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 aeruginosa* 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.

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**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-
age, which has been implicated in several patho-
logical disorders, such as ischemia-reperfusion
injury, coronary atherosclerosis, Alzheimer’s dis-
ease, carcinogenesis, and aging processes (9, 10).

The genus *Mentha* L., member of the family La-
miacaeae, 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 ge-
nus consists approximately 14-25 species (11,12).
Most of the species are characterized by a great
polyorphism, 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 con-
siderable chemical diversity in essential oil com-
position, 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 caryo-
phyllene (2.0-22.0%) and menthol (0.0-32.50%).
The genus *Mentha* clearly has marked anti-
microbial characteristics across the spectrum from
fungi and parasites, through bacteria, to vi-
ruses. 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 antioxi-
dant 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 anti-
fungal 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. *Men-
tha longifolia*, is often used as a domestic herbal
remedy, being valued especially for its antiseptic
properties and its beneficial effect on the diges-
tion as it is a well-know treatment for flatulence.
The objectives of this study were to analyze the
composition, antimicrobial and antioxidant activ-
ity 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 sub-
mittied to hydrodistillation according to Euro-
pean 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 (200ºC)
and a flame ionization detector (FID) (250ºC).
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 50ºC to 280ºC
at a rate of 10ºC/min until 130ºC and 130-280ºC at a
rate of 12ºC/min, respectively with split ratio, 1:10.
Coelution and mass spectrometry MS analysis
based on the identification of the individual com-
ounds, and the comparison of their relative reten-
tion 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 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, 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): ciprofloxacin 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>
<thead>
<tr>
<th>pick no.</th>
<th>Components</th>
<th>Retention indices (RI)</th>
<th>percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>alfa-pinene</td>
<td>938</td>
<td>0.78</td>
</tr>
<tr>
<td>2</td>
<td>Sabinene</td>
<td>974</td>
<td>0.47</td>
</tr>
<tr>
<td>3</td>
<td>beta-pinene</td>
<td>978</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>beta-myrcene</td>
<td>992</td>
<td>0.69</td>
</tr>
<tr>
<td>5</td>
<td>Terpinolene</td>
<td>1008</td>
<td>0.07</td>
</tr>
<tr>
<td>6</td>
<td>Limonene</td>
<td>1035</td>
<td>0.06</td>
</tr>
</tbody>
</table>

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

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.
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 yield 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):

$$\text{RSC (\%)} = 100 \times \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}$$  \hspace{1cm} (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 EC50 value and the necessary time to reach the EC50 (TEC50), according to the following Equation (2):

$$\text{AE} = \frac{1}{\text{EC}_{50} \times \text{TEC}_{50}}$$ \hspace{1cm} (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 Mentha longifolia plants collected in Bosnia and Herzegovina (Table 1). A total of the 36 chemical constituents representing 98.17% of the total content.

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

<table>
<thead>
<tr>
<th>source</th>
<th>organism</th>
<th>10 %</th>
<th>5 %</th>
<th>1%</th>
<th>0.5%</th>
<th>0.1%</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 6633</td>
<td>Bacillus subtilis</td>
<td>11±0.81</td>
<td>9.5±0.80</td>
<td>8±0.71</td>
<td>-</td>
<td>-</td>
<td>32±0.70 penicilin</td>
</tr>
<tr>
<td>ATCC 6538</td>
<td>Staphylococcus aureus</td>
<td>13.6±1.52</td>
<td>14±1.62</td>
<td>8±0.71</td>
<td>-</td>
<td>-</td>
<td>10.5±0.00 gentamycine</td>
</tr>
<tr>
<td>ATCC 11228</td>
<td>Staphylococcus epidermidis</td>
<td>12±2.11</td>
<td>10.9±1.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.2±0.00 gentamycine</td>
</tr>
<tr>
<td>ATCC 8739</td>
<td>Escherichia coli</td>
<td>19±0.71</td>
<td>17±1.22</td>
<td>13±0.61</td>
<td>9±0.51</td>
<td>7±0.33</td>
<td>17±0.22 gentamycine</td>
</tr>
<tr>
<td>ATCC 9027</td>
<td>Pseudomonas aeruginosa</td>
<td>25±2.12</td>
<td>22±1.71</td>
<td>19±0.77</td>
<td>19±0.87</td>
<td>11±1.85</td>
<td>28±0.85 ciprofl oxacine</td>
</tr>
<tr>
<td>NCTC 6017</td>
<td>Salmonella enterica subsp.enterica serotype ABONY</td>
<td>20±0.33</td>
<td>15±0.56</td>
<td>10±0.99</td>
<td>-</td>
<td>-</td>
<td>20±0.22 tetracycline</td>
</tr>
</tbody>
</table>

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).
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.

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 $10.58*10^{-3}$. 

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

**FIGURE 2.** Antimicrobial activity against *Staphylococcus aureus*

**FIGURE 3.** Antimicrobial activity against *Staphylococcus epidermidis*
**FIGURE 4.** Antimicrobial activity against *Bacillus subtilis*

**FIGURE 5.** Antimicrobial activity against *Escherichia coli*

**FIGURE 6.** Antimicrobial activity against *Pseudomonas aeruginosa*
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 than 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 (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 as *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 on concentration). 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 ≤ 1·10^{-3} – low antiradical activity
1·10^{-3} < AE ≥ 5·10^{-3} – medium antiradical activity
5·10^{-3} < AE ≥ 10·10^{-3}– high antiradical activity
AE > 10·10^{-3}– very high antiradical activity

It was found that AE of tested essential oil was 10.58*10^{-3}, which places it into groupe 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 deterioration 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, antimicrobial and antioxidant activity essential oil of wild mint species from the area of Bosnia and Herzegovina.

**Competing interests**

Authors declare no conflict of interest.

**References**


15. European Pharmacopeia, 7th ed.; Council of Europe European (COE) - European Directorate for the Quality of