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Chemical composition and antibacterial activity of spice essential oils against *Escherichia coli* and *Salmonella* Typhimurium

Zusammensetzung und antibakterielle Wirkung von ätherischen Pflanzenölen gegen Escherichia coli und Salmonella Typhimurium

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Summary

The aim of the present study was to investigate the chemical composition of spice EOs from the family of *Lamiaceae* (basil, mentha, winter savory) and *Apiaceae* (dill) as well as their antibacterial activity against food-borne pathogenic bacteria *Escherichia coli (E. coli)* and *Salmonella* Typhimurium (*S.* Typhimurium). Chemical composition of the EOs was identified by gas chromatography coupled with mass spectrometer detector (GC-MS). The results showed that linalool (in basil EO), menthol (in mentha EO), p-cymene (in winter savory EO) and carvone (in dill EO) were the main compounds. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined by broth microdilution method. All EOs possessed significant antibacterial effects on both tested bacterial strains. Dill EO had the highest antibacterial activity against tested bacterial strains with MIC/MBC values of 3.55 µL/mL, while mentha had the lowest antibacterial activity with MIC/MBC values of 14.20 µL/mL against *E. coli* and 56.81 µL/mL against *S.* Typhimurium. The MIC/MBC values of basil and winter savory EOs were 7.10 µL/mL for both tested bacteria. Based on the present study, the EOs from spice herbs possess antimicrobial activity against tested food-borne pathogens and thus can be a good source of natural antimicrobials agents.

Keywords: essential oils, antibacterial activity, chemical composition

Zusammenfassung

Das Ziel der vorliegenden Studie war, die chemische Zusammensetzung von ätherischen Pflanzenölen aus der Familie der *Lamiaceae* (Basilikum, Minze, Winterbohnenkraut) und *Apiaceae* (Dill) sowie deren antibakterielle Wirkung gegen die durch Lebensmittel übertragbaren pathogenen Bakterien *Escherichia coli (E. coli)* und *Salmonella* Typhimurium (*S.* Typhimurium) zu untersuchen. Die chemische Zusammensetzung der Pflanzenöle wurde durch Gaschromatographie sowie mittels Massenspektrometer (GC-MS) identifiziert. Als Hauptkomponenten in untersuchten ätherischen Ölen (ÄÖ) wurden Linalool (im ÄÖ von Basilikum), Menthol (im ÄÖ der Minze), p-Cymol (im ÄÖ des Winterbohnenkrauts). und Carvon (im ÄÖ des Dills). Die Minimale Hemmkonzentration (MHK) und die minimale bakterizide Konzentration (MBK) wurden durch die Bouillon-Mikrodilutionsmethode bestimmt. Alle ÄÖ besaßen bedeutende antibakterielle Wirkungen auf die beiden getesteten Bakterienstämme. Dill ÄÖ hatte die höchste antibakterielle Wirkung gegen die getesteten Bakterienstämme mit MHK/MBK-Werten von 3,55 µl/ml, während das ÄÖ der Minze die niedrigste antibakterielle Aktivität zeigte, mit MHK/MBK-Werten von 14,20 µl/ml gegen *E. coli* und 56,81 µl/ml gegen *S.* Typhimurium. Die MHK/MBK-Werte von Basilikum und Winterbohnenkraut ÄÖ waren 7,10 µl/ml für beide getesteten Bakterienspezies. Die vorliegende Studie zeigt, dass die ätherischen Pflanzenöle eine antibakterielle Aktivität gegen die getesteten Pathogene aufweisen und sie können somit eine gute Quelle von natürlichen antimikrobiellen Mitteln sein.

Schlüsselwörter: ätherische Öle, antibakterielle Wirkung, chemische Zusammensetzung

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Introduction

Food safety is a fundamental interest for both consumers and food producers alike. Consumer awareness and concern that synthetic chemical additives may have some toxic or even carcinogenic effects, has increased the demand for high-quality, minimally processed foods with extended shelf-life, preferably without or with a reduced level of added chemical antimicrobial agents. However, the minimally processed foods and natural foods may be more susceptible to growth of food-borne pathogens than the conventional food version (Shahbazi, 2015). Therefore, there is growing interest in the use natural compounds, such as plant extracts and essential oils (EOs) with antibacterial properties, as a replacement or alternative to synthetic compounds for the food preservation (Özcan, 1998; Smid and Gorris, 1999). Plant EOs have been long recognized for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. They are widely used in medicine and the food industry due to these properties. The antibacterial activity of plant EOs is applied by mankind for various purposes since ancient times. They are used as spices in food preparation, forming the sensory profile and increasing the shelf life of foods (Teneva et al., 2016). Chemically, EOs also known as volatile oils, are complex mixtures of terpenoides, monoterpenes, sesquiterpenes and possibly diterpenes with different groups of aliphatic constituents, such as hydrocarbons, acids, alcohols, aldehydes, ketones, acyclic esters or lactones (Bakkali et al., 2008; Kocić-Tanackov et al., 2017). Many studies investigated antibacterial activity of spice herbs EOs. These data are useful for future investigations but, this information is difficult to compare directly due to a number of factors, such as variability in chemical composition or content of active compounds between plants due to origin from various geographical regions, harvesting seasons, growth and drying conditions, or using plant material of different maturity (Senatore et al., 2005; Hussain et al., 2011; Witkowska et al., 2013; Özcan and Ozkan, 2015). EOs recovered from the *Lamiaceae* and *Apiaceae* species have application against different diseases in folk medicine, in food industry as substitutes for chemical preservatives and as a flavoring agent (Ilić et al., 2019). According to the geographical origin of basil varieties and on the basis of their major constituents, they are classified in four chemotypes: (1) European chemotype, the oil of which is characterized by high amounts of linalool (35–50%) and estragole (15–25%); (2) reunion chemotype (estragole basil) whose main EO component is estragole (80% or more); (3) tropical chemotype (cinnamon basil), the oil of which is dominated by methyl cinnamate; and (4) eugenol chemotype whose major oil component is eugenol (Koutsos et al., 2009). The genus *Mentha,* is one of the most important members of the *Lamiaceae* family. EOs of mentha plants are mainly composed of monoterpenes and sesquiterpenes, which content and composition varies (Kumar et al., 2011). According to the EO composition, has been reported nine chemotypes for the mentha plants: (1) geraniol and/or geranyl acetate; (2) linalool and/or linalool acetate, (3) carvone/dihydrocarvone, (4) piperitone/piperitone oxide; (5) piperitenone oxide/piperitone oxide; (6) menthofuran; (7) pulegone/isopulegone; (8) pulegone/menthone/isomenthone; (9) menthone/isomenthone (Mimica-Dukić and Božin, 2008). Other explanation for the differences in oil content and composition may be attributed to factors related to ecotype, phenophases, temperature, relative humidity, photoperiod, irradiance, genotype, and agronomic conditions (harvesting time, plant age, crop density) (Selles et al., 2018).

Satureja montana L., is known as winter savory, from *Lamiaceae* family has been traditionally used as a culinary and medicinal herb. EO derived from plants of *Satureja* species possess strong antibacterial activities of different extents against microorganisms of importance to food spoilage and poisoning, such as *Salmonella* spp., *Listeria* spp., *Escherichia* spp. and *Staphylococcus* spp. Various biologically active constituents from triterpene and flavonoid categories consisting of many components such as carvacrol, thymol, δ-terpinene, γ-terpinene, p-cymene, and α- and β-pinene have been found in winter savory. Depending on the prevalence of phenols or monoterpene alcohols two main chemotypes can be described, namely A and B, each of them including several subtypes such as A (thymol), A (carvacrol), B (linalool), B (geraniol) (Rezvanpanah et al., 2011; Trifan et al., 2015). Due to high levels of phenols (mainly carvacrol), Serbian *Satureja* montana EO belongs to chemotype A (carvacrol) as well as EOs from former Yugoslavia, Italy or Portugal (Serrano et al., 2011; Hassanein et al., 2014).

Dill belongs *Apiaceae* family. According to the EO chemical composition has been reported several chemotypes of dill. They are different on the basis of the predominant EO components. The major components of the chemotypes are: (1) limonene-carvone-myristicin-n-dill apiole; (2) limonene-carvone-dill apiole; (3) limonene-carvone; (4) carvone; (5) α-phellandrene-limonene-anethofuran (Badoc and Lamarti 1991; Radulescu et al., 2010).

Food contamination by pathogenic bacteria is a public health problem. Foodborne diseases are increasing worldwide and deserve a great deal of concern due to morbidity and the high mortality rate, particularly in the developing countries (Hoelzer et al., 2018). A wide spectrum of pathogens and food vehicles has been documented in produce-associated outbreaks (Berger et al., 2010). In recent years there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance (Tomičić et al., 2018). The role of herbs and spices in human life and their multiple uses as ingredients in food, alcoholic beverages, perfumery, cosmetics, medicine and coloring agents are well established (Tassov, 2006). Considering the above, the aim of this study was to determine chemical composition of EOs from the family of *Lamiaceae* (*Mentha piperita* L., *Ocimum basilicum* L. and *Satureja montana* L.) and *Apiaceae* (*Anethum graveolens* L.) orginated from Province of Vojvodina, Serbia, as well as to examine their antimicrobial activity against bacteria *E. coli* and *S.* Typhimurium. Given that so far, in literature has not been evidenced research conducted on plant species with similar geographical origin, thus result of this research represented original contribution to science.

Materials and Methods

Extraction of essential oils

The plant materials (*Anethum graveolens* L., *Mentha piperita* L., *Ocimum basilicum* L. and *Satureja montana* L.) used in this study were obtained from the Field and Vegetable Crops Institute of Novi Sad, Serbia which numbers of voucher specimens are 2-1440, 2-1550, 2-1441 and 2-1561, respectively. Voucher specimens were confirmed and depo-

sited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium) Faculty of Sciences, University of Novi Sad. Aerial parts of plants were collected from the locality Province of Vojvodina, Serbia. Dried samples of each plant species were prepared by hydrodistillation using Clevenger-type apparatus (ATICO Medical Pvt., Ambala, India) to extract EOs according to the method outlined by the European Pharmacopoeia (2004). The samples were milled, homogenized and made in fine powder. In order to extract the EOs, 100 g of the powder was placed in 1 l conical flask and connected to the Clevenger apparatus. 500 mL of distilled water was added to the flask and heated to the boiling point. The steam in combination with the EOs was distilled into a graduated cylinder for 4 h and then separated from aqueous layer. The oil was kept refrigerated at 4°C until required for further analysis. The oils (100% pure EO) were stored in dark bottles and kept refrigerated according to the manufacturer's recommendation until use.

GC/MS analysis

The EOs composition was determined by gas chromatography-mass spectrometry (GC-MS) technique. The gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph (Agilent, Santa Clara, USA) coupled with an Agilent 5973 Network mass selective detector (MSD) (Agilent, Santa Clara, USA), in positive ion electron impact (EI) mode. The separation was effected using Agilent 19091S-433 HP-5MS 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 index and by comparison with reference spectra (Wiley and NIST databases).

Preparation of bacterial strains

All tests in this study were performed using *S.* Typhimurium ATCC 14028 (American Type Culture Collection) and *E. coli* strain isolated from pork meat The strain *E. coli* used in this study was obtained from Scientific Veterinary Institute of Novi Sad, Serbia. Working culture was prepared from a subculture and grown in Tryptone Soya Broth medium at 37°C for 24 h. (TSB, HiMedia Laboratories Pvt. Ltd., Mumbai, India).

Broth microdilution method

Antimicrobial activity of EOs was performed by broth microdilution method according to the Clinical Laboratory Standards (CLSI, 2012) with slight modification. Inoculum suspension were prepared using overnight cultures and adjusted to 0.5 McFarland standard turbidity (corresponding to 1×10^8 CFU/mL), using a densitometer DEN-1 (Biosan, Riga, Latvia). A serial doubling dilutions of the tested EOs were prepared in a 96/well microtiter plate (Greiner Bio-One GmbH, Kremsmünster, Austria) over the range from 454.4 to 0.22 µL/mL, containing inoculated Mueller-Hinton broth (MHB, HiMedia Laboratories Pvt. Ltd., Mumbai, India) supplemented with 0.5% Tween 80® (Polyoxyethylenesorbitan monooleate, HiMedia Laboratories Pvt. Ltd., Mumbai, India). The final volume in each well was 110 µL/mL and the final microbial concentration was 10^6 CFU/mL. The plate was incubated for 24 h at 37°C. In all tests growth control (MHB + test organism) and a sterility control (MHB + test oil) were included. Microbial growth was determined by adding 10 µL of 0.01% resazurin (HiMedia Laboratories Pvt. Ltd., Mumbai, India) aqueous solution. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (blue colored pellet on the bottom of the wells after the addition of resazurin) (Elshikh et al., 2016). Based on previously obtained MIC results, the wells that showed a complete absence of growth were identified and 100 µL solutions from each well was transferred on the surface Mueller-Hinton agar (MHA, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37°C for 24 hours. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the EOs at which 99.9% of the inoculated microorganisms were killed. Each experiment was repeated four times. The application of the micro-well dilution method is useful to compare the antibacterial effect between EOs and to identify the minimal concentrations of EO that did not allow bacterial replication (Mazzarrino et al., 2015).

MIC Test Strip

The MIC Test Strip was used for determination of the minimal inhibitory concentrations of gentamicin and streptomycin against *E. coli* and *S.* Typhimurium. This test was performed according to the procedures of manufacturer Liofilchem (Via Scozia, Zona Industriale-Reseto Italy).

Shortly, 200 µL of each tested microorganisms suspension (ca. 1×10^6 cells/mL) was added to a sterile petri dish and 20 mL of MHA was poured, homogenized and left to tighten.

After drying the surfaces of the plates, a sterile porous strip (Liofilchem, Italy) with a predefined concentration gradient of antibiotics gentamicin and streptomycin was applied on the surface of each inoculated agar surface.

After 24 hours incubation at 37°C, a symmetric ellipsoid of inhibition is formed along the strip. The MIC was read directly from the scale in terms of µg/mL at the point where the edge of the inhibition ellipse intersects the strip MIC Test Strip.

Results and Discussion

Antibacterial properties of EOs from the spices such as cinnamon, anise, dill, pepper, basil, clove, savory, mentha, thyme and oregano have been previously reported. In addition to antibacterial activity, some spice EOs were reported to possess anti-free radical activities. Due to these properties, spice EOs may be used as natural antioxidants and antibacterial agents in conventional and organic food products (Nanasombat and Wimuttigosol, 2011).

TABLE 1: *Antibacterial activity of essential oils expressed as MIC/MBC, determined by the broth microdilution method.*

Plant species	Common name	*E. coli* MIC/MBC (µL/mL)	*S.* Typhimurium MIC/MBC (µL/mL)
Anethum graveolens L.	dill	3.55	3.55
Mentha piperita L.	mentha	14.20	56.81
Ocimum basilicum L.	basil	7.10	7.10
Satureja montana L.	winter savory	7.10	7.10

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According to the results obtained by the broth microdilution assay (Table 1), tested EOs moderately differ in their activity against bacterial strains. The highest antibacterial activity showed dill EO with MIC/MBC values of 3.55 µL/mL. On the other hand, *E. coli* and *S.* Typhimurium displayed high resistance to mentha EO with MIC/MBC values of 14.20 µL/mL, and 56.81 µL/mL respectively. Considering the antibacterial activity of EOs against *E. coli* and *S.* Typhimurium dill EO was the most effective followed by basil, winter savory and mentha. It is interesting that basil and winter savory EOs exhibited an identical antibacterial activity to the tested bacteria with MIC/MBC values of 7.10 µL/mL. Keeping in mind that the MIC value depends on the initially used concentration of EOs. Usually, the initially used concentration is diluted for two orders of magnitude, which allows obtaining a series of 1:2 dilutions. Thus, in the theoretical view MIC would be corresponding to the median of the concentration range. The larger the concentration range is the lower accuracy of the MIC estimation. In the case of the obtained results in this study, the identical values of MIC and MBC can be explained as a consequence of the use a series of 1:2 dilutions of EOs (Saad et al., 2013). In the future research we will correct range of using concentration of EOs.

E. coli and *S.* Typhimurium are often exposed to low doses of antibiotics, since antibiotics are used in large amounts both in human and animal medicine (Aarestrup, 2012). In the present study, the effect of antibiotics gentamicin and streptomycin was also examined as presented in Table 2. The results showed that gentamicin had a stronger antibacterial activity against both bacterial strains. Further, *S.* Typhimurium was more susceptible bacteria to both antibiotics in comparison to *E. coli.* Similar results were presented in study conducted by Roldán et al. (2010).

The antimicrobial activity of the herbs and spice EOs is attributed mainly to their phenolic compounds. Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity (Boyraz et al., 2006). Generally, essential oils are comprised of two or three major components in relatively high concentrations (20–95%) while other components are present in traces. The major components of EOs determine their biological properties. However, some studies have demonstrated that EOs usually have higher antibacterial activity than the mixtures of their major components, suggesting that the minor components are critical to the synergistic activity (Bassolé and Juliani, 2012). The results obtained by GC/MS analysis of the essential oils are presented in Table 3, 4, 5 and 6.

As previously mentioned, other factors that may influence the chemical composition of a particular EO are climatic, seasonal and geographic conditions (Baydar et al., 2004; Figueredo et al., 2015). Since the chemical composition of EOs have influence on their antibacterial properties, comparison among the results of different studies is not always possible. Also, the susceptibility of a microorganism to an EO depends not only on the properties of the EO but also on the microorganism itself (Roldán et al., 2010).

TABLE 2: *Antibacterial activity of antibiotics expressed as MIC, determined by the Test Strip.*

Antibiotics	*E. coli*	*S.* Typhimurium
	MIC (µL/mL)	
Gentamicin	2.00	0.50
Streptomycin	4.00	2.00

TABLE 3: *Chemical composition of Ocimum basilicum L. EO.*

No	Compound*	RI	Rt	%
1	α-Pinene	934	5.851	0.3
2	Camphene	949	6.253	0.1
3	Sabinene	973	6.927	0.1
4	β-Pinene	977	7.036	0.6
5	Myrcene	991	7.417	0.3
6	α-Terpinene	1017	8.283	0.1
7	p-Cymene	1025	8.547	0.2
8	Limonene	1028	8.685	0.2
9	1,8-Cineole	1030	8.768	5.7
10	trans-β-Ocimene	1047	9.371	0.1
11	cis-Sabinene hydrate	1067	10.087	0.1
12	cis-Linalool oxide	1073	10.286	0.2
13	trans-Linalool oxide	1090	10.907	0.3
14	Linalool	1103	11.498	69.2
15	Hotrienol	1105	11.563	0.4
16	Camphor	1143	13.157	0.7
17	Menthone	1153	13.565	0.2
18	trans-Pinocamphone	1159	13.843	0.3
19	Menthol	1171	14.365	0.4
20	cis-Pinocamphone	1172	14.425	0.7
21	Terpinene-4-ol	1175	14.574	0.4
22	α-Terpineol	1189	15.155	0.4
23	Methyl chavicol	1197	15.509	1.6
24	Linalool acetate	1254	18.026	0.3
25	Bornyl acetate	1284	19.35	0.6
26	Menthyl acetate	1293	19.72	0.1
27	α-Cubebene	1349	22.179	0.1
28	Eugenol	1356	22.56	0.5
29	α-Copaene	1375	23.331	0.3
30	β-Bourbonene	1384	23.726	0.4
31	β-Cubebene	1389	23.973	0.1
32	β-Elemene	1391	24.042	1.8
33	Methyl eugenol	1406	24.672	0.1
34	trans-Caryophyllene	1418	25.218	0.6
35	β-Copaene	1429	25.637	0.1
36	β-Gurjunene	1432	25.765	0.1
37	trans-α-Bergamotene	1435	25.923	2.3
38	α-Guaiene	1439	26.046	0.8
39	α-Humulene	1453	26.677	0.5
40	trans-β-Farnesene	1457	26.831	0.1
41	Alloaromadendrene	1461	26.991	0.1
42	epi-Bicycloseskviphelandrene	1463	27.083	0.3
43	γ-Muurolene	1477	27.669	0.1
44	Germacrene D	1481	27.857	1.9
45	NI	1486	28.013	0.1
46	β-Selinene	1486	28.063	0.2
47	Viridifloren	1496	28.478	0.2
48	Bicyclogermacrene	1497	28.511	0.3
49	Aciphyllene	1499	28.619	0.1
50	α-Bulnesene	1506	28.905	1.1
51	γ-Cadinene	1514	29.244	1.8
52	δ-Cadinene	1523	29.621	0.4
53	Maliol	1566	31.394	0.2
54	NI	1577	31.838	0.2
55	NI	1583	32.050	0.1
56	1,10-di-epi-Cubenol	1614	33.320	0.2
57	epi-α-Cadinol	1641	34.326	1.0
Σ				**99.7**

*Compounds listed in order of elution on a HP-5MS column (Rt-retention time, RI-retention index), tr-compound present less than 0.1%, NI-Unidentified compound.

The results of the GC-MS investigation of basil EO are shown in Table 3. This oil was characterized by a high percentage of oxygenated monoterpenes (79.5%) which constituted the predominant class. Other compounds were divided into four classes such as sesquiterpene hydrocarbons (13.5%), monoterpene hydrocarbons (2.5%), phenylpropanoids (2.2%) and oxygenated sesquiterpenes (1.6%). The results of our study showed that the dominant compounds in basil EO were linalool (69.2%) and 1,8-cineole (5.7%). This is consistent with a previous study where the EOs of European varieties of basil are generally characterized by a high content of linalool (Nurzyńska-Wierdak et al., 2012). Chemical analyses of two basil cultivars named 'Kasia' and 'Wala' showed that the EO extracted from the herb of 'Kasia' plants contained more linalool (67.7%) compared to cultivar 'Wala' (60.5%). In the same research, the concentration of 1,8-cineole (eucalyptol) was higher in the cultivar 'Wala' (6.4%) than in the 'Kasia' (3.9%). In contrary to results obtained in this study, Özcan et al. (2002) reported that the oil of *Ocimum basilicum* from Turkey contained as main compounds methyl eugenol (78.02%) and α-cubebene (6.17%). Kotan et al. (2007) reported that linalool possessed a wide antibacterial effect against 42 bacterial strains, compared to menthol and 1,8-cineole. Silva et al. (2015) observed that linalool showed antibacterial activity against the tested *Pseudomonas aeruginosa* strains. This supports the idea that linalool of *Ocimum basilicum* oil is the substance primarily responsible for the antibacterial activity. According to Greay and Hammer (2015), monoterpenes such as linalool interfere with the integrity and function of the cell membrane; changing the membrane potential, causing loss of cytoplasmic material, and inhibiting the respiratory chain.

GC-MS analysis of mentha EO identified 19 different components which represents 88.6% of the total oil. The identified compounds are listed in Table 4. The main compounds of mentha EO were menthol (30.8%), menthone (28.8%), 1,8-cineole (5.9%), menthofuran (5.9%) and isomenthon (5.0%). These compounds were divided into three classes such as oxygenated monoterpenes (81.5%), monoterpene hydrocarbons (4.4%) and sesquiterpene hydrocarbons (2.7%). Bassolé et al. (2010) reported that EO from leaves of *Mentha* x *piperita* contains menthol (39.3%) and menthone (25.2%) as the main compounds. Our results showed the presence of menthol (30.8%) and menthone (28.8%) as the main compounds of the EO of mentha which is similar to previous research by Mahboubi et al. (2014). Genetic and biochemical differences among specific cultivars of the same botanical species could explain these differences (Akgül et al., 1999; Tholl, 2006). These data indicate that chemical composition of mentha EO significantly varies depending on the origin, variety, climate and environmental conditions, condition on the plant (fresh or dried). Depending on the chemical composition, mentha EO exhibited different antibacterial activity against food-borne pathogens. In our study, mentha EO showed moderate activity against tested bacterial strains. In a study conducted by Zaidi and Dahiya (2015) EOs of *Mentha spicata* and *Mentha piperita* exhibited antimicrobial activity against 11 bacterial clinical isolates (including *E. coli, Acinetobacter* spp., *Klebsiella* spp., *S.* Typhi, *S.* Paratyphi, *Staphylococcus aureus, Pseudomonas aeruginosa*) and 4 fungal clinical isolates (*Aspergillus* spp., *Aspergillus niger, Candida albicans, Rhizopus nigricans*). Both EOs exhibited a varying degree of inhibitory effect against the tested bacterial strains. In our study, mentha EO had the lowest efficacy against *S.* Typhimurium with MIC/MBC values of 56.81 µL/mL. The results of previous research conducted by Roldán et al. (2010) supported this finding. Previous researches conducted by Busani et al. (2004) and Musgrove et al. (2006) were showed that antimicrobial resistance in *Salmonella* spp. is serotypically dependent. They reported that among tested *Salmonella* isolates, *S.* Typhimurium was the most prevalent serotype and demonstrated the greatest multiple resistance against tested antimicrobial drugs. The mechanism of the antimicrobial resistance in *Salmonella* spp. is complex, including its resistance at the cellular level and the adaptive resistance (Penesyan et al., 2015). The mechanism of resistance at the cellular level is related to the presence of certain genes, while adaptive resistance can be explained by the ability producing a biofilm (Corona and Martinez, 2013). *Salmonella* spp. have demonstrated the ability to form biofilms on abiotic surfaces, plant surfaces, and animal epithelial cells in several studies that were focused on characterizing biofilm-forming ability in *S.* Typhimurium clinical isolates (Steenackers et al., 2012; Čabarkapa et al., 2019). Bacteria produce and use small signaling molecules to evaluate their external environment and their internal physiological status i.e. to cell-cell communication (quorum sensing) modulating their populations. These molecules are in general known by autoinducers (Camilli and Bassler, 2006). Quorum sensing (QS) is involved in biofilm production, motility, swarming, stress resistance and virulence (Kjelleberg and Molin, 2002). The influence of QS on many essential aspects of the bacterial life makes this process an interesting target to control infections and decreasing antimicrobial resistance (March and Bentley, 2004). The investigation of the anti-QS activity of EOs or its components is in progress (Li et al., 2017).

TABLE 4: *Chemical composition of Mentha piperita L. EO.*

No	Compound*	RI	Rt	%
1	α-Pinene	921	5.801	0.8
2	Sabinene	970	6.889	0.4
3	β-Pinene	975	6.998	1.1
4	Limonene	992	8.659	1.9
5	1,8-Cineole	998	8.740	5.9
6	γ-Terpinene	1025	9.765	0.2
7	cis-Sabinene hydrate	1028	10.056	0.3
8	Menthone	1031	13.558	28.8
9	Menthofuran	1056	14.008	5.9
10	Isomenthon	1056	14.015	5.0
11	Menthol	1064	14.359	30.8
12	Terpinene-4-ol	1084	14.575	1.1
13	Isomenthol	1095	14.844	0.3
14	Pulegone	1143	17.285	0.5
15	Piperitone	1152	17.968	0.2
16	Menthyl acetate	1163	19.745	2.7
17	trans-Caryophyllene	1164	25.258	1.7
18	Germacrene D	1173	27.895	0.8
19	Bicyclogermacrene	1178	28.553	0.2
Σ				**88.6**

*Compounds listed in order of elution on a HP-5MS column (Rt-retention time, RI-retention index), tr-compound present less than 0.1%, NI-Unidentified compound.

The results of chemical composition of winter savory EO are presented in Table 5. Detected compounds were classified in four classes such as monoterpene hydrocarbons (57.7%), oxygenated monoterpenes (37.5%), sesquiterpene hydrocarbons (3.7%) and oxygenated sesquiter-

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TABLE 5: *Chemical composition of Satureja montana L. EO.*

No	Compound*	RI	Rt	%
1	α-Thujene	928	5.674	0.6
2	α-Pinene	935	5.861	1.4
3	Camphene	949	6.262	0.5
4	β-Pinene	978	7.046	1.1
5	Myrcene	992	7.421	1.3
6	α-Phellandrene	1006	7.890	0.2
7	δ-3-Carene	1012	8.086	0.1
8	α-Terpinene	1017	8.287	1.6
9	p-Cymene	1027	8.630	33.8
10	Limonene	1029	8.719	0.9
11	1,8-Cineole	1031	8.792	0.4
12	γ-Terpinene	1059	9.825	15.9
13	cis-Sabinene hydrate	1067	10.088	0.1
14	Terpinolene	1090	10.923	0.1
15	p-Cimenene	1092	10.980	0.1
16	Linalool	1101	11.336	1.4
17	Menthone	1153	13.600	0.1
18	Borneol	1164	14.089	1.2
19	Menthol	1171	14.385	0.4
20	Terpinene-4-ol	1176	14.593	0.7
21	p-Cimene-8-ol	1185	14.968	0.1
22	α-Terpineol	1190	15.191	0.1
23	Carvacrol, methyl ether	1243	17.533	0.1
24	NI	1245	17.624	0.1
25	NI	1287	19.477	0.1
26	Thymol	1294	19.747	0.1
27	NI	1297	19.909	0.2
28	Carvacrol	1305	20.318	32.9
29	NI	1322	20.987	0.1
30	α-Copaene	1375	23.356	0.1
31	β-Bourbonene	1384	23.750	0.1
32	trans-Caryophyllene	1419	25.240	1.4
33	trans-α-Bergamotene	1436	25.940	0.1
34	Aromadendrene	1439	26.077	0.1
35	α-Humulene	1454	26.698	0.1
36	γ-Muurolene	1477	27.684	0.2
37	β-Selinene	1487	28.092	0.1
38	Viridifloren	1496	28.469	0.1
39	β-Bisabolene	1509	29.025	0.9
40	γ-Cadinene	1515	29.254	0.2
41	δ-Cadinene	1524	29.639	0.3
42	Caryophyllene oxid	1582	32.054	0.3
43	NI	1931	44.769	0.1
Σ				**99.8**

*Compounds listed in order of elution on a HP-5MS column (Rt-retention time, RI-retention index), tr-compound present less than 0.1%, NI-Unidentified compound.

penes (0.3%). The main compounds of winter savory EO were p-cymene (33.8%), carvacrol (32.9%) and γ-terpinene (15.9%). The essential oil content and the variability of composition in genus *Satureja* were frequently investigated (Biavati et al., 2004). Miladi et al. (2013) reported that EO of *Satureja montana* L. is characterized by a high content of the oxygenated monoterpene carvacrol (53.35%). Further, other important compounds were monoterpene hydrocarbons γ-terpinene (13.54%) and p-cymene (13.03%). However, Skočibušić et al. (2004) reported that EOs of the aerial parts of *Satureja montana* L. collected in Croatia contained an important percentage of carvacrol (45.7%). Various isolates of winter savory from Croatia, Bosnia, and Herzegovina had carvacrol (up to 84.19%) as the main constituent. The results presented in Table 1. showed that the EO of winter savory had significant antimicrobial activity against *E. coli* and *S.* Typhimurium. Strong antibacterial activity of winter savory EO has been attributed to oxygenated monoterpenes carvacrol which had synergistic effect with other compounds such as monoterpene hydrocarbons p-cymene and γ-terpinene. On the other hand p-cymene is a very weak antibacterial compound but it swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism p-cymene probably enables carvacrol to be more easily transported into the bacterial cell so that a synergistic effect is achieved when both compounds are simultaneously present (Roldán et al., 2010). Thereby, carvacrol does not directly act as outer membrane permeabilising agent (Nazzaro et al., 2013).

The results of GC-MS analysis of dill EO are shown in Table 6. Identified compounds were classified into two classes such as oxygenated monoterpenes (70.3%) and monoterpene hydrocarbons (29.3%). The major compounds were carvone (66.6%) and limonene (25.3%). In accordance with previous research, the chemical composition was similar but different in the relative quantity of chemical compounds found in the EO (Peerakam et al., 2014). Radulescu et al. (2010) and Vokk et al. (2011) reported that the EO from seeds of *Anethum graveolens* L. which grew in Romania and Estonia contained two major compounds, carvone (45.9-75.2%) and limonene (18.4-21.6%). Yili et al. (2009) and Peerakam et al. (2014) also revealed that major compounds of EO from seeds of *Anethum graveolens* growing in Uzbekistan were carvone and limonene. Our results showed that the most abundant chemical compounds of dill EO collected from Serbia are in accordance with previous studies. In contrast with this data, chemical composition of EO of *Anethum graveolens* that was cultivated in Thailand is contained dillapiole (48.90%), D-limonene (26.97%) and D-carvone (18.05%) as the main compounds (Ruangamnart et al., 2015). Previous researchers reported that dill EO possesses antibacterial and antifungal activity (Yiğit et al., 2000; Derakhshan et al., 2017). The results obtained in this study showed that dill EO possesses a significant antibacterial activity against *E. coli* and *S.* Typhimurium. Antimicrobial activity of dill EO in our study probably originates from active constituents such as monoterpens carvone and limonene. The mechanism of antibacterial activity of carvone is not completely understood. It has been demonstrated that the mechanism of action of carvone on the growth of microorganisms

TABLE 6: *Chemical composition of Anethum graveolens L. EO.*

No	Compound*	RI	Rt	%
1	α-Pinene	936	5.886	0.3
2	α-Phellandrene	1007	7.905	1.3
3	NI	1008	7.972	0.3
4	p-Cymene	1025	8.566	2.4
5	Limonene	1029	8.704	25.3
6	Dill ether	1185	14.974	0.5
7	cis-Dihydro carvone	1196	15.459	1.1
8	trans-Dihydro carvone	1203	15.777	2.1
9	NI	1227	16.893	0.1
10	Carvone	1243	17.549	66.6
Σ				**100.0**

*Compounds listed in order of elution on a HP-5MS column (Rt-retention time, RI-retention index), tr-compound present less than 0.1%, NI-Unidentified compound.

includes the destabilization of the phospholipid bilayer structure, interaction with membrane enzymes and proteins, it acts as a proton exchanger reducing the pH gradient across the membrane (Selles et al., 2018). Other terpenes, such as limonene, α-pinene, β-pinene, γ-terpinene δ-3-carene, (+)-sabinene and α-terpinene showed a very low or no antimicrobial activity against food-borne pathogens when used as singular compounds (Nazzaro et al., 2013). The high content of carvone and limonene with numerous biological properties, indicates the possible application of dill EO in food industry, as a food protective product of natural origin. Other components of the oil probably also contribute to this activity (Bakkali et al., 2008; Stanojević et al., 2016).

Briefly, the mechanism of action of EOs depends on their chemical composition, and their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell; together, these properties are referred to as the "essential oils versatility" (Nazzaro et al., 2013).

Although essential oils have been shown to be promising alternative to chemical preservatives, they present special limitations that must be solved before their application in food systems (Fernández-Lopéz et al., 2018). Previous studies have revealed that food composition and structure have a significant effect on the final outcomes of antimicrobial activity of EOs. However, numerous conducted researches have shown that antimicrobial activity of EOs might be reduced by certain food components (fats, carbohydrates, proteins, water, salt, antioxidants, preservatives, other additives) and pH (Čabarkapa et al., 2016). One of the main limitations of application of EOs as antimicrobial agents is intense flavor and odour. Recent advances that refer to new forms of application to avoid these problems are currently under study. Their application into packaging materials and coated films but also directly into the food matrix as emulsions, nano emulsions, and coating are some of their new applications among others (Fernández-Lopéz et al., 2018).

Conclusion

In conclusion, alternative preservation techniques based on the use of naturally derived ingredients are under investigation for their application in food products. The negative consumer perceptions of chemical preservatives, imposes the use natural products as alternatives, especially plant extracts, including the EOs and essences of plant extracts. The results obtained from this study established that the essential oils obtained from *Lamiaceae* and *Apiaceae* family showed interesting biological potentiality. The assessment of antimicrobial properties is an excellent basis for further *in vitro* assays that could be used to define these EOs as potential candidates for natural biopreservatives in combination with or in substitution to synthetic chemical ones. Furthermore, additional studies should be undertaken in order to understand their potential in real food samples. Further research should be done particularly aim to establish the most effective EO concentration depending on the food matrix, its organoleptic properties, and the microorganisms it should inhibit. Anyhow, these results represent a valid basis for future evaluations and enriched current understanding about the specificity *Lamiaceae* and *Apiaceae* plant species and their EOs.

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Conflict of interest

The authors declare that no conflict of interest among authors.

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