Original Research Article

Chemical Composition and Antibacterial Activity of Essential oil of *Mentha longifolia* Leaf from Albaha Area Southern Saudi Arabia

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ABSTRACT

The essential oil obtained by hydrodistillation from the leaves of Saudi Arabia native *Mentha longifolia* (*Lamiaceae*) was analyzed by gas chromatography-mass spectrometry GC/MS. Forty six compounds were analysed, it was characterized by a high percentage of oxygenated monoterpenes (30.40%) with Piperitone as the major one (30.77%), followed by sesquiterpen hydrocarbons (26.08%) in which Caryophellene (5.58%) as the main, while monoterpene hydrocarbons (23.90%) with gamma-Terpinene (1.36%) as a main. The principal components of *M. longifolia* oil was the piperitone with chemical formula $C_{10}H_{14}O$. The antibacterial activity of the essential oil of *M. longifolia* L. leaf were evaluated in this study. The essential oil showed strong antibacterial activity against *Bacillus subtilis* with inhibition zone (25mm) and *Escherichia coli* (24mm), It seems that the essential oils derived from *the Mentha longifolia* L. oil could be used as a natural source of antimicrobial agents.

Key words: *Mentha longifolia*, GC/MS analysis, Essential oil, Antibacterial, Monoterpenes, Saudi Arabia.

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INTRODUCTION

Medicinal and aromatic plants constitute a large part of natural flora and are considered an important resource in various fields such as the pharmaceutical, flavor and fragrance, perfumery and cosmetic industries.[1]

In medicinal and aromatic plants, essential oils generally accumulate in the secretary canals or cavities and glandular trichomes and sometimes in the epidermal cells.[2] Essential oils and their chemical constituents exhibit more bioactivity when present in the oxygenated or active form. In general, the chemical composition of essential oils is relatively complex and about 20 to 60 different bioactive components are observed in many of these essential oils.

Natural products are the source of synthetic and traditional herbal medicine. About 80% of the population worldwide use traditional medicine, which has compounds derived from medicinal plants.[3] These plants are also used for stabilizes in several food items from deterioration. They contributed a major amount for the treatment of key disorders of the body.

Traditional folk remedies from plants have always guided scientists to search for new medications in order to maintain and promote healthy life for human and animals.[4] The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal[5] medicinal practitioners.

Mentha longifolia L. "Wild-mint" is an aromatic plant belonging to the *Lamiaceae* family. It is native plant in Mediterranean region and South Africa. It is a fast growing perennial herb strongly aromatic. The leaves formed in pairs opposite each other along the square shaped

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stem. The leaves are usually coarsely hairy and the edges sparsely toothed. The colour of the leaves varies from light and dark green to grey. Wild-mint is a popular traditional medicine. It is mainly used for respiratory ailments, for coughs, colds, stomach cramps, asthma, flatulence and headache.[6,7] *Mentha* ssp. has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative, colitis and liver complaints due to its anti-inflammatory, carminative and antioxidant activities.[8] Externally, they have been applied for the treatment of acne, loss of smell, insect stings, snake bites and skin infections.[9] The activity is chiefly attributed to a variety of phenolic compounds and composition of essential oil. The main compounds responsible for typical aroma are chavicol methyl ether (estragol), linalool, eugenol, 1, 8- cineole and methyl cinnamate.[10] The family is also famous for the presence of diterpenoids in its members. However, the biological activity of the oil depends upon the chemical constituents in the oil, which vary greatly with geographical regions. For example, essential oil rich in piperitenone oxide (from Lithuania and Jordan), carvone and cis-carveol (from Iran), pulegone (from Israel) and diosphenol (from Spain) have been reported.[11-16] Many plants from the *Myrtaceae* family are reported to have antibacterial or antifungal activities.

The present study was aimed to examine the chemical composition of *M. longifolia* and to investigate the possible inhibitory activity of *M. longifolia* oil extract against various micro-organisms.

MATERIALS AND METHODS

Plant material

Mentha longifolia was obtained from a local market in Al-Baha city and authenticated by D. Haidar Abd-Elgadirin Al-Baha University, Faculty of Science, Department of Biology.

Isolation of the essential oil

Essential oil was extracted from the dried leaves parts of *M. longifolia* by hydro-distillation using an apparatus of Clevenger type[17] according to European Pharmacopoeia. The extraction was carried out for 8 h to mixture 300 g of leaves in distilled water (1000 ml). The collected oil was dried over anhydrous sodium sulphate and stored in sealed vials at 15^{O}C until analysis. Chemical identification of the oil composition was conducted by GC-MS analyses. Antibacterial potential was characterized against two micro-organisms, signifying Gram positive and Gram-negative bacteria.

Gas Chromatography–Mass Spectrometry (GC/MS)

The qualitative and quantitative analysis of the oil sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japan Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30mx0.25mmx0.25um). The sample was injected by using split mode, instrument operating in EI mode at 70eV. Helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50°C with rate 7°C/min to 180°C then the rate was changed to 10°C/min reaching 300°C as final temperature degree, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 30 min.

Identification of components

Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library, the National Institute of Standards and Technology (NIST), results were recorded.

Antibacterial activity

Antibacterial activity was assessed by using a wide range of Gram-positive and Gram-negative bacteria. The zone of inhibition against the microbes was determined using disc diffusion method. Microorganisms were grown in nutrient broth medium and incubated, with shaking, at 37°C and at 33°C, for bacteria.

RESULTS

The components in essential oil in the leaves of *M. longifolia* are presented in Table 1. The essential oil obtained from leaves of *M. longifolia* growing in Saudi. GC/MS analysis of essential oil revealed the presence of 46 different compounds (Table 1). The retention time RT, molecular mass and the relative percentages of the compounds present in leaves of *M. longifolia* were recorded. The GC/MS chromatogram spectrum (Figure 1) confirmed the present compounds. Interpretation of mass spectrum GC/MS was conducted using the database of National Institute Standard and Technique (NIST). The oil is characterized by a high concentration of monoterpenes (54.30%) including mostly oxygenated monoterpenes (30.40%). Concerning the high content of sesquiterpene hydrocarbons, 26.08% (Table 2). The prevailing compounds are piperitone (30.77%), eucalyptol (14.85%), $C_{10}H_{16}O$ (13.68%) and caryophellene (5.58%).

Table 1: Composition of the essential oil obtained from the leaf of *M. longifolia*.

Peak #	Compound	RT (min)	Area (%)	Molecular mass	Chemical formula
1	Alpha-thujene	4.565	0.03	136	$C_{10}H_{16}$
2	Alpha-pinene	4.697	0.56	136	$C_{10}H_{16}$
3	Camphene	4.970	0.12	136	$C_{10}H_{16}$
4	(+)-Sabinene	5.399	0.57	136	$C_{10}H_{16}$
5	2(10)-pinene	5.475	1.21	136	$C_{10}H_{16}$
6	Beta-Myrcene	5.682	0.26	136	$C_{10}H_{16}$
7	(+)-2-Carene	6.203	0.83	136	$C_{10}H_{16}$
8	O-Cymene	6.366	0.09	134	$C_{10}H_{14}$
9	D-Limonene	6.442	0.48	136	$C_{10}H_{16}$
10	Eucalyptol	6.512	14.85	154	$C_{10}H_{18}O$
11	Gamma-Terpinene	7.033	1.36	136	$C_{10}H_{16}$
12	Cis-sabinene hydrate	7.244	0.71	154	$C_{10}H_{18}O$
13	Cyclohexene,4-methyl	7.634	0.32	136	$C_{10}H_{16}$
14	Linalool	7.860	1.30	154	$C_{10}H_{18}O$
15	Cis-para-menth-2-en-1-ol	8.349	0.22	154	$C_{10}H_{18}O$
16	Trans-(-)-pinocarveol	8.730	0.54	152	$C_{10}H_{16}O$
17	Cis-Verbenol	8.843	0.20	152	$C_{10}H_{16}O$
18	Pinocarvone	9.199	0.33	150	$C_{10}H_{14}O$
19	Isoborneol	9.289	3.45	154	$C_{10}H_{18}O$
20	(-)-Terpinen-4-ol	9.491	3.48	154	$C_{10}H_{18}O$
21	alpha-terpineol	9.769	3.88	154	$C_{10}H_{18}O$
22	Unidentified	11.051	13.68	152	$C_{10}H_{16}O$
23	D-carvone	11.401	1.93	150	$C_{10}H_{14}O$
24	2-pinen-7-one	11.820	0.29	150	$C_{10}H_{14}O$
25	Beta-elemene	12.600	0.77	204	$C_{15}H_{24}$
26	Piperitone	12.788	30.77	150	$C_{10}H_{14}O$
27	Isopropylidenecyclohexanone	13.222	0.79	138	$C_{9}H_{14}O$
28	(-)-beta-Bourbonene	13.547	0.18	204	$C_{15}H_{24}$
29	Unidentified	13.645	0.28	204	$C_{15}H_{24}$
30	Caryophellene	14.205	5.58	204	$C_{15}H_{24}$
31	alpha-Cubebene	14.663	0.14	204	$C_{15}H_{24}$
32	Cis-beta-Farnesene	14.735	0.14	204	$C_{15}H_{24}$
33	Humulene	14.823	0.31	204	$C_{15}H_{24}$
34	1H-Cyclopenta[1,3]cyclopropa[1,2] benzene	14.983	0.21	204	$C_{15}H_{24}$
35	1,6-Cyclodecadiene,1-methyl cyclodecadiene	15.312	3.84	204	$C_{15}H_{24}$
36	Germacrene B	15.589	1.74	204	$C_{15}H_{24}$
37	gamma-Muurolene	15.880	0.57	204	$C_{15}H_{24}$
38	Gamma-cadinene	16.015	0.20	204	$C_{15}H_{24}$
39	Spatulenol	17.031	0.32	220	$C_{15}H_{24}O$
40	Caryophellene oxide	17.127	0.28	220	$C_{15}H_{24}O$
41	Cubenol	17.630	0.17	222	$C_{15}H_{26}O$
42	tau-Cadinol	18.047	1.88	222	$C_{15}H_{26}O$
43	alpha-Cadinol	18.283	0.11	222	$C_{15}H_{26}O$
44	Prasterone-3-sulfate	23.587	0.44	368	$C_{19}H_{28}O_5$
45	Unidentified	23.954	0.45	308	$C_{20}H_{36}O2$
46	Denatonium saccharide	27.660	0.15	446	$C_{28}H_{34}N_2O_3$

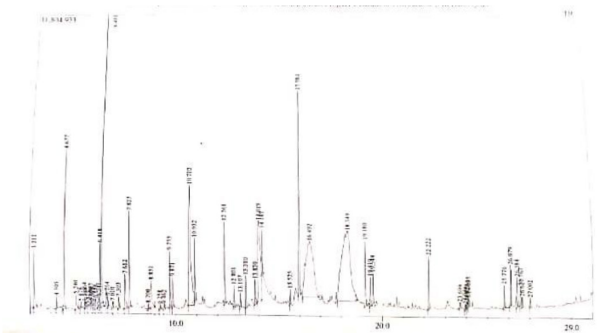

Figure 1: GC/MS Chromatogram Spectrum of *M. longifolia* oil extract.

Table 2: Components of essential oil from the leaf of *M. longifolia*.

Compounds	%
Monoterpene Hydrocarbons (MH)	54.30%
Oxygenated Monoterpenes (OM)	30.40
Sesquiterpene Hydrocarbons (SH)	26.08

Table 3: Antibacterial Activity of *M. longifolia* oil (leaves).

Bacteria	Concentration (mg/ml)			
	100	50	25	12.5
Staphylococcus aureus	14	13	15	14
Bacillus subtilis	25	21	22	18
Escherichia coli	24	22	21	20
Pseudomonas aeruginosa	13	15	16	15

M D I Z = Mean diameter of growth inhibition zone = average of two replicates in millimeters
Inhibition zone: 10-12 weak, 13-15 moderate, 16-18 strong, ≥ 18 highly strong.

The oil was screened against Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). The essential oil showed strong antibacterial activity against *Bacillus subtilis* with inhibition zone (25mm) and *Escherichia coli* (24mm), It seems that the essential oils derived from the *Mentha longifolia* L. oil could be used as a natural source of antimicrobial agents.

Standard bacteria

Staphylococcus aureus (ATCC 25923), *Bacillus subtilis* (NCTC 8236)

Escherichia coli (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853),

Most essential oils are composed of terpenes, terpenoids and other aromatic and aliphatic constituents with low molecular weights. Terpenes or terpenoids are synthesized within the cytoplasm of the cell through the mevalonic acid pathway.[23] Terpenes are composed of isoprene units and are generally represented by the chemical formula $(C_5H_8)n$. Terpenes can be acyclic, monocyclic, bicyclic, or tricyclic.[24] Terpenes are classified into several groups such as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$) and triterpenes ($C_{30}H_{40}$). The major component (more than 90%) of bioactive essential oils is constituted of monoterpenes.[25] Some of the major compounds include monoterpene hydrocarbons (limonene, α-pinene, p-cymene and -terpinene), oxygenated monoterpenes (thymol, carvacrol, eugenol and camphor), diterpenes (kaurene and camphorene), sesquiterpene hydrocarbons (caryophyllene, germacrene D and humulene), oxygenated sesquiterpenes (spathulenol, caryophyllene oxide), monoterpene alcohols (geraniol, linalool and nerol), sesquiterpene alcohol (patchoulol), aldehydes (citral, cuminal), acids (geranic acid, benzoic acid), ketones (acetophenone, benzophenone), lactones (bergapten), phenols (eugenol, thymol, carvacrol and catechol), esters (bornyl acetate, ethyl acetate) and coumarins (fumarin, benzofuran).[1,2,26-29]

The essential oil of leaves from *M. longifolia* was examined for its antibacterial activity potential against a panel of pathogenic microorganisms including Gram positive, Gram negative, (Table 3). *M. longifolia* demonstrated variable level of antimicrobial activity against all examined micro-organisms.

Essential oil extracted from *Mentha longifolia* oil showed the highest activity against *Bacillus subtilis* and *Escherichia coli* with the strongest inhibition zone of 25 and 24mm respectively, these in agree with the study by Hafedn *et al.*[30] they proved that the essential oil of *M. longifolia* has shown interesting antimicrobial activity against *E. coli, S. typhimurium*. The most effective antibacterial activity was expressed by the essential oil against the Gram-negative bacteria, *Escherichia coli*.

CONCLUSION

The present study was aimed to analyze the essential oil of the *M. longifolia* leaves (Saudi Arabia variety) to identify their composition of volatile oil and antibacterial activity against bacteria .The essential oil showed strong antibacterial activity against *Bacillus subtili* and *Escherichia coli* (25, 24 mm), It seems that the essential oils derived from *the Mentha longifolia* L. oil could be used as a natural source of antimicrobial agents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

KSA: Kingdom of Saudi Arabia; **M. longifolia:** *Mentha longifolia*; **GC/MS:** Gas chromatography-mass spectrometry; **mm:** Millimeter; **NIST:** National Institute of Standards and Technology; **RT:** Retention Time; **MH:** Monoterpene Hydrocarbons; **OM:** Oxygenated Monoterpenes; **SH:** Sesquiterpene Hydrocarbons; **MDIZ:** Mean diameter of growth inhibition zone = average of two replicates in millimeters; **IM:** Inhibition zone.

SUMMARY

The essential oil of the *Mentha longifolia* L. could be used as a natural source of antimicrobial agents.

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