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GC–MS characterisation and chemometric differentiation of volatile fractions from inflorescences of selected *Echinacea* and *Rudbeckia* species

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Abstract: Volatile fractions obtained by hydrodistillation from inflorescences of *Echinacea purpurea*, *E. paradoxa*, *Rudbeckia fulgida*, *R. hirta* and *R. laciniata* cultivated in Estonia were analysed by GC–MS. In total, 104 compounds were identified and semi-quantified. The highest yields were recorded for *E. purpurea* (0.62%) and *R. hirta* (0.53%). The volatile profiles showed clear interspecific differentiation. *E. purpurea* was characterised by a predominance of monoterpene hydrocarbons (notably *o*-cymene, α-pinene and camphene), whereas *Rudbeckia* species were dominated by sesquiterpene hydrocarbons, particularly germacrene D and eremophilene. *E. paradoxa* exhibited a distinct profile enriched in oxygenated sesquiterpenes, including *(E)*-α-bergamotene and α-cadinol. PCA and HCA consistently separated the taxa studied by their volatile composition and showed high within-species similarity. To the best of our knowledge, this is the first report on the volatile composition of *E. paradoxa* inflorescences. The results highlight pronounced chemical differentiation among the examined taxa and support the use of volatile constituents as auxiliary chemotaxonomic markers within the tribe Heliantheae.

Keywords: Asteraceae; essential oil; gas chromatography; chemometric analysis.

INTRODUCTION

The close botanical relationship between *Echinacea* and *Rudbeckia* has long been recognised in classical classifications of the Asteraceae.[1] Subsequent molecular phylogenetic studies based on nuclear and chloroplast DNA markers have confirmed their placement within a coherent clade of the Heliantheae alliance.[2] In this context, comparative investigation of volatile secondary metabolites may provide additional chemotaxonomic information and contribute to the evaluation of chemical differentiation within and between these genera.

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Among the species of *Echinacea*, *E. purpurea* (L.) Moench is widely known as a medicinal plant with documented antioxidant, immunomodulatory, anti-inflammatory and antimicrobial properties.[3] Its fresh herb is recognised by the European Medicines Agency as a well-established herbal medicinal product for short-term prevention and treatment of the common cold,[4] while *E. angustifolia* radix is accepted as a traditional herbal medicinal product.[5,6] The chemistry and pharmacology of *Echinacea* species have been extensively reviewed, [7–10] and extracts have been studied for their effects on immune responses and viral infections, including SARS-CoV-2.[11–13] Owing to its well-documented secondary metabolite diversity, the genus continues to attract interest in phytochemistry.[14]

The volatile composition of *E. purpurea* has been previously studied by several authors.[15–21] In contrast, data on volatile fractions of other *Echinacea* and *Rudbeckia* species remain limited. The essential oil of *R. laciniata* has been reported by Miyazawa *et al*. (1981)[22] and Lawson *et al*. (2020)[23], and leaf oils of *R. hirta* and *R. fulgida* were analysed by Stewart *et al*. (2014).[24] However, comparative data on volatile fractions of inflorescences of *E. paradoxa*, *R. fulgida* and *R. hirta* are lacking.

The aim of the work was a comparative analysis of volatile compounds in some *Echinacea* and *Rudbeckia* species cultivated in Estonia, and to evaluate their chemotaxonomic significance within and between the genera. To the best of our knowledge, this is the first time that we have investigated the volatiles, including the terpenoid composition, in flowers of *E. paradoxa, R. fulgida,* and *R. hirta*. To the best of our knowledge, the volatile profile of *E. paradoxa* has not been previously studied.

EXPERIMENTAL

Plant material

Inflorescences of *Echinacea* and *Rudbeckia* spp cultivated in Estonia were collected from private gardens in the summer of 2025 (Table I). The studied plant species were identified by their collectors based on the Estonian Plant Identifier[25] and a special app (FloraIncognita, Ilmenau, Germany), and the identity was additionally checked based on photographs by professors O. Koshovyi and A. Raal. Voucher specimen is deposited at the Institute of Pharmacy, University of Tartu (Asteraceae/Ech1-2 and Asteraceae/Rud1-3). The plant material was dried for 10 days at room temperature, protected from the sun, and stored in paper bags before distillation.

Hydrodistillation of volatile fractions

Immediately before distillation, the plant material was ground in a coffee grinder (particle size 1-2 mm). Volatile profiles were obtained by hydrodistillation of *Echinacea* and *Rudbeckia* samples using the European Pharmacopoeia apparatus of essential oil.[26] The plant materials (30 g) were hydrodistilled with 300 mL of purified water in a 1000 mL round-bottom flask for 3 hours (2–3 mL/min). Hexane (0.5 mL) was added to a graduated tube to remove the distilled oil. The yield of volatile fractions was determined according to the European Pharmacopoeia method, using xylene as a measuring solvent.[26]

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TABLE I. The plant material cultivated and collected from private gardens in Estonia

Species	Cultivated and collected from and by	Photos*
Echinacea paradoxa Britton	Tartu	
Echinacea purpurea L.	Tartu	
Rudbeckia fulgida Aiton	Kaika village, Antsla parish, Võru county	
Rudbeckia hirta L.	Sirvaste village, Kanepi parish, Põlva county	
Rudbeckia laciniata L.	Ollepa village, Türi parish, Järva county	

*Photos by O. Koshovyi

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GC-MS analysis of volatile fractions

The samples of volatile profile were analysed on an Agilent 6890/5973 GC-MS system, run using MSD Chemstation. 1 µL of the sample was introduced into Agilent HP-5MSUI column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) using split mode (5:1). The injector temperature was 280 ºC and the carrier gas (He) flow was kept constant 1 mL min^{-1} throughout the whole analysis. The oven was held at 50 °C for 2 minutes, followed by a ramp of 4 °C/min to a final temperature of 280 °C, which was maintained for 5 minutes.

The MSD was operated in EI mode at 70 eV, scanning across the mass range of 29–400 *m/z* with a delay time of 4 minutes and a scan speed of 3.8 scans per second. The data were analysed using the Agilent MassHunter Software package, applying a deconvolution algorithm at different window size factors. Resulted compounds were identified by using NIST23 library with Match Factor ≥ 90 and by retention indexes (relative to n-alkanes C8 – C30) or obtained by the analysis of the reference compounds. The area percentages of each peak were calculated from the total areas in the chromatograms without using correction factors.[27] The study was conducted on cultivated specimens collected from single, defined locations; therefore, the results reflect species-specific chemical profiles under the given cultivation conditions.

Statistical analysis

Statistical analysis of the volatile profiles was performed using species-level mean relative abundances (%) of selected major volatile constituents. For each sample, GC–MS analyses were carried out in triplicate, and results are expressed as mean values with standard deviations (SD) calculated from three parallel determinations. Prior to multivariate analysis, the data were autoscaled (mean-centred and unit-variance scaled) to minimise the influence of highly abundant compounds. Principal component analysis (PCA) was applied as an unsupervised multivariate method to explore interspecific similarities and differences in volatile composition. PCA results were visualised using a biplot that combined species scores and compound loadings, enabling identification of volatile constituents that contributed most strongly to species discrimination. To complement the PCA results, hierarchical cluster analysis (HCA) was performed using Euclidean distance and Ward's linkage method to evaluate similarities among the investigated taxa based on their volatile profiles. Because the study was based on species-specific volatile profiles obtained from single cultivated accessions, the statistical treatment was restricted to exploratory chemometric methods.

RESULTS AND DISCUSSION

Yield of volatile fractions

The highest yields of volatile fractions were observed (Table SI) in the inflorescences of *E. purpurea* and *R. hirta* (0.62% and 0.53%, respectively), in other plants, it was several times lower (0.12-0.18%). As previously shown, the average yield of five stem-distilled volatile fractions from *E. purpurea* was 0.13%.[15] Pellati *et al*.[20] distilled from aerial parts of *E. purpurea* about 0.03% of essential oil. In the roots of *E. purpurea*, the yield of essential oil was 0.13-0.30% depending on the month of collection.[21] Nyalambisa *et al*.[19] reported that the essential oil yields from leaf and root were rather similar (0.24 and 0.29%, respectively). The aerial parts of *E. laciniata* yielded 0.03% of essential oil.[22] The relatively high yield of volatile fractions in inflorescences of *E. purpurea*

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cultivated in Estonia may reflect both organ-specific accumulation and the influence of northern European growing conditions.

Content of volatile fractions from studied plants

Initially, 138 compounds were identified in the volatile fractions of the studied plant species (Table SI). Of these, 104 plant-synthesized compounds were semi-quantified, excluding substances resulting from contamination and compounds with a content below 0.1%. The volatile profiles showed pronounced interspecific differences in the relative abundance of individual constituents and chemical classes.

At the level of individual constituents, *E. purpurea* was dominated by monoterpene hydrocarbons, particularly *o*-cymene (11.98%), α-pinene (9.28%) and camphene (9.28%), which together accounted for a substantial proportion of its volatile fraction. In *E. paradoxa*, sesquiterpene and oxygenated sesquiterpene constituents prevailed, with germacrene D (8.94%), *(E)*-α-bergamotene (8.51), α-cadinol (7.34%) and perhydrofarnesyl acetone (6.62%) occurring at comparatively high levels. The volatile profiles of *Rudbeckia* species were characterised by the quantitative dominance of sesquiterpene hydrocarbons germacrene D (12.49-16.55%), and eremophiliene (7.75-10.23%), which are the major components in *R. hirta* and *R. fulgida*. *R. laciniata* shared this sesquiterpene-rich pattern but additionally showed elevated levels of limonene (14.63%) and β-pinene (5.51%). The classification of volatile constituents into pharmacognostic groups revealed clear interspecific differences in chemical profiles (Table S1). The volatile fractions of *Echinacea* spp. are chemically diverse and dominated by mono- and sesquiterpenes, oxygenated terpenes and aliphatic aldehydes. These constituents exhibit recognisable biological activities but do not account for the primary therapeutic effects traditionally and clinically associated with *Echinacea*.

The PCA biplot (Fig. 1) revealed a clear separation of the investigated taxa based on their volatile profiles. The first two principal components accounted for 92.0% of the total variance (PC1 = 62.2%, PC2 = 29.8%), indicating that the ordination adequately represented the major patterns in the dataset. *Echinacea purpurea* was positioned on the positive side of PC1 and was associated with higher loadings of α-pinene, *o*-cymene, and camphene. In contrast, *Echinacea paradoxa* was separated along the positive side of PC2 and was aligned with α-cadinol and *(E)*-α-bergamotene. *Rudbeckia laciniata* and *Rudbeckia fulgida* occupied the negative region of both PC1 and PC2 and were associated with germacrene D and eremophilene, whereas *Rudbeckia hirta* formed a separate group in the negative region of PC1. These results demonstrate substantial interspecific variation in volatile composition and support the chemotaxonomic differentiation of the investigated taxa.

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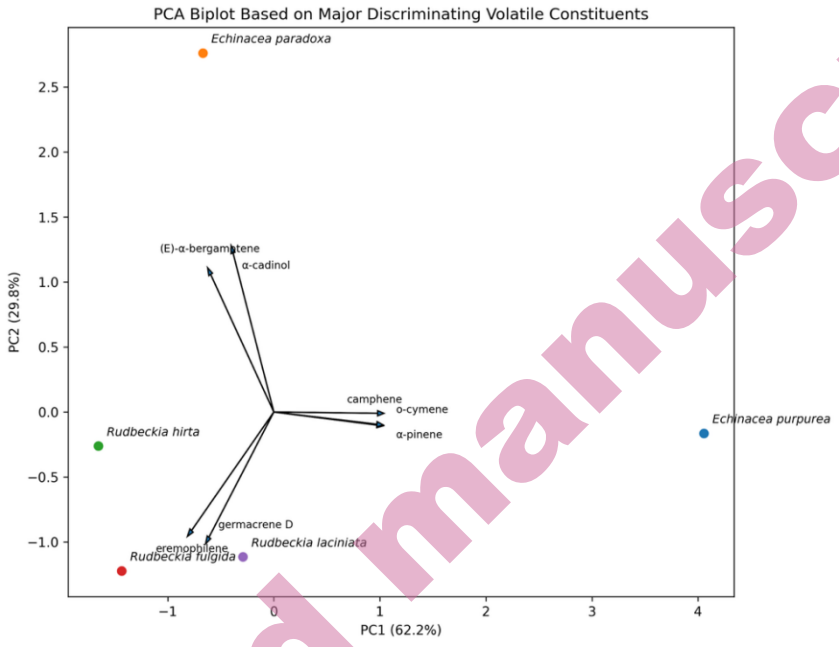

Figure 1. PCA biplot of the major discriminating volatile constituents identified in the aerial parts of *Echinacea* and *Rudbeckia* species. The first principal component (PC1) accounted for 62.2% of the total variance, whereas the second principal component (PC2) explained 29.8%. Arrows represent the loading vectors for the volatile.

Hierarchical cluster analysis (Fig. 2) corroborated the PCA results and revealed a clear separation among the investigated species. Biological replicates clustered tightly within their respective species, indicating high within-species consistency of the volatile profiles. *Echinacea purpurea* formed the most distinct cluster and was separated from all other taxa at the greatest Euclidean distance. *Echinacea paradoxa* also formed a well-defined species-specific cluster, distinct from both *E. purpurea* and the *Rudbeckia* taxa. The three *Rudbeckia* species are grouped into a single major cluster, with *R. fulgida* and *R. laciniata* exhibiting the highest similarity, while *R. hirta* forms a separate subcluster. The concordance between PCA and HCA indicates that the observed differences in volatile composition are robust and reflect clear taxonomic relationships among the studied taxa.

Previous studies have reported germacrene D, α-phellandrene, β-caryophyllene and nerolidol as major constituents of *E. purpurea* essential oils, with marked variation depending on plant organ, developmental stage and analytical approach[19,18,21,22,28,30-32] In contrast, the present study identified *o*-cymene, germacrene D, α-pinene, and camphene as the principal compounds in *E. purpurea* inflorescences cultivated in Estonia.

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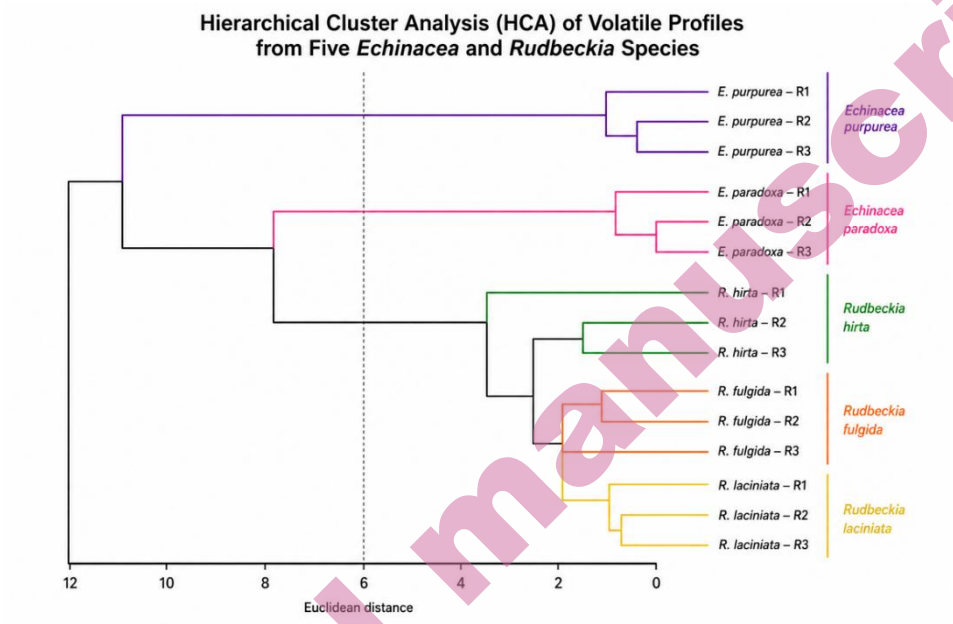

Figure 2. Hierarchical cluster analysis (HCA) dendrogram of the volatile profiles of *Echinacea* and *Rudbeckia* species based on the relative abundances (%) of identified volatile constituents. Clustering was performed using Euclidean distance and Ward's linkage method.

In *E. laciniata*, limonene was the dominant volatile constituent, whereas α- and β-pinene occurred at lower levels than previously reported for aerial plant parts.[22–24] In *R. fulgida* and *R. hirta*, germacrene D was the major component, with species-specific differences reflected by higher proportions of α-cadinol and α-muurolene, respectively, highlighting organ- and condition-dependent variability in volatile composition.

Thus, the major constituents identified in the present study differ in part from those reported previously for *Echinacea* and *Rudbeckia* species. These differences are likely attributable to the use of inflorescences rather than other plant organs, cultivation under northern European climatic conditions, and methodological differences in volatile isolation and analysis. Such variability reflects the well-recognised plasticity of volatile metabolism in Asteraceae rather than data inconsistency.

The observed interspecific differences in volatile composition reflect distinct distributions of monoterpene and sesquiterpene biosynthetic products within the investigated taxa. The predominance of monoterpene hydrocarbons in *E. purpurea* contrasts with the sesquiterpene-rich profiles of the studied *Rudbeckia* species, suggesting divergent regulation of terpene synthase pathways. The enrichment of oxygenated sesquiterpenes in *E. paradoxa* further indicates species-specific

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metabolic differentiation. Such patterns highlight the chemical plasticity of terpenoid metabolism in Asteraceae and support the view that volatile constituents serve as indicators of metabolic diversity rather than as direct functional or pharmacological equivalents.

Overall, this comparative analysis highlights clear chemotaxonomic differentiation between *Echinacea* and *Rudbeckia* species, provides the first detailed volatile profile of *E. paradoxa*, and supports the relevance of volatile constituents as indicators of chemical differentiation within the investigated taxa.

CONCLUSION

The volatile fractions of the investigated *Echinacea* and *Rudbeckia* species exhibited clear interspecific differences, which were consistently confirmed by both PCA and hierarchical cluster analysis. The volatile fractions of the investigated *Echinacea* and *Rudbeckia* species exhibited clear, statistically significant interspecific differences, reflected in both the distribution of individual constituents and the relative proportions of major terpenoid classes. *E. purpurea* was characterised by a predominance of monoterpene hydrocarbons, whereas the studied *Rudbeckia* species were dominated by sesquiterpene hydrocarbons. *E. paradoxa* displayed a distinct profile enriched in oxygenated sesquiterpenes. The present study provides the first detailed GC–MS characterisation of the volatile composition of *E. paradoxa* inflorescences cultivated under northern European conditions. The observed chemical differentiation supports the chemotaxonomic distinction of the investigated taxa within the Heliantheae.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13780, or from the corresponding author on request.

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GC–MS КАРАКТЕРИЗАЦИЈА И РАЗЛИКОВАЊЕ НА ОСНОВУ ХЕМОМЕТРИЈСКИХ МЕТОДА ИСПАРЉИВИХ ФРАКЦИЈА ЦВЕТНОГ ДЕЛА ОДАБРАНИХ ВРСТА *ECHINACEA* И *RUDBECKIA*

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Испарљиве фракције добијене дестилацијом воденом паром, из цветних делова врста *Echinacea purpurea*, *E. paradoxa*, *Rudbeckia fulgida*, *R. hirta* и *R. laciniata* узгајаних у Естонији, испитане су помоћу GC–MS анализе. Укупно су идентификована 104 једињења која су делимично квантификована. Највећи принос забележен је код *E. purpurea* (0,62%) и *R. hirta* (0,53%). Профил испарљивих компоненти јасно показује интерспецифичне разлике. *E. purpurea* карктеришу доминантно монотерпенски угљоводоници (истичу се *o*-цимен, α-пинен, и камфен), док код врста *Rudbeckia* доминирају сесквитерпенски угљоводоници, посебно гермакрен Д и еремофилен. Код *E. paradoxa* је посебан профил, обогаћен сесквитерпенима који садрже кисеоник, укључујући *(E)*-α-бергамотен и α-кадинол. Применом ПЦА и ХЦА анализа могуће је доследно разликовати изучаване врсте преко њихових испарљивих компоненти при чему је показана велика сличност у оквиру врста. Колико је нама познато, ово је први извештај о испарљивом саставу цветног дела *E. paradoxa*. Добијени резултати посебно истичу разлике у хемијском саставу изучаваних врста и подржавају коришћење испарљивих компоненти као помоћног хемотаксономског маркера унутар трибуса Heliantheae.

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