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Research Article

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *MENTHA LONGIFOLIA* L OF MEKELE, ETHIOPIA

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Abstract

The constituents of essential oil isolated by hydro distillation of the aerial parts of *Mentha longifolia* L. Lamiaceae family, from Mekele, Northern Ethiopia was examined by GC-MS. A total of 16 chemical constituents representing 91.6 % in the essential oil of *Mentha longifolia* were identified by GC-MS analysis. Epizonarene with contribution of 29.7 % was found to be the principal constituent. Other important components identified were: caryophyllene (11.3 %), dimethyl malonate (7.5 %), β-eudesmol (10.3 %) β-cubebene (6.1 %), α-cadinol (6.9 %), β-bourbonene (4.9 %) and α-guaiene (3.8 %). The essential oil of *Mentha longifolia* showed biological activities against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria.

Keywords: *Mentha longifolia* L. Lamiaceae, essential oil, antibacterial activity.

INTRODUCTION

The genus of *Mentha*, includes 20 species that spread all over the world. *Mentha longifolia* or wild mint in the Lamiaceae family is a fast-growing perennial herb is distributed in various regions of Africa. Mint is used as a traditional medicine for stomach ache, anti asthmatic anti spasmodic, digestive and carminative[1-3]. It is mostly the leaves that are used, usually to make a tea that is drunk for coughs, colds, stomach cramps, asthma, flatulence, indigestion and headaches[4-5]. It is also used externally to treat wounds and swollen glands[6-8]. Mint extracts are commonly used as food flavoring additive and are generally considered safe to use as they provide good defense against oxidative damage and health benefits[9]. The essential oil of *Mentha* species was reported to have fungicidal, anti-inflammatory, antimicrobial and antioxidant activities[10-14].

MATERIALS AND METHODS

Plant Material

The aerial parts of *Mentha longifolia* plant were collected during the month of December from Mekele, Northern Ethiopia in 2011 and was identified by the authors and a herbarium sheet was deposited at the Chemistry department, Mekele University, Mekele, Ethiopia.

Chemical Reagents

All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

Essential Oil Extraction

The shade dried aerial parts of *Mentha longifolia* plant collected (1 Kg) was subjected to hydro distillation in a Clevenger apparatus for 3 h. The essential oil was separated from aqueous layer using a 100 mL capacity separator funnel. The collected essential oil was dried over anhydrous sodium sulfate and filtered using a Whatman filter paper no. 40. The extracted essential oil was light yellow liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis. The yield of the oil was found to be 1.56 % (w/w) in relation to the dry weight basis.

GC and GC-MS Analysis

GC analysis were carried out in Agilent Technology 6890N gas Chromatograph data handling system equipped with a split-split less injector and fitted with a FID using N2 as carrier gas. The column was HP-5capillary column (30 m x 0.32 mm, 0.25 µm film thickness) and temperature program was used as follows: initial temperature of 60^0C (hold: 2 minutes) programmed at a rate of 3^0C/min to a final temperature of 220^0C (hold: 5 minutes). Both the temperature of injector and FID were maintained at 210^0C. The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a split-split less injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 min x 0.32 mm, 0.25 µm film thicknesses). Helium was used as carrier gas at a flow rate of 1.0 ml/min. The GC was interfaced with Perkin Elmer 500 mass detector operating in EI^+ mode. The mass spectra was recorded over 40-500 amu and revealed the Total Ion Current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210^0 C and that of ion source at 200^0C. Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds[16].

Antibacterial Activity

The study was conducted by using standard disc diffusion method. In each experiment, microorganisms were cultured at 37°C for 24 h and prepared to turbidity which is equivalent to

0.5 McFarland standards (National Committee of Clinical Laboratory Standards)[17-19]. Mueller-Hinton (MH) agar 38 g was dissolved in 1000 ml of distilled water. Then it was boiled on heating mantle to dissolve the media completely and then sterilized by autoclaving at 15 lbs. and 121^{0}C for 15 minutes. After it was autoclaved at indicated conditions, it was poured to the sterilized petridishes and allowed to set at room temperature until the agar has solidified. It was then incubated at 37^{0}C for 24 h to be ready for susceptibility test. The stock solution of the crude *Mentha longifolia* oil in Chloroform (20 mg/ml) and test discs were prepared from Whatman filter paper. A 0.5 McFarland standard was prepared as described in National Committee of Clinical Laboratory Standards (NCCLS)[20-21]. One percent V/V solution of sulfuric acid and 1.175 % W/V solution of barium chloride were prepared and made it turbidity standard. A small volume of this turbid solution was transferred to a screw capped tube and vigorously shaken on a mechanical vortex mixer to have a uniform turbid appearance and stored in the dark at room temperature. Purely cultured Mueller-Hinton agar petridishes were labeled with different names of bacteria. Then 5 ml of sterile Normal Saline Solution (NSS) was pipette out into a three different sterile screw-cap tubes. These tubes were labeled according to the type and number of bacteria used to test (*E. coli* and *S. aureus)*. To prepare inoculums, 3 well isolated colonies of the same morphological types were selected from an agar plate culture. The top of each colony is touched with a loop, and growth was transferred into a tube containing 5 ml of NSS that corresponds to each bacterium names. These inoculums containing tubes were mixed by using mechanical vortex mixer and their turbidity was compared accurately. The sterile discs which were prepared by office perforator were inserted in to different concentrations of *Mentha longifolia* oil with stock solution of 20 mg/ml. It was impregnated in to negative and positive controls petroleum ether and chloroform, and amoxicillin respectively. After that, discs with different concentrations were placed on the inoculated plates using a pair of sterile forceps. Seven discs were placed on a 90 cm diameter petridish plate and the space between each disc was given as 24 mm gap from center of the disk to the center of petridish. The pressed discs were completely stacked the agar surface, plates were inverted and placed in an incubator at 37^{0}C for 24 h. After overnight incubation, the diameter of each zone (including the diameter of the disc) were measured and recorded.

Table 1: Chemical composition of essential oil of *Mentha longifolia*

Peak No	RI	Compounds Identified	Formula	% Composition
1	986	Dimethyl malonate	$C_5H_8O_4$	7.5
2	1011	γ- Elemene	$C_{15}H_{24}$	2.1
3	1024	Caryophyllene	$C_{15}H_{24}$	11.3
4	1048	Copaene	$C_{15}H_{24}$	1.2
5	1030	(-) β-Bourbonene	$C_{15}H_{24}$	4.9
6	1130	α-Guaiene	$C_{15}H_{24}$	3.8
7	1140	β-Maaliene	$C_{15}H_{24}$	1.8
8	1390	β -Eudesmol	$C_{15}H_{26}O$	10.3
9	1423	β - Cubebene	$C_{15}H_{24}$	6.1
10	1428	Epizonarene	$C_{15}H_{24}$	29.7
11	1448	Agarospirol	$C_{15}H_{26}O$	0.7
12	1480	Cubenol	$C_{15}H_{26}O$	2.3
13	1520	α -Cadinol	$C_{15}H_{26}O$	6.9
14	1560	Bicyclo germacrene	$C_{15}H_{24}$	1.6
15	1620	α -Humulene	$C_{15}H_{24}$	0.2
16	1640	Androstenol	$C_{19}H_{30}O$	1.2

Table 2: *In vitro* antibacterial test of *Mentha longifolia* oil

Bacterial strain	Concentration of oil (mg/ml)					Negative control	Positive control
	100	50	25	10	St	chloroform	Amox (30 µg/disk)
E. coli	11.5 ± 0.5	9.5 + 0.5	7.24 + 0.12	6.56 + 0.05	4.85 + 0.04	-	11.53 + 0.5
S. aureus	9.04 + 0.12	7.93 + 0.2	3.99 + 0.77	3.02 + 0.6	2.09 + 0.33	-	9.06 + 0.95

All the values are given as mean ± STD which were analyzed in triplicate, St: - Stock solution, - Has no activity, AM: - Amoxicillin, *S. aureus:- Staphylococcus aureus, E. coli:- Escherichia coli*

RESULTS AND DISCUSSION

The composition of *Mentha longifolia* oil of is shown in the Table 1. A total of 91.6 % was identified. The major components identified were Epizonarene (29.7 %), Caryophyllene (11.3 %), dimethyl malonate (7.5 %), β-eudesmol (10.3 %) β – cubebene (6.1 %), α-cadinol (6.9 %), β-bourbonene (4.9 %) and α-guaiene (3.8 %). Analysis of the oil of *Mentha longifolia* from Italy and Lithuania revealed piperitenone oxide as the main component[22-23]. Many studies also have shown that piperitenone, piperitone and pulegone generally co-exist in the typical sequiterpene of different varieties of this species[15,23]. But a report from Poland showed that the essential oil of *Mentha longifolia* contained mainly limonene and carvone[24]. The constituents of *Mentha longifolia* in the present study are similar to a report of *Mentha* species from South Africa[25]. It is found that sesquiterpene components are the main constituents in the present study and also androstenol, dimethyl malonate and β-eudesmol are the newly reported compounds present in the oil of *Mentha longifolia*. This variation of essential oil contents in similar chemo types may be attributed to different climatic and geographical condition of the regions. The essential oil of *Mentha longifoli*a showed moderate antibacterial activity against gram negative (*E. coli*) and gram positive bacteria (*S. aureus*).

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