg mL^{-1}). Commercial oral antiseptic, Hexoral, showed lower anti-

fungal effect on tested *Candida* strains (*MIC*s and *MFC*s in range 1–4 mg mL^{-1}) (Table III). Tested *Candida* strains proved to be more sensitive compared to clinical isolates of all bacteria, which could be principally addressed to the differences in their cell organizations.

TABLE III. Anticandidal (*C. albicans*, *C. krusei* and *C. glabrata*) efficacy of tested aromatic samples and antibiotics (*MIC*s and *MFC*s in mg mL^{-1})

No.	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
1	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
2	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
3	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
4	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
5	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
6	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
7	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	1.00
8	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
9	0.003	0.007	0.03	0.06	0.03	0.06	0.13	0.25	1.00	2.00
10	0.003	0.007	0.01	0.02	0.03	0.06	0.06	0.12	1.00	2.00
11	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
12	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
13	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
14	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
15	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
16	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
17	0.003	0.007	0.06	0.12	0.03	0.06	0.13	0.20	1.00	2.00
18	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	2.00	4.00
19	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
20	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
21	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
22	0.003	0.007	0.01	0.02	0.06	0.13	0.13	0.25	1.00	2.00
23	0.003	0.007	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
24	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
25	0.003	0.007	0.06	0.12	0.06	0.13	0.13	0.25	1.00	2.00
26	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
27	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
28	0.003	0.007	0.06	0.12	0.06	0.13	0.13	0.25	1.00	2.00
29	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
30	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
31	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
32	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
33	0.003	0.007	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
34	0.003	0.007	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
35	0.003	0.007	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
36	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
37	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00

TABLE III. Continued

No.	Essential oils						Absolute		Hexoral®	
	C. martini var. *motia*		*P. graveolens*		*L. petersonii*		*R. centifolia*			
	MIC	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*	*MIC*	*MFC*
38	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
39	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
40	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	1.00	2.00
41	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
42	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
43	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
44	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
45	0.007	0.015	0.01	0.02	0.06	0.13	0.06	0.12	2.00	4.00
46	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
47	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
48	0.003	0.007	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
49	0.007	0.015	0.06	0.12	0.06	0.13	0.06	0.12	1.00	2.00
50	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
51	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
52	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	2.00	4.00
53	0.007	0.015	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00
54	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
55	0.003	0.007	0.03	0.06	0.06	0.13	0.13	0.25	1.00	2.00
56	0.003	0.007	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
57	0.003	0.007	0.03	0.06	0.03	0.06	0.13	0.25	1.00	2.00
58	0.007	0.015	0.06	0.12	0.03	0.06	0.06	0.12	1.00	2.00
59	0.007	0.015	0.03	0.06	0.03	0.06	0.06	0.12	1.00	2.00
60	0.003	0.007	0.03	0.06	0.06	0.13	0.06	0.12	1.00	2.00

Although there are recent findings[30] on the anticandidal activity of natural products that were used in the current study, this study presents the first comprehensive investigation on various *Candida* strains, the referent ATCC strains and the clinically isolated ones from the human oral cavities. The obtained results indicated that natural, terpene-rich products, investigated herein, are good candidates for further development of topical anticandidal preparations, which make them particularly interesting for application in the pharmaceutical industry.

The results of antibiofilm activity of tested commercial aromatic samples are presented in Table IV. The process of biofilm formation was inhibited by samples in which *S. mutans*, *P. aeruginosa* and *C. albicans* were engaged. Tested sub*MIC* (1/2*MIC*) concentrations of all aromatic samples inhibited biofilm formation of *S. mutans* and *P. aeruginosa* to the extent ranging 81.22–86.07 and 70.98–86.79 %, respectively. Lower inhibition values were achieved by antibiotic: streptomycin and ampicillin (49.40 and 69.16 %, respectively).

The inhibition values for *C. albicans* biofilm range 81.22–86.07 %, while that of the standard antimycotic drug fluconazole was even lower 73.00 %. The

best inhibition capacity is observed with the use of *R. centifolia* absolute while the lowest with *L. petersonii* EO (Table IV).

TABLE IV. Antibiofilm activity of tested aromatic samples (biofilm inhibition ± *SD*, %) applied in sub*MIC*s compared to standard antibiotics

Antibiofilm agent	Microorganism		
	S. mutans	*P. aeruginosa*	*C. albicans*
C. martini var. *motia*	84.56±1.80[a]	86.79±2.21[a]	84.56±2.30[a]
P. graveolens	81.55±1.40[a]	87.50±3.25[a]	81.55±2.10[a]
R. centifolia	81.22±2.10 [a]	70.98±2.58[b]	81.22±1.80[a]
L. petersonii	86.07±2.30[a]	81.07±2.50[a]	86.07±2.00[a]
Streptomycin	49.40±0.90[c]	49.40±1.80[c]	–
Ampicillin	69.16±1.10[b]	69.16±1.20[b]	–
Fluconazole	–	–	73.00±2.30[b]

Nevertheless, it should be noted that antibiofilm investigations conducted in our study represents one of the first reports on antibiofilm activities of four different aromatic products against several oral pathogenic microbes.

In order to observe inhibitory effect of *L. petersonii* and *P. graveolens* EOs, in particular the changes they cause to *C. albicans* cells, SEM micrographs were recorded. Applied at determined MIC value, both EOs achieved strong inhibition of *C. albicans* cells growth (Figs. 1 and 2), though the activity of *L. petersonii* was stronger.

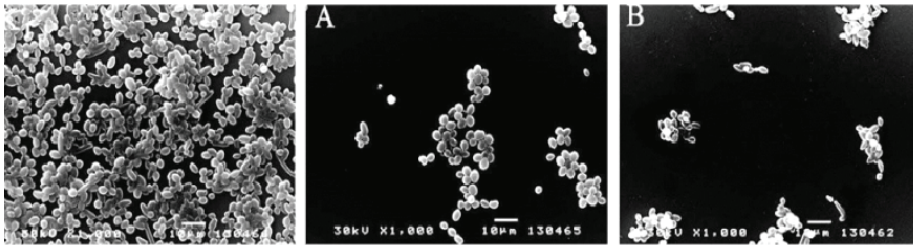

Fig. 1. SEM of *C. albicans* biofilm untreated control (K), treated with *P. graveolens* EO following 6 (A) and 24 h (B).

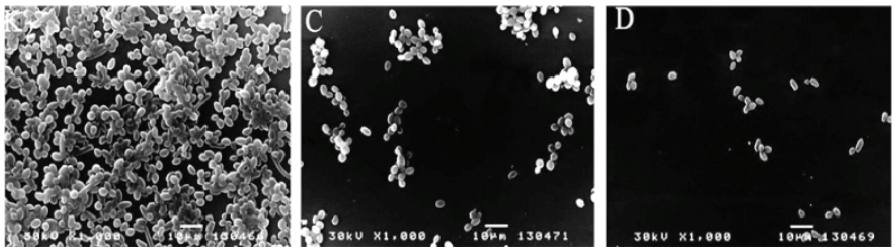

Fig. 2. SEM of untreated control *C. albicans* biofilm (K), treated with *L. petersonii* EO following 6 (C) and 24 h (D).

Six hours following the incubation (Figure 1A and 2C), both EOs significantly reduced the initial phase of biofilm formation, while 24 h following the incubation, the reduction was much higher (Fig. 1B and D). Morphological changes were observed in the cells shape, as well in their size and number. The induced modifications may be attributed to the interference of EOs constituents with enzymatic reactions of the cell wall synthesis which affects the fungal morphogenesis and growth. The results of this experiment indicate that EOs of *L. petersonii* and *P. graveolens* affect cells of *C. albicans*, thereby leading to clearly detectable number of cells and morphological changes. Although their mechanism of action is not fully explained yet, taking into account the obtained results, we assume that they have a great potential in eliminating *C. albicans* biofilm.

Cytotoxic activity

The cytotoxic effects of the commercial aromatic samples on several human tumour cells lines (NCI-H460, MCF-7, HCT-15, HeLa and HepG2) and on non-tumour cells (PLP2), represented as the concentrations that inhibit 50 % of cell growth (GI_{50}), are summarized in Table V.

Table V. Cytotoxicity of tested aromatic samples ($GI_{50} \pm SD$, μg mL^{-1}) compared to standard cytotoxic drug

Cell line	Essential oils			Absolute	Ellipticine
	P. graveolens	*Rosa centifolia*	*C. martini* var. *motia*	*Rosa centifolia*	
MCF7	116.66±9.62[a]	47.70±2.34[c]	54.51±4.47b[c]	80.80±1.28[b]	0.91±0.04
NCI-H460	81.47±2.03[b]	24.38±0.01[d]	60.65±0.92[c]	92.45±0.95[a]	1.42±0.00
HCT15	63.72±1.39[a]	5.60±0.37[c]	39.23±0.65[b]	65.48±2.20[a]	1.91±0.06
HeLa	70.96±0.04[a]	9.01±2.21[d]	58.47±0.14[b]	49.55±1.29[c]	1.14±0.21
HepG2	93.91±2.99[a]	29.58±2.59[c]	53.84±3.16[b]	90.94±1.26[a]	3.22±0.67
PLP2	>400	316.83±2.63	358.67±3.49[a]	>400	2.06±0.03

This is the first comparative report summarizing cytotoxic potential of EOs of *P. graveolens*, *L. petersonii*, *C. martinii* and *R. centifolia* absolute. Our results indicated that essential oils and absolute should be safe for use since expressing low or no-cytotoxicity to primary liver cells. On the other side, further *in vivo* studies are needed to confirm our findings.

CONCLUSION

This work suggests that the studied essential oils and absolute are naturally occurring antimicrobials that could be assumed as promising agents in prevention and treatment of a range of bacteria and fungi related contaminations and infections. In addition, the samples showed cytotoxic effects in human tumour cell lines, while low or no toxicity to primary liver cells. The antimicrobial activity was confirmed against a range of Gram-positive and Gram-negative bacteria and *Candida* species. In particular, data from the present study illustrate the ways in

which *P. graveolens* and *L. petersonii* EOs inhibit and kill bacteria and fungi; possessing very good anti-tumour potentials at the same time, ultimately making them applicable in the development of new procedures for the treatment of various oral conditions. However, this is the first comparative report summarizing antimicrobial activity against pathogenic microbes isolated from human oral cavity; providing an insight into the modes of antimicrobial action; and at the same time describing their cytotoxicity against tumour cell lines. Further *in vivo* studies are necessary to support our data.

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ИЗВОД

ХЕМИЈСКИ САСТАВ И БИОЛОШКЕ ОСОБИНЕ ЕТАРСКИХ УЉА *Pelargonium graveolens*, *Leptospermum petersonii* И *Cymbopogon martinii* VAR. *motia* И АРОМАТИЧНЕ ВОДЕ *Rosa centifolia*

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У овом раду испитиван је хемијски састав и биолошка активност етарских уља (EO) *Pelargonium graveolens*, *Leptospermum petersonii*, *Cymbopogon martinii* var. *motia* и ароматичне воде *Rosa centifolia*. Главна једињења у *P. graveolens* EO били су монотерпенски алкохоли цитронелол, гераниол и линалол; у *L. petersonii* EO монотерпенски алдехиди гераниал, нерал и цитронелал; у *C. martinii* var. *motia* EO монотерпенски алкохол гераниол и естар геранил-ацетат, док је у ароматичној води *R. centifolia* био доминантан алкохол 2-фенилетанол. EO *L. petersonii* показало је најснажније антибактеријско дејство док је EO *C. martinii* var. *motia* испољило најјачи антифунгални потенцијал. Најбољи инхибиторни капацитет биофилма забележен је за ароматичну воду *R. centifolia*. Резултати анализе скенирајуће електронске микроскопије (SEM) указали су да EO *L. petersonii* и *P. graveolens* смањују број и мењају морфологију ћелија квасца *Candida albicans*. *L. petersonii* EO имало је највећи цитотоксични потенцијал према хуманим туморским ћелијским линијама. Ово је први упоредни извештај који сумира ефикасност проучаваних ароматичних узорака против патогених микроба, пружајући дубљи увид у начине антимикробног деловања и истовремено описујући њихову цитотоксичност према ћелијским линијама.

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A study towards the synthesis of (–)-*atrop*-abyssomicin C core•

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Abstract: An attempt to synthesize the cyclohexane core of antibiotic abyssomicin C is described. The initial, protecting group-free approach (relying on internal protection) failed and had to be modified, in order to allow for efficient deprotection of the acid-sensitive cyclization precursor in the penultimate synthetic step. Thus, a pyranoside structural unit was used as a latent lactone/ester functionality, which was deprotected *via* thioacetalization/hydrolysis/oxidation sequence, to give the δ-valerolactone-type cyclization precursor. Unfortunately, the key cyclization reaction was not feasible, even after structural modification of the cyclization precursor. Reluctance towards cyclization turned out to be a general property of (at least some) Δ^7-unsaturated esters, which required the development of a new strategy for this type of transformation.

Keywords: organic synthesis; cyclization; protecting groups; pyranoside; natural products.

INTRODUCTION

Some time ago we embarked on the total synthesis of (–)-*atrop*-abyssomicin C (**1**) – a naturally occurring antibiotic with intricate molecular architecture and a new mechanism of action.[1] Our retrosynthetic analysis, displayed in Scheme 1, relied on incremental topological simplification of the polycyclic target and involved a cyclohexane derivative **2** as a synthetic intermediate. This compound contains 5 stereogenic centers out of 6 carbon atoms (constituting cyclohexane ring; the stereochemistry of the ester-bound carbon atom is irrelevant for the course of synthesis, though), which oriented retrosynthetic analysis of **2** toward stereoselective transforms. In addition, the presence of reactive functional groups required protection: we planned to use internal protection, *i.e.*, to interconnect

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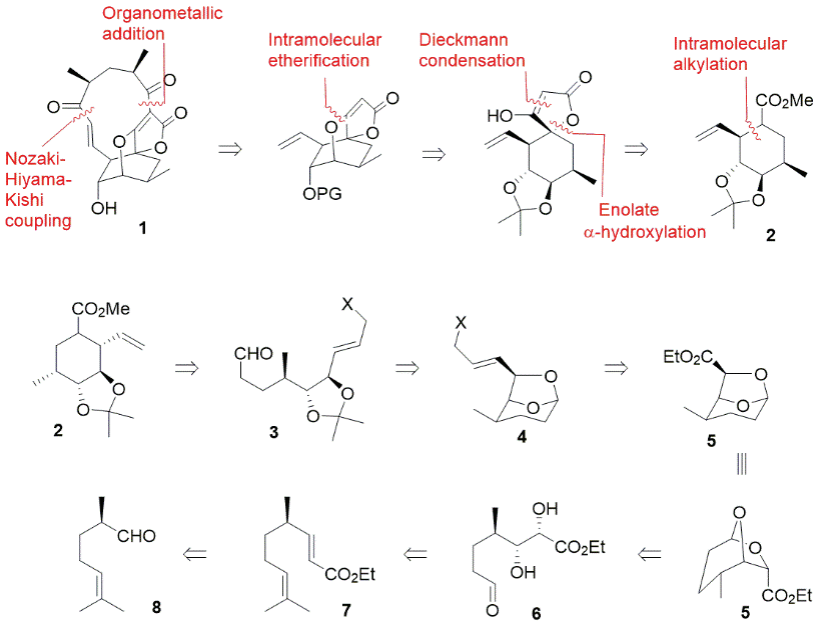
• Dedicated to the outstanding researcher, excellent teacher and dear colleague, Professor Emeritus Slobodan Milosavljević, on the occasion of his 80th birthday.

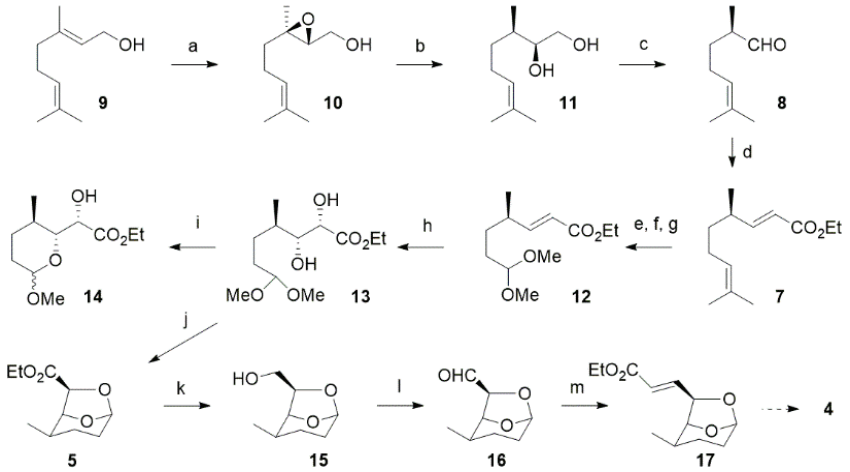
functional groups already present in the molecule, so as to achieve maximum atom-economy (*i.e.*, no introduction of additional atoms, save those constituting the target molecule). This ambition would be accomplished by proceeding *via* intermediate **5**, where vicinal diol and aldehyde functionalities are both present in latent form as bicyclic acetal. Retrosynthetic "hydrolysis" of this compound gives **6**, on its turn obtainable by Sharpless asymmetric dihydroxylation (AD) of diene **7**. Aldehyde **8** (also known as melonal) could be prepared in the optically pure form from geraniol, as previously described (Scheme 2).



Scheme 1. Retrosynthetic analysis of (–)-*atrop*-abyssomicin C.

The synthesis commenced with Sharpless-Katsuki asymmetric epoxidation of geraniol (**9**). Curiously, the optical purity of our product **10** was 85 % (as compared to 95 % *ee* in the literature;[2] we performed the reaction several times, with reproductive results). Reductive opening of epoxide **10** with $NaBH_3CN$,[3] followed by oxidative cleavage of the intermediary diol **11** provided optically enriched (–)--melonal (**8**),[4] which was converted into diene **7** by the Horner–Wadsworth––Emmons modification of the Wittig reaction.[5] Regioselective conversion of this compound into acetal **12** was accomplished by Malaprade–Johnson–Lemieux reaction sequence, with subsequent acetalization.[6] Sharpless asymmetric dihydroxylation of **12** with AD-mix-*β* afforded diol **13**, which was expected to undergo acid-catalyzed cyclization into bicyclic acetal **5**. Upon exposure of **13** to catalytic amounts of *p*-TsOH in chloroform at rt, a monocyclization occurred with the

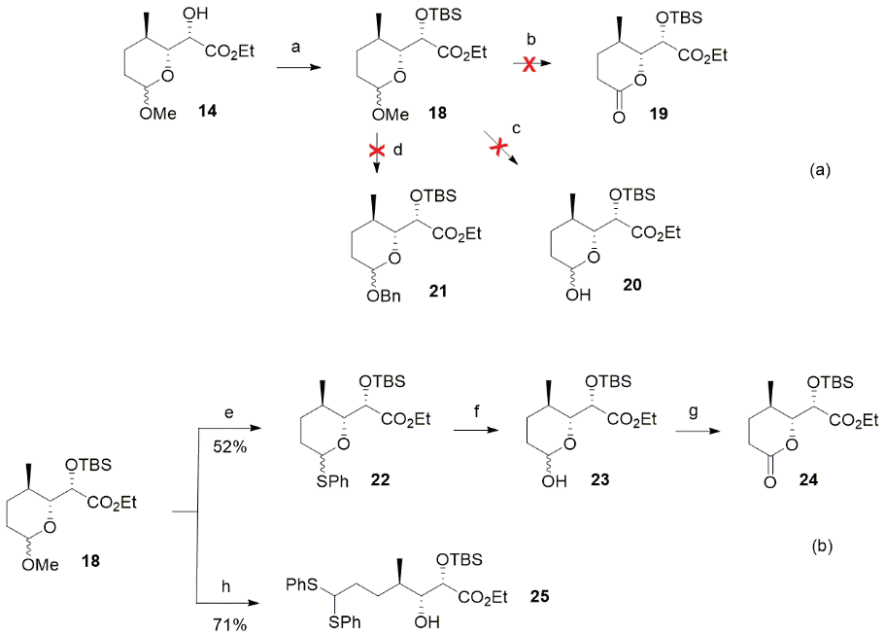
formation of acetal **14** (obtained as an equimolar mixture of stereoisomers). However, performing the reaction at reflux afforded **5** in 62 % yield. The plan called for the conversion of **5** into the allylic intermediate **4**, which would be deprotected (*i.e.*, **4** → **3**) to allow for cyclization. We were aware that the deprotection might be non-trivial so, before continuing along the path leading to **4**, we examined the acid-catalyzed deprotection of its structurally simpler predecessor **5**. Indeed, the compound turned out to be capricious toward deprotection (as we feared), as no reaction was observed with methanol, under Lewis, or protic, acid-catalyzed conditions. This was a bad predicament, as the allylic derivative of type **4** was supposed to be more sensitive to acid-catalyzed side-reactions, with respect to **5**. In addition, aldehyde **16** was difficult to purify (aldehydes with an oxygen substituent at the α-position are often hydrated and trail at TLC and silica column). Also, Wittig olefination of aldehyde **16** proceeded sluggishly and (in addition to the desired product **17**) afforded an unidentified side product. For all these reasons, we modified the initial plan and decided to proceed *via* monocyclic acetal **14**, where the loss in atom-economy would be compensated by better control of reactivity.



Scheme 2. Reagents and conditions: a) $Ti(OiPr)_4$, (–)-DET, *t*-BuOOH, ms 4Å, CH_2Cl_2, –20 °C; b) $NaCNBH_3$, $BF_3{\cdot}Et_2O$, THF; c) $NaIO_4$, SiO_2, H_2O, CH_2Cl_2; d) $(i\text{-PrO})_2P(O)CH_2CO_2Et$, LiBr, Et_3N, THF; e) OsO_4 (cat.), NMO, *t*-BuOH, H_2O, 87 %; f) $NaIO_4$, MeOH, H_2O, 89 %; g) $CeCl_3{\cdot}7H_2O$, $HC(OMe)_3$, 87 %; h) $(DHQD)_2PHAL$, OsO_4, AD-mix-β, $MsNH_2$, *t*-BuOH, H_2O, 0 °C, 81 %; i) *p*-TsOH (cat), $CHCl_3$, rt, 94 %; j) *p*-TsOH (cat), $CHCl_3$, reflux, 62 %; k) $LiAlH_4$, THF, rt, 88 %; l) DMP, CH_2Cl_2, rt; m) $Ph_3P{:}CHCO_2Et$, CH_2Cl_2, rt, 35 %. DET = diethyl tartrate; NMO = *N*-methylmorpholine *N*-oxide; $(DHQD)_2PHAL$ = hydroquinidine 1,4-phthalazinediyl diether; *p*-TsOH = *para* toluenesulfonic acid.

After the secondary hydroxyl group in **14** was protected as TBS-ether, we tested (on the model compound **18**) the conditions for the conversion of the cyclic acetal into the corresponding lactone **19**. Here again, compound **18** proved reluc-

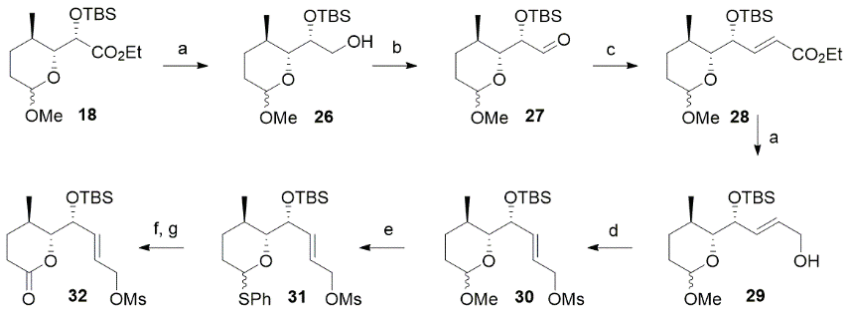
tant towards Jones oxidation (to **19**), hydrolysis (to **20**),[7] or alkoxy-group exchange (to **21**; Scheme 3, part a). Therefore, a two-step maneuver was applied, comprising the conversion of **18** into monothioacetal **22**, followed by its deprotection into hemiacetal **23** (Scheme 3, part b). Our initial attempt to catalyze the first reaction (**18** → **22**) with $LiClO_4$ failed;[8] however, using equimolar amount of $TiCl_4$ at low temperature provided **22** in 52 % yield.[9] Upon exposure to $Hg(ClO_4)_2$,[10] monothioacetal **22** was instantaneously converted into hemiacetal **23** (73 %). This result indicated that the deprotection of the advanced synthetic intermediate should be feasible under the similar reaction conditions. Interestingly, with two equivalents of thiophenol, acetal **18** was smoothly converted into dithioacetal **25** (71 %).



Scheme 3. Reagents and conditions: a) TBSOTf, 2,4,6-collidine, CH_2Cl_2, 0 °C, 94 %; b) Jones reagent; c) H_2O, DME, reflux; d) *p*-methoxybenzyl alcohol, *p*-TsOH (cat.), ms 4Å, $CHCl_3$, rt; e) PhSH (1 eq), $TiCl_4$, $CHCl_3$, –20 °C→rt, 52 %; f) $Hg(ClO_4)_2$, $CaCO_3$, THF, H_2O, rt, 73 %; g) Jones reagent; h) PhSH (2 eq), $TiCl_4$, $CHCl_3$, –20 °C→rt, 71 %. TBSOTf = *t*-butyldimethylsilyl trifluoromethanesulfonate; ms = molecular sieves; DME = dimethoxyethane.

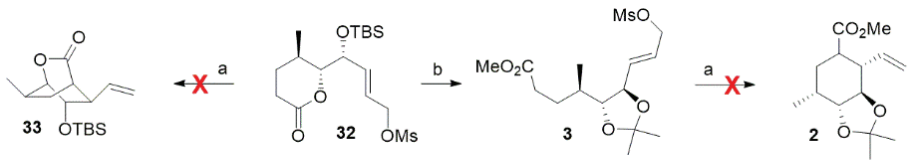
An attempt to obtain aldehyde **27** by direct, low-temperature (–78 °C) reduction of ester **18** with DIBALH was not successful, as the alcohol **26** was formed immediately (Scheme 4). Therefore, ester **18** was first reduced to alcohol **26** (DIBALH, 81 %), then oxidized to aldehyde **27** by DMP (92 %). Wittig reaction with **27** proceeded without event (93 %), providing the conjugated ester **28** which was further reduced to allylic alcohol **29** (DIBALH, 82 %) and converted into mesylate **30** (81 %) via sulfene.[11] The conversion of acetal **30** into lactone

32 was accomplished *via* a previously developed protocol (*i.e.*, **18** → **22** → **23** → **24**) in 23 % overall yield, thus setting up the stage for the pivotal cyclization reaction (*i.e.*, **32** → **33**).



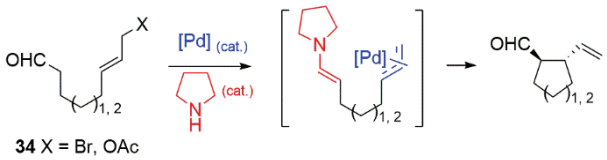
Scheme 4. Reagents and conditions: a) DIBALH, Et_2O, –40 °C, 81 % (for **26**); 82 % (for **29**); b) DMP, CH_2Cl_2, rt, 92 %; c) Ph_3P:$CHCO_2Et$, CH_2Cl_2, rt, 93 %; d) MsCl, Et_3N, CH_2Cl_2, -20 °C, 81 %; e) PhSH (1 eq), $TiCl_4$, $CHCl_3$, –15 °C, 46 %; f) $Hg(ClO_4)_2$, $CaCO_3$, THF, H_2O, –15 °C; g) Jones reagent, 62 % from **31**. DIBALH = diisobutylaluminum hydride; DMP = Dess–Martin periodinane; MsCl = methanesulfonyl chloride.

The cyclization was attempted with LDA as a base, in the presence of HMPA, at –78 °C (Scheme 5). Unfortunately, no reaction was observed, neither at –78 °C, nor at rt. When the temperature was raised to 65 °C, the starting material decomposed (Scheme 5). We reasoned that the strain increase associated with the formation of a bridged bicyclic system might have hampered the reaction. In that case, the cyclization would be facilitated if a condensed, rather than bicyclic, system would be closed. Therefore, we exposed lactone **32** to the action of 2,2-dimethoxypropane, methanol and catalytic amount of CSA,[12] to convert it into ester **3** – hopefully a superior cyclization precursor. Surprisingly, in this reaction an unfavorable equilibrium was established, and we were not able to shift it in favor of the desired compound **3**, even with a considerable excess of 2,2-dimethoxypropane. However, we isolated enough of **3** for the cyclization trial. This experiment was performed under the same reaction conditions as attempted cyclization of **32**, unfortunately with the identical result.



Scheme 5. Reagents and conditions: a) LDA, THF, HMPA, –78 °C → 65 °C; b) 2,2-dimethoxypropane, CSA (cat.), MeOH, rt, 26 % (brsm). HMPA = hexamethylphosphoramide; CSA = camphorsulfonic acid.

Subsequent study showed that, quite surprisingly, esters containing allylic (pseudo)halide moiety are not good substrates for the 6-membered ring closure, and we had to find another way to effect the related 6-*exo*-cyclization and prepare synthetic equivalent of **2**. The solution was found in the development of double-catalyzed cyclization, where synergistic action of both pyrrolidine and organotransition metal catalyst on aldehydes of type **34** resulted in 5-, or 6-membered ring closure. The newly developed method has general synthetic applicability and it has eventually allowed us to accomplish the total synthesis of (–)--*atrop*-abyssomicin C.[13] However, that study is above the scope of this paper and has been described elsewhere.[14]



Scheme 6. Ring closure by double catalysis.

EXPERIMENTAL

General experimental details

All chromatographic separations were performed on Silica (SDS, 60 Å, 40–63 μm). Standard techniques were used for the purification of reagents and solvents. NMR spectra were recorded on a Bruker Avance III 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz), a Bruker Avance III 300 (^{1}H-NMR at 300 MHz, ^{13}C-NMR at 75 MHz) and a Varian Gemini 200, (^{1}H-NMR at 200 MHz, ^{13}C-NMR at 50 MHz). Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (J) are in Hz.

Compounds **10**[2] and **11**[3] were prepared according to literature procedures.

Ethyl (*R,E*)-4,8-dimethylnona-2,7-dienoate (**7**) was obtained from (*R*)-melonal, using the procedure described in the literature for another compound.[5] Starting from 3.04 g of melonal, 1.326 g (88 %) of compound **7** was obtained as a colorless oil.

The physical data of the synthesized compounds are given in Supplementary material to this paper.

*Ethyl (*R,E*)-7,7-dimethoxy-4-methylhept-2-enoate (**12**)*

A) Dihydroxylation of compound **7**: A mixture of diene **7** (1.36 g; 6.47 mmol), OsO_4 (1.3 mL of 0.1 M solution in *t*-BuOH; 0.13 mmol), NMO (0.555 mL of 60 % solution in H_2O; 3.235 mmol), *t*-BuOH (27 mL) and H_2O (13.5 mL) was stirred at rt. After 20 min additional NMO (0.555 mL of 60 % solution in H_2O; 3.235 mmol) was added. After 4 h (as the reaction was not complete) additional OsO_4 (0.5 mL of 0.1 M solution in *t*-BuOH; 0.05 mmol) was added and the reaction mixture was stirred for an additional 5 h. The reaction was quenched by the addition of celite (500 mg), H_2O (7 mL) and $NaHSO_3$ (1 mL of 37.5 % aqueous solution), stirred for 15 min, filtered, extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated at rotavap, to afford 1.5 g of the crude product. Purification by dry-flash chromatography (SiO_2; eluent: heptane/EtOAc = 1/1, followed by pure EtOAc) afforded 1.38 g (87 %) of the product as colorless oil.

B) Oxidative fragmentation of the diol: A solution of the product from the previous step (1.38 g) in MeOH (70 mL) was added to the solution of $NaIO_4$ (4.23 g) in H_2O (45mL), with stirring. After 2 min precipitation occurred, and after 6 min TLC indicated the completion of the reaction. The reaction mixture was diluted with CH_2Cl_2 and H_2O, extracted with CH_2Cl_2, washed with H_2O, dried over anh. $MgSO_4$ and concentrated at rotavap to afford 925 mg (89 %) of the crude aldehyde which was used in the next step without purification.

C) Acetalization of the aldehyde: The crude product from the previous step (870 mg) was treated with $CeCl_3 \cdot 7H_2O$ (122 mg), $HC(OMe)_3$ (5 g; 5.16 ml) and MeOH (12 mL), according to the literature procedure,[6] to give 948 mg (87 %) of the title compound **12** as colorless oil.

*Ethyl (2*S*,3*R*,4*R*)-2,3-dihydroxy-7,7-dimethoxy-4-methylheptanoate (**13**)*

Compound **13**: dihydroxylation of compound **12** was accomplished according to the modified literature procedure;[13] the modification consists in increasing the quantities of OsO_4 and the chiral ligand ($(DHQD)_2PHAL$), as the reaction with the commercial AD-mix is too slow. Thus, a mixture of compound **12** (795 mg), $(DHQD)_2PHAL$ (161 mg), OsO_4 (1.73 mL of the 0.1 M solution in *t*-BuOH), AD-mix-β (4.83 g), methanesulfonamide (328 mg), *t*-BuOH (15.5 mL) and H_2O (17.3 mL) was stirred at 0 °C for 11.5 h. Work-up as described in the literature procedure, followed by purification of the crude product by dry-flash chromatography (SiO_2; gradient elution by heptane/EtOAc = 3/1 → 1/1) afforded 730 mg (81 %) of the title compound **13**, as a colorless oil (a mixture of isomers in 3.7:1 ratio).

*Ethyl (2*S*)-2-hydroxy-2-((2*R*,3*R*)-6-methoxy-3-methyltetrahydro-2*H*-pyran-2-yl)acetate (**14**)*

Compound **14**: a solution of compound 13 (200 mg; 0.757 mmol) and *p*-TsOH (1 mg) in $CHCl_3$ (5 mL) was stirred at rt for 1 h 15 min. Solid K_2CO_3 was added to the reaction mixture, followed by H_2O. Extraction with $CHCl_3$, followed by drying over anh. $MgSO_4$ and concentration at reduced pressure gave 166 mg (94 %) of the title compound **14** as a colorless oil (a nearly equimolar mixture of isomers).

*Ethyl (1*S*,2*R*,5*S*,7*S*)-2-methyl-6,8-dioxabicyclo[3.2.1]octane-7-carboxylate (**5**)*

Compound **5**: a solution of compound **13** (60 mg; 0.23 mmol) and *p*-TsOH (7.7 mg; 0.04 mmol) in $CHCl_3$ (6 mL) was heated to reflux for 8 h. After cooling to rt, solid K_2CO_3 was added, followed by aqueous solution of K_2CO_3 and H_2O. The reaction mixture was extracted with $CHCl_3$, washed with water, dried over anh. Na_2SO_4 and concentrated under reduced pressure, to afford 43 mg of the crude product. Purification by flash-chromatography (SiO_2; eluent: heptane/Et_2O = 3/2) afforded 28 mg (62 %) of the title compound **5**, as a colorless oil (a mixture of isomers in 2.5:1 ratio).

*((1*S*,2*R*,5*S*,7*R*)-2-methyl-6,8-dioxabicyclo[3.2.1]octan-7-yl)methanol (**15**)*

Compound **15**: a mixture of compound **5** (26 mg; 0.13 mmol), $LiAlH_4$ (7 mg; 0.184 mmol) and THF (1 mL) was stirred, initially at 0 °C, then at rt, until TLC indicated the complete conversion. A usual work-up afforded 18 mg (88 %) of the title compound **15**, as a colorless oil (a mixture of isomers in 2.8:1 ratio).

*(Ethyl (*E*)-3-((1*S*,2*R*,5*R*,7*R*)-2-methyl-6,8-dioxabicyclo[3.2.1]octan-7-yl)acrylate (**17**)*

A) Oxidation of alcohol **15** to aldehyde **16**: A mixture of compound **15** (18 mg; 0.114 mmol), DMP (144.8 mg; 0.34 mmol) and CH_2Cl_2 (1 mL) was stirred at rt for 1 h. Standard work-up, followed by flash-chromatography (SiO_2; eluent: heptane/EtOAc = 2/1) afforded 6 mg (33 %) of the title compound **16**. Note: the aldehyde is hydrated and does not have a well-

defined Rf value at TLC plates: with *n*-heptane/EtOAc = 3/1, *Rf* = 0.1–0.25; with *n*-heptane/EtOAc = 1/1, *Rf* = 0.2–0.4. Aldehyde **16** was immediately used for the next step.

B) Wittig reaction with aldehyde **16**: a mixture of aldehyde **16** (6 mg; 0.0384 mmol; from the previous step) and ethoxycarbonylmethylene triphenylphosphorane (67 mg; 0.192 mmol; 5 equiv.) in CH_2Cl_2 (0.5 mL) was stirred overnight. Standard work-up, followed by purification by flash chromatography (SiO_2; eluent: *n*-heptane/EtOAc = 12/1) afforded 3 mg (35 %) of the title compound **17** (equimolar mixture of stereoisomers), as a colorless oil (an equimolar mixture of isomers).

Ethyl (*2S*)-2-((*tert*-butyldimethylsilyl)oxy)-2-((*2R,3R*)-6-methoxy-3-methyltetrahydro-2*H*-pyran-2-yl)acetate (**18**) was prepared from compound **14**, applying the procedure described in the literature.[15] Starting from 152 mg of **14**, 213 mg (94 %) of compound **18** was obtained, as a colorless oil (a mixture of isomers in 1.5:1 ratio).

*Attempts to convert compound **18** into compounds **19**, **20** and **21***

Attempt to convert compound **18** into lactone **19** was done according to the literature procedure.[16] TLC monitoring indicated a very slow reaction with substantial decomposition of starting material.

Attempt to convert compound **18** into hemiacetal **20** was performed according to the literature procedure.[17] No reaction was observed, and the starting material was recovered unchanged.

Attempt to convert compound **18** into compound **21**: a mixture of compound **18** (10 mg; 0.029 mmol), *p*-methoxybenzyl alcohol (8 mg; 0.058 mmol), *p*-TsOH (1 mg), molecular sieves (4 Å) and $CHCl_3$ (0.3 mL) was stirred at rt. No reaction could be observed after 2 h. After 24 h slow formation of 2 products was observed.

Ethyl (*2S*)-2-((*tert*-butyldimethylsilyl)oxy)-2-((*2R,3R*)-3-methyl-6-(phenylthio)tetrahydro-2*H*-pyan-2-yl)acetate (**22**) was prepared according to the modified literature procedure:[9] the modification is in that 1 equiv. of $TiCl_4$ was used (instead of 10 mol. %, as described in the reference). Starting from 11 mg of compound **18**, after purification by dry-flash chromatography (SiO_2; eluent: *n*-heptane/EtOAc = 18/1), 7 mg (52 %) of compound **22** was obtained, as a colorless oil (a mixture of isomers in 5: 1 ratio).

Ethyl (*2S*)-2-((*tert*-butyldimethylsilyl)oxy)-2-((*2R,3R*)-6-hydroxy-3-methyltetrahydro-2*H*-pyran-2-yl)acetate (**23**) was prepared according to literature procedure.[10a] The reaction is immediate, however, the reaction mixture was left additional 45 min at r.t. to verify the stability of the product under the reaction conditions. The crude product was dissolved in minimum quantity of CH_2Cl_2, this solution was diluted with the same amount of *n*-heptane and applied to the SiO_2 column for dry-flash purification (eluent: *n*-heptane/EtOAc = 4/1) to afford 4 mg (73 %) of the title compound **23** which spontaneously crystallizes (a mixture of isomers in 2: 1 ratio).

Ethyl (*2S,3R,4R*)-2-((*tert*-butyldimethylsilyl)oxy)-3-hydroxy-4-methyl-7,7-bis(phenylthio)heptanoate (**25**): A solution of compound **18** (10 mg; 0.029 mmol), thiophenol (0.06 mL of 1 M solution in $CHCl_3$; 0.06 mmol) and $TiCl_4$ (0.03 mL of 1 M solution in $CHCl_3$; 0.03 mmol) in $CHCl_3$ (0.3 mL) was stirred at –15 °C. After 2 h additional thiophenol (0.03 mL of 1 M solution in $CHCl_3$; 0.03 mmol) was added and the reaction mixture was allowed to reach rt with stirring, for 3 h. Standard work-up, followed by purification by dry-flash chromatography (SiO_2; gradient elution with: *n*-heptane/EtOAc = 12/1 → 9/1) afforded 11 mg (71 %) of the title compound **25**, as a viscous oil.

(*2R*)-2-((*Tert*-butyldimethylsilyl)oxy)-2-((*2R,3R*)-6-methoxy-3-methyltetrahydro-*2H*-pyran-2-yl)ethan-1-ol (**26**): DIBALH (6.4 mL of 1.5 M solution in toluene; 9.6 mmol; 4 equiv.) was added over 10 min to a cold (–40 °C) solution of compound **18** (840 mg; 2.424 mmol) in

Et_2O (25 mL). The reaction is virtually instantaneous. The reaction mixture was diluted with a mixture of concentrated aqueous solution of Rochelle's salt and Et_2O, stirred for 1 h at rt, extracted with ether, dried over anh. $MgSO_4$, concentrated at rotavap and purified by flash chromatography (SiO_2, eluent: *n*-heptane/EtOAc = 5/1) to give 600 mg (81 %) of the title compound **26**, which spontaneously crystallizes (a mixture of isomers in 1.4:1 ratio).

(*2S*)-2-((*Tert*-butyldimethylsilyl)oxy)-2-((*2R,3R*)-6-methoxy-3-methyltetrahydro-2*H*-pyran-2-yl)acetaldehyde (**27**): a mixture of compound **26** (110 mg; 0.36 mmol) and DMP (300 mg; 0.71 mmol) in CH_2Cl_2 (7 mL) was stirred at rt for 1 h. Work-up as described in the literature[18] afforded 100 mg (92 %) of the title compound **27** as a colorless oil (an equimolar mixture of isomers).

Ethyl (*4R,E*)-4-((*tert*-butyldimethylsilyl)oxy)-4-((*2R,3R*)-6-methoxy-3-methyltetrahydro-*2H*-pyran-2-yl)but-2-enoate (**28**): a solution of compound **27** (100 mg; 0.33 mmol) and ethoxycarbonyl triphenylphosphorane (244 mg; 0.7 mmol) in CH_2Cl_2 (5 mL) was stirred at rt. After 40 h, additional phosphorane reagent (120 mg) was added, and stirring was continued for 47 more hours. The reaction mixture was concentrated at rotavap, dissolved in EtOH (5 mL), $NaBH_4$ (3 mg) was added and the mixture was stirred for 15 min at rt, followed by addition of aqueous solution of NH_4Cl and CH_2Cl_2. The organic extract was washed with water, dried over anh. $MgSO_4$, concentrated at rotavap and purified by flash chromatography (SiO_2, eluent: *n*-heptane/EtOAc = 12/1) to give 115 mg (93 %) of the title compound **28**, as a colorless oil (a mixture of (*E*)-isomers in 1.4: 1 ratio, with 10 % of (*Z*)-isomers).

(*4R,E*)-4-((*Tert*-butyldimethylsilyl)oxy)-4-((*2R,3R*)-6-methoxy-3-methyltetrahydro-*2H*-pyran-2-yl)but-2-en-1-ol (**29**) was obtained according to the procedure described for the preparation of compound **26** (above). Starting from 365 mg (0.981 mmol) of compound **28**, 315 mg of the crude product **29** was obtained. Purification by flash chromatography (SiO_2; gradient elution with: *n*-heptane/EtOAc = 5/1 → 3/1) afforded 265 mg (82 %) of the title compound **29**, as a colorless oil. A fraction of pure (*Z*)-isomers was isolated (less polar than (*E*)-isomers) and separately characterized by NMR.

(*4R,E*)-4-((*Tert*-butyldimethylsilyl)oxy)-4-((*2R,3R*)-6-methoxy-3-methyltetrahydro-2*H*-pyran-2-yl)but-2-en-1-yl methanesulfonate (**30**): the mesylation of compound **29** was accomplished according to the literature procedure.[11] Starting from 68 mg (0.206 mmol) of compound **29**, 68 mg (81 %) of the title compound **30** was obtained as colorless oil (a mixture of isomers in 1.3:1 ratio).

(*4R,E*)-4-((*Tert*-butyldimethylsilyl)oxy)-4-((*2R,3R*)-3-methyl-6-(phenylthio)tetrahydro-2*H*-pyran-2-yl)but-2-en-1-yl methanesulfonate (**31**): a solution of compound **30** (35 mg; 0.086 mmol), thiophenol (0.086 mL of 1 M solution in $CHCl_3$; 0.086 mmol) and $TiCl_4$ (0.086 mL of 1 M solution in $CHCl_3$; 0.086 mmol) in $CHCl_3$ (1 mL) was stirred at –20 to -15 °C, for 30 min. The reaction mixture was diluted with water and $CHCl_3$, the organic extract was washed with H_2O, dried over anh. $MgSO_4$, concentrated and purified by flash chromatography (SiO_2; eluent: *n*-heptane/EtOAc = 5/1) to give 19 mg (46 %) of the title compound **31**, as a viscous oil (a mixture of isomers in 5:1 ratio).

*(*R,E*)-4-((Tert-butyldimethylsilyl)oxy)-4-((2*R,*3*R*)-3-methyl-6-oxotetrahydro-2*H*-pyran-2-yl)-but-2-en-1-yl methanesulfonate (**32**)*

A) The conversion of compound **31** into the corresponding hemiacetal was accomplished according to the procedure described for the preparation of compound **23** (see above), starting from 133 mg (0.27 mmol) of compound **31**. The crude hemiacetal was used in the next step without purification.

B) Oxidation of hemiacetal to lactone **32**: A solution of the product from the previous step in acetone (28 mL) was treated with a solution of Jones reagent (1 mL of 2.7 M solution; 2.7 mmol) at 0 °C. The reaction is instantaneous. Standard work-up afforded 110 mg of the crude product **32**. The product is unstable in contact with SiO_2, so the purification was accomplished by very fast dry flash chromatography (13 g SiO_2; gradient elution with: 12×10 mL of *n*-heptane/EtOAc = 2/1, followed by 12×10 mL of *n*-heptane/EtOAc = 1/1), to afford 67 mg (62 % over 2 steps) of the title compound **32** as a colorless oil.

Attempted cyclization of compound **32** (attempted synthesis of **33**): a solution of compound **31** (35 mg; 0.089 mmol) in THF (0.3 mL) was added dropwise to a cold (–78 °C) solution of LDA (0.1 mmol) in THF (1 mL), with stirring under an argon atmosphere. As no reaction was observed at TLC, HMPA (72 mg; 0.070 mL; 0.4 mmol) was added, and the reaction mixture was allowed to reach rt with stirring. As no reaction was observed by TLC, the reaction mixture was heated to 65 °C; after 30 min, TLC indicated total decomposition of the starting product, without any well-defined product.

Methyl (*R*)-4-((*4R,5R*)-2,2-dimethyl-5-((*E*)-3-((methylsulfonyl)oxy)prop-1-en-1-yl)-1,3-dioxolan-4-yl)pentanoate (**3**): a solution of compound **32** (35 mg; 0.089 mmol), 2,2-dimethoxypropane (0.4 mL), MeOH (6 mg; 0.078 mL; 0.19 mmol) and camphorsulfonic acid (CSA, 12 mg; 0.054 mmol) was stirred at rt. As the conversion was very slow (according to TLC), additional CSA (8 mg), 2,2-dimethoxypropane (0.2 mL) and MeOH (0.01 mL) were added and the reaction was stirred for 2 h. Although the reaction was not complete, TLC indicated the formation of degradation products, so the reaction was interrupted. Work-up as described previously,[12] afforded 35 mg of the crude product. Purification by flash chromatography afforded 4 mg (26 %, based on the recovered starting compound) of the title compound **3** (less polar), followed by 18 mg of the unreacted, recovered starting compound **32**.

Attempted cyclization of compound **3** (attempted synthesis of **2**): this attempt was performed as described for the attempted synthesis of compound **33** (see above), with the same result.

ИЗВОД

СТУДИЈА СИНТЕЗЕ ЦИКЛОХЕКСАНСКОГ ЈЕЗГРА АНТИБИОТИКА (–)-*атрој*-АБИСОМИЦИНА С

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У раду је описан покушај синтезе циклохексанског језгра антибиотика абисомицина С. Иницијални покушај, да се овај задатак оствари без коришћења заштитних група (коришћењем интерне заштите), морао је бити модификован, како би се обезбедила ефикасна депротекција циклизационог прекурсора у претпоследњем кораку синтезе. Стога је као латентни синтетички еквивалент естарске, односно лактонске функционалне групе коришћен дериват пиранозида, чија је депротекција у дериват δ-валеролактона извршена секвенцом: тиоацеталицација/хидролиза/оксидација. Нажалост, кључна реакција циклизације није остварена, чак ни са структурно модификованим прекурсором. Показало се да су Δ^7-незасићени естри изненађујуће лоши супстрати за циклизацију, што нас је подстакло на развој нове стратегије која би омогућила овај тип трансформације.

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SUPPLEMENTARY MATERIAL TO

A study towards the synthesis of (–)-*atrop*-abyssomicin C core

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Physical data for **7**:

$[\alpha]_D^{23}$-33.5 (c 1.11, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.86 (dd, J = 15.5, 7.9 Hz, 1H), 5.76 (d, J = 15.6 Hz, 1H), 5.06 (t, J = 7 Hz, 1H), 4.18 (q, J = 7.3 Hz, 2H), 2.36-2.25 (m, 1H), 1.95 (dd, J = 15, 7.3, 2H), 1.67 (s, 3H), 1.57 (s, 3H), 1.30-1.47 (m, 2H), 1.27 (t, J = 7.3 Hz, 3H), 1.03 (d, J = 6.7 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.9, 154.5, 131.9, 124.0, 119.7, 60.2, 36.1, 25.7, 19.4, 17.7, 14.3. **IR** (ATR) ν_{max}: 2964, 2914, 1716, 1650, 1454, 1367, 1347, 1299, 1264. **HRMS** (ESI) Calcd. for $C_{13}H_{22}O_2Na^+$ $[M+Na]^+$: 233.1517, found: 233.1531.

Physical data for **12**:

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.84 (dd, J = 15.6, 7.9 Hz, 1H), 5.78 (d, J = 15.8 Hz, 1H), 4.33 (t, J = 5.6 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.30 (s, 3H), 3.31 (s, 3H), 2.36-2.25 (m, 1H), 1.61-1.55 (m, 2H), 1.46-1.40 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ 166.9, 153.9, 120.2, 104.5, 60.3, 53.9, 52.8, 36.4, 30.8, 30.3, 19.5, 14.3. **IR** (ATR) ν_{max}: 2955, 2830, 1715, 1651, 1455, 1367, 1301, 1264. **HRMS** (ESI) Calcd. for $C_{12}H_{22}O_4Na^+$ $[M+Na]^+$: 253.1416, found: 253.1439.

Physical data for **13** (in the NMR spectra, *min* stands for the minor, and *maj* - for the major diastereoisomer):

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.36 (t, J = 5.1 Hz, 1H *maj*), 4.37-4.32 (m, 1H *min*), 4.30-4.24 (m, 2H+2H*), 3.62-3.53 (m, 1H *min*), 3.56 (t, J = 8.8 Hz, 1H *maj*), 3.31 (s, 12H), 3.17-3.12 (m, 2H), 2.23 (d, J = 9.4 Hz, 1H *maj*), 2.17 (d, J = 9.1 Hz, 1H *min*), 1.82-1.65 (m, 6H), 1.62-1.49 (m, 2H), 1.30 (t, J = 7.2 Hz, 6H), 1.33-1.19 (m, 2H), 1.02 (d, J = 6.7 Hz, 3H *min*), 0.95 (d, J = 6.6 Hz, 3H *maj*). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 174.3, 174.1, 105.0, 104.9, 76.5, 76.2, 71.5, 71.3, 62.2, 53.0, 53.0, 52.9, 52.8, 36.1, 35.8, 30.0, 29.7, 28.0, 27.6, 27.6, 15.9, 15.3, 14.3. **IR** (ATR) ν_{max}: 3452, 2936, 2831, 1732, 1453, 1384, 1267, 1216. **HRMS** (ESI) Calcd. for $C_{12}H_{24}O_6Na^+$ $[M+Na]^+$: 287.1471, found: 287.1468.

Physical data for **14**:

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70-4.66 (m, 1H), 4.40-4.10 (m, 7H), 3.74 (dd, J = 10.4, 1.7 Hz, 1H), 3.40 (dd, J = 9.7, 1.9 Hz, 1H), 3.36 (s, 3H), 3.25 (m, 3H), 2.93 (d, J = 9.1 Hz, 1H), 2.85 (d, J = 8.2 Hz, 1H), 1.97-1.65 (m, 6H), 1.61-1.39 (m, 3H), 1.34-1.22 (m, 1H), 1.31 (td, J = 7.1, 1.9 Hz, 6H), 0.94 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.3 Hz, 3H). **^{13}C NMR**

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(75 MHz, $CDCl_3$) δ 173.7, 173.2, 103.7, 98.6, 82.2, 75.6, 70.9, 70.6, 61.8, 61.6, 56.0, 54.4, 31.4, 31.0, 31.1, 31.1, 29.9, 26.5, 17.5, 16.6, 14.5, 14.3. **IR** (ATR) ν_{max}: 3482, 2931, 1738, 1460, 1370, 1378, 1270. **HRMS** (ESI) Calcd. for $C_{11}H_{20}O_5Na^+$ $[M+Na]^+$: 255.1208, found: 255.1195.

Physical data for **5**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 5.72 (s, 1H *min*), 5.68 (s, 1H *maj*), 4.48 (s, 1H *maj*), 4.47 (s, 1H *min*), 4.38 (s, 1H *maj*), 4.46 (d, J = 2.7 Hz, 1H *min*), 4.25-4.18 (m, 2H *min*), 4.22 (q, J = 7.1 Hz, 2H *maj*), 2.12-2.03 (m, 1H *min*), 2.03-1.94 (m, 2H), 1.80 (dd, J = 13.7, 5.9 Hz, 1H *min*), 1.77-1.58 (m, 4H), 1.50 (dd, J = 13.7, 6.1 Hz, 1H *min*), 1.35-1.22 (m, 3H), 1.18 (d, J = 7.0 Hz, 3H *min*), 0.92 (d, J = 6.8 Hz, 3H *maj*). ^{13}**C NMR** (125 MHz, $CDCl_3$) δ 171.5, 171.3, 104.0, 103.1, 82.2, 82.2, 72.9, 61.4, 32.4, 31.9, 30.5, 26.9, 24.5, 21.8, 17.1, 17.0, 14.2. **IR** (ATR) ν_{max}: 2958, 2360, 1758, 1726, 1462, 1370, 1343, 1286. **HRMS** (ESI) Calcd. for $C_{10}H_{16}O_4Na^+$ $[M+Na]^+$: 223.0946, found: 223.0941.

Physical data for **15**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 5.55 (s, 1H *min*), 5.51 (s, 1H *maj*), 4.18-4.14 (m, 2H), 4.00 (s, 1H *maj*), 3.97 (s, 1H *min*), 3.61-3.51 (m, 4H), 2.08-1.97 (m, 1H *maj*), 1.95 (s, 2H), 1.80 (dd, J = 13.7, 5.9 Hz, 1H *maj*), 1.77-1.59 (m, 3H), 1.50 (dd, J = 13.7, 6.1 Hz, 1H *maj*), 1.46-1.36 (m, 1H *min*), 1.35-1.22 (m, 3H), 1.17 (d, J = 7.1 Hz, 3H *maj*), 0.85 (d, J = 7.0 Hz, 3H *min*). ^{13}**C NMR** (125 MHz, $CDCl_3$) δ 101.8, 101.4, 78.9, 79.1, 76.1, 64.3, 31.4, 31.0, 29.5, 27.2, 24.5, 22.1, 16.8, 16.7.

Physical data for **17**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 6.92-6.84 (m, 1H), 6.10-6.07 (m, 1H), 6.07-6.04 (m, 1H), 5.67-5.64 (m, 1H), 5.64-5.61 (m, 1H), 4.66-4.61 (m, 1H), 4.25-4.18 (m, 2H), 4.02-3.99 (m, 1H), 2.13-2.02 (m, 2H), 1.87-1.77 (m, 3H), 1.72-1.64 (m, 2H), 1.60-1.53 (m, 1H), 1.51-1.41 (m, 1H), 1.39-1.32 (m, 1H), 1.33-1.28 (m, 6H), 1.19 (d, J = 7.1 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H).

Physical data for **18**:

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 4.75-4.72 (m, 1H *min*), 4.41-4.39 (m, 2H), 4.30-4.10 (m, 5H), 3.77 (dd, J = 10.3, 2.1 Hz, 1H *min*), 3.40 (dd, J = 9.8, 2.4 Hz, 1H *maj*), 3.35 (s, 3H *maj*), 3.24 (s, 3H *min*), 1.95-1.62 (m, 6H), 1.62-1.38 (m, 3H), 1.33-1.23 (m, 7H), 0.96-0.82 (m, 24H), 0.16 (s, 3H *maj*), 0.14 (s, 3H *min*), 0.06 (s, 3H *maj*), 0.04 (s, 3H *min*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 172.9, 172.6, 103.9, 98.4, 83.1, 76.5, 72.9, 72.7, 61.1, 60.8, 55.9, 54.4, 31.3, 31.3, 29.8, 29.7, 29.4, 26.6, 26.1, 18.6, 17.7, 16.8, 14.5, 14.4. **IR** (ATR) ν_{max}: 2954, 2930, 2853, 2358, 1745, 1471, 1461, 1444, 1371, 1348, 1285. **HRMS** (ESI) Calcd. for $C_{17}H_{34}O_5SiNa^+$ $[M+Na]^+$: 369.2073, found: 369.2058.

Physical data for **22**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 7.45-7.42 (m, 1H *min*), 7.38-7.33 (m, 3H), 7.25-7.18 (m, 4H), 7.15-7.10 (m, 2H), 5.77-5.74 (m, 1H *maj*), 4.67 (dd, J = 11.5, 1.8 Hz, 1H *min*), 4.43 (d, J = 2.0 Hz, 1H *maj*), 4.38 (d, J = 2.1 Hz, 1H *min*), 4.22 (dd, J = 10.3, 2.0 Hz, 1H *maj*), 4.14-4.01 (m, 2H *min*), 3.97 (dq, J = 10.7, 7.1 Hz, 1H *maj*), 3.73 (dq, J = 10.7, 7.2 Hz, 1H *maj*), 3.47 (dd, J = 9.8, 2.2 Hz, 1H *maj*), 2.21-2.11 (m, 1H *maj*), 2.01-1.78 (m, 4H), 1.78-1.62 (m, 3H), 1.59-1.48 (m, 1H *maj*), 1.37-1.20 (m, 1H *min*), 1.20-1.15 (t, J = 7.4 Hz, 3H *min*), 0.99 (t, J = 7.1 Hz, 3H *maj*), 0.97-0.93 (m, 21H), 0.88-0.84 (m, 3H *min*), 0.18 (s, 3H *maj*), 0.16 (s, 3H *min*), 0.06 (s, 3H *maj*), 0.05 (s, 3H *min*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 129.3, 128.6, 125.9, 84.9, 77.3, 72.5, 60.9, 31.2, 30.0, 28.2, 26.0, 13.8, -4.0, -5.6.

Physical data for **23**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 5.34-5.31 (m, 1H *maj*), 4.65-4.60 (m, 1H *min*), 4.41 (d, J = 2.0 Hz, 1H *maj*), 4.37 (d, J = 2.4 Hz, 1H *min*), 4.33-4.23 (m, 2H), 4.22-4.13 (m, 2H), 4.00

(dd, *J* = 10.3, 1.9 Hz, 1H *maj*), 3.49 (dd, *J* = 9.8, 2.2 Hz, 1H *min*), 2.74 (d, *J* = 5.9 Hz, 1H *min*), 2.19 (s, 1H *maj*), 1.90-1.82 (m, 2H *maj*), 1.82-1.74 (m, 2H *min*), 1.74-1.66 (m, 3H), 1.66-1.55 (m, 2H), 1.45-1.12 (m, 7H), 1.00-0.75 (m, 24H), 0.16 (s, 3H *maj*), 0.16 (s, 3H *min*), 0.06 (s, 6H).

Physical data for **25**:

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.50-7.40 (m, 4H), 7.34-7.24 (m, 6H), 4.38 (t, *J* = 6.5 Hz, 1H), 4.28 (d, *J* = 2.3 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.52-3.42 (m, 1H), 2.18 (d, *J* = 9.8 Hz, 1H), 2.15-1.75 (m, 3H), 1.65-1.41 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.93-0.88 (m, 9H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.13 (s, 3H), 0.05 (s, 3H).

Physical data for **26**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 4.75-4.72 (m, 1H *min*), 4.26 (dd, *J* = 9.6, 1.9 Hz, 1H *maj*), 3.90 (td, *J* = 5.1, 2.4 Hz, 1H *maj*), 3.86 (td, *J* = 4.9, 2.0 Hz, 1H *min*), 3.82-3.70 (m, 4H), 3.48 (dd, *J* = 10.3, 1.9 Hz, 1H *min*), 3.45 (s, 3H *maj*), 3.34 (s, 3H *min*), 3.16 (dd, *J* = 9.8, 2.3 Hz, 1H *min*), 2.44-2.40 (m, 1H *min*), 2.38 (t, *J* = 5.8 Hz, 1H *maj*), 1.86-1.65 (m, 5H), 1.56-1.39 (m, 3H), 1.27-1.16 (m, 2H), 0.94-0.88 (m, 18H), 0.88 (s, *J* = 6.5 Hz, 3H *min*), 0.87 (s, *J* = 6.5 Hz, 3H *maj*), 0.12 (s, 3H *min*), 0.11 (s, 3H *maj*), 0.10 (s, 3H *maj*), 0.09 (s, 3H *min*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 104.0, 98.4, 83.9, 77.0, 72.4, 72.2, 65.3, 64.9, 56.2, 54.7, 31.7, 31.4, 30.6, 30.3, 29.9, 27.0, 26.1, 26.0, 17.9, 17.2, -3.9, -4.0, -4.7, -4.8. **IR** (ATR) ν_{max}: 3468, 2952, 2929, 2856, 2359, 1462, 1387, 1251, 1127. **HRMS** (ESI) calcd. for $C_{15}H_{32}O_4SiNa^+$ $[M+Na]^+$: 327.1968, found: 327.1935.

Physical data for **27**:

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 9.75 (d, *J* = 2.3 Hz, 1H), 9.75 (d, *J* = 2.1 Hz, 1H), 4.70 4.66 (m, 1H), 4.20 (dd, *J* = 9.5, 2.1 Hz, 1H), 4.16-4.14 (m, 1H), 4.13 (dd, *J* = 2.6, 1.2 Hz, 1H), 3.78 (dd, *J* = 10.2, 2.1 Hz, 1H), 3.46-3.39 (m, 1H), 3.38 (s, 3H), 3.23 (s, 3H), 1.94-1.63 (m, 8H), 1.62-1.40 (m, 2H), 0.97-0.91 (m, 18H), 0.89-0.82 (m, 6H), 0.14 (s, 3H), 0.12 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H).

Physical data for **28**:

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 15.7, 4.3 Hz, 1H *maj*), 7.12 (dd, *J* = 15.6, 4.4 Hz, 1H *min*), 6.06 (dt, *J* = 15.7, 1.9 Hz, 2H), 4.74-4.70 (m, 1H *min*), 4.49-4.44 (m, 1H *maj*), 4.43-4.38 (m, 1H *min*), 4.30-4.14 (m, 5H), 3.47 (s, 3H *maj*), 3.42-3.36 (m, 1H *min*), 3.31 (s, 3H *min*), 3.09 (dd, *J* = 9.9, 3.0 Hz, 1H *maj*), 1.85-1.63 (m, 5H), 1.62-1.35 (m, 4H), 1.30 (t, *J* = 7.1 Hz, 6H), 1.34-1.14 (m, 1H *maj*), 0.96-0.82 (m, 24H), 0.09 (s, 3H *min*), 0.09 (s, 3H *maj*), 0.08 (s, 6H). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 166.6, 153.0, 148.7, 148.2, 121.0, 120.8, 117.7, 103.6, 98.4, 84.9, 83.6, 77.0, 74.2, 74.0, 68.4, 60.4, 56.0, 54.5, 31.9, 31.7, 31.6, 31.4, 31.4, 31.2, 29.9, 27.3, 26.1, 26.0, 26.0, 18.3, 18.3, 17.7, 16.9, 16.6, 14.4. **IR** (ATR) ν_{max}: 2953, 2928, 2855, 1724, 1707, 1643, 1472, 1462, 1387, 1259. **HRMS** (ESI) calcd. for $C_{19}H_{36}O_5SiNa^+$ $[M+Na]^+$: 395.2230, found: 395.2212.

Physical data for **29-*E*** (a mixture of isomers in 2.4: 1 ratio):

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 5.98-5.76 (m, 4H), 4.73-4.69 (m, 1H *maj*), 4.32-4.20 (m, 3H), 4.19-4.10 (m, 4H), 3.44 (s, 3H *min*), 3.32-3.24 (m, 1H *maj*), 3.27 (s, 3H *maj*), 2.98 (dd, *J* = 9.8, 2.7 Hz, 1H *min*), 1.93 (s, 1H *min*), 1.80-1.53 (m, 6H), 1.52-1.30 (m, 4H), 1.27-1.08 (m, 1H *min*), 0.94-0.80 (m, 24H), 0.06 (m, 3H *maj*), 0.05 (s, 3H *min*), 0.03 (s, 3H *maj*), 0.02 (s, 3H *min*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 132.2, 131.6, 129.6, 129.4, 103.6, 98.2, 84.1, 77.5, 74.1, 73.7, 63.2, 55.8, 54.3, 31.7, 31.2, 30.8, 30.8, 29.8, 27.1, 25.9, 25.9, 18.2, 17.6, 16.8, -4.1, -4.2, -5.0, -5.1. **IR** (ATR) ν_{max}: 3412, 2950, 2928, 2855, 1462, 1386, 1251. **HRMS** (ESI) calcd. for $C_{17}H_{34}O_4SiNa$ $[M+Na]^+$: 353.2124, found: 353.2119.

Physical data for **29-*Z*** (a mixture of isomers in 3.9: 1 ratio):

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 5.97-5.79 (m, 2H), 5.77-5.61 (m, 2H), 4.77-4.67 (m, 2H), 4.66-4.59 (m, 1H *min*), 4.34 (dd, J = 9.4, 1.8 Hz, 1H *maj*), 4.22-4.10 (m, 2H), 4.00-3.87 (m, 2H), 3.50 (s, 3H *maj*), 3.40 (dd, J = 10.2, 2.7 Hz, 1H *min*), 3.33 (s, 3H *min*), 3.15 (dd, J = 9.9, 3.0 Hz, 1H *maj*), 2.92 (dd, J = 9.5, 3.5 Hz, 1H *maj*), 2.65 (dd, J = 7.6, 4.4 Hz, 1H *min*), 1.87 1.46 (m, 6H), 1.47-1.13 (m, 4H), 1.03-0.80 (m, 24H), 0.85-0.55 (m, 6H), 0.05 (s, 3H *min*), 0.04 (s, 3H *maj*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 134.5, 134.2, 130.2, 129.4, 103.8, 98.5, 83.9, 77.6, 70.9, 70.3, 58.1, 57.8, 56.6, 54.6, 32.0, 31.4, 31.2, 29.9, 27.4, 25.8, 18.1, 18.0, 17.4, -4.3, -5.0.

Physical data for **30**:

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 6.22-6.08 (m, 2H), 5.94-5.81 (m, 2H), 4.79-4.75 (m, 4H), 4.74-4.71 (m, 1H *min*), 4.39-4.34 (m, 1H *maj*), 4.34-4.29 (m, 1H *min*), 4.29-4.23 (m, 1H *maj*), 3.40 (s, 3H *maj*), 3.38-3.31 (m, 1H *min*), 3.32 (s, 3H *min*), 3.08-3.02 (m, 1H *maj*), 3.03 (s, 3H *maj*), 3.03 (s, 3H *min*), 1.83-1.60 (m, 4H), 1.60-1.33 (m, 5H), 1.30-1.13 (m, 1H *maj*), 0.95-0.90 (m, 18H), 0.89 (d, J = 6.4 Hz, 3H *min*), 0.89 (d, J = 6.4 Hz, 3H *maj*), 0.10 (m, 3H *min*), 0.09 (s, 3H *maj*), 0.07 (s, 3H *min*), 0.06 (s, 3H *maj*). **IR** (ATR) ν_{max}: 2930, 2856, 1462, 1355, 1253. **HRMS** (ESI) calcd. for $C_{18}H_{36}O_6SiNaS$ $[M+Na]^+$: 431.1900, found: 431.1898.

Physical data for **31**:

^{1}**H NMR** (500 MHz, $CDCl_3$) δ 7.47-7.40 (m, 4), 7.30-7.24 (m, 5), 7.24-7.18 (m, 1H *min*), 6.10 (dd, J = 15.3, 5.0 Hz, 1H *min*), 5.93 (dd, J = 15.5, 5.5 Hz, 1H *maj*), 5.89-5.80 (m, 1H *min*), 5.80-5.72 (m, 1H *maj*), 5.69 (d, J = 5.2 Hz, 1H *maj*), 4.73 (d, J = 6.6 Hz, 2H *min*), 4.70 (d, J = 9.7 Hz, 1H *min*), 4.64-4.56 (m, 2H *maj*), 4.37-4.33 (m, 1H *min*), 4.26 (d, J = 5.3 Hz, 1H *maj*), 3.81 (dd, J = 10.0, 2.3 Hz, 1H *maj*), 3.08 (dd, J = 9.9, 2.7 Hz, 1H *maj*), 3.01 (s, 3H *min*), 2.95 (s, 3H *maj*), 2.14-2.04 (m, 1H *maj*), 1.98-1.91 (m, 1H *maj*), 1.89-1.79 (m, 2H *min*), 1.78-1.69 (m, 1H *maj*), 1.69-1.62 (m, 2H), 1.57-1.49 (m, 2H), 1.32-1.24 (m, 1H *min*), 0.96-0.82 (m, 24H), 0.07 (s, 3H *min*), 0.04 (s, 6H), 0.01 (s, 3H *maj*). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 138.6, 131.1, 128.8, 126.7, 122.4, 85.3, 78.0, 73.3, 70.0, 38.2, 31.2, 31.1, 28.7, 26.0, 26.0, 18.4, 17.7, -4.0, -4.9. **IR** (ATR) ν_{max}: 2930, 2856, 1584, 1462, 1439, 1358, 1252. **HRMS** (ESI) calcd. for $C_{23}H_{38}O_5SiNaS_2$ $[M+Na]^+$: 509.1828, found: 509.1820.

Physical data for **32**:

$[\alpha]_D^{23}$-5.44 (c 1.7, $CHCl_3$). ^{1}**H NMR** (300 MHz, $CDCl_3$) $\boldsymbol{\delta}$ 6.10 (ddt, J = 15.5, 5.9, 1.1 Hz, 1H), 5.90 (dtd, J = 15.6, 6.3, 1.2 Hz, 1H), 4.79-4.73 (m, 2H), 4.41-4.35 (m, 1H), 3.94 (dd, J = 9.6, 2.5 Hz, 1H), 3.05 (s, 3H), 2.61 (ddd, J = 17.9, 5.9, 3.7 Hz, 1H), 2.43 (ddd, J = 17.8, 11.2, 6.4 Hz, 1H), 2.05-1.81 (m, 2H), 1.66-1.48 (m, 1H), 1.08 (d, J = 6.6 Hz, 3H), 0.92 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H). ^{13}**C NMR** (75 MHz, $CDCl_3$) δ 171.2, 136.4, 124.6, 88.0, 72.7, 69.5, 38.3, 29.9, 29.1, 27.9, 25.9, 17.8, -4.1, -4.8. **IR** (ATR) ν_{max}: 2930, 2857, 2360, 1736, 1584, 1462, 1354, 1251. **HRMS** (ESI) calcd. for $C_{17}H_{32}O_6SiNaS$ $[M+Na]^+$: 415.1587, found: 415.1589.

Physical data for **3**:

^{1}**H NMR** (200 MHz, $CDCl_3$) δ 6.05-5.82 (m, 2H), 4.73 (d, J = 6.7 Hz, 2H), 4.23 (dd, J = 7.9, 5.5 Hz, 1H), 3.67 (s, 3H), 3.58 (dd, J = 7.9, 6.6 Hz, 1H), 3.03 (s, 3H), 2.50-2.35 (m, 2H), 2.01-1.85 (m, 1H), 1.08-1.63 (m, 1H), 1.62-1.44 (m, 1H), 1.44 (s, 3H), 1.39 (s, 3H), 0.92 (d, J = 6.8 Hz, 3H). ^{13}**C NMR** (50 MHz, CDCl) δ 174.1, 135.1, 125.9, 108.9, 84.6, 78.9, 69.0, 51.5, 38.1, 35.1, 31.6, 29.6, 28.1, 27.0, 26.8, 15.7. **IR** (ATR) ν_{max}: 2950, 2925, 2880, 1736, 2840, 2355, 2334, 1738, 1460, 1439, 1360, 1256.

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