SUPPLEMENTARY MATERIAL TO

# Contribution to the knowledge of the chemical composition, biological activities and activity concentration of $^{40}$K, $^{137}$Cs, $^{226}$Ra and $^{232}$Th *of* the lichen *Evernia prunastri*

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*Lichen material*

Lichen *E. prunastri*, was collected in October 2015 at Vlasina visoravan, (1213 m above sea level, coordinates N 42°41' and E 22°20' Serbia) from *Prunus domestica* bark. The voucher specimen has been deposited in the Herbarium collection at the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš under the acquisition number 10892. The lichen material was air-dried without exposure to direct sunlight for 10 days and stored at ambient temperature (25° ± 2°C) prior further treatment. Taxonomical identifications was performed by dr Bojan Zlatković Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš.

*Preparation of lichen extracts*

For HPLC-UV, GC-MS and GC-FID analysis extracts were prepared according to procedure described by Stojanović et al. (2017).[1] In order to obtain dry acetone extract prior to further testing of biological activities finely ground dry lichen thalli (10 g) was extracted as previously described.[2] The extract yield was 6.59 ±0.5 % (w/w).

*HPLC-UV analysis*

HPLC-UV analysis was performed according the experimental procedure used in a previous research by Stojanović et al. (2017).[1] Identification was conducted using UV spectra and retention time of isolated components.

35 *GC-MS analysis*

36 The volatile components of the acetone, ether, ethyl acetate and dichloromethane extracts of
37 *E. prunastri* were investigated by GC-MS (in triplicate), which were carried out using a
38 7890/7000B GC-MS/MS triple quadrupole system (Agilent Technologies, USA, equipped
39 with a Combi PAL auto sampler) fallowing the experimental conditions described by
40 Stojanović et al. (2017).[1] The percentage composition was computed from the GC-FID peak
41 areas.

42 Constituents were identified by comparison of their linear retention indices (relative to C8 –
43 C40 alkanes on the HP-5MS column) with literature values and their MS with those from
44 Wiley 6, NIST02 and Mass Finder 2.3, by the application of the AMDIS software (the
45 Automated Mass Spectral Deconvolution and Identification System, Ver. 2.1, DTRA/NIST,
46 2011).

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48 *Cytokinesis-block micronucleus assay (CBMN)*

49 The human *in vitro* micronucleus (MN) test is one of the widely used genotoxicity test for
50 monitoring of chromosome damage in human populations. Cytokinesis–block micronucleus
51 assay was performed as previously described.[3,4]

52

53 *Total phenolic content and antioxidant activity*

54 Total phenolic content (TPC) and four antioxidant assays: DPPH and ABTS scavenging
55 radical capacity, CUPRAC (cupric reducing antioxidant capacity) and TRP (total reducing
56 power) were performed as previously described.[5,6] The amount of total phenolic content
57 (TPC) was expressed as mg galic acid equivalents per g of dry extract weight (mg GAE $g^{-1}$
58 dw). The result of the total reducing power assay (TRP) was expressed as mg ascorbic acid
59 equivalents per g of dry extract weight (mg AAE $g^{-1}$ dw) while the result obtained by
60 CUPRAC method was expressed as mg Trolox equivalents per g of dry extract weight (mg
61 TE $g^{-1}$ dw) The abilities of the extract to inhibit DPPH and ABTS radical-cations were
62 expressed as percentage (%).

63

64 *Cholinesterase activity*

65 Assessment of extract effect on cholinesterase activity was performed as previously
66 described.[1,7]

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68 *Antibacterial activity*

Antibacterial activity against two *Gram-positive (Bacillus spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three *Gram-negative* bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017) was examined according to the NCCLS.[8] Experimental procedure was as previously described.[1]

Activity concentrations of $^{40}$K, $^{137}$Cs, $^{226}$Ra and $^{232}$Th

*Sample preparation and counting*

The homogenized lichens samples were dried in the oven at the temperature of 105ºC until constant weight, placed in the plastic Marinelli beakers, sealed and left for 4 weeks to reach radioactive equilibrium.[9] Each prepared sample was put into the HPGe detector and measured for 86000 s. Gamma background was determined prior to the sample measurement by measuring an empty Marinelli baker under identical measurement conditions. The counting time for background measurement was 240000s. It has been later subtracted from the measured gamma spectra of each sample.

*Activity concentration determination*

Activity measurement of the samples was done using a high-resolution coaxial semiconductor detector with high-purity germanium crystal *HPGe ORTEC GEM 50*, with 50% relative efficiency at 1332 keV. The detector was shielded by lead in order to achieve a background level as low as possible. Calibration of the energy and efficiency calibration was done before the measurement. The calibration source used was a commercially available gamma standard, with mixed radionuclides-type MBSS 2 in Marinelli geometry of 0.5 l, developed by the *Inspectorate for Ionizing Radiation Czech Metrological Institute*, with the isotopes $^{241}$Am, $^{109}$Cd, $^{57}$Co, $^{139}$Ce, $^{113}$Sn, $^{85}$Sr, $^{137}$Cs, $^{88}$Y, $^{203}$Hg, and $^{60}$Co. The energy of gamma lines of these radionuclides is very suitable for the calibration and covers the region of interest, i.e. from 30 to 3000 keV. The quality assurance of measurements was carried out by daily efficiency and energy calibration, repeating each sample measurement. Correction of the radioactive decay, and the background, as well as the analysis of results, were obtained using dedicated software program *ORTEC Gamma Vision-32 Model A66-B32 Version 6.01.*

The activities of $^{226}$Ra were determined by its decay products: $^{214}$Pb (295.22 keV, 351.93 keV) and $^{214}$Bi (609.31 keV, 1120.29 keV). In the case of $^{232}$Th two photopeaks of $^{228}$Ac (911.20 and 698.97keV) were used. The activities of $^{40}$K and $^{137}$Cs were derived from the 1460.83 keV and 661.66 keV gamma lines, respectively.

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