Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.5.2017J7-108

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

Nagarjuna Reddy Desam[1*], Abdul Jabbar Al-Rajab[1], Mukul Sharma[1], Mary Moses Mylabathula[2], Ramachandra Reddy Gowkanapalli[3], Albratty Mohammed[4]

[1]*Center for Environmental Research and Studies, Jazan University, Jazan, Saudi Arabia*

[2]*Department of Biotechnology, College of Science, Krishna University, Machilipatnam, A.P., India*

[3]*Department of Polymer science and Technology, College of Science, Anatapuram, A.P., India*

[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jazan University, Jazan, Saudi Arabia*

ARTICLE INFO

Article history:
Received 19 Jun 2017
Received in revised form 11 Aug 2017
Accepted 27 Aug 2017
Available online 26 Sep 2017

Keywords:
Chemical composition
Essential oil
Antibacterial
Antifungal
Mentha longifolia L.
Menthone
Isopulegone
Eucalyptol

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

*Corresponding author: Nagarjuna Reddy Desam, Center for Environmental Research and Studies, Jazan University, Jazan, Saudi Arabia.
Tel: +966-537839837
E-mail: dndnrchem@gmail.com
The journal implements double-blind peer review practiced by specially invited international editorial board members.

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 (IKA 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 C_5–C_{24} *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* (*B. cereus*) (ATCC 10876), *Bacillus macerans* (*B. macerans*)(M58), *Bacillus megaterium* (*B. megaterium*) (M3), *Bacillus subtilis* (*B. subtilis*) (ATCC 6633), *Brucella abortus* (*B. abortus*) (A77), *Burkholderia cepacia* (*B. cepacia*) (A255), *Enterobacter cloacae* (*E. cloacae*) (ATCC 13047), *Enterococcus faecalis* (*E. faecalis*) (ATCC 49452), *Listeria monocytogenes* (*L. monocytogenes*) (ATCC 15313), *Staphylococcus aureus* (*S. aureus*) (ATCC 25923), *Micrococcus flavus* (*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 freundi* (*C. freundi*)

(ATCC 13311), *Enterobacter aerogenes* (*E. aerogenes*) (ATCC 13048), *Salmonella enteritidis* (I K27), *Proteus vulgaris* (*P. vulgaris*) (A 161), *Pseudomonas syringae* (*P. syringae*) (A 35), and *Xanthomonas campestris* (*X. campestris*) (A 235). The fungi strains used comprised *Alternaria alternaria* (MNHN 843390), *Aspergillus flavus* (*A. flavus*) (MNHN 994294), *Aspergillus fumigates* (MNHN 566), *Candida albicans* (ATCC 26790), *Cladosporium herbarum* (MNHN 3369), *Fusarium oxyporum* (MNHN 963917), *Aspergillus variecolor*, *Fusarium acuminatum*, *Fusarium solani*, *Fusarium tabacinum*, *Moliniana fructicola*, *Penicillum* spp, *Rhizoctonia solani*, *Sclerotinia minor*, *Sclerotinia sclerotiorum*, *Trichophyton mentagrophytes* (*T. mentagrophytes*), and *Trichophyton rubrum*. All microorganisms were stored at 4 °C.

2.6. Determination of zone of inhibition by disc diffusion method

The antibacterial and antifungal activity of *M. longifolia* volatile oil were investigated using the agar disc diffusion method. Microorganisms used were of referred laboratory standards. The culture suspensions were diluted so that 100.0 µL of the tested microorganisms contained 10^8 CFU/mL of bacteria and 10^6 CFU/mL of fungi strains. We utilized a Sabouraud dextrose agar medium to spread the microorganisms. The discs were impregnated with 10.0 µL of the extracted essential oil before being placed on the agar medium containing the tested microorganisms. The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Gentamicin for Gram-positive, mikacin for Gram-negative and amphotericin B for fungi served as reference compounds, allowing comparison of the antibacterial and antifungal activity exhibited by the extracted essential oil and principle components from laboratory standards. Each test was carried out in triplicate.

2.7. Determination of MIC values

The MIC values were determined using the microbroth dilution assay method, as recommended by NCCLS 2001. All tests were performed in Sabouraud dextrose broth and Mueller-Hinton broth, for bacteria and fungi, respectively. The *M. longifolia* essential oil was dissolved in 10% dimethylsulfoxide. The suspensions were prepared to contain 5.0×10^5 and 2.0×10^6 CFU/mL of bacteria and fungi, respectively. The standard strains of these suspensions were soaked onto the microplates, which were subsequently incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungi. The MIC was defined as the lowest concentration of the compound required for inhibiting the microorganism growth, and the results obtained were used to compare the antibacterial and antifungal activity of the oil with the aforementioned reference standards.

2.8. Statistical analysis

All experiments performed as a part of the present study were conducted in triplicate, and the results were presented as mean ± SD. The statistical analysis was conducted by ANOVA using the SPSS 21 software package.

3. Results

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%), isopulegone (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
Chemical constituents of the essential of *M. longifolia*.

Retention index	Components	% Composition
966	β-Pinene	2.38
1 042	o-Cymene	0.44
1 059	Eucalyptol	10.38
1 082	Linalool	2.41
1 148	Menthone	39.55
1 164	Menthol	1.52
1 175	α-Terpineol	3.14
1 179	Isopulegone	30.49
1 195	Verbenone	0.53
1 228	Nerol	0.47
1 400	β-Cubebene	1.12
1 413	Longifolene	2.47
1 418	β-Caryophyllene	1.15
1 470	γ-Muurolene	0.34
1 549	Diepicedrene-1-oxide	0.12
1 561	Caryophyllene oxide	0.51
1 576	Spathulenol	1.61
1 645	Cubenol	0.15
1 640	τ-Cadinol	0.93
	Total	99.71

HP-5ms capillary column (30 m × 0.25 mm × 0.25 µm), Helium carrier gas, FID detector for *n*-alkane series C_5–C_{24}.

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 ($P \leqslant 0.01$) 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 ($P \leqslant 0.01$), respectively.

Finally, minimum activity was observed against *E. cloacae*, *M.*

Table 2

Antibacterial activity of *M. longifolia*.

Microbial strains	Zone of inhibition (mm[a])					MIC[b] (μg/mL)			
	EO	RA	Ment	Isopul	Eucal	EO	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	1.00 ± 0.01	2.00 ± 0.01	3.50 ± 0.02	6.00 ± 0.04
B. macerans	25.05 ± 0.12	20.28 ± 0.05	16.20 ± 0.01	15.00 ± 0.02	8.00 ± 0.04	0.86 ± 0.04	2.50 ± 0.02	3.50 ± 0.02	6.00 ± 0.02
B. megaterium	11.03 ± 0.05	18.02 ± 0.16	10.40 ± 0.02	9.00 ± 0.01	8.00 ± 0.01	0.75 ± 0.08	2.50 ± 0.02	3.50 ± 0.04	5.00 ± 0.04
B. subtilis	10.12 ± 0.11	12.10 ± 0.08	9.00 ± 0.04	7.00 ± 0.02	6.00 ± 0.02	1.00 ± 0.14	2.00 ± 0.02	3.50 ± 0.02	6.00 ± 0.04
B. abortus	25.06 ± 0.01	9.02 ± 0.02	20.00 ± 0.04	18.00 ± 0.04	10.00 ± 0.06	2.50 ± 0.02	2.00 ± 0.06	3.00 ± 0.04	5.00 ± 0.04
B. cepacia	9.06 ± 0.10	12.45 ± 0.03	8.00 ± 0.02	8.20 ± 0.06	5.00 ± 0.02	1.50 ± 0.06	2.50 ± 0.04	3.00 ± 0.04	5.00 ± 0.06
E. cloacae	14.18 ± 0.15	16.28 ± 0.06	10.00 ± 0.04	10.00 ± 0.04	6.00 ± 0.04	1.00 ± 0.14	2.00 ± 0.02	3.50 ± 0.02	5.00 ± 0.06
E. faecalis	32.12 ± 0.12	15.32 ± 0.14	20.00 ± 0.02	19.00 ± 0.06	9.00 ± 0.02	1.50 ± 0.18	2.00 ± 0.06	3.50 ± 0.02	5.00 ± 0.06
L. monocytogenes	15.11 ± 0.04	12.23 ± 0.12	10.00 ± 0.02	8.00 ± 0.02	8.21 ± 0.04	1.00 ± 0.06	2.50 ± 0.06	3.50 ± 0.02	6.00 ± 0.04
S. aureus	35.24 ± 0.13	25.42 ± 0.16	25.00 ± 0.06	22.00 ± 0.04	14.00 ± 0.02	1.00 ± 0.03	2.00 ± 0.04	3.00 ± 0.02	6.00 ± 0.04
M. flavus	14.01 ± 0.10	10.28 ± 0.06	12.40 ± 0.04	10.00 ± 0.02	7.00 ± 0.06	2.00 ± 0.07	2.50 ± 0.02	3.50 ± 0.02	6.00 ± 0.04
S. epidermidis	15.14 ± 0.08	16.32 ± 0.05	12.00 ± 0.02	12.00 ± 0.01	6.00 ± 0.04	1.53 ± 0.07	2.50 ± 0.06	3.00 ± 0.02	5.00 ± 0.04
C. michiganense	22.04 ± 0.04	15.03 ± 0.08	20.00 ± 0.02	20.40 ± 0.02	12.00 ± 0.03	2.10 ± 0.12	2.00 ± 0.02	3.00 ± 0.02	5.00 ± 0.02
S. pyogenes	23.02 ± 0.07	9.04 ± 0.12	22.00 ± 0.04	15.00 ± 0.02	6.00 ± 0.04	1.04 ± 0.12	2.00 ± 0.02	3.00 ± 0.02	6.00 ± 0.04
A. baumannii	14.08 ± 0.18	18.12 ± 0.12	7.00 ± 0.05	5.00 ± 0.02	5.00 ± 0.04	1.50 ± 0.03	2.50 ± 0.02	3.50 ± 0.02	5.00 ± 0.04
E. coli	25.08 ± 0.13	15.24 ± 0.34	20.00 ± 0.06	20.00 ± 0.02	10.00 ± 0.02	0.20 ± 0.09	2.00 ± 0.04	3.50 ± 0.02	6.00 ± 0.04
K. pneumonia	16.04 ± 0.09	12.22 ± 0.02	10.00 ± 0.04	14.00 ± 0.06	10.00 ± 0.02	0.50 ± 0.14	2.50 ± 0.02	3.50 ± 0.02	6.00 ± 0.02
P. mirabilis	15.12 ± 0.12	14.36 ± 0.06	12.00 ± 0.08	10.00 ± 0.02	7.00 ± 0.04	1.80 ± 0.17	2.50 ± 0.02	3.50 ± 0.04	6.00 ± 0.02
S. typhimurium	7.06 ± 0.06	11.03 ± 0.06	4.00 ± 0.06	4.00 ± 0.02	2.00 ± 0.02	1.50 ± 0.15	3.00 ± 0.02	3.00 ± 0.02	5.00 ± 0.04
C. freundi	5.04 ± 0.02	8.04 ± 0.03	3.00 ± 0.03	4.00 ± 0.02	2.00 ± 0.02	2.00 ± 0.19	3.00 ± 0.04	3.00 ± 0.02	5.00 ± 0.04
E. aerogenes	9.02 ± 0.07	12.24 ± 0.02	7.00 ± 0.02	6.00 ± 0.02	2.00 ± 0.06	1.40 ± 0.12	2.50 ± 0.06	3.50 ± 0.06	5.00 ± 0.04
Salmonella enteritidis	10.12 ± 0.12	15.36 ± 0.12	6.00 ± 0.04	6.00 ± 0.02	2.00 ± 0.02	1.58 ± 0.02	2.00 ± 0.02	3.00 ± 0.06	6.00 ± 0.02
P. vulgaris	3.09 ± 0.01	12.02 ± 0.04	5.00 ± 0.04	3.00 ± 0.06	3.00 ± 0.02	1.25 ± 0.02	3.00 ± 0.04	3.00 ± 0.04	6.00 ± 0.02
P. syringae	2.16 ± 0.04	17.09 ± 0.06	3.00 ± 0.02	3.00 ± 0.04	2.00 ± 0.02	2.05 ± 0.16	3.00 ± 0.04	3.00 ± 0.04	6.00 ± 0.02
X. campestris	2.12 ± 0.03	6.04 ± 0.02	2.00 ± 0.04	2.00 ± 0.04	2.00 ± 0.02	100.00 ± 0.12	3.00 ± 0.02	3.00 ± 0.04	5.00 ± 0.02

[a]: Values represent mean ± SD for triplicate experiments; [b]RA: Reference of antibiotics, gentamicin for Gram-positive bacteria and mikacin for Gram-negative bacteria used 30 μg/disc; EO: Essential oil; Ment: Menthone; Isopul: Isopulegone; Eucal: Eucalyptol.

Table 3

In vitro antifungal activity of essential oil.

Strains	Essential oil[a]		Amphotericin[b]	
	Disc diameter (mm)	MIC (μg/mL)	Disc diameter (mm)	MIC (μg/mL)
Alternaria alternaria (MNHN 843390)	35.26 ± 0.12	8.50 ± 0.04	24.02 ± 0.04	1.00 ± 0.02
A. flavus (MNHN 994294)	38.02 ± 0.06	15.80 ± 0.06	27.07 ± 0.08	3.50 ± 0.01
Aspergillus fumigates (MNHN 566)	30.08 ± 0.08	7.50 ± 0.03	20.06 ± 0.05	3.00 ± 0.02
Candida albicans (ATCC 26790)	26.34 ± 0.26	12.50 ± 0.12	15.08 ± 0.03	2.30 ± 0.09
Cladosporium herbarum (MNHN 3369)	20.23 ± 0.12	2.50 ± 0.16	18.02 ± 0.12	2.50 ± 0.12
Fusarium oxyporum (MNHN 963917)	30.64 ± 0.06	4.50 ± 0.12	23.04 ± 0.04	3.50 ± 0.14
Aspergillus variecolor	15.23 ± 0.04	5.50 ± 0.01	20.04 ± 0.02	3.50 ± 0.17
Fusarium acuminatum	20.56 ± 0.01	4.50 ± 0.03	18.24 ± 0.04	1.25 ± 0.02
Fusarium solani	26.41 ± 0.05	8.00 ± 0.03	22.30 ± 0.03	1.25 ± 0.01
Fusarium tabacinum	30.24 ± 0.03	9.50 ± 0.02	20.25 ± 0.06	4.35 ± 0.24
Moliniana fructicola	16.32 ± 0.03	3.50 ± 0.08	25.03 ± 0.06	3.50 ± 0.21
Penicillum spp	34.14 ± 0.02	3.50 ± 0.01	21.10 ± 0.01	3.65 ± 0.01
Rhizoctonia solani	23.12 ± 0.12	3.50 ± 0.12	12.04 ± 0.16	3.65 ± 0.01
Sclerotinia minor	12.34 ± 0.16	3.50 ± 0.18	18.65 ± 0.13	3.50 ± 0.01
Sclerotinia sclerotiorum	17.58 ± 0.04	9.80 ± 0.14	20.03 ± 0.24	2.50 ± 0.03
T. mentagrophytes	9.56 ± 0.06	10.50 ± 0.13	15.36 ± 0.14	6.50 ± 0.05
Trichophyton rubrum	12.34 ± 0.03	5.00 ± 0.01	16.59 ± 0.01	3.03 ± 0.01

[a]: Essential oil impregnated with 10 μL/disc; [b]: Amphotericin B impregnated with 20 μg/disc.

flavus, *A. baumannii*, *B. megaterium*, *B. subtilis*, *B. cepacia*, *E. aerogenes*, *S. typhimurium*, *C. freundi*, *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 ($P \leqslant 0.01$), 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 (0.20 ± 0.09) to (100.00 ± 0.12) μg/mL.

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 ($P \leqslant 0.01$). Maximum and minimum inhibition concentration values were in the range of (9.56 ± 0.06) and (38.02 ± 0.06) μg/mL [(2.50 ± 0.16) and (15.80 ± 0.06) μg/mL] for yeast (fungi).

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 Yanmis *et al.*[30] found isopulegone (15.66%), cis-piperitone epoxide (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, Yanmis *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.

Acknowledgments

The authors are grateful to Dr. Muralidara Rao, Head of the Microbiology Laboratory, Sri Krishnadevaraya University, India, for providing the infrastructure necessary for conducting this research.

References

[1] Abreu AC, McBain AJ, Simões M. Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep* 2012; **29**(9): 1007-21.

[2] Affronti A, Orlando A, Cottone M. An update on medical management of Crohn's disease. *Expert Opin Pharmacother* 2015: **16**: 63-78.

[3] Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta* 2013; **1830**(6): 3670-95.

[4] Murad HA, Abdallah HM, Ali SS. *Mentha longifolia* protects against acetic-acid induced colitisin rats. *J Ethnopharmacol* 2016; **190**: 354-61.

[5] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012; **75**(3): 311-35.

[6] Orel R, Kamhi Trop T. Intestinal microbiota, probiotics and prebiotics in inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 11505-24.

[7] Singh R, Shushni MAM, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arab J Chem* 2015; **8**: 322-8.

[8] Saharkhiz MJ, Motamedi M, Zomorodian K, Pakshir K, Miri R, Hemyari K. Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *ISRN Pharm* 2012; **2012**:

718645.

[9] Mahboubi M, Kazempour N. Chemical composition and antimicrobial activity of peppermint (*Mentha piperita* L.) essential oil. *Songklanakarin J Sci Technol* 2014; **36**(1): 83-7.

[10] Derwich E, Benziane Z, Taouil R, Senhaji O, Touzani M. Aromatic plants of morocco: GC/MS analysis of the essential oils of leaves of *Mentha piperita*. *Adv Environ Biol* 2010; **4**(1): 80-5.

[11] Kizil S, Hasimi N, Tolan V, Kilinc E, Yuksel U. Mineral content, essential oil components and biological activity of two *Mentha* species (*M. piperita* L., *M. spicata* L.). *Turk J Field Crops* 2010; **15**(2): 148-53.

[12] Tyagi AK, Malik A. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of *Cymbopogon citratus*. *BMC Complement Altern Med* 2010; **10**: 65.

[13] Gorrasi G. Dispersion of halloysite loaded with natural antimicrobials into pectins: characterization and controlled release analysis. *Carbohydr Polym* 2015; **127**: 47-53.

[14] Biddeci G, Cavallaro G, Di Blasi F, Lazzara G, Massaro M, Milioto S, et al. Halloysite nanotubes loaded with peppermint essential oil as filler for functional biopolymer film. *Carbohydr Polym* 2016; **152**: 548-57.

[15] Kashiri M, Cerisuelo JP, Domínguez I, Lopez-Carballo G, Muriel-Gallet V, Gavara R, et al. Zein films and coatings as carriers and release systems of *Zataria multiflora* Boiss. essential oil for antimicrobial food packaging. *Food Hydrocoll* 2017; **70**: 260-8.

[16] Baliga MS, Rao S. Radioprotective potential of mint: a brief review. *J Cancer Res Ther* 2010; **6**(3): 255-62.

[17] Sandasi M, Leonard CM, Viljoen AM. The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. *Lett Appl Microbiol* 2010; **50**(1): 30-5.

[18] Guptaa AK, Mishraa R, Singh AK, Srivastavaa A, Lal RK. Genetic variability and correlations of essential oil yield withagro-economic traits in *Mentha* species and identification of promising cultivars. *Ind Crops Prod* 2017; **95**: 726-32.

[19] El-Badry AA, Al-Ali KH, El-Badry YA. Activity of *Mentha longifolia* and *Ocimum basilicum* against *Entamoeba histolytica* and *Giardia duodenalis*. *Sci Parasitol* 2010; **11**: 109-17.

[20] Shah AJ, Bhulani NN, Khan SH, Ur Rehman N, Gilani AH. Calcium channel blocking activity of *Mentha longifolia* L. explains its medicinal use in diarrhoea and gut spasm. *Phytother Res* 2010; **24**(9): 1392-7.

[21] Iqbal S, Nisar ur Rehman, Kortz U, Iqbal J. Development of a fast and efficient CE enzyme assay for the characterization and inhibition studies of α-glucosidase inhibitors. *J Sep Sci* 2013; **36**: 3623-8.

[22] Mokaberinejad R, Zafarghandi N, Bioos S, Dabaghian FH, Naseri M, Kamalinejad M, et al. *Mentha longifolia* syrup in secondary amenorrhea: a double-blind, placebo-controlled, randomized trials. *Daru* 2012; **20**(1): 97.

[22] Raza H, John A, Shafarin J, Howarth FC. Exercise-induced alterations in pancreatic oxidative stress and mitochondrial function in type 2 diabetic Goto-Kakizaki rats. *Physiol Rep* 2016; **4**(8): e12751.

[23] Razavi SM, Zarrini G, Molavi G. The evaluation of some biological activity of *Mentha longifolia* (L.) huds growing wild in Iran. *Pharmacologia* 2012; **3**: 535-8.

[24] Viuda-Martos M, Mohamady MA, Fernández-López J, Abd ElRazik KA, Omer EA, Pérez-Alvarez JA, et al. *In vitro* antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. *Food Control* 2011; **22**: 1715-22.

[25] Singh S, Das SS, Singh G, Perotti M, Schuff C, Catalán C. Comparative study of chemistry compositions and antimicrobial potentials of essential oils and oleoresins from dried and fresh *Mentha longifolia* L. *J Coast Life Med* 2015; **3**(12): 987-91.

[26] Robert P, Adams. *Identification of essential oil components by gas chromatography/mass spectroscopy*. 4 ed. Carol Stream: Allured Pub Corp; 2007.

[27] Weerakkody NS, Caffin N, Turner MS, Dykes GA. *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control* 2010; **21**: 1408-14.

[28] Asekun OT, Grieson DS, Afolayan AJ. Effects of drying methods on the quality and quantity of the essential oil of *Mentha Longifolia* L. subsp. *Capensis*. *Food Chem* 2007; **101**: 995-8.

[29] Oyedeji OA, Afolayan AJ. Chemical composition and antibacterial activity of the essential oil isolated from South African *Mentha longifolia* (L.) L. subsp. *capensis* (Thunb.) Briq. *J Essent Oil Res* 2006; **18**: 57-60.

[30] Yanmis D, Gormez A, Bozari S, Orhan F, Gulluce M, Agar G, et al. Determination of chemical composition and antibacterial properties of essential oil of *Mentha longifolia* ssp. *longifolia* against phytopathogenic bacteria. *Microbes Appl Res* 2012: 531-5.

[31] Nikšić H, Bešović EK, Makarević E, Durić K. Chemical composition, antimicrobial and antioxidant properties of *Mentha longifolia* (L.) Huds. essential oil. *J Health Sci* 2012; **2**(3): 192-200.

[32] Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F, Romdhane M. Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. *J Food Sci* 2009; **74**(7): M358-63.

[33] Ghasemi M, Radjabalizade K, Gharari K, Yaghoobi H, Asgarpoor D. Chemical composition and *in vitro* antibacterial activity of native *Mentha longifolia* essential oil from Ardabil, Iran on *Streptococcus mutans*, *Lactobacillus rhamnosus* and *Actinomyces viscosus*. *Infect Epidemiol Med* 2016; **2**(1): 11-4.

[34] Hussain A, Anwar F, Nigam PS, Ashraf M, Gilani AH. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *J Sci Food Agric* 2010; **90**(11): 1827-36.

[35] Pajohi Alamoti MR, Tajik H, Akhondzadeh A, Gandomi H, Ehsani A. A study on chemical composition and antimicrobial activity of essential oil of *Mentha longifolia* L. and *Cuminum cyminum* L. in soup. *Iranian J Food Sci Technol* 2012; **9**(36): 33-45.

[36] Reddy DN, Al-Rajab AJ. Chemical composition, antibacterial and antifungal activities of *Ruta graveolens* L. volatile oils. *Cogent Chem* 2016; **2**: 1220055.

[37] Ben-Bnina E, Hammami S, Daamii-Remadi M, Ben-Jannet H, Mighri Z. Chemical composition and antimicrobial effects of Tunisian *Ruta chalepensis* L. essential oils. *J Soc Chim Tunis* 2010; **12**: 1-9.

[38] Angienda PO, Onyango DM, Hill DJ. Potential application of plant essential oils at sub-lethal concentrations under extrinsic conditions that enhance their antimicrobial effectiveness against pathogenic bacteria. *Afr J Microbiol Res* 2010; **4**: 1678-84.

[39] Gilles M, Zhao J, An M, Agboola S. Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. *Food Chem* 2010; **119**: 731-7.