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Antiradical and Antibacterial Activity of Essential Oils from the *Lamiaceae* Family Plants in Connection with their Composition and Optical Activity of Components

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Abstract: Antiradical activity of essential oils of korean mint (*Agastache rugosa* (Fisch. et Mey)), blue giant hyssop (*Agastache foeniculum* (Pursh) Kuntze), hyssop (*Hyssopus officinalis* L.), lavander (*Lavandula angustifolia* L.), peppermint (*Mentha piperita* L.), lemon mint (*Mentha piperita* var. *citrata* (Ehrh.) Briq), monarda (*Monarda fistulosa* L.), oregano (*Origanum vulgare* L.), common sage (*Salvia officinalis* L.), clary (*Salvia sclarea* L.), and winter savory (*Satureja montana* L.) cultivated in the Central Botanical Garden of NAS of Belarus was investigated in the reaction with the cation-radicals of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$). The most pronounced antiradical activity was observed for the essential oils with a high content of phenolic compounds: winter savory and monarda. Antiradical properties of the essential oils and individual phenolic and terpene compounds (eugenol, carvacrol, thymol, citral, (+)-pulegone) in the reaction with $ABTS^{+\bullet}$ significantly differ in aqueous solutions and ethanol-water mixtures.

High antibacterial activity of selected components of essential oils from the *Lamiaceae* plants, carvacrol, citral, and linalool, towards test organisms *Sarcina lutea, Escherichia coli, Staphylococcus saprophyticus, Pseudomonas fluorescens, Bacillus megaterium, Pseudomonas putida* was shown. The antibacterial activity of enantiomers of pinene and limonene was determined. The dextrorotary isomer of α-pinene possesses a significantly higher level of activity as compared with the levorotary one. S-(-)-limonene proves itself as a more active antimicrobial component towards *Sarcina lutea* and *Staphylococcus saprophyticus* than R-(+)-limonene. Both enantiomers show comparable activity towards *Escherichia coli*. Due to the high antibacterial activity the essential oils from *Satureja montana* and *Monarda fistulosa* can be considered as effective antibacterial agents.

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1. INTRODUCTION

The consumption of aromatic plants in the pharmaceutical, cosmetic, and food industries has been increasing steadily. The *Lamiaceae* (Lindl.) family consists of about 3500 species

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widely distributed all over the world and many plants are of interests as a source of essential oils, because they diverge sufficiently in the level of accumulation and variety of fragrant substances [1].

Essential oils distilled from plants show a wide spectrum of biological activities that depends on their composition and content of individual constituents [1-2]. Some aspects of the activity, such as antimicrobial or anticonvulsant properties, are affected by chirality of compounds biosynthesized in selected plants and their distribution in a given oil [3,4]. In its turn, the composition of essential oils originated from plants depends on many factors, such as difference in chemotypes, plant growing conditions, technologies of production and storage of vegetative raw materials [1,2]. The evaluation of antioxidant activity of essential oils frequently produces sufficiently different results that do not correlate with each other [5,6]. One of the reasons is the specificity of interaction of essential oil compounds with different media (solubility, hydrogen bonding with solvent molecules and other constituents, etc.). Antimicrobial activity of essential oils is mainly due to the content of specific components and their interaction with selected parts of bacterial cell [7,8].

We report on high antioxidant and antibacterial activity of selected essential oils distilled from raw plant material of the *Lamiaceae* family species cultivated in the central region of Belarus. The essential oil composition and properties of individual compounds are the main factors responsible for a certain type of oil biological activity. The comparison of antiradical activity of essential oil in two different solvents, water and 80 % ethanol, allows for qualitative evaluation of its major antioxidant constituents. For antibacterial activity, both essential oil composition and the content of enantiomers in it are important.

2. METHOD

2.1. Plant material

The aerial parts of korean mint (*Agastache rugosa* (Fisch. et Mey)), blue giant hyssop (*Agastache foeniculum* (Pursh) Kuntze), hyssop (*Hyssopus officinalis* L.), lavander (*Lavandula angustifolia* L.), peppermint (*Mentha piperita* L.), lemon mint (*Mentha piperita var. citrata* (Ehrh.) Briq), monarda (*Monarda fistulosa* L.), oregano (*Origanum vulgare* L.), common sage (*Salvia officinalis* L.), clary (*Salvia sclarea* L.), winter savory (*Satureja montana* L.), basil (*Ocimum basilicum* L.) plants were collected from the Herbs and Spice Collection of the Central Botanical Garden, National Academy of Sciences of Belarus. A half of the collected sample was immediately investigated and the other half was dried in shade at room temperature for two days and then analyzed.

2.2. Extraction and isolation of essential oils

The plant material was subjected to hydrodistillation in a Ginsburg type apparatus (0.2 kg per each loading) for 1 h. The collected samples of essential oils were dried over anhydrous Na_2SO_4 for 24 h and stored at 4-8 ºC until analyzed.

2.3. Chromatography

A Tsvet-800 gas chromatograph (TsvetChrom LTd., Russia) equipped with a HP-5 column (Agilent Technologies Inc., 5% phenyl 95% methylpolysiloxane: equivalent to USP Phase G27, 30 m x 0.25 mm, film thickness 0.25 μm) and a flame ionization detector (FID) with N_2 as a carrier gas was used. The column temperature was ranged from 50 to 200 ºC with an increase programmed at 3 ºC/min. The characterization of essential oil composition was achieved on the basis of retention indices (RI) using a homologous series of *n*-alkanes (Supelco) and standard substances.

The enantioselective capillary GC analysis was conducted on a Cyclosil B (Agilent Technologies Inc.) capillary column (30 m x 0.32 mm, film thickness 0.25 μm, 30% hepatkis

(2,3-di-O-methyl-6-O-t-butyl dimethylsilyl)-ß-cyclodextrin in DB-1701). The column temperature was ranged from 70 to 200 ºC in five consecutive steps: a 5 min isotherm at 70 ºC, an 3 ºC/min increase to 115 ºC, an isotherm for 20 min, a 4 ºC/min increase to 200 ºC and an isotherm for 10 min. Nitrogen was used as a carrier gas at the linear speed of 30 cm/s, the split ratio was 1:50. The identification of terpene enantiomers was performed on the basis of retention indices of the substances ((−)-α-pinene (P7408, Sigma), (+)-α-pinene (W290238, Kosher, Aldrich), (−)-β-pinene (402753, Aldrich), (+)-β-pinene (80607, Fluka), (−)-limonene (62128, Fluka), (+)-limonene (62118, Fluka)) in a standard mixture. Triplicate analysis of each oil sample was performed and quantitative results were presented as a mean of the data derived from the GC-FID analyses. The relative amounts of individual components (in %) were calculated on the basis of GC peak areas without using correction factors.

2.4. Antiradical activity

The antiradical activity of individual compound and essential oil was evaluated using its reaction with the cation-radicals of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$) [9, 10]. In a typical experiment, a solution of individual compound or essential oil under investigation (5–200 μL) was added to 3.0 mL of $ABTS^{+\bullet}$ solution at 25 °C. The change of absorbance at 734 нм ($A_0 - A$) was measured 6 min after mixing.

The comparative evaluation of antiradical activity of different compounds and essential oils was carried out using values of antiradical parameter (AP) and antiradical activity (ARA). The value of AP was considered as a tangent of linear dependences of $A_0 - A$ on the concentration of individual compound or essential oil. ARA was calculated as a quotient of AP of the substance under investigation and AP of the standard antioxidant compound Trolox. ARA is equivalent to the amount of Trolox that has the same effect in the reaction with $ABTS^{+\bullet}$ as 1 mole of individual compound or 1 mL of essential oil. To avoid and quantify solvent effect we evaluated AP and ARA values of selected essential oils and individual terpene and phenolic compounds found in the oils in two different media, pure water and aqueous ethanol with 80 % content of alcohol.

2.5. Antibacterial activity

Antibacterial activity of essential oils was evaluated using the agar disc-diffusion method [11]. The method consists of determining the diameter of growth inhibition zone of test-cultures on meat-peptone agar medium (MPA) which is formed under the action of the substance diffusing in the medium from a disc impregnated with a substance that presumably has antimicrobial activity.

MPA (20 mL) was placed in a Petri dish under aseptic conditions. The bacterial culture was added to the top of agar plates as a continuous lawn with a standardized inoculum of the test microorganism using a Drigalski spatula. Then, sterile filter paper discs of 8 mm in diameter were placed on the inoculated with a test microorganism agar surface. An aliquot of essential oil (10 μL) was placed on every disc. The Petri dish was incubated under suitable condition for 48 h. The diameters of inhibition growth zones for test-cultures were measured to the nearest millimeter. For liquid individual compounds, the experiments were carried out in the similar way.

3. RESULTS AND DISCUSSION

3.1 Essential oil composition

The identified active substances and their content in essential oils obtained from different plants are summarized in Table 1. Many terpene and phenolic compounds are found in all species but some are specific for certain plants.

Table 1. Major components (%) in essential oils

Compound	RI	Agastache rugosa	Agastache foeniculum	Hyssopus officinalis	Lavandula angustifolia	Mentha piperita	Mentha piperita var. citrata	Monarda fistulosa	Ocim basilic
α-thujene	927					0.05/0.05		1.24	
camphene	950					0.08/0.05		0.11	
sabinene	971					0.15/0.18		0.18	
α-pinene	976	0.47/0.02		0.50	0.12	0.35/0.43	0.31/0.40	0.49	0.4
β-myrcene	992					0.22/0.31	0.99/1.31	1.87	
1,8-cineole	1017	0.13/0.26		0.53	1.43	0.42/0.46		23.36	7.6
β-pinene	982.1	0.06		8.52	0.10	0.27/0.47	0.31/0.44	1.14	0.4
3-octanol	977.6								
α-phellandrene	996					0.03/0.06		0.68	
β-phellandrene	1028								
p-cymene	1029					0.42/0.36			
limonene	1033.2	8.63/10.02	13.5		1.61	1.79/2.86	8,25/9.73	1.44	0.1
cis-ocimene	1035								
trans-ocimene	1050							0.46	
γ-terpinene	1063							3.49	
trans-sabinenehydrate	1074					0.18/0.11			
γ-terpinolene	1078							0.45	
cis-sabinenehydrate	1089							0.16	
α-terpinene	1106							0.27	
linalool	1108	0.93/0.61		0.84	43.65		0.17/0.20	1.8	44.1
α-thujone	1114								
β-thujone	1124				1.02				
camphor	1155		0.09		0.37				
menthone	1160	7.50/4.53	4.28	0.44	0.71	23.24/23.56	0.63/0.46		
pinocamphone isomer (T)	1168								
isomenthone	1156	38.8/27.60				3.56/3.95			
borneol	1176							0.42	
menthol	1168					51.49/51.77			
isomenthole	1184					2.45/2.34			
terpinen-4-ol	1173							0.93	
α-terpineol	1148							0.53	

Table 1. Continuation of Table-1

Compound	RI	Agastache rugosa	Agastache foeniculum	Hyssopus officinalis	Lavandula angustifolia	Mentha piperita	Mentha piperita var. citrata	Monarda fistulosa	Ocim basilic
methyl chavicol (estragol)	1207.5	33.37/34.10							
carvone	1221					0.17/0.16			
geraniol	1229							9.12	
thymol	1273		0.11		0.18			28.46	
carvacrol	1288							14.01	
citral (neral B)	1248		0.16	0.16			82.74/80.38		
pulegone	1250	3.59/20.8				1.21/0.81			
piperitone	1251					0.99/1.46	0.32/0.28		
linalyl acetate	1253				17.90			0.42	
bornyl acetate	1275							0.42	
menthyl acetate	1289					0.96/1.81			
eugenol	1356		0.19	0.22					3.4
ceranyl acetate	1363							0.02	
α-copaene	1391								
carvacryl acetate	1370							0.04	
β-bourbonene	1391	3.53/2.02				0.26/0.23	0.16/0.14	0.11	
β-elemene	1399					0.27/0.24	1.28/0.87	0.2	
β–caryophyllene	1436	0.84/1.46	0.88	0.38	0.75		1.55/1.33	0.8	
aromadendrene	1443					1.78/1.70			
α–caryophyllene	1455								
α-humulene	1472								
γ–muurolene	1483							0.03	
germacrene D	1490							1.87	
γ-cadinene	1505							0.11	
α-muurolene	1518								
α-cadinene	1524							0.18	
α–farnesene	1537								
viridiflorol	1595								
caryophyllene oxide	1605								
T–muurolol	1636								
Other components									

RI- for column 1, * - fresh/dried

β-Myrcene is the main representative of the group of acyclic triene monoterpenes in most investigated essential oils. The essential oil of *O. vulgare* is distinguished by a high content of another compound of this group – *cis*-ocimene. At the same time, only the essential oil of *M. fistulosa* contains *trans*-ocimene. Among the functional derivatives of acyclic monoterpenes, linalool found in eight essential oils is the most common component. Linalool and linalyl acetate dominate in oils distilled from *L. angustifolia* and *S. sclarea* (61.55 and 57% of total composition). Citral is the major component in *M. piperita var. citrata* but it is also found in the essential oils from *S. sclarea*, *H. officinalis*, and *A. rugosa*. Geraniol, another derivative of acyclic monoterpenes, was observed exclusively in the *M. fistulosa* oil.

Monocyclic monoterpenes of the essential oils differ in variety. Among the investigated plants, 1,8-cineole is found in a high quantity in the essential oils from *M. fistulosa* (the second most abundant component) and *S. officinalis* (the third one). The central metabolite of the biosynthesis of monocyclic monoterpenes of the menthane group, limonene, is found in practically all essential oils with the exception of *H. officinalis*, *O. vulgare*, and *S. montana*. Limonene is contained in high amounts in *A. rugosa, A. foeniculum,* and *M. var. citrata* oils. Menthone is the second most abundant constituent after menthol in peppermint essential oil. The sum content of mentone and isomenthone exceeds 46% in *A. rugosa* oil. Monoterpene ketone pulegone in high amounts presents in essential oil from the *A. rugosa* dried materials, while no traces of the compound is found in the oil from *A. foeniculum*. Pulegone is also observed in minor amounts in *M. piperita* and *S. officinalis*. Another monoterpene ketone piperitone was found in both *Mentha* sp.

Bicyclic monoterpenes present in practically all of the studied essential oils. The sum of pinenes is high in essential oils from hyssop and common sage, but still their combined content is lower than 10%. Thujones and camphor prevail in the essential oil from *S. officinalis*, their sum reaches 56.03% and 53.34 % in the oils from fresh and dried plant materials accordingly.

The amount of sesquiterpenic hydrocarbons is the largest in the *O. vulgare* essential oil, but did not exceed 18% of all compounds. The presence of bicyclic sesquiterpene β–caryophyllene is confirmed in all analyzed oils with rare exception, its high content is found in the oils of *M. piperita var. citrata, O. vulgare,* and *S. officinalis*.

Among the investigated essential oils the highest content of phenolic compounds was observed in ones from monarda and winter savory, thymol and carvacrol combined give 42.61% of the oil in the first plant, and carvacrol alone constitutes up to 73.4% in the second with a light admixing of thymol. One more phenolic compound eugenol is found only in minor amounts in the oils from *A. rugosa, H. officinalis*, and *S. officinalis*.

The composition of essential oils from spice and medicinal plants of the *Lamiaceae* family varies significantly indicating the complexity of synthetic pathways of secondary plant metabolites in every species.

3.2. Distribution of enantiomers in essential oils

(-)-α-Pinene is found as the dominating form in the essential oils from all investigated *Mentha* and *Agastache sp*., while the dextrorotary form of the compound prevails in the oils of *M. fistulosa, L. angustifolia*, and all *Salvia sp*. (Table 2).

Table 2. Enantiomer components (%) in essential oils from dried plants

Compound	RI	*Agastache rugosa*	*Agastache foeniculum*	*Hyssopus officinalis*	*Lavandula angustifolia*	*Mentha piperita*	*Mentha piperita var. citrata*	*Mo fist*
1*S*-(-)-α-pinene	985	0.02		0.35	0.02	0.26	0.26	0
1*R*-(+)-α-pinene	989	-		0.15	0.10	0.14	0.16	0
1*R*-(+)-β-pinene	1031	0.01		1.72	-	0.29	0.44	
1*S*-(-)-β-pinene	1036	0.05		6.80	0.10	0.21	0.32	
4*S*-(-)-limonene	1068	0.20	0.16	-	0.94	2.09	5.66	
4*R*-(+)-limonene	1077	9.82	13.34	-	0.67	0.19	2.36	6
(-)-linalool	1225	0.61	-	-	43.65	-	-	
(+)-linalool	1229			0.84	-			0
S-(-)-camphor	1267		0.09	-	0.17			
R-(+)-camphor	1269				0.20			
(–)–menthone	1240	4.53		0.44	0.12	21.10	0.83	
(+)–menthone	1246		4.28		0.59		-	

RI – for column 2 (chiralic).

For β-pinene, the (-)-enantiomer is found in the dominant amount in the *Hyssopus* oil. A higher content of this form as compared with the (+)-isomer is observed in essential oils from *Lavandula* and *A. rugosa*, where the terpene is detected in trace amounts. At the same time, the (+)-enantiomer is prevalent in both *Mentha* species, *Monarda*, and *S. officinalis*. Limonene exists exclusively as the (+)-form in *Agastashe*, while in the essential oils from the *Mentha* kind the (-)-form is abundant.

In the *Lavandula* essential oil, the sole presence of (-)-linalool is detected while in the essential oil from *S. sclarea* it is accompanied by (+)-linalool, the amount of the later was twice as low. In the oil from *S. officinalis*, the (+)-isomer of camphor dominates while in the oil of *L. angustifolia* the excess of (+)-camphor over (-)-isomer was minor.

3.3 Antiradical activity of individual compounds

The choice of phenolic and terpene compounds for testing antiradical activity in the $ABTS^{+\bullet}$ system is based on the chromatographic analysis of essential oils from the *Lamiaceae* family species, as indicated in Table 1, and availability of pure individual components. AP and ARA of the individual compounds are summarized in Table 3.

Table 3. Antiradical activity of selected compounds

Compound	AP, L/mol		ARA, mol Trolox/mol	
	water	80% ethanol	water	80% ethanol
eugenol	$2.5 \cdot 10^5$	$2.0 \cdot 10^5$	65.8	52.6
carvacrol	$3.5 \cdot 10^4$	$5.5 \cdot 10^3$	9.2	1.5
thymol	$1.5 \cdot 10^4$	$7.0 \cdot 10^2$	4.0	$18 \cdot 10^{-2}$
(+)–pulegone	$3.7 \cdot 10^3$	0	1.0	0
(±)–citral	0	$0.2 \cdot 10^2$	0	$5.3 \cdot 10^{-3}$

Individual phenolic constituents of essential oils, such as eugenol, thymol, and carvacrol, show extraordinary antiradical activity exceeding that of Trolox (Table 3). It is evident that the specificity of ARA of the phenolic compounds is determined by their structure. The activity of phenolic compounds in radical reactions depends on two factors: the strength of O–H bond and the presence of bulk substituents in the ortho-position of phenol ring that create steric hindrance [12]. Thus, shifting bulk isopropyl substituent from meta-position relatively to –OH group as in carvacrol molecules into ortho-position as in thymol results in a decrease of ARA by a factor of 2.3 in aqueous and by a factor of 8.1 in ethanol medium. Eugenol shows ARA that exceeds that of carvacrol 7.1 times in water and 36.3 times in ethanol.

Among the investigated monoterpenoids, pulegon solely demonstrates the ability to react with $ABTS^{+\bullet}$ in water, while citral does in ethanol. The later discolors the cation-radical solution in the concentrations of the order of 1 mmol/L. Both substances have α, β – unsaturated carbonyl moiety, from which they derive the potential properties to act as radical scavengers, covalently bind to target proteins, or act as antioxidants, for example, by thiol trapping [13].

The significant difference of ARA of the compounds in different media presumably can be explained by the interaction of individual components of essential oils with solvent molecules, by specific and non-specific solvation in particular. Phenols can form hydrogen bonds with molecules containing hetero-atoms or π-bonds [12]. In such solvents, phenolic-type inhibitor (InH) exists in two forms: as free molecule and associated with solvent through hydrogen bond (InH и $InH...OH_2$ accordingly). Free radicals attack phenolic O–H bond which is not involved into such complexation, and lower value of rate constants in such solvents is due to decreasing concentration of free, and thus more active, molecules InH. Depending on

the phenol structure the association can be different. Moreover, the cation-radicals $ABTS^{+\bullet}$ themselves are highly polar particles and presumably form complexes with alcohols.

By ascending ARA, the investigated compounds can be arranged in the following orders: in water: linalool = 1,8–cineole = linalyl acetate = menthone = limonene = β–caryophyllene = citral (zero activity) < pulegone < thymol < carvacrol < eugenol; in 80% ethanol: linalool = 1,8–cineole = linalyl acetate = menthone = limonene = β-caryophyllene = pulegone (zero activity) < citral < thymol < carvacrol < eugenol. The values of ARA of the compounds in aqueous solutions are higher than those in the medium with a high ethanol content, but their ranking is similar in both media.

3.4 Antiradical activity of essential oils

As one can see from Table 4, the investigated essential oils vary in antiradical activity. Taking into account the presence of radical scavenging compounds among the constituents of different essential oils and the character of ARA changes in water and ethanol aqueous media the investigated essential oils can be arranged in three nominal groups with carvacrol/thymol, eugenol/pulegone, and citral dominating types of antiradical activity.

Table 4. Antiradical activity of essential oils

Essential oil	AP, L/mL		ARA, mol Trolox/mL	
	water	80% ethanol	water	80% ethanol
Agastache foeniculum	0.04	0.02	0.01	$5 \cdot 10^{-3}$
Agastache rugosa	3.5	1.5	0.9	0.4
Hyssopus officinalis	4.3	0.4	1.1	0.1
Lavandula angustifolia	-	0.02	-	$5 \cdot 10^{-3}$
Mentha piperita	4.6	0.6	1.2	0.2
Mentha piperita var. citrata	1.8	1.4	0.5	0.4
Monarda fistulosa	42.9	17.6	11.3	4.6
Ocimum basilicum	21.1	2.5	5.6	0.7
Origanum vulgare	7.9	2.6	2.1	0.7
Salvia sclarea	2.2	1.9	0.6	0.5
Salvia officinalis	1.8	0.8	0.5	0.2
Satureja montana	182.4	108.3	48.0	28.5

The essential oil of *S. montana* shows the highest ARA both in water and 80% ethanol due to the domination of phenols, carvacrol and thymol, in this oil. Two other oils with a high ARA value, *M. fistulosa* and *O. vulgare*, also contain carvacrol and thymol in sufficient amounts. The comparative analysis of ARA values shows that in aqueous ethanol, as compared with pure water, the essential oils of *S. montana*, *M. fistulosa*, and *O. vulgare* decrease their activity towards $ABTS^{+\bullet}$ similar to pure carvacrol and thymol, but to a lesser degree than these individual compounds.

A tendency of 2-10 times decreasing ARA in ethanol solutions was also observed for the oils with intermediate ARA values, such as *M. piperita*, *H. officinalis*, *A. rugosa*, *S. officinalis*, and *A. foeniculum* containing eugenol and pulegone/piperitone in reasonable amounts. The essential oil of *O. basilicum* with the highest eugenol content among investigated oils [14] shows relativly high activity in the discoloration of $ABTS^{+\bullet}$ in water and 8 times lower ARA value in ethanol. In the oils of *Lavandula* and *A. foeniculum*, a low content of eugenol and

thymol is combined with a high concentration of easily oxidazable terpenes, thus their ARA is extremely low in both solvents.

Despite the fact that citral alone has no antiradical activity in pure water, the oils of *S. sclarea* and *M. citrata* containing high concentrations of citral retain comparable ARA level both in water and aqueous ethanol solutions. Piperitone found in moderate amounts in the *M. citrata* oil can also host some antiradical activity.

The results above suggest a complex character of the interaction of $ABTS^{+\bullet}$ with essential oils which are multicomponent systems of interacting with each other components. The properties of essential oils as a whole are sufficiently different from simple additive mixtures of individual constituents.

3.5. Antimicrobial activity of essential oil components

Many constituents of essential oils are reported to have high antibacterial activity. Besides being related to physicochemical characteristics of the compounds (such as lipophilicity, water solubility, molecule polarity), antibacterial effect appears to be dependent on the lipid composition and net surface charge of the bacterial membranes. Furthermore, the active compounds might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity [11,15].

According to the obtained experimental results carvacrol completely inhibits proliferation of *E. coli, S. lutea*, and *S. saprophyticus* bacteria (Figure 1). High antimicrobial activity of carvacrol and compounds with similar structure (thymol, cymene, carvacrol methyl ester) is well known and associated with the presence of system of delocalized electrons and enhanced by phenol hydroxyl group. Carvacrol presumably adsorbs in phospholipid bilayer, thus fluidizes the cytoplasmic membrane that weakens and expands [11,15]. As a week acid, carvacrol disturbs the pH gradient and ionic transport across the cytoplasmic membrane. This results in collapse of the proton motive force and depletion of the ATP pool and eventually lead to cell death [8]. Eugenol showed lower activity against microorganisms than carvacrol and thymol, due to its capacity to make intramolecular hydrogen bonding with the neighboring ether groups; therefore, it has diminished ability to make intermolecular hydrogen bonding [16].

Oxygenated monoterpenes exhibit strong antimicrobial activity while hydrocarbon derivatives possess lower antimicrobial properties, as their low water solubility and limited hydrogen bonding capacity decrease their diffusion through the medium. Ketones, aldehydes, and alcohols are considered as more active antimicrobial compounds, but with varying specificity and levels of activity. Their activity is related to the presence of functional groups and influenced by hydrogen bonding parameters in all cases [16, 17]. As compared to antibiotic streptomycin, thymol, carvacrol, and menthol reportedly showed higher antibacterial activity, while linalool, linalyl acetate, camphor, and 1,8-cineole showed the same or slightly higher level of activity than the antibiotic [7]. The sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone that have traditionally been used as flavorants and aroma compounds in the food and perfume industries enhance bacterial permeability and susceptibility of *Lactobacillus fermentum*, *Staphylococcus aureus*, and *Escherichia coli* to a number of exogenous antimicrobial compounds [18].

Our experimental results prove citral being an effective antimicrobial agent toward all test-organisms while linalool was highly active toward *E. coli* and *S. lutea*, and less active against *S. saprophyticus.* However, other terpenes show a sufficiently lower level of activity. (+)-Pulegone is half as active as citral. (-)- Carvone and (+)-menthofuran inhibit slightly the proliferation of two types of bacteria, while (-)-thujon was active exclusively against *E. coli*. 1,8-cineole shows no antibacterial properties under experimental conditions (Fig. 1).

S-(-)-limonene is more active towards *S. lutea* and *S. saprophyticus*, while both enantiomers of the compound were equally effective against *E. coli*. (R)-(+)-limonene is known to be especially effective in inhibiting the proliferation of a variety of microorganisms that cause crop damage or food spoilage, including *Aspergillus niger*, *Colletotrichum falcatum*, *Bacillus subtilis*, and *Staphylococcus aureus* even though some studies declare that it exerts almost no antimicrobial activity [19].

The agar diffusion test shows that levorotary isomers of pinenes do not have any bacteriostatic properties while both (+) - α- and β-pinenes are active toward *S. lutea*, and (+)-α-pinene - towards *E. coli*. It is in good agreement with other observations that only the dextrorotary enantiomers of the α- and β-isomers of pinene are microbiologically active [20].

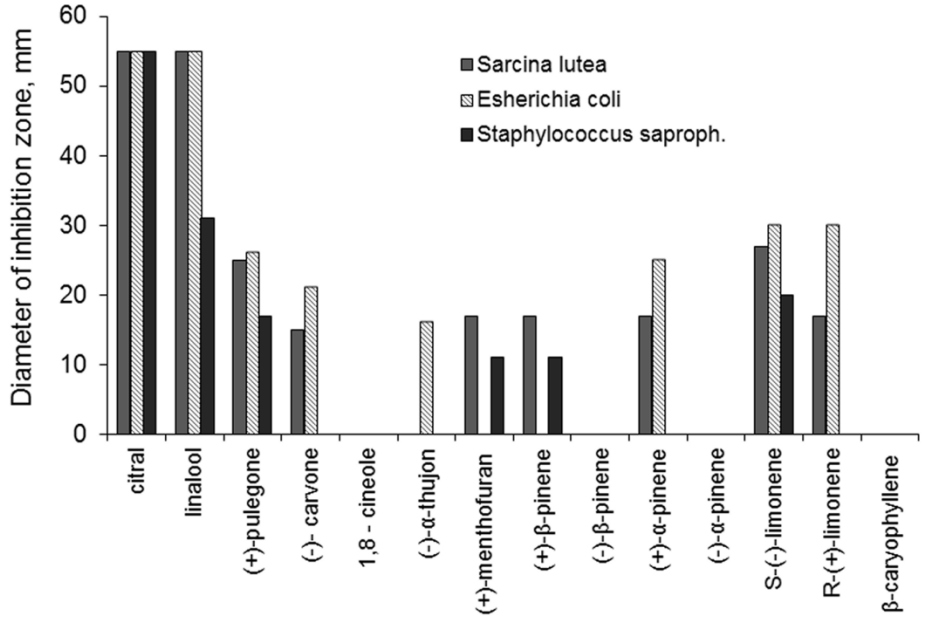

Figure 1. Antimicrobial activity of selected components of essential oils.

3.6. Antimicrobial activity of essential oils

In the used assay the oils of *S. montana, L. angustifolia, M. fistulosa, H. officinalis,* and *S. officinalis* are highly active toward *Sarcina lutea.* The oil of *S. montana* appears to be the most effective among them ensuring a proliferation inhibiting zone on a *Sarcina lutea* continuous lawn as high as 69 mm (Figure.2). The oils from *M. fistulosa* and *S. montana* were the most effective among investigated plants towards *Pseudomonas putida*. A higher efficacy of the essential oils towards gram-positive *S. lutea* as compared with gram-negative *Pseudomonas putida* can be explained by specific structure of cell walls of gram-positive and gram-negative cultures. The presence of additional outer membrane supports to higher resistivity of gram-negative bacteria to bactericidal agents of different nature.

The essential oil of *S. montana* also shows the highest activity towards *P. fluorescens* supporting the zone of proliferation inhibition of about 26 mm. Other oils were much less active or show no activity at all against specific bacteria. With the exception to *M. citrata* and *S. officialis,* all oils were effective towards *Bacillus megaterium.*

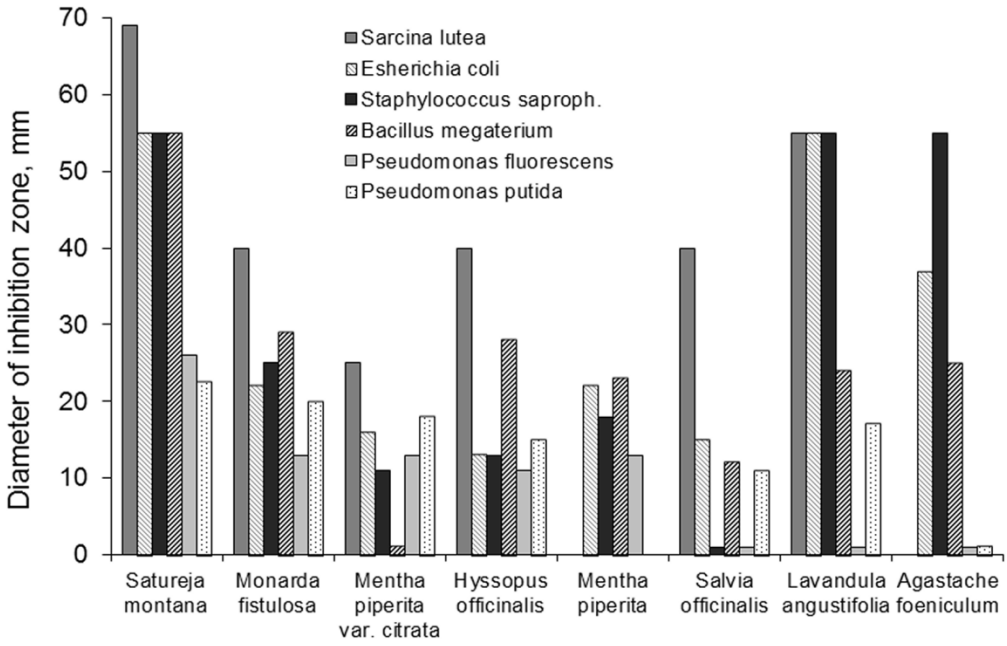

Figure 2. Antimicrobial activity of essential oils.

4. CONCLUSIONS

The antiradical and antibacterial activity of essential oils distilled from raw plant material of the *Lamiaceae* family species in model systems varies significantly and depends on their composition and properties of individual compounds.

Antiradical properties of essential oils and individual phenolic and terpene compounds that are components of the oils significantly differ in aqueous solutions and ethanol-water mixtures. The comparative analysis of antiradical activity of essential oils in the two different media, water and 80 % ethanol, allows us to distinguish three nominal types of essential oils related with their major antioxidant constituents. The highest antiradical activity in the reaction with the ABTS cation-radicals was observed for essential oils with high content of carvacrol and thymol: winter savory and monarda. The intermediate ARA values that are 2-10 times lower in ethanol than in water are associated with an eugenol/pulegon type of activity in peppermint, hyssops, korean mint, and common sage. The citral-related type of activity with comparable ARA levels in water and 80% ethanol is observed for clary and lemon mint oils. The comparison of antiradical activity in water and ethanol-water mixtures can be used as a tool for preliminary analysis of essential oils of unknown composition in order to evaluate the presence of certain classes of constituents in the mixture.

For antibacterial activity, both the essential oil composition and the content of enantiomers in it are important. A significantly higher antibacterial activity of the dextrorotary isomer of α-pinene as compared with the levorotary one was found. S-(-)-limonene proves itself as more active antimicrobial component towards *Sarcina lutea* and *Staphylococcus saprophyticus* than R-(+)-limonene, while both enantiomers show comparable activity towards *Escherichia coli*. Essential oils from the *Lamiaceae* family plants with a high content of carvacrol, citral, and linalool are traditionally chosen for high antibacterial activity towards a wide spectrum of bacteria. Due to high antibacterial activity essential oils from winter savory and monarda can be considered as the most effective antibacterial agents among the investigated essential oils.

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