Bioactivity of essential oils from cultivated winter savory, sage and hyssop

MILICA AČIMOVIĆ1,*, MARINA TODOSIJEVIĆ2, ANA VARGA3, BILJANA KIPROVSKI1, VELE TEŠEVIC2, IVANA ČABARKAPA3, AND VLADIMIR SIKORA1

1Institute of Field and Vegetable Crops Novi Sad, Maksima Gorkog 30, 21000 Novi Sad, Serbia
2University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia
3University of Novi Sad, Institute of Food Technology Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
*Corresponding author: milica.acimovic@ifvcns.ns.ac.rs

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Species of the Lamiaceae family have enjoyed a rich tradition of use for flavoring, food preservation, and medicinal purposes, due to their curative and preventive properties. Cultivated winter savory (Satureja montana L.), sage (Salvia officinalis L.) and hyssop (Hyssopus officinalis L.) are produced for seed, herb, and essential oil. Dominant compounds in S. montana essential oil were carvacrol (43.2%) and thymol (28.4%), while cis-thujone (27.1%) and camphor (19.3%), followed by trans-thujone and 1,8-cineole were the major compounds in S. officinalis essential oil. As for H. officinalis essential oil, cis- and trans-pinocamphone (41.1% and 20.5%, respectively) were the most abundant compounds, followed by β-pinene. S. montana essential oil exhibit the highest antimicrobial properties, as well as antioxidant capacity, compared to other tested essential oils. Furthermore, H. officinalis essential oils showed higher antioxidant activity than that of S. officinalis. The aim of this investigation was to determine the composition and bioactivity of essential oils of mentioned varieties. Presented results show that S. montana essential oil could be proposed as a valuable source of natural preservatives.

Key words: Satureja montana; Salvia officinalis; Hyssopus officinalis; antibacterial; antioxidant

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1. INTRODUCTION

Species of the family Lamiaceae have a long and a rich tradition of use for flavoring, food preservation, and medicinal purposes, due to their curative and preventive properties (Carović-Stanko et al., 2016). The majority of aromatic species belong to the Lamiaceae family, which is one of the largest families among the dicotyledons (Venkateshappa and Sreenath, 2013).

Winter savory (Satureja montana) is well known as a medicinal herb, mainly as a muscle pain reliever, tonic, and carminative agent in order to treat stomach and intestinal disorders such as cramps, nausea, indigestion, and diarrhea (Tepe and Cilkiz, 2016). It has a strong and spicy taste, and therefore it is used as a flavoring agent in salads, soups, sauces, stews, and lentil dishes (Wesołowska et al., 2014).

Sage (Salvia officinalis) has been used for culinary purposes as spice and preservative throughout history, but today it is commonly used to flavor meat, seafood, and cheese (Mapes and Xu, 2014). In traditional medicine, sage has been used to treat mild dyspepsia (such as heartburn and bloating), excessive sweating, age-related cognitive disorders, and inflammations in the throat and skin (Ghorbani and EsmaeiliZadeh, 2017).

Sage leaf (Salvia officinalis folium) has been listed in European Pharmacopoeia and many others (Ph. Eur. 7.0., 2010). Despite its slightly bitter taste and minty flavor, hyssop (Hyssopus officinalis) is commonly used for centuries to produce flavors and fragrances in food, mainly sauces, and seasonings, and in bitters and liqueurs (Dehghanzadeh et al., 2012). Apart from this, it is used in folk medicine as a carminative, tonic, antiseptic, expectorant and cough reliever (Fathiazad et al., 2011).

Because of bioactive components in their essential oils, characterized by specific taste and fragrance, mentioned plants are popular today in the concept of functional food. In recent years, many research studies have been conducted to find new biological effects of plants including antioxidant, antimicrobial, anticancer, hypoglycemic and hypolipidemic effects. Sage represents a most common medicinal plant that is cultivated and collected from natural habitats. However, S. montana and especially, H. officinalis, are rarely cultivated and their natural habitats are constricted to sparse population area. Due to the extensive harvest of these plants in their natural habitats, comparison of quality and biological activity of essential oil between cultivated and wild plants is important to
all parties which use these species.

Bearing in mind numerous properties of savoury, sage and hyssop, the aim of this paper was to determine chemical composition and content, as well as biological activity of essential oils of these plants grown in our collection and to compare these findings to available literature data.

2. MATERIALS AND METHODS

2.1. Plant material

As a part of medicinal plants collection of the Institute of Field and Vegetable Crops in Novi Sad, located in Bački Petrovac, at the Department for Alternative Crops and Organic Production, winter savoury (S. montana L., variety "Domaci"), sage (S. officinalis L., variety "Primorska") and hyssop (H. officinalis L., variety "Domaci ljubičasti") are produced for essential oil extraction. Above-ground parts of selected plants were collected in July 2017. Voucher specimens were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Sciences, University of Novi Sad. Voucher specimens were referenced as 2-1561 (S. montana), 2-1548 (S. officinalis) and 2-1567 (H. officinalis).

2.2. Essential oil extraction

Dried samples of winter savoury, sage and hyssop were subjected to hydro-distillation using an all-glass Clevenger-type apparatus to extract essential oils according to the method outlined by the European Pharmacopoeia (Ph. Eur. 7.0, 2010). In order to extract the essential oils, 100 g of the plant material was placed in 1 L conical flask and connected to the Clevenger apparatus. Distilled water was added to the flask (500 mL) and heated to the boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer. Essential oils were kept refrigerated until further analysis.

2.3. GC and GC-MS analysis

The gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network transmission quadrupole mass spectrometer (Agilent, Santa Clara, USA), in positive ion-electron impact (EI) mode. The separation of individual compounds was achieved using non-polar HP-5 fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 3 °C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 mL/min at 210 °C. Injector temperature: 250 °C; injection mode: splitless. MS scan conditions: MS source temperature, 230 °C; MS Quad temperature, 150 °C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out on the basis of retention indices followed by comparison with reference spectra (Wiley and NIST databases) and literature data.

2.4. Antibacterial activity

The antimicrobial activity was evaluated using control strains obtained from the American Type Culture Collection. Four Gram-positive bacteria: Bacillus cereus (ATCC 11778), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 25923) and Staphylococcus epidermidis (ATCC 12228), and four Gram-negative bacteria: Escherichia coli (ATCC 8739), Pseudomonas aeruginosa (ATCC 27853), Salmonella enteritidis (ATCC 13076), and Proteus hauseri (ATCC 13315). The activity of essential oils was tested by a modified broth microdilution method according to the National Committee for Clinical Laboratory Standards (CLSI, 2012). A serial doubling dilution of the tested essential oils was prepared in a 96-well microtiter plates over

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### Table 1. The essential oil composition of winter savoury (S. montana).

| # | Compound namea | RIb | %m/m |
|---|----------------|-----|------|
| 1 | α-thujene | 924 | 0.6 |
| 2 | α-pinene | 932 | 0.6 |
| 3 | n.i. | 942 | tr |
| 4 | camphene | 946 | 0.3 |
| 5 | 1-ocen-3-ol | 975 | 0.4 |
| 6 | β-pinene | 975 | 0.2 |
| 7 | myrcene | 989 | 1.1 |
| 8 | α-phellandrene | 1004 | 0.2 |
| 9 | δ-3-carene | 1010 | 0.1 |
| 10 | α-terpinene | 1015 | 1.7 |
| 11 | p-cymene | 1024 | 8.9 |
| 12 | limonene | 1027 | 0.5 |
| 13 | 1,8-cineole | 1029 | 0.3 |
| 14 | cis-β-ocimene | 1036 | tr |
| 15 | γ-terpinene | 1057 | 7.5 |
| 16 | cis-sabinene hydrate | 1065 | 0.5 |
| 17 | terpinolene | 1088 | tr |
| 18 | n.i. | 1099 | 0.1 |
| 19 | linalool | 1100 | 0.5 |
| 20 | cis-thujone | 1106 | tr |
| 21 | camphor | 1143 | tr |
| 22 | trans-pinocamphone | 1159 | tr |
| 23 | borneol | 1163 | 0.4 |
| 24 | cis-pinocamphone | 1172 | 0.1 |
| 25 | terpinen-4-ol | 1175 | 0.5 |
| 26 | α-terpineol | 1189 | 0.1 |
| 27 | carvacrol, methyl ether | 1242 | 0.4 |
| 28 | n.i. | 1282 | tr |
| 29 | thymol | 1292 | 28.4 |
| 30 | carvacrol | 1301 | 43.2 |
| 31 | α-copaene | 1375 | tr |
| 32 | β-bourbonene | 1384 | tr |
| 33 | trans-caryophyllene | 1419 | 1.4 |
| 34 | β-copaene | 1429 | tr |
| 35 | aromadendrene | 1439 | tr |
| 36 | α-humulene | 1453 | tr |
| 37 | γ-murolene | 1477 | 0.1 |
| 38 | viridiflorene | 1496 | 0.1 |
| 39 | n.i. | 1501 | tr |
| 40 | β-bisabolene | 1510 | 0.7 |
| 41 | γ-cadinene | 1515 | 0.1 |
| 42 | δ-cadinene | 1524 | 0.2 |
| 43 | n.i. | 1578 | tr |
| 44 | caryophyllene oxide | 1583 | 0.3 |
| 45 | n.i. | 1904 | tr |
| 46 | n.i. | 1931 | tr |
| 47 | n.i. | 2147 | tr |
| 48 | n.i. | 2164 | tr |

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*a* n.i. stands for not identified compounds; *tr* - traces.

*b* RI, retention indices as determined on HP-5 column using homologous series of C₅-C₂₀ alkanes.
Table 2. The essential oil composition of sage *S. officinalis*.

| #  | Compound namea | RI | %m/m |
|----|----------------|----|------|
| 1  | cis-salvane    | 846| 0.4  |
| 2  | trans-salvane  | 856| tr   |
| 3  | n.i.           | 918| tr   |
| 4  | tricycene      | 922| 0.1  |
| 5  | α-thujene      | 925| 0.1  |
| 6  | α-pinene       | 932| 3    |
| 7  | camphene       | 947| 4.6  |
| 8  | sabineone      | 972| 0.1  |
| 9  | β-pinene       | 976| 1.6  |
| 10 | myrcene        | 989| 0.8  |
| 11 | α-phellandrene | 1005| tr |
| 12 | α-terpinene    | 1016| 0.2 |
| 13 | p-cymene       | 1024| 0.4 |
| 14 | limonene       | 1028| 4.4 |
| 15 | 1,8-cineole    | 1031| 11.5|
| 16 | γ-terpinene    | 1057| 0.4 |
| 17 | cis-sabineone  | 1066| 0.1 |
| 18 | terpinolene    | 1088| 0.2 |
| 19 | trans-sabineone| 1103| 0.1 |
| 20 | linalool       | 1108| 0.3 |
| 21 | cis-thujone    | 1110| 27.1|
| 22 | trans-thujone  | 1119| 12.3|
| 23 | α-campholenal  | 1126| tr |
| 24 | iso-3-thujanol | 1138| 0.1 |
| 25 | n.i.           | 1143| tr |
| 26 | camphor        | 1147| 19.3|
| 27 | trans-pinocamphene | 1159| tr |
| 28 | bornol         | 1164| 0.9 |
| 29 | terpinen-4-ol  | 1175| 0.4 |
| 30 | α-terpinol     | 1188| tr |
| 31 | n.i.           | 1196| tr |
| 32 | bornyl acetate | 1284| 0.5 |
| 33 | trans-sabinylacetate | 1291| 0.1 |
| 34 | trans-carylacetate | 1337| tr |
| 35 | trans-caryophyllene | 1419| 1.7 |
| 36 | n.i.           | 1438| tr |
| 37 | α-humulene     | 1454| 2.4 |
| 38 | 9-epi-trans-caryophyllene | 1461| tr |
| 39 | viridiflorene  | 1496| tr |
| 40 | caryophyllene oxide | 1582| 0.3 |
| 41 | viridiflorol   | 1591| 3.4 |
| 42 | n.i.           | 1598| 0.1 |
| 43 | humulene epoxide II | 1609| 0.7 |
| 44 | n.i.           | 1630| tr |
| 45 | n.i.           | 1673| 0.1 |
| 46 | n.i.           | 1781| tr |
| 47 | n.i.           | 1806| tr |
| 48 | isopimara-9(11),15-diene | 1913| tr |
| 49 | n.i.           | 1932| tr |
| 50 | n.i.           | 2001| tr |
| 51 | manool         | 2061| 1.9 |
| 52 | n.i.           | 2094| tr |

Norterpnes: 0.4
Monoterpenic hydrocarbons: 15.9
Oxigenated monoterpenes: 72.7
Sesquiterpene hydrocarbons: 7.5
Oxigentepid sesquiterpene: 1
Diterpene hydrocarbon: tr
Oxigenated diterpene: 1.9

Total identified 99.6

a n.i. stands for not identified compounds; tr - traces.
b RI, retention indices as determined on HP-5 column using homologous series of C10-C30 alkanes.

c Sage essential oil had 52 compounds, where the major compounds were cis-thujone with 27.1% and camphor with 19.3%, followed by trans-thujone with 12.3%, and 1,8-cineole with 11.5% (Table 2). Other abundant compounds presented in amount above 1% were: camphene (4.6%), limonene (4.4%), viridiflorol (3.4%), α-pinene (3.0%), α-humulene (2.4%), manool (1.9%), trans-caryophyllene (1.7%) and β-pinene (1.6%). Qualitative analysis of the essential oil showed that *S. officinalis* variety “Primorska” belonged to Chemotype A, which is rich in cis-thujone and camphor (Cvetković et al., 2015). However, the sum of toxic thujones, cis- and trans-thujone, was high (39.4%) according to the Perry et al. (1999) who classified in the range of 454.4-0.22 μL/mL in inoculated Mueller-Hinton broth (MHB, HiMedia). The mixture was discharged from the last well in a row (100 μL). The test was performed in a total volume of 110 μL/mL with a final microbial concentration of 106 CFU/mL per well. The plate was incubated for 24 h at 37 °C. The same tests were performed simultaneously for growth control (MHB + test organism), sterility control (MHB + test oil), and positive control (MHB + gentamicin+ test organism). Gentamicin was prepared in sterile water and diluted in MHB to obtain concentrations in a range of 16 to 0.016 μg/mL. Additionally, susceptibility to gentamicin was confirmed using a quantitative assay for determining the MIC (gentamicin Test Strip Liofilchem®) according to the manufac-

2.5. Antioxidant activity

Total potential antioxidant activity of tested essential oils was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH-test) free radicals (Panda 2012). Re-

3. RESULTS AND DISCUSSION

3.1. Chemical characterization of essential oils

Gas chromatographic and mass spectrometric analysis of the essential oil of winter savory (*S. montana*) showed 48 compounds, which represent 99.5% of the essential oil. The dominant compounds (higher than 5%) in the essential oil were carvacrol with 43.2% and thymol with 28.4%, followed by p-cymene (8.9%) and γ-terpinene (7.5%) (Table 1). Nevertheless, γ-terpinene and p-cymene are precursors of thymol and carvacrol and therefore some authors proposed that they are produced by a similar mechanism (Papadatou et al., 2015). Variety “Domaci” in general can be classified as phenol-rich chemotype since carvacrol and thymol are the most abundant compounds (Bezić et al., 2009).
Research Article

The essential oil composition of sage

Table 3. The essential oil composition of sage S. officinalis.

| # | Compound namea | RI\textsuperscript{b} | %m/m |
|---|----------------|----------------------|------|
| 1 | n.i.           | 881                  | tr   |
| 2 | α-thujene      | 868                  | tr   |
| 3 | β-thujene      | 853                  | tr   |
| 4 | terpinen-4-ol  | 821                  | tr   |
| 5 | thujene 2,4(10)-diene | 812 tr | |
| 6 | sabine 2       | 812                  | tr   |
| 7 | δ-cadinene     | 812                  | tr   |
| 8 | γ-cadinene     | 812                  | tr   |
| 9 | δ-cadinene 4   | 812                  | tr   |
| 10 | δ-cadinene 5   | 812                  | tr   |
| 11 | δ-cadinene 6   | 812                  | tr   |
| 12 | δ-cadinene 7   | 812                  | tr   |
| 13 | δ-cadinene 8   | 812                  | tr   |
| 14 | δ-cadinene 9   | 812                  | tr   |
| 15 | δ-cadinene 10  | 812                  | tr   |
| 16 | δ-cadinene 11  | 812                  | tr   |
| 17 | δ-cadinene 12  | 812                  | tr   |
| 18 | δ-cadinene 13  | 812                  | tr   |
| 19 | δ-cadinene 14  | 812                  | tr   |
| 20 | δ-cadinene 15  | 812                  | tr   |
| 21 | δ-cadinene 16  | 812                  | tr   |
| 22 | δ-cadinene 17  | 812                  | tr   |
| 23 | δ-cadinene 18  | 812                  | tr   |
| 24 | δ-cadinene 19  | 812                  | tr   |
| 25 | δ-cadinene 20  | 812                  | tr   |
| 26 | δ-cadinene 21  | 812                  | tr   |
| 27 | δ-cadinene 22  | 812                  | tr   |
| 28 | δ-cadinene 23  | 812                  | tr   |
| 29 | δ-cadinene 24  | 812                  | tr   |
| 30 | δ-cadinene 25  | 812                  | tr   |
| 31 | δ-cadinene 26  | 812                  | tr   |
| 32 | δ-cadinene 27  | 812                  | tr   |
| 33 | δ-cadinene 28  | 812                  | tr   |
| 34 | δ-cadinene 29  | 812                  | tr   |
| 35 | δ-cadinene 30  | 812                  | tr   |
| 36 | δ-cadinene 31  | 812                  | tr   |
| 37 | δ-cadinene 32  | 812                  | tr   |
| 38 | δ-cadinene 33  | 812                  | tr   |
| 39 | δ-cadinene 34  | 812                  | tr   |
| 40 | δ-cadinene 35  | 812                  | tr   |
| 41 | δ-cadinene 36  | 812                  | tr   |
| 42 | δ-cadinene 37  | 812                  | tr   |
| 43 | δ-cadinene 38  | 812                  | tr   |
| 44 | δ-cadinene 39  | 812                  | tr   |
| 45 | δ-cadinene 40  | 812                  | tr   |
| 46 | δ-cadinene 41  | 812                  | tr   |
| 47 | δ-cadinene 42  | 812                  | tr   |
| 48 | δ-cadinene 43  | 812                  | tr   |
| 49 | δ-cadinene 44  | 812                  | tr   |
| 50 | δ-cadinene 45  | 812                  | tr   |
| 51 | δ-cadinene 46  | 812                  | tr   |
| 52 | δ-cadinene 47  | 812                  | tr   |
| 53 | δ-cadinene 48  | 812                  | tr   |
| 54 | δ-cadinene 49  | 812                  | tr   |
| 55 | δ-cadinene 50  | 812                  | tr   |
| 56 | δ-cadinene 51  | 812                  | tr   |
| 57 | δ-cadinene 52  | 812                  | tr   |
| 58 | δ-cadinene 53  | 812                  | tr   |
| 59 | δ-cadinene 54  | 812                  | tr   |

The results of antimicrobial activity of essential oil from the leaves of S. officinalis grown in Serbia confirmed the activity against B. subtilis, S. aureus, E. coli and S. enteritidis in two different concentrations; 1% and 2%, in comparison to ampicillin (Miladinović and Miladinović, 2000). While, the essential oil of S. officinalis from Portugal showed very weak antimicrobial activity (Miguél et al., 2011). The same was concluded for S. officinalis essential oil from Greece that was lacking a notice-able antibacterial action since the MIC values recorded against all pathogens (Klebsiella oxytoca, K. pneumonia and E. coli) were above 150 µg/mL (Fournomiti et al., 2015).

H. officinalis has been traditionally used for its antiseptic prop-

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\textsuperscript{a} n.i. stands for not identified compounds; tr - traces.

\textsuperscript{b} RI, retention indices as determined on HP-5 column using homologous series of C\textsubscript{15}-C\textsubscript{30} alkanes.
Table 4. Antimicrobial properties of essential oils, MIC and MBC [µL/mL].

|                | S. montana | S. officinalis | H. officinalis | Streptomycin | Gentamicin |
|----------------|------------|----------------|----------------|--------------|------------|
|                | MIC        | MBC            | MIC            | MBC          | MIC        | MIC        |
| Bacillus cereus (ATCC 11778) | 1.77       | 3.55           | 113.62         | 227.25       | 14.20      | 28.40      | 1           | 0.19       |
| Escherichia coli (ATCC 8739)   | 1.77       | 3.55           | 56.81          | 113.62       | 227.25     | 227.25     | 4           | 2          |
| Enterococcus faecalis (ATCC 29212) | 1.77     | 7.10           | 454.50         | 454.50       | 454.50     | 454.50     | 96          | 8          |
| Pseudomonas aeruginosa (ATCC 27853) | 3.55     | 3.55           | 113.62         | 227.25       | 454.50     | 454.50     | 16          | 1          |
| Salmonella enteritidis (ATCC 13076) | 3.55     | 7.10           | 113.62         | 113.62       | 227.25     | 227.25     | 2           | 0.5        |
| Staphylococcus aureus (ATCC 25923) | 3.55     | 3.55           | 113.62         | 113.62       | 227.25     | 227.25     | 3           | 0.38       |
| Staphylococcus epidermidis (ATCC 12228) | 3.55     | 3.55           | 113.62         | 113.62       | 227.25     | 227.25     | >1024.00    | 0.094      |
| Proteus hauseri (ATCC 13315)   | 3.55       | 3.55           | 454.50         | 454.50       | 227.25     | 454.50     | 6           | 1          |

Properties in the treatment of infectious disorders (Mahboubi et al., 2011). Some previous studies showed the significant activity of H. officinalis essential oil against Gram-positive bacteria (Baj et al., 2018; De Martino et al., 2009; Mahboubi et al., 2011). Other reported that H. officinalis essential oil MIC ranged from 15.625 to 250 µL/mL depending on bacterial strains (Özer et al., 2006).

3.3. Antioxidative activity

S. montana showed the highest antioxidant capacity when compared to other tested species (Table 5). H. officinalis and S. officinalis followed with 1.5 and 3-fold lower antioxidant activity, respectively. All available literature mostly performed DPPH-test on extracts of analyzed plants and only a few of them used essential oils, as it was the case in our research.

Table 5. Antioxidative activity of cultivated savory, sage and hyssop essential oils.

| Species               | IC₅₀ [µg/mL] |
|-----------------------|-------------|
| Satureja montana      | 17.0±0.1    |
| Salvia officinalis    | 50.0±0.4    |
| Hyssopus officinalis  | 24.0±0.2    |

If compared with literature data, S. montana essential oil tested in this study has greater antioxidant capacity than other S. montana plants collected in nature (Cavar et al., 2008; Coutinho de Oliveira et al., 2012), or compared with other Satureja species: 32 µg/mL in S. ciliaca (Ozkan et al., 2007) and 185.5 µg/mg in S. cuneifolia (Oke et al., 2007). DPPH-test of S. officinalis essential oil showed lower antioxidant capacity than those from published data which reported IC₅₀ activity as 1.78 µg/L (Bozin et al., 2007). Results from other studies varied for H. officinalis: 16.37 µg/mL (Kizil et al., 2010) and 156.6 mg/mL (Džamić et al., 2013).

CONCLUSION

With on-going use of an artificial preservative in the food industry, in addition to the challenge of microbial resistance, there is growing concern over side effects of these compounds. Alternatives such as the use of herbal essential oils in food preservation that have no side effects and sometimes even positive effects have to be considered. According to the presented results, S. montana essential oils could be proposed as an invaluable source of natural preservatives. Despite cultivation practice, our results showed that essential oils obtained from commercially grown varieties have high biological activity and could be used instead of plants grown in nature. In this way, more raw materials could be produced without effect on natural gene pool and habitats of these species.

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