Chemical composition, antibacterial and antifungal activities of Saudi Arabian *Mentha longifolia* L. essential oil

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**ABSTRACT**

**Objective:** To investigate the chemical composition, *in vitro* antibacterial and antifungal activity of *Mentha longifolia* L. essential oils using aqueous extract.

**Methods:** *Mentha longifolia* L. essential oils were extracted using hydrodistillation with Clevenger apparatus for 3 h and the yield of the essential oil was calculated. Essential oils were qualitatively and quantitatively analyzed with gas chromatography and gas chromatography-mass spectrometer. For these essential oils, antibacterial and antifungal activity against human pathogens were evaluated.

**Results:** Nineteen chemical constituents representing 99.72% of the essential oil were found, comprising menthone (39.55%), isopulegone (30.49%), eucalyptol (10.38%), and α-terpineol (3.15%); these were major components, and others were minor components. The essential oil showed strong antibacterial activity against *Staphylococcus aureus* [(35.24 ± 0.13) mm], *Enterococcus faecalis* [(32.12 ± 0.12) mm] and *Bacillus cereus* [(30.06 ± 0.04) mm], as well as antifungal activity against *Aspergillus flavus* [(38.02 ± 0.06) mm], *Alternaria alternaria* [(35.26 ± 0.12) mm], and *Penicillum* spp [(34.14 ± 0.02) mm].

**Conclusions:** It seems that the essential oils derived from the *Mentha longifolia* L. species could be used as a natural source of antimicrobial agents.

1. Introduction

Plant diversity is an essential and rich source of pharmaceutically active chemical compounds[1-6]. Foodborne diseases present serious health risk worldwide. The safety and quality of food products can be compromised by various harmful substances and microorganisms[7]. Since ancient times, natural herbs have been used for storing and preserving food, as well as for enhancing its flavor. More recently, essential oils derived from various medicinal plant species have been applied as antibacterial agents, food preservatives, pharmaceutical agents, and spices in a wide range of fields, such as alternative medicine, cosmetics, aromatherapy, phototherapy, and nutrition[8].

Essential oils of *Mentha* species are most widely used[9], primarily because of the active compounds (menthone and isopulegone). The essential oils of *Mentha longifolia* L. (*M. longifolia*) have been extensively investigated, as evident in a large number of studies focusing on their antiviral, antimicrobial, antifungal[10-12], antibiofilm formation[13-15], radioprotective[16,17], antioedema, analgesic and antioxidant properties[13].

Mint is an important medicinal and aromatic plant, comprising of 25–30 species of the genus *Mentha* (commonly known as mint or pudina), belonging to the Lamiaceae family. The genus thrives in tropical and subtropical climates along the banks of streams and rivers, in gardens, on mountains, etc., and can be found throughout North America and Europe, as well as in China, Brazil, India, and many other countries. India is the leading producer, consumer, and exporter of *Mentha* oil in the world with an annual production of around 14000 tons[18]. However, to date, no attempt has been made
to study the chemical constituents or antibacterial and antifungal activity of volatile oils extracted from *M. longifolia* growing in the Jazan area, Saudi Arabia.

In recent years, traditional medicine has gained more importance in modern medical practice worldwide. *M. longifolia* (wild mint), known as habek in Jazan area, is one of such medicinal herbs with a wide range of applications. The plant is mainly used for the treatment of common diseases, such as coughs, colds, stomach cramps, asthma, flatulence, indigestion, and headaches[19-21]. However, it is also effective in the treatment of wounds and swollen glands[22-24]. While the properties and applications of this plant have been extensively researched, a thorough review of pertinent literature revealed no studies focusing on *M. longifolia* growing in the Jazan region, Saudi Arabia. This work aims to fill this gap in the extant knowledge by examining the chemical constituents as well as antibacterial and antifungal activity of the essential oil extracted from *M. longifolia*.

2. Materials and methods

2.1. General information

The major instruments used were Clevenger apparatus, gas chromatography (GC) and gas chromatography-mass spectrometer (GC-MS) (Agilent Technology 6890N). Microbial culture media (Merck) and chemicals (menthone, isopulegone and eucalyptol) used were of analytical grade for antibacterial and antifungal activity.

2.2. Plant material and essential oil extraction

*M. longifolia* was collected as a whole plant from Jazan, Saudi Arabia, during the flowering stage in October of 2016. The collected plant material was authenticated by a senior plant taxonomist, Dr. Ramesh Moochikkal, of the Department of Biology. Voucher specimen (JAZUH 1147) was deposited at Jazan University Herbarium, Jazan, Saudi Arabia. As the aerial parts of the plant are used as flavoring agents and in traditional medicine, they were the focus of the investigation. Aerial parts were thus separated from the remaining plant material and were washed to remove soil particles, before being left to dry in shade at room temperature, after which they were ground by the grinder (IKW Werke MF 10 basic). Following the procedure described by Singh et al.[25], the ground material (100 g of substance dissolved in 400 mL of double-distilled water in round-bottom flask of 1.000 mL volume) was hydrodistillation for 3 h using Clevenger apparatus (Klaus Hofmann GmbH, Germany). Moisture was removed from the extracted oil using anhydrous sodium sulfate and the resultant oil was stored at 4 °C until required for further analysis.

2.3. GC-MS analysis conditions

The composition of the volatile oil was analyzed using GC and GC-MS. The phytochemical analysis was carried out using the Agilent Technology 6890N instrument that allows for both GC and GC-MS. The gas chromatographer was equipped with a flame ionization detector (FID), split/splitless injector (260 °C) and an HP-5ms 5% capillary column with a film of 30 m × 0.25 mm × 0.25 μm dimensions. The gradient temperature program was used for GC. The chromatographic run was carried out at a column temperature in the 50–280 °C range, whereby 10 °C/min increments were used for temperatures below 140 °C, and 12 °C/min for 140–280 °C. Helium (99.99%) was used as carrier gas at a flow rate of 1.0 mL/min. Sample injection temperature was maintained at 260 °C. The split ratio was 1:10 and the sample was injected manually. On the basis of peak area, percentage was performed by the FID signal. By using the aforementioned Agilent Technology 6890N instrument, along with the predetermined system parameters, the chemical constituents of the essential oil were detected, quantified, and characterized. The mass detector was operated in the electron impact ionization mode to record the mass spectra at 70 eV, in the 50–500 m/z range. GC conditions were the same as described above. Mass spectrometry analysis was performed to identify individual compounds, and the comparison of relative retention time (homologues series of C₅–C₃₄ n-alkane obtained from HP-5ms 5% capillary column) with those of standard reference samples was performed. When specific reference compounds were not available, the identification was performed by matching their retention time and mass spectra with those obtained from the samples. The mass spectra were interpreted using the reference library of the National Institute of Standards and Technology, US, along with Wiley 5 and mass finder, as well as data reported by Robert and Adams[26]. The constituent percentages were measured based on the peak area.

2.4. Antimicrobial activity

Antimicrobial activity was assessed by using a wide range of Gram-positive and Gram-negative bacteria and fungi. The zone of inhibition against the microbes was determined using disc diffusion method, while the minimum inhibitory concentration (MIC) against each microorganism was determined using a microbroth dilution assay.

2.5. Microbial strains

The extracted oil and principle components were tested against a set of microorganisms. The bacteria and fungi strains were obtained from the Laboratory of Microbiology, Sri Krishnadevaraya University, India. The Gram-positive micro-organisms used included *Bacillus cereus* (ATCC 10876), *Bacillus macerans* (B. macerans)(M58), *Bacillus megaterium* (B. megaterium) (M3), *Bacillus subtilis* (B. subtilis) (ATCC 6633), *Brucella abortus* (B. abortus) (AT7), *Burkholderia cepacia* (B. cepacia) (A255), *Enterobacter cloacae* (E. cloaceae) (ATCC 13047), *Enterococcus faecalis* (E. faecalis) (ATCC 49452), *Listeria monocytogenes* (L. monocytogenes) (ATCC 15313), *Staphylococcus aureus* (S. aureus) (ATCC 25923), *Micrococcus flava* (M. flavus) (ATCC 9341), *Staphylococcus epidermidis* (S. epidermidis) (A 233), *Clavibacter michiganense* (C. michiganense) (A 277), and *Streptococcus pyogenes* (S. pyogenes) (ATCC 176). We also used the following Gram-negative microorganisms: *Acinetobacter baumannii* (A. baumannii) (ATCC 19606), *Escherichia coli* (E. coli) (ATCC 25922), *Klebsiella pneumonia* (K. pneumonia) (ATCC 27853), *Proteus mirabilis* (P. mirabilis) (ATCC 35659), *Salmonella typhimurium* (S. typhimurium) (ATCC 13311), *Citrobacter freundii* (C. freundi)
The essential oil was extracted from the aerial parts of *M. longifolia* by hydrodistillation with a 1.35% (w/v) yield. Its chemical constituents were separated by GC and were characterized by GC-MS, as shown in Table 1. Through GC-MS analysis, 19 chemical components were separated and characterized, with monoterpenes as the major constituent, at 99.59%. The principal components were menthone (39.55%), isopulegol (30.49%), and eucalyptol (10.38%). Other compounds in the essential oil included α-terpineol (3.14%), longifolene (2.47%), linalool (2.41%), and β-pinene (2.38%), while the others were found in minimal concentrations only.

**Table 1**

| Retention index | Components               | % Composition |
|-----------------|--------------------------|---------------|
| 966             | β-Pinene                 | 2.38          |
| 1042            | α-Cymene                 | 0.44          |
| 1059            | Eucalyptol               | 10.38         |
| 1082            | Linalool                 | 2.41          |
| 1148            | Menthone                 | 39.55         |
| 1164            | Menthol                  | 1.52          |
| 1175            | α-Terpineol              | 3.14          |
| 1179            | Isopulegol               | 30.49         |
| 1195            | Verbenone                | 0.53          |
| 1228            | Nerol                    | 0.47          |
| 1400            | β-Cubebene               | 1.12          |
| 1413            | Longifolene              | 2.47          |
| 1418            | β-Caryophyllene          | 1.15          |
| 1470            | γ-Murolene               | 0.34          |
| 1549            | Diepicedrene-1-oxide     | 0.12          |
| 1561            | Caryophyllene oxide      | 0.51          |
| 1576            | Spathulenol              | 1.61          |
| 1645            | Cubenol                  | 0.15          |
| 1640            | τ-Cadinol                | 0.93          |
| Total           |                          | 99.71         |

The antibacterial and antifungal activities of the extracted *M. longifolia* essential oil were examined and reported as a zone of inhibition in mm and MIC values in µg/mL. The results pertaining to the antibacterial activity are shown in Table 2, while those of the antifungal assay are reported in Table 3.

The zone of inhibition was measured using the disc diffusion method, which was described elsewhere[27]. The *M. longifolia* essential oil analyzed in the present study exhibited stronger antibacterial activity than any of the major components against all bacteria tested. The highest antibacterial activity was measured against *S. aureus*, *E. faecalis* and *B. cereus* with a zone of inhibition (*P* ≤ 0.01) of (35.24 ± 0.13), (32.12 ± 0.12) and (30.06 ± 0.04) mm respectively, when compared with gentamicin. Moderate activity was observed against *B. macerans* (M58), *B. abortus* (A77), *E. coli*, *S. pyogenes*, *C. michiganense*, *K. pneumonia*, *P. mirabilis*, *S. epidermidis*, and *L. monocytogenes* with (25.05 ± 0.12), (25.06 ± 0.01), (25.08 ± 0.13), (23.02 ± 0.07), (22.04 ± 0.04), (16.04 ± 0.09), (15.12 ± 0.12), (15.14 ± 0.08), and (15.11 ± 0.04) mm inhibition zones (*P* ≤ 0.01), respectively.

Finally, minimum activity was observed against *E. cloacae*, *M.*
Table 2
Antimicrobial activity of *M. longifolia*.

| Microbial strains | EO | RA | Ment | Isopul | Eucal |
|-------------------|----|----|------|--------|-------|
| *B. cereus* | 30.06 ± 0.04 | 26.44 ± 0.12 | 22.00 ± 0.02 | 18.20 ± 0.01 | 12.00 ± 0.02 |
| *B. macerans* | 25.05 ± 0.12 | 20.28 ± 0.05 | 16.20 ± 0.01 | 15.00 ± 0.02 | 8.00 ± 0.04 |
| *B. megaterium* | 11.03 ± 0.05 | 18.02 ± 0.16 | 10.40 ± 0.02 | 9.00 ± 0.01 | 8.00 ± 0.01 |
| *B. subtilis* | 10.12 ± 0.11 | 12.10 ± 0.08 | 9.00 ± 0.04 | 7.00 ± 0.02 | 6.00 ± 0.02 |
| *B. abortus* | 25.06 ± 0.01 | 9.02 ± 0.02 | 20.00 ± 0.04 | 18.00 ± 0.04 | 10.00 ± 0.06 |
| *B. cepacia* | 9.06 ± 0.10 | 12.45 ± 0.03 | 8.00 ± 0.02 | 8.20 ± 0.06 | 5.00 ± 0.02 |
| *E. cloacae* | 14.18 ± 0.15 | 16.28 ± 0.06 | 10.00 ± 0.04 | 10.00 ± 0.04 | 6.00 ± 0.04 |
| *E. faealis* | 32.12 ± 0.12 | 15.32 ± 0.14 | 20.00 ± 0.02 | 19.00 ± 0.06 | 9.00 ± 0.02 |
| *L. monocytogenes* | 15.11 ± 0.04 | 12.23 ± 0.12 | 10.00 ± 0.02 | 8.00 ± 0.02 | 8.21 ± 0.04 |
| *M. flavus* | 35.24 ± 0.13 | 25.42 ± 0.16 | 25.00 ± 0.06 | 22.00 ± 0.04 | 14.00 ± 0.02 |
| *S. epidermidis* | 15.14 ± 0.08 | 16.32 ± 0.05 | 12.00 ± 0.02 | 12.00 ± 0.01 | 6.00 ± 0.04 |
| *S. pyogenes* | 32.04 ± 0.04 | 15.03 ± 0.08 | 20.00 ± 0.02 | 20.40 ± 0.02 | 12.00 ± 0.03 |
| *S. typhimurium* | 10.07 ± 0.02 | 12.4 ± 0.00 | 8.00 ± 0.02 | 8.2 ± 0.06 | 5.00 ± 0.02 |
| *S. vulgaris* | 14.01 ± 0.10 | 10.28 ± 0.06 | 12.40 ± 0.04 | 10.00 ± 0.02 | 7.00 ± 0.06 |
| *S. aureus* | 9.02 ± 0.07 | 12.24 ± 0.02 | 7.00 ± 0.02 | 6.00 ± 0.02 | 2.00 ± 0.06 |
| *Salmonella enteritidis* | 10.12 ± 0.12 | 15.36 ± 0.12 | 6.00 ± 0.04 | 6.00 ± 0.02 | 2.00 ± 0.02 |
| *P. vulgaris* | 3.09 ± 0.01 | 12.02 ± 0.04 | 5.00 ± 0.04 | 3.00 ± 0.06 | 3.00 ± 0.02 |
| *P. syringae* | 2.16 ± 0.04 | 17.09 ± 0.06 | 3.00 ± 0.02 | 3.00 ± 0.04 | 2.00 ± 0.02 |
| *X. campestris* | 2.12 ± 0.03 | 17.09 ± 0.06 | 3.00 ± 0.02 | 3.00 ± 0.04 | 2.00 ± 0.02 |

| MIC (µg/mL) |
|-------------|
| 1.00 ± 0.02 |

Table 3
In vitro antifungal activity of essential oil.

| Strains | Disc diameter (mm) | Essential oil* |
|---------|--------------------|----------------|
| *Alternaria alternata* (MNHN 843390) | 35.26 ± 0.12 | 8.50 ± 0.04 |
| *A. flavus* (MNHN 994294) | 38.02 ± 0.06 | 15.80 ± 0.06 |
| *Aspergillus fumigatus* (MNHN 566) | 30.08 ± 0.08 | 7.50 ± 0.03 |
| *Candida albicans* (ATCC 26970) | 32.46 ± 0.26 | 12.50 ± 0.12 |
| *Cladosporium herbarum* (MNHN 3369) | 20.23 ± 0.12 | 2.50 ± 0.16 |
| *Fusarium oxysporum* (MNHN 963917) | 30.64 ± 0.06 | 4.50 ± 0.12 |
| *Aspergillus variecolor* | 15.23 ± 0.04 | 5.50 ± 0.01 |
| *Fusarium alpinum* | 20.56 ± 0.01 | 4.50 ± 0.03 |
| *Fusarium culmorum* | 26.41 ± 0.05 | 8.00 ± 0.03 |
| *Fusarium tubaevacuum* | 30.24 ± 0.03 | 9.50 ± 0.02 |
| *Molniarectica fructicola* | 16.32 ± 0.03 | 3.50 ± 0.08 |
| *Penicillium spp* | 34.14 ± 0.02 | 3.50 ± 0.01 |
| *Rhizoctonia solani* | 23.12 ± 0.12 | 3.50 ± 0.12 |
| *Sclerotinia minor* | 12.34 ± 0.16 | 3.50 ± 0.18 |
| *Sclerotinia sclerotiorum* | 17.58 ± 0.04 | 9.50 ± 0.14 |
| *T. mentagrophytes* | 9.56 ± 0.06 | 15.50 ± 0.13 |
| *Trichophyton rubrum* | 12.34 ± 0.03 | 5.00 ± 0.01 |

| MIC (µg/mL) |
|-------------|
| 1.00 ± 0.02 |

* Essential oil impregnated with 10 µL/disc; 1: Amphotericin B impregnated with 20 µg/disc.

The essential oil exhibited the strongest antifungal activity against yeast and fungi species, and the comparison of findings relative to the standard drug amphotericin is given in Table 3. Strong antifungal activity was noted against *A. flavus* ([38.02 ± 0.06] mm), while the lowest antifungal activity was recorded against *T. mentagrophytes*, at (9.56 ± 0.06) mm zone of inhibition (*P* ≤ 0.01). Maximum and minimum inhibition concentration values were in the range of (9.56 ± 0.06) and (38.02 ± 0.06) µg/mL ([2.5 ± 0.16] and [15.80 ± 0.06] µg/mL for yeast (fungi)).

*flavus, A. baumannii, B. megaterium, B. subtilis, B. cepacia, E. aerogenes, S. thyphimurium, C. freundii, P. vulgaris, P. syringae* and *X. campestris*, with (14.18 ± 0.15), (14.01 ± 0.10), (14.08 ± 0.18), (11.03 ± 0.05), (10.12 ± 0.11), (9.06 ± 0.10), (9.02 ± 0.07), (7.06 ± 0.06), (5.04 ± 0.02), (3.09 ± 0.01), (2.16 ± 0.04) and (2.12 ± 0.03) mm inhibition zones (*P* ≤ 0.01), respectively. These results indicated that the essential oil obtained in the present study was a strong antibacterial agent, with MIC values in the range of (0.2 ± 0.09) to (100.00 ± 0.12) µg/mL.
4. Discussion

Present study compared with the percentage contributions of these major components which were lower than those reported by Asekun et al.[28] for M. longifolia oil from South Africa, where menthone (47.6%), eucalyptol (16.4%), and isopulegone (18.4%) were the principal components. In another study on M. longifolia oil from South Africa, Oyedeji and Afolayan[29] reported menthone (50.9%), isopulegone (19.3%), and eucalyptol (11.9%) as the principal constituents, while Yannmis et al.[30] found isopulegone (15.66%), cis-piperitnine oxide (26.52%), piperitenone oxide (26.40%), and menthone (1.17%) in a study conducted in Turkey.

M. longifolia oil extracted from the plant material sourced from Herzegovina was shown by Nikšić et al.[31] comprising piperitenone oxide (68.39%), eucalyptol (12.03%), and caryophyllene oxide (4.33%). M. longifolia essential oil from Tunisia, on the other hand, contained primarily isopulegone (54.41%), followed by isomenthone (12.02%) and piperitenone oxide (3.19%), with menthone (0.18%) being only a minor constituent[32]. In a study conducted in Iran, Ghasemi et al.[33] identified isopulegone (31.78%), eucalyptol (15.99%), menthofuran (11.25%), cis-isopulegone (10.5%), and p-menth-3-en-8-ol (6.85%) as the major constituents of M. longifolia essential oil. On the other hand, Singh et al.[25] reported that M. longifolia contained 94.0% and 98.1% of the fresh and dry mint essential oil, respectively. Hussain et al.[34] examined differences in the wild growing M. longifolia constituents during the summer and winter season, reporting that piperitenone oxide comprised 79.9% and 88.5% of fresh and dry mint essential oil, respectively. However, the authors noted some seasonal variations, as 60.10% of the essential oil comprised piperitenone oxide in the summer, while this figure increased to 64.60% in winter. Similarly, 6.37% and 1.97% was measured for piperitenone, and 5.13% and 5.97% for germacrene D. Finally, Pajohi Alamoti et al.[35] reported isopulegone (31.54%) as the major essential oil constituent, noting that all remaining constituents were found in negligible amounts.

The findings yielded by the present study for the principal components (menthone, isopulegone and eucalyptol) were compared to those reported by other authors. For example, while at 39.55% menthone was identified as the major component in the present study, Reddy and Ben-Bnina[36,37] reported 47.6% and 50.9%, respectively. On the other hand, Yannmis et al.[30] identified menthone as a minor constituent, with 1.17% and 7.9% contribution, respectively. Finally, in most other studies reviewed as a part of this work, menthone quantities were negligible[25,31-35]. As can be seen from the above, the findings yielded by the present study do not support those reported by other authors.

The findings reported in pertinent literature indicated that the mint essential oil is usually more effective against Gram-positive than Gram-negative bacteria. The results yielded by the present study are in accordance with the previous reports[36,38,39].

M. longifolia is widely cultivated throughout the world, as its essential oil has many commercial applications, such as in confectionary, as a flavoring agent, in perfumes, and in traditional medicine. Several reports on the antimicrobial activity[36], the percentage of chemical compounds, and the major constituents of the essential oil are available. The antibacterial and antifungal activity of the essential oil is primarily due to its chemical constituents, such as terpenes containing heteroatoms such as oxygen[37]. Monoterpenes are the major phytoconstituents of the essential oil, and they exhibit more potent antibacterial properties relative to those of the normal hydrocarbon plant constituents. As was shown in this work, the essential oil extracted from the aerial parts of M. longifolia is the potential antibacterial and antifungal agent, as it demonstrated superior performance against a wide range of microorganisms when compared to synthetic drugs.

M. longifolia shows strong antibacterial and antifungal activity against various Gram-positive and Gram-negative bacteria, as well as against several strains of fungi. The essential oil extracted from the aerial parts of the plant is mainly composed of monoterpenes, to which the antimicrobial properties are attributed. However, further investigations are required to identify the individual compounds responsible for the antibacterial and antifungal activity. In addition, the findings reported here should be validated in in vivo conditions. Nonetheless, we postulate that, owing to its demonstrable antibacterial and antioxidant activity, M. longifolia may be used for preservation of raw or processed foods, as well as in pharmaceuticals and in natural therapy. This work will be extended to fully analyze the antioxidant activity of this essential oil.

Conflict of interest statement

We declare that we have no conflict of interest.

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