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**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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Abstract: This study reports the effect of an acetone extract of *Evernia prunastri* on the micronucleus distribution of human lymphocytes and effect on cholinesterase activity. Furthermore, the antioxidant activity (estimated via DPPH, ABTS, TRP and CUPRAC assays), as well as total phenolic compounds (TPC) and antibacterial activity (against two Gram-positive and three Gram-negative bacteria) were determined. Chemical profiling of four *E. prunastri* extracts (acetone, diethyl ether, ethyl acetate and dichloromethane) was realized by GC–MS and HPLC analysis. In addition, the activity concentrations of ^{40}K , ^{137}Cs , ^{226}Ra and ^{232}Th were established.

Keywords: radionuclides; micronucleus test; cholinesterase test; antioxidant assays; antibacterial assay.

INTRODUCTION

Evernia is a genus of lichenised fungi in the phylum Ascomycota within the family Parmeliaceae. Lichen *Evernia prunastri* (L.) Ach., with the common name oakmoss, belongs to the foliose type of lichens. It is widespread on deciduous trees and conifers, especially on oak trees. The thallus appears fruticose and the colour varies from green to greenish white when dry and sometimes pale yellow–green, indicating the presence of usnic acid.^{1,2}

Major secondary metabolites of *E. prunastri* are evernic acid located in the medulla, and usnic acid, atranorin and chloroatranorin detected in the upper cortex.^{3,4} The chemical composition of *E. prunastri* has been the subject of several thorough studies due to its widespread utilisation in the fragrance industry as a

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fixative base, as documented in a review by Joulain and Tabacchi (2008).⁵ Furthermore, allergic reaction on oakmoss that contains atranol and chloroatranol has also been reported.⁶

The vast majority of lichen secondary metabolites have different biological roles in lichen itself, such as protection against different pathogens, herbivores and insects.⁷ According to published data, acetone and methanol extracts of *E. prunastri* and isolated evernic acid have manifested significant antioxidant, antimicrobial, anticancer and antiproliferative activities.^{2,8,9}

Lichens and mosses have been reported to accumulate a great variety of radionuclides, such as ⁷Be, a light element of cosmogenic origin, ⁴⁰K, a natural radionuclide, anthropogenic fallout nuclides such as ⁵⁵Fe, ⁹⁰Sr, ¹³⁷Cs, ¹⁰⁶Ru, ¹⁴⁴Ce, ¹²⁵Sb and ²³⁹Pu from nuclear power plants and nuclear tests and, finally, naturally occurring heavy metal nuclides such as ²³⁸U and its decay products.^{10–12} Lichens depend on nutrients from the atmosphere, since they have no root system or cuticle as vascular plants and are therefore unlikely to accumulate significant levels of radioactive nuclide and other contaminants from the substrate. The lichen *E. prunastri* was used as bioindicator of radioactive isotope pollution in several biomonitoring studies^{12,13} in order to determine the radioactive fallout after the accident due to their ability to intercept and retain long-lived radionuclides such as ¹³⁷Cs.

Although *E. prunastri* has been the subject of many studies, there are unexplored aspects worthy of examination that should be considered. Regarding the aforementioned, the aim of the present study was to evaluate for the first time the effect of an acetone extract of *E. prunastri* on micronucleus distribution in human lymphocytes and on cholinesterase activity. Additionally, the antioxidant activity and the antibacterial activity against two Gram-positive (*Bacillus subtilis* subsp. *spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella enterica* subsp. *enterica* serotype Abony NCTC 6017) were determined. The GC and HPLC profiles of acetone, ether, ethyl acetate and dichloromethane extracts were investigated as well as the activity concentrations of radionuclides ⁴⁰K, ¹³⁷Cs, ²²⁶Ra and ²³²Th.

EXPERIMENTAL

Details about the lichen material and preparation of the lichen extracts are given in the Supplementary material to this paper.

For HPLC–UV, GC–MS and GC–FID analysis, extracts were prepared according to the procedure described by Stojanović *et al.*¹⁴ In order to obtain dry acetone extract prior to further testing of biological activities, dry finely ground lichen thalli (10 g) was extracted as previously described.¹⁵ The extract yield was 6.59±0.50 mass %.

HPLC–UV analysis

HPLC–UV analysis was performed according to the experimental procedure used in previous research by Stojanović *et al.*¹⁴ Identification was conducted using the UV spectrum and retention time of the isolated components.

GC–MS analysis

The volatile components in the acetone, diethyl ether, ethyl acetate and dichloromethane extracts of *E. prunastri* were investigated by GC–MS (in triplicate), which was realised using a 7890/7000B GC–MS/MS triple quadrupole system (Agilent Technologies, USA, equipped with a Combi PAL auto sampler) following the experimental conditions described by Stojanović *et al.*¹⁴ The percentage composition was computed from the GC-FID peak areas.

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

Cytokinesis-block micronucleus assay (CBMN)

The human *in vitro* micronucleus (MN) test is one of the widely used genotoxicity tests for monitoring chromosome damage in human populations. The cytokinesis-block micronucleus assay was performed as previously described.^{9,16}

Total phenolic content and antioxidant activity

The total phenolic content (TPC) was determined by four antioxidant assays, *i.e.*, DPPH and ABTS scavenging radical capacity, CUPRAC (cupric reducing antioxidant capacity) and TRP (total reducing power), as previously described.^{17,18} The total phenolic contents (TPC) are expressed as mg gallic acid equivalents per g of dry extract weight (mg GAE g⁻¹ dw). The results of the total reducing power assay (TRP) are expressed as mg ascorbic acid equivalents per g of dry extract weight (mg AAE g⁻¹ dw), while the results obtained by the CUPRAC method are expressed as mg trolox equivalents per g of dry extract weight (mg TE g⁻¹ dw). The abilities of the extract to inhibit DPPH and ABTS radical-cations are expressed as percentage (%).

Cholinesterase activity

Assessment of the effect of an extract on cholinesterase activity was made as previously described.^{14,19}

Antibacterial activity

The antibacterial activities of the extracts against two Gram-positive (*Bacillus subtilis* subsp. *spizizenii* ATCC 6633 and *Staphylococcus aureus* ATCC 6538) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella enterica* subsp. *enterica* serotype Abony NCTC 6017) were examined according to the NCCLS.²⁰ The experimental procedure was as that described previously.¹⁴

Activity concentrations of ⁴⁰K, ¹³⁷Cs, ²²⁶Ra and ²³²Th

Sample preparation and counting. The homogenized lichens samples were dried to constant weight in an oven at a temperature of 105 °C, placed in plastic Marinelli beakers, sealed and left for 4 weeks to reach radioactive equilibrium.²¹ Each prepared sample was put into an HPGe detector and measured for 86000 s. The gamma background was determined prior to sample measurement by measuring an empty Marinelli beaker under identical measurement

conditions. The counting time for the background measurement was 240,000 s, and it was 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 a 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 were realised before the measurements. 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 the gamma lines of these radionuclides is very suitable for the calibration and covers the region of interest, *i.e.*, from 30 to 3000 keV. Quality assurance of the measurements was performed by daily efficiency and energy calibrations, repeating each sample measurement. Correction of the radioactive decay, and the background, as well as the analysis of results, were obtained using the 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 and 1120.29 keV). In the case of ^{232}Th , two photopeaks of ^{228}Ac (911.20 and 698.97 keV) were used. The activities of ^{40}K and ^{137}Cs were derived from the 1460.83 and 661.66 keV gamma lines, respectively.

RESULTS AND DISCUSSION

HPLC–UV analysis

High performance liquid chromatography coupled with a UV detector (HPLC–UV) enabled the identification of the following lichens metabolites: evernic acid, evernic acid, usnic acid, atranorin and chloroatranorin (Table I and Fig. 1).

TABLE I. Chemical composition of the acetone (A), diethyl ether (E), ethyl acetate (EA) and dichloromethane (DCM) extracts of *E. prunastri* (as percentages of the total absorbance of the HPLC chromatograms recorded at 254 nm); *SD*: standard deviation

Compound	Absorbance maxima, nm	Mean relative abundance \pm <i>SD</i> , %			
		A	E	EA	DCM
Evernic acid	220, 260, 300	0.5 \pm 0.0	–	0.4 \pm 0.0	0.1 \pm 0.0
Evernic acid	220, 270, 310	31.3 \pm 0.6	61.4 \pm 1.5	29.2 \pm 0.9	15.4 \pm 0.9
Usnic acid	232, 282	1.2 \pm 0.1	1.1 \pm 0.2	1.0 \pm 0.1	0.8 \pm 0.2
Atranorin	215, 252, 320	52.6 \pm 1.1	26.3 \pm 0.8	50.0 \pm 1.2	64.0 \pm 1.9
Chloroatranorin	215, 255, 320, 355	11.8 \pm 0.9	5.9 \pm 0.4	13.9 \pm 0.7	17.8 \pm 1.1

The most abundant components of the acetone and ethyl acetate extracts of *E. prunastri* were atranorin and evernic acid, while in the diethyl ether extract, the amount of evernic acid was double that of atranorin. Atranorin was the predominant component in dichloromethane extract, the second was chloroatranorin followed by evernic acid. The relative abundance of chloroatranorin was variable in the examined extracts. The relative quantity of usnic acid was similar in all

extracts and amounted to about 1 %, while evernic acid was represented by below 1 %, except for the ether extract in which it was not detected. A previous study of Culberson emphasized that evernic acid (depside of the medulla), and atranorin and usnic acid (metabolites of the cortex) were obligatory metabolites of *E. prunastri*.³ Fifteen phenolic compounds were isolated from a methanol extract of *E. prunastri* by Gonzalez *et al.*²² among them were usnic acid, atranorin and evernic acid, which is consistent with the present results. However, it was shown that more than 170 components were identified in oakmoss extracts, including 47 depsides in a comprehensive review but there is no information concerning the relative abundance of the individual components.⁵ According to Kosanić *et al.*,² the aforementioned compounds were identified alongside physodic acid, which is not in consistence with the present results.

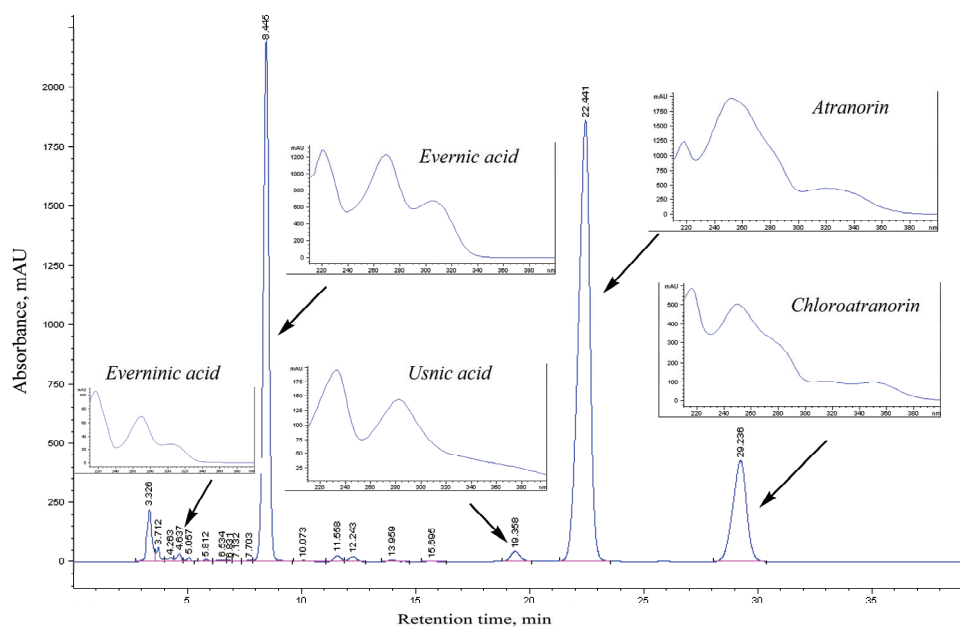


Fig. 1. HPLC chromatogram of the ethyl acetate extract of *E. prunastri* and the UV spectra of the identified constituents.

The intensity of the HPLC signals revealed that the extraction ability of the solvents decreased in the following order: acetone, ethyl acetate, diethyl ether, dichloromethane (ratio of HPLC signals intensity A:E:EA:DCM = 1.6:1.3:1.3:1), similar to the results of a previous study of lichen extracts.²³

GC-MS analysis

Qualitative composition, retention index and relative abundance of the volatiles are given in Table II.

TABLE II. Volatile compounds of acetone (A), ether (E), ethyl acetate (EA) and dichloromethane (DCM) extracts of *E. prunastri*; *SD* – standard deviation, *RI* – experimental retention indices relative to C8–C40 alkanes; *RI*_{lit.} – reference retention indices, t – trace amount (<0.05 %); –, not found

No.	<i>RI</i>	<i>RI</i> _{lit.}	Constituent	Mean amount ± <i>SD</i> , %			
				A	E	EA	DCM
1.	1318	1317 ^a	3-Methoxy-5-methylphenol (<i>syn.</i> orcinol monomethyl ether)	15.1±0.9	6.3±0.3	3.7±0.2	0.7±0.1
2.	1369.4	1369 ^a	5-Methylbenzene-1,3-diol (<i>syn.</i> orcinol)	15.7±0.9	11.5±0.7	3.9±0.2	0.5±0.1
3.	1417	1419 ^b	2,5-Dimethyl-1,3-benzenediol (<i>syn.</i> β-orcinol)	2.1±0.2	0.9±0.1	–	0.3±0.0
4.	1498	1494 ^a	3-Chloro-2,6-dihydroxy-4-methylbenzaldehyde <i>syn.</i> chloroatranol	4.5±0.3	3.9±0.2	6.4±0.4	7.8±0.4
5.	1555	1549 ^b	2,6-Dihydroxy-4-methylbenzaldehyde <i>syn.</i> atranol	14.8±0.8	6.8±0.3	12.2±0.6	11.7±0.5
6.	1585	1580 ^a	Methyl 2-hydroxy-4-methoxy-6-methylbenzoate (<i>syn.</i> sparassol)	0.5±0.0	0.9±0.1	t	0.2±0.0
7.	1658	1659 ^b	Methyl 2,4-dihydroxy-6-methylbenzoate (<i>syn.</i> methyl orsellinate)	0.5±0.0	0.7±0.1	0.7±0.1	0.5±0.0
8.	1668	1666 ^c	Methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (<i>syn.</i> methyl haematommate)	0.2±0.0	2.0±0.2	0.8±0.1	4.0±0.3
9.	1685	–	2-Hydroxy-4-methoxy-6-methylbenzoic acid (<i>syn.</i> evernicic acid)	–	24.2±0.8	4.5±0.2	–
10.	1712	1706 ^b	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (<i>syn.</i> methyl β-orcinolcarboxylate; atraric acid)	45.0±1.0	21.1±0.9	51.1±1.2	52.3±1.2
11.	1743	1745 ^a	2,4-Dihydroxy-6-methylbenzoic acid (<i>syn.</i> orsellinic acid)	–	1.2±0.2	0.9±0.1	1.6±0.2

TABLE II. Continued

No.	RI	RI _{lit}	Constituent	Mean amount \pm SD, %			
				A	E	EA	DCM
12.	1752	1746 ^b	Ethyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (<i>syn.</i> ethyl haematommate)	–	4.9 \pm 0.3	1.8 \pm 0.2	–
13.	2734	2738 ^b	2,6-Diacetyl-7,9-dihydroxy-8,9 b -dimethyl-1,3(2 <i>H</i> ,9 <i>bH</i>)-dibenzofurandione (<i>syn.</i> usnic acid)	0.1 \pm 0.0	2.5 \pm 0.2	5.7 \pm 0.3	3.9 \pm 0.2
14.	2817.9	–	Ergosta-5,8,22-trien-3 β -ol (<i>syn.</i> lichesterol)	–	0.1 \pm 0.0	0.6 \pm 0.0	0.1 \pm 0.0

^aStojanović *et al.*²⁵, ^bMitrović *et al.*²⁴, ^cMitrovic *et al.*²⁶

Mono-aryl compounds, such as orcinol, orcinol monomethyl ether and atranol, are present in similar amounts (about 15 %) in the acetone extract. On the other hand, evernicic acid and orcinol were the major monocyclic aromatic derivatives present in the diethyl ether and ethyl acetate extracts. It is well known that hydrolysis of evernin and atranorin produces methyl β -orcinolcarboxylate.^{5,24} Orcinol monomethyl ether, which was identified in different amounts in all extracts, could be formed during the decarboxylation of evernicic acid.⁵

Evernicic acid is represented in similar amounts as atraric acid in the diethyl ether extract. Atraric acid followed by atranol and chloroatranol are the major components of the ethyl acetate and dichloromethane extracts and are represented in similar quantities. Furthermore, atraric acid is the major component of the acetone extract. It could be assumed that the identified atraric acid is likely to be an artifact generated during the GC–MS analysis by the thermal degradation of atranorin. Haematommic acid is also a hydrolytic product of atranorin and its decarboxylation yields atranol. However, haematommic acid was not detected but significant amount of atranol were. Usnic acid is the only dibenzofuran detected in all extracts but in different quantities. Joulain and Tabacchi reported similar volatile compositions together with the presence of terpenoides and steroids.⁵ The GC–MS profile of the acetone-soluble fraction of the methanol extract of *E. prunastri* revealed the presence of atraric acid (30.1 %) and orcinol (25 %) as the main compounds.²⁴

Cytokinesis-block micronucleus assay (CBMN)

The acetone extract of *E. prunastri* at concentrations of 1.0, 2.0 and 3.0 $\mu\text{g mL}^{-1}$ was tested for *in vitro* protective effect on chromosome aberrations in peripheral human lymphocytes using the cytochalasin-B blocked micronucleus assay (CBMN). The results are presented in Table III.

TABLE III. Incidence of MN, cytokinesis-block proliferation index, distribution of MN per cells and frequency of MN in cell cultures of human lymphocytes treated with different concentrations of an acetone extract of *E. prunastri* (EP) and previously published results for isolated lichens metabolites; MMC – mitomycin C, MN – micronuclei, Bn – binucleated cells, CBPI – cytokinesis-block proliferation index, *SD* – standard deviation. The statistical significance of the difference between the data pairs was evaluated by analysis of variance (one-way ANOVA) followed by the Tukey test. A statistically difference was considered significant at $p < 0.01$; $p < 0.05$

Sample (<i>c</i> / $\mu\text{g mL}^{-1}$)	Mean number of MN/1000 Bn cells \pm SD	Mean amount of Bn cells with MN, % \pm SD	Mean number of MN/Bn cell \pm SD	Mean value of CBPI \pm SD	Frequency of MN, relative to control, %
Untreated cells	26.3 \pm 0.5	2.17 \pm 0.07	1.22 \pm 0.04	1.64 \pm 0.03	100.0
Amifostin (1)	23.3 \pm 0.4 ^{a*}	1.91 \pm 0.07	1.22 \pm 0.07	1.61 \pm 0.04	88.6
MMC (0.1)	33.4 \pm 1.8 ^{a;b*}	2.99 \pm 0.17	1.15 \pm 0.04	1.71 \pm 0.07	127.0
EP (1)	24.8 \pm 0.7 ^{c*}	2.07 \pm 0.06	1.20 \pm 0.05	1.66 \pm 0.01	94.3
EP (2)	23.6 \pm 1.1 ^{a;c*}	1.63 \pm 0.15	1.39 \pm 0.08	2.04 \pm 0.31	89.7
EP (3)	25.0 \pm 1.7 ^c	1.87 \pm 0.30	1.39 \pm 0.13	1.66 \pm 0.04	95.1
Atranorin (2) ^d	24.9 \pm 0.9 ^{a*;c}	2.1 \pm 0.1	1.2 \pm 0.1	1.6 \pm 0.0	88.9
Evernic acid (2) ^d	18.8 \pm 0.6 ^{a,c}	1.6 \pm 0.0	1.1 \pm 0.0	1.6 \pm 0.0	67.1
Usnic acid (2) ^d	14.3 \pm 1.4 ^{a,b,c}	1.2 \pm 0.1	1.2 \pm 0.0	1.7 \pm 0.1	51.1

^aCompared with control groups, statistically significant difference $p < 0.01$; ^{a*}compared with control groups, statistically significant difference $p < 0.05$; ^bcompared with amifostine – WR 2721, statistically significant difference $p < 0.01$; ^{b*}compared with amifostine – WR 2721, statistically significant difference $p < 0.05$; ^ccompared with mitomycin C, statistically significant difference $p < 0.01$; ^{c*}compared with mitomycin C, statistically significant difference $p < 0.05$; ^dStojanović *et al.*⁹

Among the tested extract of *E. prunastri*, the highest activity was at concentration of 2.0 $\mu\text{g mL}^{-1}$. The demonstrated activity is slightly less than the activity of the commercial radioprotectant amifostine. The tested extracts at concentration of 1.0 and 3.0 $\mu\text{g mL}^{-1}$ caused a similar decrease in the MN frequency.

In a previous study, the *in vitro* protective effects of three lichen secondary metabolites were evaluated. Among them, usnic acid exhibited the most prominent effect decreasing the frequency of MN by 48.9 %, evernic acid by 32.9 %, while atranorin demonstrated the weakest activity 11.1 %.⁹ Since the number of micronuclei serves as an indicator of DNA damage, these results indicate that the examined extract of *E. prunastri* at a concentration of 2.0 $\mu\text{g mL}^{-1}$ protects DNA but significantly less than usnic acid, which could be explained by its low content.

The total phenolic content and antioxidant activity

In the present study, the *in vitro* antioxidant potential of an acetone extract of *E. prunastri* was estimated by determining its total phenolic content (*TPC*), its ability to scavenge DPPH and ABTS radicals, its total reducing power (*TRP*) and its cupric reducing antioxidant capacity (*CUPRAC*).

The total phenolic content (*TPC*) is expressed as mg gallic acid equivalents (GAE) per g of dry extract weight. The *TPC* analyses indicated a high content of phenolic compounds in the acetone extract of *E. prunastri*, 611.0 ± 63 mg GAE g⁻¹. The obtained value for the total phenol content is considerably higher than those previously published by Mitrović *et al.*⁸ and Stojanović *et al.*²⁷ for a methanol extract (80.73 ± 1.25 mg GAE g⁻¹ dw and 18.24 ± 0.27 µg GAE mg⁻¹ dw, respectively). The present results could not be compared with those of Kosanić *et al.*² because they expressed the amount of total phenolic compounds as the pyrocatechol equivalent, 34.05 ± 1.065 µg PE mg⁻¹. The assessment of DPPH and ABTS scavenging activity demonstrated that the extract reduced the concentration of DPPH and ABTS radicals by 85.8 and 91.2 %, respectively, while the *IC*₅₀ values were 8.74 mg mL⁻¹ for DPPH and 8.22 mg mL⁻¹ for the ABTS assay, approximately ten times higher than those obtained previously by Stojanović *et al.*²⁷ (727.7 ± 0.60 µg mL⁻¹). Namely, Kosanić *et al.*² reported *IC*₅₀ values of the DPPH assay for the *E. prunastri* acetone extract of 663.12 µg mL⁻¹ and for evernic acid, a value of 322.44 µg mL⁻¹. Mitrović *et al.*⁸ obtained a value higher than 1000.00 µg mL⁻¹ for the DPPH activity of *E. prunastri* methanol extract.² The result of the total reducing power assay (*TRP*, ability of antioxidants to reduce hexacyanidoferrate(III) to hexacyanidoferrate(II), was 0.6013 ± 0.009 mg AAE g⁻¹ dw. The value of the *TRP* assay for methanol extract of *E. prunastri* was 35.5 ± 0.2 µg AAE mg⁻¹ dw.²⁷ The result obtained by CUPRAC method for *E. prunastri* was 20.7 ± 0.5 mg TE g⁻¹ dw. This result was similar to previously obtained values for the acetone extract of *Umbilicaria crustulosa* (Ach.) Frey of 19.76 ± 0.02 µg TE mg⁻¹ dw.²⁸

Antibacterial activity

The results of the antibacterial assay of the acetone extract of *E. prunastri* against five different bacteria are given in Table IV. The maximal inhibition zone for the tested bacteria was 25 mm for *Bacillus subtilis* subsp. *spizizenii*. The antibacterial activity of the tested extract towards *Staphylococcus aureus* was slightly weaker, with an inhibition zone of 20 mm. The Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* subsp. *enterica* serotype Abony were resistant. The antimicrobial activities were compared with those of the standard antibiotics, streptomycin and chloramphenicol, which had a stronger activity than the tested samples. The obtained results are not entirely in accordance with those previously published. According to Kosanić *et al.*,² the acetone extract of *E. prunastri* and isolated evernic acid manifested prominent antimicrobial activity against the tested microorganisms. Mitrović *et al.*⁸ reported strong antifungal activity of the methanol extract of *E. prunastri* and significant antibacterial activity against the tested bacteria.

TABLE IV. Bactericidal activity (in mm including disc diameter of 12.7 mm) of *E. prunastri* acetone extract (1 mg per disc) and the antibiotics, streptomycin (10 µg per disc diameter of 6 mm) and chloramphenicol (30 µg per disc diameter of 6 mm); dash (–) stands for no activity. The results are given as mean ± SD

Sample	Diameter of bactericidal zones, mm				
	<i>E. coli</i>	<i>B. subtilis</i> subsp. <i>spizizenii</i>	<i>P. aeruginosa</i>	<i>S. enterica</i> subsp. <i>enterica</i> serotype Abony	<i>S. aureus</i>
<i>E. prunastri</i>	–	25±0.3	–	–	20±0.2
Chloramphenicol	22±0.1	32±0.3	17±0.2	28±0.1	29±0.3
Streptomycin	13±0.2	22±0.3	18±0.1	16±0.3	20±0.2

Cholinesterase activity

The inhibition by the acetone extract of *E. prunastri* on cholinesterase activity was dose-dependent. The extract at a concentration of 1.0 mg mL⁻¹ manifested an insignificant inhibition of cholinesterase of 1.8 %, while a more concentrated extract of 10 mg mL⁻¹ exhibited a higher inhibition effect (22.2 %) on pooled human serum cholinesterase. In the conducted experiment, neostigmin bromide (as a standard cholinesterase inhibitor) inhibited cholinesterase to extent of 98.6 %.

Activity concentration of radionuclides ⁴⁰K, ¹³⁷Cs, ²²⁶Ra and ²³²Th

The activity of ⁴⁰K was 108 Bq kg⁻¹, while the activities of ¹³⁷Cs, ²²⁶Ra and ²³²Th were below the minimal detectable activities, which are 3 Bq kg⁻¹ for ¹³⁷Cs, 20 Bq kg⁻¹ for ²²⁶Ra and 15 Bq kg⁻¹ for ²³²Th. The uncertainty is presented at a 95 % level of confidence.

Caesium-137 is reported to be the main source of long term contaminations after atmospheric tests of atomic bombs and nuclear accidents. It is one of the most problematic fission products because it easily spreads in nature due to the high water solubility of its chemical compounds.²¹ Lichen has been recognized for their ability to retain long lived radionuclides such as ¹³⁷Cs due to the large surface area per unit mass ratios (the surface effect)¹². Potassium-40 is a natural radionuclide with a long half-life of 1.25×10⁹ years and a biological half-life of 30 days. It gives the greatest contribution to natural radioactivity. Natural thorium-232 is also a long lived radionuclide (half-life 1.6×10⁶ years) and its radioactive decay is important as a source of radiogenic heat on Earth. The half-life of radium-226 is 1600 years and its biological half-life is up to 45 years. Radium-226 occurs in the decay chain of ²³⁸U and it can be found in soils and rocks containing natural uranium.²¹

A year and a half after Chernobyl, during November 1987, activity concentrations of ¹³⁷Cs and ⁴⁰K in the samples of *E. prunastri* collected in northern Greece were determined and reported, *i.e.*, for sample 1 ¹³⁷Cs 8436±8 Bq kg⁻¹ and ⁴⁰K 71±8 Bq kg⁻¹, for sample 2 ¹³⁷Cs 3242±7 Bq kg⁻¹ and ⁴⁰K 50±6 Bq kg⁻¹, and for sample 3 ¹³⁷Cs 3250±23 Bq kg⁻¹ and ⁴⁰K 50±13 Bq kg⁻¹.¹² The activity

concentrations of radionuclides in the lichen *E. prunastri* sampled on the territory of former Yugoslavia measured in the period of 01.07.1987 until 31.12.1987 was 13610 Bq kg^{-1} as an average activity of $^{135+137}\text{Cs}$, while during 1993, a level of 815 Bq kg^{-1} was reported. Moreover, during 1991-to-1994 on the territory of south Serbia, decreasing ^{137}Cs activity concentrations (from 2621 to 1902 Bq kg^{-1}) were reported. In the year 2002, the activity of ^{137}Cs and ^{40}K determined in samples collected in Montenegro were 373 Bq kg^{-1} and 281 Bq kg^{-1} , respectively, while the ratio $^{137}\text{Cs}/^{40}\text{K}$ was 1.3.¹³ During the period 1996–98, the activity concentrations of natural radionuclides in the region of Eastern Serbia were 281 ± 193 for ^{40}K , 54 ± 29 for ^{226}Ra and 19 ± 11 for ^{232}Th , while in the period 2006-to-2010, activities of natural radionuclides were lower and amounted 207 ± 51 for ^{40}K , 12 ± 5 for ^{226}Ra and 9 ± 2 for ^{232}Th .²¹ In the year 2006, the activity of ^{137}Cs in a sample collected from Sokobanja was $14\pm 9 \text{ Bq kg}^{-1}$, while in the period 2008–2010 on the locality of Djerdap, $283\pm 86 \text{ Bq kg}^{-1}$ was reported.²⁹ Although the specific activities of radionuclides in lichen samples, especially ^{137}Cs were extremely higher after the Chernobyl accident, it has been reported that these values have significantly decreased over time.^{12,13,29} As shown in the present experiments, the activity concentration of ^{137}Cs was below 3 Bq kg^{-1} . The activity concentration of ^{40}K in the sample of *E. prunastri* obtained in this study was in accordance with the values published for activity concentrations of ^{40}K in the same species of lichen taken from sampling points in the area of the Serbia and Montenegro.³⁰

CONCLUSION

This research has enriched the great fund of scientific facts about *E. prunastri* indicating that the ethyl acetate extract contains approximately two or more times lower concentration of atranorin and chloratranorin and two or more times higher concentration of evernic acid, which is not negligible due to allergic reactions to atranorin and chloratranorin. Furthermore, the acetone extract at a concentration of $2.0 \mu\text{g mL}^{-1}$ reduces the number of micronuclei in human lymphocytes to the same extent as amifostine. Tests of the antioxidant activity showed activity similar to other previously examined lichens. Analysis showed that the acetone extract had an inhibition effect towards Gram-positive bacteria and no effect on Gram-negative bacteria. Inhibition of cholinesterase activity was about 4.5 times lower than the activity of neostigmine bromide. Activities of ^{137}Cs , ^{226}Ra and ^{232}Th were below the detection limits.

SUPPLEMENTARY MATERIAL

Additional experimental data are available electronically at the pages of the journal website: <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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ИЗВОД

ДОПРИНОС ПОЗНАВАЊУ ХЕМИЈСКОГ САСТАВА, БИОЛОШКЕ АКТИВНОСТИ И СПЕЦИФИЧНЕ АКТИВНОСТИ ^{40}K , ^{137}Cs , ^{226}Ra И ^{232}Th ЛИШАЈА *Evernia prunastri*

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У овом раду је испитан утицај ацетонског екстракта *Evernia prunastri* на дистрибуцију микронуклеуса у људским лимфоцитима и холинестеразну активност. Такође, одређени су антиоксидативна активност (коришћењем DPPH, ABTS, TRP и CUPRAC тестова) и укупни садржај фенола као и антибактеријска активност према двама Грам-позитивним и трима Грам-негативним бактеријама. Хемијски профил четири екстракта *E. prunastri* (ацетонских, етарских, етил-ацетатних и дихлорметанских) одређен је GC-MS и HPLC анализом. Одређене су специфичне активности за ^{40}K , ^{137}Cs , ^{226}Ra и ^{232}Th .

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