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Chemical characterization, antimicrobial and antioxidant properties of *Mentha spicata* L. (Lamiaceae) essential oil

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Abstract: The aim of the present study was to examine chemical composition, antibacterial and antioxidative activity of essential oil extracted from the leaf of *Mentha spicata*. The essential oil composition was investigated by GC/MS. Thirty three components were identified, accounting for 98,9% of the essential oil. Dominant components were carvone (56,4%), limonene (16,2%), 1,8 cineole (7%), β-pinene (2,4) and α- terpinene (2,3%). Agar disk diffusion assay was used to evaluate antibacterial activity of essential oil. The essential oil exhibited significant level of antibacterial activity against all tested bacterial strains. In general, Gram-negative bacteria were more susceptible to *M. spicata* essential oil than Gram-positive bacteria. *E coli* was the most sensitive of the microorganisms to the antibacterial activity of *M. spicata* essential oil. The 2,2-diphenyl-l-1-phthydrazide (DPPH) radical removal method was used for evaluating the antioxidant potential of the essential oils.. The result showed a considerable level of antioxidant activities of the essential oil investigated with (IC50 = 41, 23 µg/mL). Based on our results, *M. spicata* essential oil with a strong antioxidant and antibacterial activities, could serve as a safe natural antioxidant and antiseptic supplements in the pharmaceutical and food industry.

INTRODUCTION

Many plant species from the Lamiaceae family have been used for centuries as folk remedies and today's medicine accepts the fact that these plants have healing properties. Genus Mentha is one of most important in *Lamiaceae* family with the essential oils of a high economic value. The chemical composition of essential oil depends on many factors, including geographical region, harvest period, climatic and soil conditions, (Harley, 1972; Gobert et al., 2002).

Mentha spicata, spearmint is a hybrid commercially cultivated aromatic plant, characterized by high amount essential oil, but it is still wild species in Balkan region.

Two main groups of constituents in essential oil are monoterpene hydrocarbonates and oxygenated monoterpenes. The most abundant compound in *M. spicata* essential oil is carvone that gives to essential oil distinctive smell. Both groupes of supstances possess powerful antimicrobial and antioxidant properties (Boukhebti et al., 2011). The essential oil of *M. spicata* is used as a flavoring in the perfume production, food and pharmaceutical industry. In addition, essential oil of spearmint shows strong antifungal, fumigant and antioxidant activity (Lawrence 2007).

Leading problem of massive use of antibiotics has resulted in resistance against pathogenic Gram positive and Gram negative bacteria. Clinical efficacy of many existing synthetic antibiotics has been reduced due to the developed bacterial resistance, so the pharmaceutical industry is looking for alternative new sources of antimicrobial agents (Sulieman et al. 2011). Nowdays there has been an increasing interest in the use of plant extracts and essential oils as a novel antimicrobial compounds for the treatment of various infectious diseases. Essential oils are sources that can provide a huge range of complex supstances with antifungal, antibacterial, and antiviral properties and can serve as a powerful tool to reduce the bacterial resistance (Burt, 2004).

Beside antimicrobial activity of many essential oils nowadays, a large number of research is focused on determining antioxidant activity that these natural products possess (Dahiya, and Manglik 2013). Based on these assumptions, this research was aimed to determine *in vitro* antimicrobial and antioxidative activity and describe the qualitative and quantitative composition of essential oil of *M. spicata* native toBosnia and Herzegovina.

EXPERIMENTAL

Chemicals and Reagents: (-)–α Thujone (≥ 96% GC) analytical standard (Sigma Germany)(No. 89231); (-)- β Pinene 99+% (Sigma Germany); R -/+) – Limonene analytical standard (Fluka Germany) (No.62118); Eucalyptol analytical standard (Fluka Germany) (No. 29210); Linalool analytical standard (Fluka Germany) (No. 51782); (+)-Carvone analytical standard (Fluka Germany) (No. 22070); Thimol standard (Fluka Germany) (No.50409); (−)-trans-Caryophyllene ≥98.5% (sum of enantiomers, GC) (Sigma Germany) (No. 22075).

Plant Material

Leaf of wild growing flowering plants of Mentha spicata L. during flowering stage were collected on the bank of the Jablanicko lake in Bosnia and Herzegovina in 2016. Voucher specimen of collected plant No.1059/2 after were confirmed by an independent expert and deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Sarajevo.

Isolation of the Essential Oil

The leaves of *Mentha spicata* were shade dried (15 days) at room temperature. Air-dried leaf of *Mentha spicata* were submitted to hydrodistillation according to European Pharmacopoeia 4th Ed., using Clevenger apparatus (Klaus Hofmann GmbH, Germany). The essential oil samples of each phenophase were dried over anhydrous sodium sulfate. The quantity of predistilled essential oils was determined volumetrically (Council of Europe, 2002).

Essential Oil Analysis

Qualitative and quantitative analyses of the essential oils were carried out by using gas chromatography/mass spectrometry system (GC-MS, Agilent Tecnologies series 6890N/5975B United States of America) at electron energy of 70eV, equipped with a split-splitless injector (200°C) and a flame ionization detector (FID) (250°C). As a carrier gas helium (1 mL/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°C to 280°C at a rate of 10°C/min until 130°C and 130-280°C at a rate of 12°C/min, respectively with split ratio, 1:10. Co-elution and 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 (Adams, 2007).

Evaluation of Antibacterial Activity.

Antimicrobial activity of essential oils, isolated from *Mentha spicata L*., using diffusion method was performed in this study. A collection of six 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, subcultured 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 with n-hexane as a solvent. 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. Each test point of antimicrobial activity was performed in quadruplicate in minimum three individual experiments. (Niksic et al. 2012).

Chemicals and apparatus used: 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 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).

Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method we used in our earlier study (Niksic et al. 2012). The reduction of the radical is followed by a decrease in the absorbance at 517 nm. A volume of 2 mL of a n-hexanic stock solution of the essential oils was put into test tubes and 2 mL of 90 μM DPPH solution was added. The tubes were covered with parafilm and kept again in the dark for 1.5 h. Five working standards of trolox (0.05-0.1nM) is prepared. Absorbance at 515 nm was measured with a spectrophotometer UV-VIS. Free radical scavenging capacity in percent (RSC (%)) was calculated by Eq. [1]:

RSC (%)=100*(Ablank -Asample) / Ablank [1]

The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated from graphs for solutions of the essential oils with n-hexane as a solvent with six different concentrations.

RESULTS AND DISCUSSION

Essential oil content and chemical composition

The yield of *M. spicata* essential oils were 1.67%-2.02% v/w in dry leaf. Our results showed higher content then *M. spicata* grown in south Europe (Greece) and north Africa (Tunisia) ranging from 0.1-1.8 % (Asekun et al. 2007). A total of 34 compounds were identified, representing 98.9% of the total content in the essential oils extracted from *M. spicata* leaves collected in Bosnia and Herzegovina. The identified compounds are listed in Table 1 in elution order from the HP-5 MS column, along with the percentage composition of each component. The essential oil contains 71.2% oxygenated monoterpenes, 21.1% monoterpene hydrocarbons, and 5.2% of sesquiterpene hydrocarbons. The main constituents were carvone (56.4%) and limonene (16.2%) followed by 1,8-cineole (7.0%), terpinen-4-ol (3.0) β-pinene (2.4%), α-terpineol (2.3%) and E-caryophyllene (1.5%). The investigated oil can be classified to the carvone/limonene chemotype. Generally, there is a variation in the chemical composition of *M. spicata* essential oil wild and cultivated, around the world. Different chemotypes have been described in previous studies classified as chemotypes: carvone, limonene, carvone/limonene, pulegone/menthone/isomenthone, and pulegone/piperitone. (Telci et al. 2004; Telci et al. 2010). Carvone content also varies in the essential oil of *M. spicata* growing in different regions: Egypt (46.4%–68.55%) (Foda 2010), Canada (59%–74%) (Zheljazkov et al., 2010), Colombia (61.53%) (Roldán et al. 2010); Turkey (78.35%–82.2%) (Telci et al. 2005) and China (55.45%–74.6%) (Hua et al. 2011). *M. spicata* essential oil from Iran was characterized by a low amount of carvone 22.4% (Hadjiakhoondi et al. 2000). A linalool chemotype (82.8%) was also reported from Turkey. A low content of limonene (5.7%) was reported in essential oil from Serbia (Sokovic et al. 2009). However, differences in the yield and chemical composition of *M. spicata* essential oil can be attributed to several factors such as the geographic region, harvesting period, climatic and soil conditions, and phenophases in collection time.

Antimicrobial Activity

The antibacterial activity of essential oil against a range of Gram-positive and Gram-negative microrganisams is shown in Table 2. Significant antimicrobial activity was exhibited by essential oil against all tested Gram positive and Gram-negative microorganisms. Especially considerable is that the highest sensitivity to essential oil of *M. spicata* was observed by *Escherichia coli* ATCC 8739 producing the maximum zone of inhibition (11,8 - 21 mm), *Salmonella enterica* (8-18mm) and *Pseudomonas aeruginosa* (10-16mm). Gram-negative bacteria appear to be more sensitive to the different examined essential oils than Gram-positive bacteria. Gram positive stains showed moderate antimicrobial activity in concentrations 1%, 5% and 10%, *Staphylococcus aureus* maximum zone of inhibition (8-13mm), *Staphylococcus epidermidis* (10,1-11,2mm) and *Bacillus subtilis* (9-11,5mm). According to the literature, *M. spicata* essential oil showed an antibacterial effect on the growth of both Gram-negative and Gram-positive bacteria (Lorenzi et al. 2009). Mahboubi and Haghi, reported that the essential oil of *M. spicata* exhibited a high antibacterial activity in the range of 8-21 against *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Escherichia coli*. Recently, Mimica-Dukic et al. (2004) reported that the *M. spicata* essential oil was active against Gram-negative bacteria, Pse*udomonas aeruginosa, Escherichia coli, Salmonella enteritidis, Salmonella typhi,* and *Shigella* strains. However, antibacterial activities of essential oils are likely related to the percentage of oxygenated monoterpenes and monoterpene hydrocarbons (Dahiya et al. 2013). In conclusion, there was a close relationship between antimicrobial activity and carvone and limonene levels in essential oils.

Table 1. Chemical Composition of *M. spicata* Essential Oil

Components	RI[a]	percent %	Identification method [b]
monoterpene hydrocarbons		**21,1**	
α-pinene	**938**	1,2	RT GC MS
camfene	**954**	0,2	RT* MS
sabinene	**974**	0,6	RT* MS
β-pinene	**978**	2,4	RT GC MS
limonene	**1035**	16,2	RT* MS
E-β-ocimene	**1050**	0,2	RT* MS
γ−terpinene	**1063**	0,1	RT GC MS
terpinolene	**1080**	0,2	RT GC MS
oxygenated monoterpenes		**71,2**	
1.8-cineole	**1036**	7	RT GC MS
Z-sabinene hydrate	**1067**	0,1	RT GC MS
E-sabinene hydrate	**1098**	0,1	RT GC MS
terpinen-4-ol	**1178**	3	RT GC MS
α-terpineol	**1188**	2,3	RT GC MS
dihydrocarveol	**1194**	0,6	RT GC MS
E-carveol	**1217**	0,9	RT GC MS
carvone	**1243**	56,4	RT GC MS
bornyl acetate	**1288**	0,2	RT GC MS
Z-jasmone	**1395**	0,3	RT GC MS
sesquiterpene hydrocarbons		**5,2**	
β-burbonene	**1383**	1,2	RT GC MS
β-cubebene	**1390**	0,1	RT GC MS
β-elemene	**1391**	0,6	RT GC MS
α-gurjunene	**1410**	0,2	RT GC MS
E- caryophyllene	**1419**	1,5	RT GC MS
α-humulene	**1452**	0,1	RT GC MS
γ-gurjunene	**1477**	tr.	RT GC MS
germacrene D	**1490**	1,1	RT* MS
bicyclogermacrene	**1501**	0,2	RT* MS
γ−cardinene	**1514**	0,2	RT GC MS
δ-cadinene	**1523**	0,1	RT GC MS
oxygenated sesquiterpenes		**0,7**	
spathulenol	**1578**	0,3	RT GC MS
caryophyllene oxide	**1582**	0,2	RT* MS
α-cadinol	**1654**	0,1	RT GC MS
aliphatic compounds		**0,7**	
3-octanol	**991**	0,7	RT GC MS
total identified		**98,9**	

[a] Retention indices relative to C9-C24 n-alkanes on the HP 5MS column

[b] RT, comparison with pure standard retention time; GC, gas chromatographic coelution with pure standard; MS, mass spectrometry; RT∗, comparison of the relative retention time with those obtained from the NIST/NBS, Wiley libraries spectra and those reported by Adams[14]

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

source	*organism*	10 %	5 %	1%	0,5%	0,1%	Positive control
ATCC 6633	*Bacillus subtilis*	11,5±0,61	10,1±0,70	9±0,71	-		32±0,70 penicilin
ATCC 6538	*Staphylococcus aureus*	13±1,52	10,5±1,62	8±0,71	-	-	10,5±0,00 gentamycine
ATCC 11228	*Staphylococcus epidermidis*	11,2±1,61	10,1±1,92	-	-	-	15,2 ±0,00 gentamycine
ATCC 8739	*Escherichia coli*	21±0,90	19±1,22	11,8±0,6	-	-	17 ±0,22 gentamycine
ATCC 9027	*Pseudomonas aeruginosa*	16±1,9	14±1,71	11±0,77	10±0,87	-	28 ±0,85 ciprofloxacine
NCTC 6017	*Salmonella enterica subsp. enterica serotype ABONY*	18±1,33	17±0,50	14±1,23	8±1,22	-	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).

Antioxidant Activity

This study, also, determined the antioxidant activity of *M. spicata* essential oil. The results indicate that the hexane solution of the essential oil exhibited high antioxidant activity with a value of IC50 41.2 μg / mL (Table 3). Based on obtained results appeared to be an association between antioxidant and antimicrobial activity. Oxygenated monoterpenes and monoterpene hydrocarbons are mainly responsible for the antioxidant activity of essential oil. However, spearmint essential oil are mixture of different types of terpenoids and we can not attribute the antioxidant effect of a total essential oil only to the major compounds,because of synergistic or antagonistic interaction between supstances can occur, thus having effect on antioxidative activity.

Essential oils of *M. spicata* native from Turkey showed higher antioxidative activity IC50 77.40 μg/mL (Kizil et al. 2010). Also higher antioxidative activity was reported in spermint essential oil from Iran with the IC50 87.89 μg/mL (Nickavar et al. 2008) The lower antioxidative activity of investigated *M. spicata* essential oils might be due to lower presence of some components that have antioxidant activity such 1,8 cineole.

Table 3 . Percentage of neutralization of DPPH. Of essential oil of M. spicata and trolox as positive control) in DPPH assay

Source	Concentracion (μg/ml)				
	7,40	14,80	29,60	44,40	IC50
M. spicata	24,27	30,10	42,72	51,55	41,23
Trolox					3,53

CONCLUSION

The essential oil of *M. spicata* was characterized by GC-MS and 34 compounds were identified as carvone chemotype. The main components were carvone (56,4%), limonene (16,2%), 1,8 cineole (7%), β-pinene and α-terpinene (2,3%). The results obtained from *M. spicata* essential oil showed high antibacterial activity, especially against Gram-negative bacteria. The antibacterial activity of the essential oil could be attributed to the presence of mixture of various group of compounds, such as oxygenated monoterpenes and monoterpene hydrocarbons as dominant group of constituents. The examined essential oil exhibited high antioxidative activity that was attributed to majority constituents carvone, limonene and 1.8 cineole. Present study indicated that essential oil of *M. spicata* possess important volatile compounds with antimicrobial and antioxidative activity. These results indicate that *M. spicata* essential oil could serve as safe natural flavor agent with antioxidant and antiseptic activity in pharmaceutical and food industry.

REFERENCES

Adams, R., Adams, R. (2007*). Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. (1st Ed.) Carol Stream, Ill.: Allured Pub. Corp.

Asekun, O. T., Grierson, D. S., Afolayan, J. (2007). Effects of drying methods on the quality and quantity of the essential oil of Mentha longifolia L. subsp. Capensis. *Food Chemistry*, 101, 995-998.

Boukhebti, H., Chaker, A.N., Belhadj, H., Sahli, F., Ramdhani, M., Laouer, H., Harzallah, D. (2011). Chemical composition and antibacterial activity of Mentha pulegium L. and Mentha spicata L. essential oils. *Der Pharmacia Lettre*, 3, 267–275.

Boukhebti, H., Chaker, A.N., Belhadj, H., Sahli, F., Ramdhani, M., Laouer, H., Harzallah, D. (2011). Chemical composition and antibacterial activity of Mentha pulegium L. and Mentha spicata L. essential oils. *Der Pharmacia Lettre*, 3, 267–275.

Burt, S. (2004). Essential oils: their antibacterial proprieties and potential application in food. *Journal of Applied Microbiology*. 94, 223-253.

Chauhan, R.S., Nautiyal, M.C., Tava, A. (2010). Essential oil composition from aerial parts of Mentha spicata L. *Journal of Essential Oil Bearing Plants*, 13, 353–356.

Council of Europe. (2002). *European Pharmacopeia*. (4th Ed.), Strasbourg Cedex, France, (p.p. 183–184).

Dahiya, P., Manglik, A. (2013). Evaluation of antibacterial, antifungal and antioxidant potential of essential oil from Amyris Balsamifera against multi drug resistant clinical isolates. *Asian Journal of Pharmaceutical and Clinical Research*, 6(5), 57-60.

Foda, M.I., El-Sayed, M.A., Hassan, A.A., Rasmy, N.M., El-Moghazy, M.M. (2010). Effect of spearmint essential oil on chemical composition and sensory properties of white cheese. *The Journal of American Science*, 6, 272–279.

Hadjiakhoondi, A., Aghel, N., Zamanizadech-Nadgar, N., Vatandoost, H. (2000). Chemical and biological study of Mentha spicata L. essential oil from Iran. *DARU Journal of Pharmaceutical Sciences*, 8, 19–21.

Hua, C.X., Wang, G.R., Lei, Y. (2011). Evaluation of essential oil composition and DNA diversity of mint resources from China. *African Journal of Biotechnology*, 10, 16740–16745.

Kizil, S., Hasimi, N., Tolan, V., Kilinç, E., Yüksel, U. (2010). Mineral content, essential oil components and biological activity of two Mentha species (M. piperita L., M. spicata L.). *Turkish Journal of Field Crops*, 15, 148–153.

Lawrence, B.M. (2007). *Mint: The Genus Mentha*. Taylor and Francis Group: Boca Raton, FL, USA.

Lorenzi, V., Muselli, A., Bernardini, A.F., Berti, L., Pagès, J.M., Amaral, L., Bolla, J.M. (2009). Geraniol restores antibiotic activities against multidrug resistant isolates from gram-negative species. *Antimicrobial Agents and Chemotherapy*, *53*, 2209–2211.

Mimica-Dukić, N., Božin, B. (2007). Essential Oils from Lamiaceae Species as Promising Antioxidant and Antimicrobial agents. *Natural Product Communications*, 2(4), 445-452.

Mimica-Dukić, N., Božin, B, Sokovic, M., Natasa Simin, N.(2004) Antimicrobial and Antioxidant Activities of Melissa officinalis L.(Lamiaceae) Essential Oil. Journal of Agriculture Food Chemistry, 52 (9), 2485–2489

Nickavar, B., Alinaghi, A., Kamalinejad, M. (2008). Evaluation of the antioxidant properties of five Mentha species. *Iranian Journal of Pharmaceutical Research*, 7, 203–209.

Niksic, H., Besovic, E., Makarevic, E. and Duric, K. (2012). Chemical composition, antimicrobial and antioxidant properties of Mentha longifolia (L.) Huds. essential oil. *Journal of Health Sciences*, 2(3), 192--200.

Roldán, L.P., Díaz, G.J., Duringer, J.M. (2010). Composition and antibacterial activity of essential oils obtained from plants of the Lamiaceae family against pathogenic and beneficial bacteria. *Revista Colombiana de Ciencias Pecuarias*, 23, 451–461.

Sokovic, M.D., Vukojevic, J., Marin, P.D., Brkic, D.D., Vajs, V., van Griensven, L.J.L.D. (2009). Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules*, 14, 238–249.

Sulieman, A.M.E., Abdelrahman, S.E., Abdel Rahim, A.M. (2011). Phytochemical analysis of local Spearmint (Mentha spicata) leaves and detection of the antimicrobial activity of its oil. *Journal of Microbiology Research*, 1(1), 1-4.

Telci, I., Demirtas, I., Bayram, E., Arabaci, O., Kacar, O. (2010). Environmental variation on aroma components of pulegone/piperitone rich spearmint (Mentha spicata L.). *Industrial Crops and Products*, 32, 588–592.

Telci, I., Sahbaz, N. (2005). Variations in yield, essential oil and carvone contents in clones selected from Carvone-scented landraces of Turkish Mentha species. *Journal of Agronomy and Crop Science*, 4, 96–102.

Telci, I., Sahbaz, N., Yilmaz, G., Tugay, M.E. (2004). Agronomical and chemical characterization of spearmint (Mentha spicata L.) originating in Turkey. *Economic Botany*, 58, 721–728.

Zheljazkov, V.D., Cantrell, C.L., Astatkies, T. (2010). Yield and composition of oil from Japanese cornmint fresh and dry material harvested successively. *Agronomy Journal*, 102, 1652–1656.

Summary/Sažetak

Cilj rada bio je ispitati hemijski sastav, antibakterijsku i antioksidativnu aktivnost eteričnog ulja iz liste vrste *Mentha spicata*. GC-MS analizom identificirane su 33 komponente, koje čine 98,9% eteričnog ulja. Dominantne komponente su karvon (56,4%), limonen (16,2%), 1,8 cineol (7%), β-pinen (2,4) i a-terpinen (2,3%). Antibakterijsko djelovanje eteričnog ulja je procijenjeno disk difuzionom metodom. Eterično ulje pokazalo je visoku razinu antibakterijskog djelovanja protiv svih testiranih mikroorganizama. Generalno Gram negativne bakterije bile su osjetljivije na ispitivano eterično ulje od Gram-pozitivnih bakterija. *E. coli* bila je najosjetljiviji soj. Također je procjenjena antioksidativna aktivnost eteričnog ulja metodom DPPH. Rezultat je pokazao značajnu antioksidativnih aktivnosti ispitivanog eteričnog ulja istraženog kao (IC50 = 41, 23 ug /mL). Na osnovu dobijenih rezultata, eterično ulje vrste *M. spicata* može poslužiti kao siguran dodatak u farmaceutskoj I prehrambenoj industriji sa antioksidativnim i antiseptičkim učinkom.