Chemical composition, antimicrobial and antioxidant properties of *Mentha longifolia* (L.) Huds. essential oil

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Abstract

Introduction: Present study describes the antimicrobial activity and free radical scavenging capacity (RSC) of essential oil from *Mentha longifolia (L.) Huds*. Aim of this study to investigate the quality, antimicrobial and antioxidant activity of wild species *Mentha longifolia* essential oil from Bosnia and Herzegovina.

Methods: The chemical profile of essential oil was evaluated by the means of gas chromatography-mass spectrometry (GC-MS) and thin-layer chromatography (TLC). Antimicrobial activity was tested against 6 bacterial strains. RSC was assessed by measuring the scavenging activity of essential oils on 2,2- diphenyl-1-picrylhydrazil (DPPH).

Results: The main constituents of the essential oil of *M. longifoliae folium* were oxygenated monoterpenes, piperitone oxide (63.58%) and 1,8-cineole (12.03%). Essential oil exhibited very strong antibacterial activity. The most important antibacterial activity essential oil was expressed on Gram negative strains: *Escherichia coli, Pseudomonas aerginosa* and *Salmonella enterica. subsp.enterica serotype* ABONY. Antioxidant activity was evaluated as a RSC. Investigated essential oil was able to reduce DPPH radicals into the neutral DPPH-H form (IC_{50}=10.5 μg/ml) and this activity was dose –dependent.

Conclusion: The study revealed significant antimicrobial activity of the investigated essential oil. The examined oil exhibited high RSC, which was found to be in correlation to the content of mainly monoterpene ketones and aldehydes. These results indicate that essential oils could serve as safe antioxidant and antiseptic supplements in pharmaceuticals. © 2012 All rights reserved

Keywords: Mentha longifolia (L.) Huds, essential oil, chemical composition, antimicrobial activity, antioxidant activity

Introduction

Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Also, herbal medicines have a great potential in the emerging nutrition industry, because these materials are often considered foods as well as medicines and are used in preventive and curative treatments throughout the world (1). Especially popular today is the concept of foods that combine nutritional and medicinal benefits, so-called "functional foods". Many natural compounds extracted from plants have demonstrated biological activities. Among these various kinds of natural substances, essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation. In fact, their effectiveness against a wide range of microorganisms has been repeatedly demonstrated (2-5). Moreover, essential oils are proved to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antiviral, and anticarcinogenic effects, etc. (6). Recently, many essential oils have been qualified as natural antioxidants (3, 5-8) and proposed as potential substitutes of synthetic antioxidants in specific sectors of food preservation. Furthermore, biologically active natural compounds are of interest to the pharmaceutical industry for the control of human diseases of microbial origin and for the prevention of lipid peroxidative dam-

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age, which has been implicated in several pathological disorders, such as ischemia-reperfusion injury, coronary atherosclerosis, Alzheimer's disease, carcinogenesis, and aging processes (9, 10).

The genus *Mentha* L., member of the family *Lamiaceae*, subfamily *Nepetoideae*, and the tribe *Mentheae* is divided into 5 sections (*Audibertia, Preslia, Pulegium, Mentha* and *Eriodontes*) (11,12). The most complex section *Mentha* further can be subdivided into the three groups, reflecting their differences in the inflorescence form (*Verticillatae, Capitatae* and *Spicatae*) (12,13). Furthermore, for the genus *Mentha* the correct number of species is still not defined. According to the authors, the genus consists approximately 14-25 species (11,12). Most of the species are characterized by a great polymorphism, which is reflected in the leaf shape, indumentum, type of flowers and inflorescences etc. In addition to the morphological variation, most of the *Mentha* species also displays a considerable chemical diversity in essential oil composition, depending on the growth location (14). Examination of the published literature on the oil composition of *M. longifolia* reveals that it can exist in a myriad of chemical forms, as can be seen from the main constituents found in these oils. The main constituents in essential oil were piperitone oxide (13.90-50.50 %), 1,8-cineole (8.18-17.80%), carvone (0.5-21.5%), beta caryophyllene (2.0-22.0%) and menthol (0.0-32.50%). The genus *Mentha* clearly has marked antimicrobial characteristics across the spectrum from fungi and parasites, through bacteria, to viruses. There is some difficulty in comparing the different results obtained by research groups across the world since so many variables exist. Antimicrobial activity along with the antioxidant effectiveness of essential oils is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin. Numerous reports suggest strong antibacterial and antifungal activities of a wide range of essential oils, especially those belonging to the Lamiaceae family (12). In general, Gram-positive strains of bacteria are more sensitive to the mint essential oils. *Mentha longifolia* (L) *Huds*. is perennial herb 40-120 cm high with musty scent. Stem white or grey-villous, sometimes sparsely hairy. Leaves are sessile or shortly petiolate usually oblong elliptical, hairs simple. Extremely variable in height, leaf size and shape, indumentum and inflorescence and complicated by the occurrence of hybrids. *Mentha longifolia*, is often used as a domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effect on the digestion as it is a well-know treatment for flatulence. The objectives of this study were to analyze the composition, antimicrobial and antioxidant activity of the essential oil of *Mentha longifolia* growing wild in Bosnia and Herzegovina.

Methods

Plant Material: Aerial parts of wild growing flowering plants of *Mentha longifolia* (L.) *Huds*. during three phenophases (before flowering, flowering and after flowering) were collected in 2011. on the bank of the Jablanicko lake, near Konjic, in Bosnia and Herzegovina.

Isolation of the Essential Oil:
Air-dried plants of *Mentha longifolia* were submitted to hydrodistillation according to European Pharmacopoeia 7ed. (15), using Clevenger apparature (Klaus Hofmann GmbH, Germany). The essential oil samples of each phenophase were dried over anhydrous sodium sulfate. The quantity of the predestilated essential oils were determined volumetrically.

Essential Oil Analysis: Qualitative and quantitative analyses of the essential oils were carried out using a gas chromatography/mass spectrometry system (GC-MS, Agilent Tecnologies series 6890N/5975B, United States of America) at electron energy=70 eV, equipped with a split-splitless injector (200^{0}C) and a flame ionization detector (FID) (250^{0}C). As a carrier gas helium (1ml/min) was used. The capillary columns (HP 5MS 30m x 0.25mm; film thickness 0.25μm Agilent Tecnologies) were used. The temperature programmes were 50^{0}C to 280^{0}C at a rate of 10^{0}C/min until 130^{0}C and 130-280^{0}C at a rate of 12^{0}C/min, respectively with split ratio, 1:10. Coelution and mass spectrometry MS analysis based on the identification of the individual compounds, and the comparison of their relative retention times (RI) with those of the reference samples

were performed. For the components, mostly sesquiterpenes and aliphatic compounds, for which reference substances were not available, the identification was performed by matching their retention times and mass spectra with those obtained from the authentic samples and/or the The National Institute of Standards and Technology, known as the National Bureau of Standards (NIST/NBS), Wiley libraries spectra as well as with literature data (16).

Evaluation of Antibacterial Activity.

Antimicrobial activity of essential oils, isolated from *Mentha longifolia* (L.) *Huds*., using diffusion method was performed in this study. A collection of 6 test organisms, including three Gram-positive and three Gram-negative bacterial strains, was used. The groups included five organisms of American Type of Culture Collection (ATCC) and one organism of National Collection of Type Cultures (NCTC). The source of the bacterial strains is shown in Table 2. All test organisms were stored at +4 °C on Mueller-Hinton (MH) agar slants, sub cultured every 2 weeks and checked for purity. Antibiotics which are therapeutically important in treating infections caused by these microorganisms were used as comparative substances (as positive control): *ciprofloxacine* for evaluation of antimicrobial activity of *Pseudomonas aeruginosa, Penicilin* for *Bacillus subtilis, Gentamycin* for *Escherichia coli, Staphylococcus aureus* and *Staphylococcus epidermidis* and *tetracycline* for *Salmonella enterica subsp.enterica serotype ABONY*. All samples were applied as solution in n-hexane. The effect of the solvent (*n*-hexane) on the microbial growth was also analyzed. On the surface of the agar, the 6 mm holes in diameter were punched. Hundred microliters of the tested essential oils (10 %, 5%, 1%, 0.5% and 0.1% solutions in n-hexane was applied to the holes. The plates were incubated overnight at 37 °C, and the diameter of the resulting zone of inhibition was measured. The evaluation of the antibacterial activities of the essential oils was carried out in three repetitions.

Antioxidant Activity.

Chemicals and Apparatus: 1,1-Diphenyl-2-picrylhydrazyl (DPPH•) as free radical form (90% purity) and 6-hydroxy-2,5,7,8 tetramethylchroman-2- carboxylic acid (Trolox) were obtained

TABLE 1. Chemical Composition of *M. longifolia* Essential Oil

pick no.	Components	RI[a]	percentage (%)
	monoterpene hydrocarbons		3,06
1	alfa-pinene	938	0,78
2	Sabinene	974	0,47
3	beta-pinene	978	0,99
4	beta-myrcene	992	0,69
5	Terpinolene	1008	0,07
6	Limonene	1035	0,06
	oxygenated monoterpenes		87,1
7	1.8-cineole	1036	12,03
8	trans-sabinene hydrate	1098	0,68
9	cis-sabinol	1143	0,16
10	Borneol	1167	0,52
11	piperitone oxide	1170	63,58
12	4-terpineol (terpinen-4-ol)	1178	0,1
13	1-alfa-terpineol	1188	0,91
14	thymol	1291	1,69
15	piperitenone	1343	1,98
16	piperitenone oxide	1369	4,81
17	cis-jasmone	1395	0,64
	sesquiterpene hydrocarbons		6,79
18	alfa-kopaene	1375	0,19
19	beta-burbonene	1383	0,54
20	beta-kubebene	1390	0,48
21	beta-elemene	1391	0,18
22	cis-Caryophyllene	1405	0,82
23	trans-Caryophyllene	1419	2,98
24	alfa-humulene	1452	0,44
25	allo-aromadendrene	1462	0,23
26	alfa-amorfene	1485	0,26
27	germacren D	1490	0,16
28	alfa-murolene	1500	0,11
29	gama-cadinene	1514	0,31
30	delta-cadinene	1523	0,09
	oxygenated sesquiterpenes		5,57
31	caryophyllene oxide	1582	4,33
32	cedrol	1601	0,51
33	tau-muurolol	1651	0,2
34	alfa-cadinol	1654	0,53
	aliphatic compounds		1,22
35	3-octanol	991	1,16
36	n-udecanol	1370	0,06
	total identified		98,17

Compounds listed in order of elution from a HP-5 MS column. Retention indices relative to C9-C24 n-alkanes on the HP-5 MS column

TABLE 2. Antibacterial Activity (Inhibition Zone Measured in mm, Including Hole 6 mm in Diameter) of Essential Oils of *Mentha longifolia*

source	organism	10 %	5 %	1%	0,5%	0,1%	Positive control
ATCC 6633	Bacillus subtilis	11±0,81	9,5±0,80	8±0,71	-	-	32±0,70 penicilin
ATCC 6538	Staphylococcus aureus	13,6±1,52	14±1,62	8±0,71	-	-	10,5±0,00 gentamycine
ATCC 11228	Staphylococcus epidermidis	12±2,11	10,9±1,12	-	-	-	15,2 ±0,00 gentamycine
ATCC 8739	Escherichia coli	19±0,71	17±1,22	13±0,61	9±0,51	7±0,33	17 ±0,22 gentamycine
ATCC 9027	Pseudomonas aeruginosa	25±2,12	22±1,71	19±0,77	19±0,87	11±1,85	28 ±0,85 ciprofloxacine
NCTC 6017	Salmonella enterica subsp.enterica serotype ABONY	20±0,33	15±0,56	10±0,99	-	-	20±0,22 tetracycline

The values shown represent the average of three determinations ± standard deviations. All essential oils were diluted in n-hexane (solvent expressed no activity on bacterial growth).

from Sigma–Aldrich Quimica (Alcobendas, Spain). N- hexane was provided by Merck (Mollet del Valle´s, Spain). All reagents were of analytical grade. Double distilled water (Millipore Co.) was used throughout. Absorbance measurements were recorded on a UV/VIS mini-1240 Spectrophotometer (Shimadzu, Japan).

DPPH Method

A hexanic solution (90 μM) of the radical DPPH• was prepared daily and protected from light. Absorbance was recorded to check the stability of the radical throughout the time of analysis. 2 mL of the stock solution of essential oil (61.92 μg/ml) was mixed with 2 mL of 90 μM DPPH. solution. Absorbance at 515 nm was recorded at different time intervals until the reaction reached an equilibrium. The initial absorbance was 0.700. The blank reference cuvette contained hexane. 1.25; 3.75; 2; 5 and 10 ml of concentrated stock solutions (61.92 μg/ml) were diluted to 10 ml with n-hexane to yiled the concentrations of 7.74; 15.48; 23.22; 30.96 and 61.92 μg/ml, respectively. Absorbance intensity of DPPH on wavelength 515 nm was measured in the test solutions that were contained 2 ml of 90 μM DPPH solution and 2 ml of tested dilutions of essential oil (from 7.74 to 61.92 μg/ml). Absorbencies intensity of the test solutions and the blank (with same chemicals, except sample) were measured at the 0 min and at the time when the steady state of the reaction between DPPH and analyzed compound was reached. 0.1 M Trolox was used as positive control. For each samples three replicates were recorded. Free radical scavenging capacity in percent (RSC (%)) was calculated by following Equation (1):

$$RSC\ (\%)=100*(A_{blank}-A_{sample})/A_{Blank} \quad (1)$$

From the obtained RSC values the EC50 values, which represent the concentration of the essential oil that caused 50% neutralization, were determined by linear regression analysis. The antiradical efficiency (AE) was calculated considering the EC_{50} value and the necessary time to reach the EC_{50} (TEC50), according to the following Equation (2):

$$AE=\frac{1}{EC_{50}*TEC_{50}} \quad (2)$$

Results

Essential oil content and chemical composition

The content of the essential oil in the flowering stage, expressed in percentage was 1.9% v/w (volume of essential oil/weight dry leaf). A total of 36 compounds were identified, grouped as classes of compounds, in the essential oils extracted from *M. longifolia* plants collected in Bosnia and Herzegovina (Table 1). A total of the 36 chemical constituents representing 98.17% of the total content.

Antimicrobial Activity

The antibacterial activity of essential oil against a range of Gram-positive (three strains) and Gram-negative (three strains) is shown in Table 2 and figures 2-7. Obtained results revealed that essential oil exhibited variable levels of antibacterial activity against all tested bacterial strains.

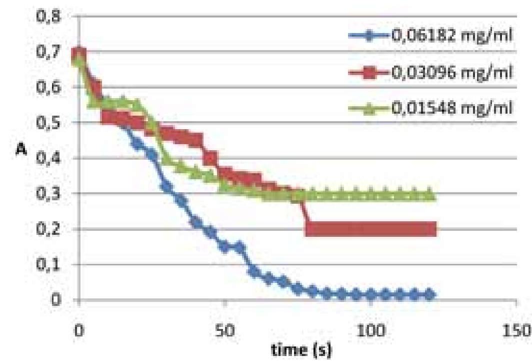
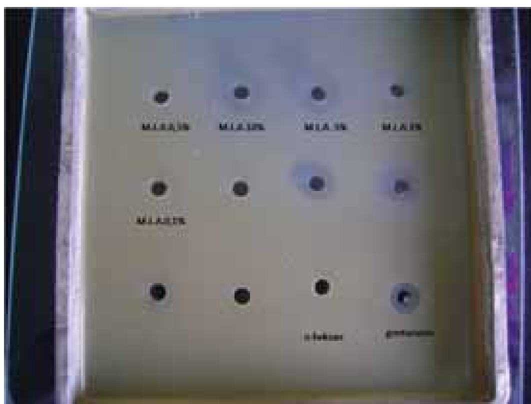


FIGURE 1. Reaction curves between 90 µM DPPH• and different solutions essential oil of *M. longifolia*.

Antioxidant Activity

This study, also, determined the antioxidant activity of one species of the family Lamiaceae. The results indicate that the hexan extract of the plant demonstrated antioxidant activity, and showed the high activity with a EC50 value of 10.5 µg/mL (Table 3). The reaction of essential oil and DPPH. is quite slow. Time at equilibrium state depends on the concentration used (Figure 1). TEC50, as the time at equilibrium reached with a concentration of essential oil equal to EC50 is 95. Calculated value of AE of tested essential oil is $10.58*10^{-3}$.



M.L.A 0,5%	*M.longifoliae aetheroleum 0,5%*
M.L.A 10%	*M.longifoliae aetheroleum 10%*
M.L.A 5%	*M.longifoliae aetheroleum 5%*
M.L.A 1%	*M.longifoliae aetheroleum 1%*
M.L.A 0,1%	*M.longifoliae aetheroleum 0,1%*

FIGURE 2. Antimicrobial activity against *Staphylococcus aureus*

M.L.A 0,5%	*M.longifoliae aetheroleum 0,5%*
M.L.A 10%	*M.longifoliae aetheroleum 10%*
M.L.A 5%	*M.longifoliae aetheroleum 5%*
M.L.A 1%	*M.longifoliae aetheroleum 1%*
M.L.A 0,1%	*M.longifoliae aetheroleum 0,1%*

FIGURE 3. Antimicrobial activity against *Staphylococcus epidermidis*

M.L.A 0,5%	*M.longifoliae aetheroleum 0,5%*
M.L.A 10%	*M.longifoliae aetheroleum 10%*
M.L.A 5%	*M.longifoliae aetheroleum 5%*
M.L.A 1%	*M.longifoliae aetheroleum 1%*
M.L.A 0,1%	*M.longifoliae aetheroleum 0,1%*

FIGURE 4. Antimicrobial activity against *Bacillus subtilis*

M.L.A 0,5%	*M.longifoliae aetheroleum 0,5%*
M.L.A 10%	*M.longifoliae aetheroleum 10%*
M.L.A 5%	*M.longifoliae aetheroleum 5%*
M.L.A 1%	*M.longifoliae aetheroleum 1%*
M.L.A 0,1%	*M.longifoliae aetheroleum 0,1%*

FIGURE 5. Antimicrobial activity against *Escherichia coli*

M.L.A 0,5%	*M.longifoliae aetheroleum 0,5%*
M.L.A 10%	*M.longifoliae aetheroleum 10%*
M.L.A 5%	*M.longifoliae aetheroleum 5%*
M.L.A 1%	*M.longifoliae aetheroleum 1%*
M.L.A 0,1%	*M.longifoliae aetheroleum 0,1%*

FIGURE 6. Antimicrobial activity against *Pseudomonas aeruginosa*

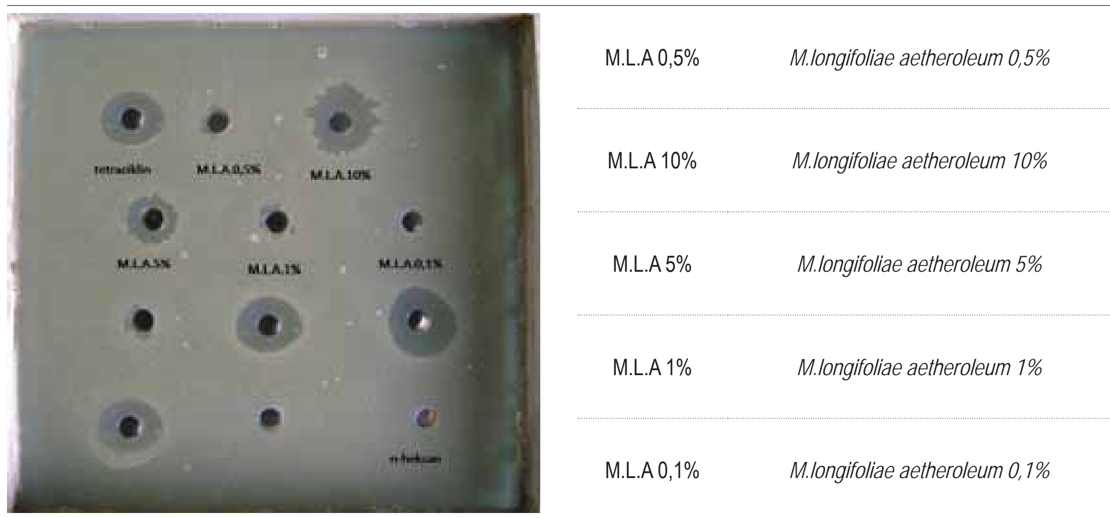


FIGURE 7. Antimicrobial activity against *Salmonella enterica subsp.enterica*

TABLE 3. Percentage of neutralization of DPPH. of essential oil of *M. longifolia* and trolox as positive control in DPPH assay

Source	Concentration [μg/ml]	RSC (%)	EC50 [μg/ml]	TEC50 [min]	AE (*10^{-3})
M. longifolia	7.74	45.22	10.50	95	10,58
	15.48	56.09			
	23.22	63.20			
	30.96	70.22			
	61.82	98.50			

Discussion

M. longifolia essential oils from other geographical locations have been extensively studied. The essential oil content (1.9% v/w in dry leaf) was in accordance with the earlier published data (3). In the oil obtained from the plants collected in the flowering stage the oxygenated monoterpenes were found to be the major class of substances (87.1%), followed by the sesquiterpene hydrocarbons (6.79%) and oxygenated sesquiterpenes (5.57%). The main constituents of the essential oil of *M. longifoliae folium* were oxygenated monoterpenes, piperitone oxide (63.58%) and 1.8-cineole (12.03%). Caryophyllene oxide (4.33%) was dominant component in class of oxygenated sesquiterpenes, and trans-caryophyllene (2.98%) and cis-caryophyllene (0.82%) were dominant components in class of sesquiterpene hydrocarbons. These results are in accordance with the previously published data except compound piperitone oxide whose concentration is a little higher then usual. Main constituents in *Mentha longifolia* samples collected at various locations: Croatia, carvone, piperitenone oxide, limonene and β-caryophyllene (17); Serbia, trans-dihydrocarvone (24%), piperitone (17%), cis-dihydrocarvone (16%) (6); Turkey, piperitone oxide (65%), piperitenone oxide (12%) (18); Iran, piperitone (44%), limonene (14%) and trans-piperitol (13%) (19); France, carvone (57%), 1,8-cineole (13%) and limonene (7%) (20); South Africa, menthone (51%), pulegone (19%), 1,8-cineole (12%) (21). Gram-negative bacteria seemed to be more sensitive to the different examined essential oils than Gram-positive bacteria. These results are partially according to the literature data (2-5). Significant antimicrobial activity of essential oil was recorded against of examined multiresistant Gram-negative pathogenic bacteria, such are *Pseudomonas aeruginosa, Salmonella enterica* and *Escherichia coli*. Especially considerable is that the highest sensitivity to essential oil of *M. longifolia* was observed by *Pseudomonas aeruginosa*

ATCC 9027 (11-25 mm depend of concetration). It is well known that the antioxidant activity of essential oil containing phenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (22). DPPH analysis is the test used to prove the ability of the components of the essential oil of *Mentha longifolia* to act as donors of hydrogen atoms. Essential oil of *Mentha longifolia* showed a significant effect in inhibiting DPPH., reaching up to 50% at concentration of 10.50 μg/ml. The antiradical efficiency (AE) is a new parameter for the measurement the free radical scavenging of samples, and it combines the potency (1/EC50) and the reaction time (TEC50) (23). According to AE samples were divided into four antiradical efficiency groups:

$AE \leq 1 \cdot 10^{-3}$ – low antiradical activity

$1 \cdot 10^{-3} < AE \geq 5 \cdot 10^{-3}$ – medium antiradical activity

$5 \cdot 10^{-3} < AE \geq 10 \cdot 10^{-3}$– high antiradical activity

$AE > 10 \cdot 10^{-3}$– very high antiradical activity

It was find that AE of tested essential oil was $10.58 * 10^{-3}$, which places it into grupe with very high antiradical activity.

Conclusion

In conclusion, the study revealed significant antimicrobial, particularly antibacterial, activity of the investigated essential oil. The examined oil exhibited high RSC, which was found to be in correlation to the content of mainly monoterpene ketones and aldehydes. These results indicate that essential oils could serve not only as flavor agents but also as safe antioxidant and antiseptic supplements in preventing d eterioration of foodstuff and beverage products and pharmaceuticals. Also, consumption of food produced with natural essential oils or aromatic plant extracts (functional foods) is expected to prevent the risk of free radical dependent diseases. This study represents the first time investigation content, chemical composition, animicrobial and antioxidant activity essential oil of wild mint species from the area of Bosnia and Herzegovina.

Competing interests

Authors declare no conflict of interest.

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