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Original article

Chemical constituents, *in vitro* antibacterial and antifungal activity of *Mentha* × *Piperita* L. (peppermint) essential oils

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ABSTRACT

This research studied the chemical constituents and antibacterial activity of essential oils from the areal parts of *Mentha* × *piperita* L. essential oil. Essential oil was subjected to hydrodistillation for 4 h using Clevenger apparatus, resulting in nineteen chemical constituents representing 100% of the essential oil, comprising menthol (36.02%), menthone (24.56%), menthyl acetate (8.95%), and menthofuran (6.88%); these are major components, and others are minor components. Essential oil shows significant antibacterial and antifungal activity than principle components. The essential oil shows significant antibacterial activity against human pathogenic micro-organisms. Further, *Staphylococcus aureus* (42.44 ± 0.10 mm), *Micrococcus flavus* (40.01 ± 0.10 mm), *Bacillus subtilis* (38.18 ± 0.11 mm), *Staphylococcus epidermidis* (35.14 ± 0.08 mm), and *Salmonella enteritides* (30.12 ± 0.12 mm) show the highest antibacterial activity against essential oils. Essential oils show significant antifungal activity against *Alternaria alternaria* (38.16 ± 0.10 mm), *Fusarium tabacinum* (35.24 ± 0.03 mm), *Penicillum* spp. (34.10 ± 0.02 mm), *Fusarium oxyporum* (33.44 ± 0.06 mm), and *Aspergillus fumigates* (30.08 ± 0.08 mm). The maximal and minimal inhibition concentration values are in the range of 10.22 ± 0.17 to 38.16 ± 0.10 and 0.50 ± 0.03 to 10.0 ± 0.14 μg/ml, for yeast and fungi respectively. The present study on essential oils deriving from the *Mentha* × *piperita* L. species could be used in antimicrobial activity as a natural source.

1. Introduction

The development of microbial resistance to antibiotics is a global concern. Natural products are a great scientific deal for finding alternative methods for food preservation (Bassolé et al., 2010; Runyoro et al., 2010). Essential oils from the plants show more antibacterial activity from oxygenated terpenoids, phenols, alcoholic compounds, and other chemical constitutes that contribute to the antimicrobial effects (Cosentino et al., 1999; Cox et al., 2001; Bajpai et al., 2008). According to the literature, the essential oils of antibacterial activity results to synergistic or antagonistic.

As per the literature, previous researchers from all over the world have investigated the traditional medicinal plant extracts of the essential oils as antibiofilm formation, antiviral, antifungal, antimicrobial, radio protective, angioedema, analgesic, food packing, biodegradable films and antioxidant activities (Giuliana Gorrasi, 2015; Derwich et al., 2010; Kizil et al., 2010; Soković et al., 2007; Tyagi and Malik, 2010; Ezzat, 2001; Behnam et al., 2006; Agarwal et al., 2008; Sandasi et al., 2010; Rasooli et al., 2008; Baliga and Rao, 2010; Yasukawa et al., 1993; Leslie, 1978; Snoussi et al., 2015; Biddeci et al., 2016; Kashiri et al., 2017; Ribeiro-Santos et al., 2017; Atares and Chiralt, 2016; Cataldo et al., 2017), clinical constituents in foods, drinks, toiletries, and cosmetics is a gaining momentum, in addition to the growing attraction of consuming constituents from natural sources and the increase in concern regarding harmful synthetic additives. The essential oils are extracted from naturally available medicinal

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plants by steam distillation or hydrodistillation (Tsai et al., 2013; Singh et al., 2015b), thereby resulting in different chemical constituents used for various biological activities, food borne pathogens, and the artificial, biologically active compounds.

Crops and food products contaminate fungi, damaging both the quantitative yield and economic losses. In order to protect fungi, synthetic chemicals have been used, resulting in an increase in crop production with deterioration in the environment, food quality, and human health (Moghaddam et al., 2013). Furthermore, synthetic chemicals, pathogens, and pesticides can kill essential microorganisms, thus, more resistance among human pathogens regarding synthetic chemicals also causes serious damage.

The Mentha species is the most widely used in terms of health and medicinal uses, mostly because of Menthol and Menthone. Normally, the *Mentha* × *piperita* L., essential oils are mainly used to relieve coughs, colds, mouth sinuses (reduced inflammation), digestive issues, menstrual symptoms (muscle relaxant), pain relief, headaches, and skin problems. Based on the literature survey, this is the first study on this plant in Jazan, Saudi Arabia. Therefore, the present study on the chemical constituents and antimicrobial activity of the volatile oil extracted from *Mentha* × *piperita* L. would be highly valuable.

2. Materials and methods

2.1. Plant material and extraction of essential oil

Peppermint (*Mentha* × *piperita* L.) plant material was purchased from a market in Jazan, Saudi Arabia during January 2017. The collected plant material was authenticated by a senior plant taxonomist, Dr. Ramesh Moochikkal, of the Department of Biology. Voucher specimen (JAZUH 1146) was deposited at Jazan University herbarium, Jazan, Saudi Arabia. Areal parts were separated and dried under shade at room temperature, after drying; the plant material was ground by the grinder. 100 g of dried plant material and 500 mL of water was subjected to hydrodistillation for 4 h by using Clevenger apparatus. It was then separated into the *Mentha* × *piperita* L. essential oil and kept in a refrigerator at 4 °C for further analysis.

2.2. GC–MS analysis conditions

The essential oils were analyzed by using gas chromatography and gas chromatography with Mass spectrometry. GC and GC–MS analysis was done by Agilent Technology 6890N. GC was equipped with a flame ionization detector (FID) and a DB-5 capillary column of 30 m × 0.25 mm × 0.25 μm film thickness. Helium (99.99%) was used as a carrier gas at a flow rate of 1.0 ml/min. For the column, the gradient temperature program was maintained at 4 °C/min. The temperature used for the column ranged from 40 °C to 260 °C. To conduct the sample injection, the temperature was maintained at 260 °C. Split ratio was used 10:1 and the sample was injected manually.

Gas chromatography with mass spectrometry (Agilent Technology 6890N) was done for essential oil qualitative and quantitative analysis using the electron impact ionization (70 eV) method and mass spectra, which recorded a range of 50 to 500 m/z with a mass detector. Gas chromatography conditions were the same as mentioned above. The components were identified based on the comparison of their relative retention index and compared to the standards of mass spectra referred to in the library National Institute of Standards and Technology (NIST), wiley 5, mass finder, and Adams (2007), as well as the percentage of constituents measured based on the peak area. See Table 1.

Table 1
Chemical constituents of *Mentha* × *piperita* L. essential oil.

RI	Chemical Constituents	% of Constituent
980	β-pinene	2.08
993	β-myrcene	1.22
1031	β-Phellandrene	1.52
1025	1,8 –cineole	5.13
1083	Terpinolene	2.02
1149	Menthol	36.02
1127	Menthone	24.56
1156	Menthofuran	6.88
1294	Menthyl acetate	8.95
1082	Linalool	0.39
1212	Pulegone	1.35
1220	Trams-carveol	1.69
1203	Cis-carveol	3.49
1645	Cubenol	0.56
1576	Spathulenol	0.10
1392	Eugenol	0.30
1632	τ- cadinol	0.12
1254	Carvone	2.30
1373	β – elemene	1.30
	Total	99.98

RI: Retention index.

2.3. Antibacterial activity

2.3.1. Microbial strains

The extracted oils were individually tested against a set of microorganisms. Gram-positive micro-organisms are stated as follows: *B. cereus* (ATCC10876), *B. macerans* (M58), *B. megaterium* (M3), *B. subtilis* (ATCC 6633), *B. abortus* (A77), *B. cepacia* (A255), *E. cloacae* (ATCC13047), *E. faecalis* (ATCC49452), *L. monocytogenes* (ATCC15313), *S. aureus* (ATCC 25923), *M. flavus* (ATCC 9341), *S. epidermidis* (A 233), *C. michiganense* (A 277), *and S. pyogenes* (ATCC 176). Gram-negative micro-organisms are stated as follows: *A. baumannii* (ATCC 19606), *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 27853), *P. mirabilis* (ATCC 35659), *S. typhimurium* (ATCC 13311), *C. freundi* (ATCC 13311), *E. aerogenes* (ATCC 13048), *S. enteritides* (I K27), *P. vulgaris* (A 161), *P. syringae* (A 35), *and X. campestris* (A 235). Fungal micro organisms are stated as thus: *A. alternaria* (MNHN 843390), *A. flavus* (MNHN 994294), *A. fumigates* (MNHN 566), *C. albicans* (ATCC 26790), *C. herbarum* (MNHN 3369), *F. oxyporum* (MNHN 963917), *A. variecolor, F. acuminatum, F. solani, F. tabacinum, M. fructicola, R. saloni, S. minor, S. selerotiorum, T. Mentagrophytes, and T. rubrum*. Strain numbers and microorganisms are given Table 3 and 4. For each assay, microorganisms were stored at 4 °C for 24 h. These bacterial and fugue strains were obtained from the laboratory of Microbiology, Sri Krishnadevaraya University, India.

2.3.2. Determination of disc diffusion method

The antibacterial activity of the *Mentha* × *piperita* L. volatile oils were investigated using the agar disc diffusion method. Micro organisms are referred laboratory standards (NCCLS, 2001; Pfaller et al., 1998). Using 100.0 μL of tested microorganisms contains 10^8 cfu/mL of bacteria and 10^6 cfu/mL fungi strains spreading Sabouraud Dextrose agar (SDA) medium, respectively. The extracted essential oil (10.0 μL) was separately impregnated on a disc and placed on the tested micro-organisms. The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Each test was carried out as a triplet.

2.3.3. Microdilution method

The determination of the minimal inhibition concentration (MIC) values determined the microorganisms using a Micro Broth dilution assay, as recommended by NCCLS (2001). All the tests were performed in Sabouraud Dextrose broth and Muller-Hilton broth,

Table 2
Comparative chemical composition of *Mentha* × *piperita* L., essential oil.

Place	Major components	Reference
Brazil	Menthol (42.32%), Menthyl acetate (35.01%), menthofuran (4.56%), menthone (4.05%) and 1,8 cineole (5.56%)	Scavroni et al. (2005)
England (Commercial oil)	Menthol (49.79%), menthone (19.08%), and menthyl acetate (5.08%)	de Sousaa et al. (2010)
Iran	Menthol (25.16%), menthofuran (6.49%), menthyl acetate (4.61%), and 1,8-cineole (2.15%)	Moghaddam et al. (2013)
Iran	Menthol (53.28%), menthyl acetate (15.1%) and menthofuran (11.18%)	Saharkhiz et al. (2012)
Iran	Menthol (36.9%), menthone (28.8%), menthyl acetate (4.54%), and 1,8-cineole (3.75%)	Mahboubi and Kazempour (2014)
Iran	α-terpiene (19.7%), pipertitinone oxide (19.3%), trans-carveol (14.5%), and isomenthone (10.3%)	Yadegarinia et al. (2006)
Taiwan	Menthol (30.35%), menthone (21.12%), trans-carveol (10.99%), and 1, 8-cineole	Tsai et al. (2013)
Burkina Faso	Menthol (39.3%), menthone (25.2%), menthofuran (6.8%), and menthyl acetate (6.7%)	Bassolé et al. (2010)
Serbia	Menthol (37.40%), menthyl acetate (17.37%), menthone (12.70%), and menthofuran (6.82%)	Soković et al., 2007
Korea	Menthol (4.30%), caryophyllene (5.50%) and eucalyptol (62.16%),	park et al. (2016)
Colombia	Isomenthol (7.23%), Isomenthone (26.15%), pulegone (44.54%) and Chrysanthenone (8.07%)	Roldán et al. (2010)
Brazil	3-octanol (10.1%), linalool (51.0%), Terpin-4-ol (8.00%), and carvone (23.42%),	Sartoratto et al. (2004)
Saudi Arabia	Menthol (36.02), menthone (24.56), menthyl acetate (8.95), and menthofuran (6.88%).	**Present study**

Table 3
Antibacterial activity of *Mentha* × *piperita* L. essential oil (1.0 μg/ml) in disc diffusion method.

Microbial strains	Zone inhibition (mm[a])				MIC[b](μg/mL)			
	Essential oil	RA[b]	menthol	menthone	essential oil	RA	menthol	menthone
B. cereus	32.08 ± 0.02	22.24 ± 0.16	23.0 ± 0.04	20.0 ± 0.02	1.0 ± 0.01	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
B. macerans	24.05 ± 0.11	22.28 ± 0.05	20.0 ± 0.02	16.0 ± 0.06	0.50 ± 0.01	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
B. megaterium	10.03 ± 0.05	14.02 ± 0.13	9.0 ± 0.06	8.0 ± 0.08	0.75 ± 0.08	1.0 ± 0.04	2.0 ± 0.04	2.0 ± 0.04
B. subtilis	38.18 ± 0.11	16.10 ± 0.08	24.2 ± 0.02	20.0 ± 0.04	1.5 ± 0.14	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
B. abortus	22.03 ± 0.01	10.02 ± 0.02	18.0 ± 0.02	15.0 ± 0.02	3.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	1.0 ± 0.02
B. cepacia	8.15 ± 0.10	14.45 ± 0.03	6.0 ± 0.02	4.0 ± 0.04	1.3 ± 0.06	0.5 ± 0.02	2.0 ± 0.04	2.0 ± 0.04
E. cloacae	16.14 ± 0.13	18.24 ± 0.06	15.0 ± 0.06	12.4 ± 0.04	1.0 ± 0.14	0.5 ± 0.02	2.0 ± 0.02	1.0 ± 0.02
E. faecalis	30.18 ± 0.12	17.32 ± 0.14	23.0 ± 0.02	18.4 ± 0.04	1.0 ± 0.17	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
L. monocytogenes	17.20 ± 0.04	13.23 ± 0.12	15.0 ± 0.04	13.0 ± 0.02	1.0 ± 0.06	0.5 ± 0.02	2.0 ± 0.04	0.5 ± 0.02
S. aureus	42.44 ± 0.10	30.22 ± 0.12	28.0 ± 0.02	22.0 ± 0.02	0.75 ± 0.03	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
M. flavus	40.01 ± 0.10	28.20 ± 0.06	28.2 ± 0.04	22.0 ± 0.02	1.0 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
S. epidermidis	35.14 ± 0.08	20.32 ± 0.05	23.0 ± 0.02	22.0 ± 0.06	1.53 ± 0.07	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
C. mmichiganense	15.05 ± 0.08	10.02 ± 0.08	13.0 ± 0.02	10.0 ± 0.04	3.10 ± 0.12	1.0 ± 0.02	1.0 ± 0.04	2.0 ± 0.04
S. pyogenes	13.26 ± 0.03	6.04 ± 0.20	10.0 ± 0.04	8.4 ± 0.04	2.04 ± 0.12	1.0 ± 0.02	1.0 ± 0.04	2.0 ± 0.04
A. baumannii	12.08 ± 0.18	20.15 ± 0.12	10.0 ± 0.06	8.0 ± 0.02	1.5 ± 0.03	0.5 ± 0.02	1.0 ± 0.04	2.0 ± 0.04
E. coli	27.02 ± 0.13	17.24 ± 0.34	16.0 ± 0.06	13.0 ± 0.02	0.20 ± 0.09	0.5 ± 0.02	0.5 ± 0.02	1.0 ± 0.02
K. pneumonia	14.24 ± 0.07	16.28 ± 0.02	10.0 ± 0.06	9.0 ± 0.04	0.50 ± 0.14	0.5 ± 0.02	1.0 ± 0.04	2.0 ± 0.04
P. mirabilis	12.13 ± 0.12	15.33 ± 0.06	11.4 ± 0.04	9.0 ± 0.04	2.03 ± 0.17	0.5 ± 0.02	2.0 ± 0.04	2.0 ± 0.04
S. typhimurium	20.06 ± 0.06	12.03 ± 0.06	17.6 ± 0.02	12.0 ± 0.04	1.5 ± 0.15	1.0 ± 0.06	0.5 ± 0.04	1.0 ± 0.02
C. freundi	6.12 ± 0.02	7.16 ± 0.03	4.0 ± 0.02	2.0 ± 0.06	2.0 ± 0.14	1.0 ± 0.06	2.0 ± 0.04	2.0 ± 0.04
E. aerogenes	12.17 ± 0.07	10.44 ± 0.02	9.0 ± 0.02	5.0 ± 0.04	1.5 ± 0.12	1.0 ± 0.06	1.0 ± 0.04	2.0 ± 0.04
S. enteritides	30.12 ± 0.12	18.36 ± 0.12	17.6 ± 0.02	10.0 ± 0.02	1.50 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
P. vulgaris	4.02 ± 0.05	10.04 ± 0.04	2.0 ± 0.06	2.0 ± 0.02	1.50 ± 0.02	1.0 ± 0.06	2.0 ± 0.04	2.0 ± 0.04
P. syringae	3.12 ± 0.02	15.02 ± 0.06	2.0 ± 0.06	2.0 ± 0.02	2.50 ± 0.16	0.5 ± 0.02	2.0 ± 0.04	2.0 ± 0.04
X. campestris	3.18 ± 0.07	8.06 ± 0.02	2.0 ± 0.06	2.0 ± 0.02	80.0 ± 0.30	1.0 ± 0.02	1.0 ± 0.04	2.0 ± 0.04

[a] Values represent means ± standard deviations for triplicate experiments.
[b] RA: Reference of antibiotics Gentamicin for bacteria used was 10 μg/disc.

respectively. The *Mentha* × *piperita* L. essential oil dissolved 10% Dimethylsulfoxide was prepared to 5.0×10^5 and 2.0×10^6 cfu/mL for bacteria and fungus, respectively. The standard strain suspensions were soaked onto micro plates. For bacteria, the plates were incubated at 37 °C for 24 h, while fungi were incubated at 30 °C for 48 h. The MIC was defined as having the lowest concentration of the compounds to inhibit the growth of micro-organisms and to compare the antibacterial and antifungal activity of the oil.

3. Results and discussion

The extracted essential oil obtained from the areal parts of *Mentha* × *piperita* L. was hydrodistillated for 3 h using Clevenger apparatus. Next, the essential oil was qualitatively and quantitatively analyzed by GC–MS. The results represented 99.98% of the essential oil, and the principle compounds were menthol (36.02), menthone (24.56), menthyl acetate (8.95), and menthofuran (6.88%). The chemical components are given in Table 1.

The *Mentha* × *piperita* L. essential oils extracted from Brazil (Scavroni et al., 2005) include menthyl acetate (35.01%), menthol (42.32%), menthofuran (4.56%), menthone (4.05%) and 1,8 cineole (5.56%) are major components, and (de Sousaa et al., 2010) oil representing (100%) of the oil shows menthol (49.79%), menthone (19.08%), and menthyl acetate (5.08%) as major components. Oils are extracted from different places of Iran show (Moghaddam et al., 2013) (99.97%) of the essential oil, with the major constituents being menthol (25.16%), menthofuran (6.49%), menthyl acetate (4.61%), and 1,8-cineole (2.15%). as shown by Saharkhiz et al. (2012), the essential oil shows (99.37%) menthol (53.28%), menthyl acetate (15.1%) and menthofuran (11.18%) as the major constituents. (Mahboubi and Kazempour, 2014) found that (99.8%) of the oil includes menthol (36.9%), menthone (28.8%), menthyl acetate (4.54%), and 1,8-cineole (3.75%) as the major constituents. Furthermore, other results from Iran (Yadegarinia et al., 2006) show that (93.58%) of the total oil include the main constituents of α-terpiene (19.7%), pipertitinone oxide (19.3%), trans-carveol (14.5%), and isomenthone (10.3%) as major components.

Table 4
In vitro antifungal activity of essential oil.

Sstrains	Essential oil[a]		Amphotericin[b]	
	DD[c]	MIC[d]	DD[c]	MIC[d]
Alternaria alternaria (MNHN 843390)	38.16 ± 0.10	1.50 ± 0.06	25.02 ± 0.04	1.50 ± 0.04
Aspergillus flavus (MNHN 994294)	20.02 ± 0.06	10.0 ± 0.06	24.07 ± 0.08	2.50 ± 0.06
Aspergillus fumigates (MNHN 566)	30.08 ± 0.08	0.50 ± 0.03	22.06 ± 0.05	1.0 ± 0.14
Candida albicans (ATCC 26790)	16.34 ± 0.26	1.50 ± 0.16	12.18 ± 0.03	5.0 ± 0.16
Cladosporium herbarum (MNHN 3369)	23.23 ± 0.12	1.50 ± 0.16	15.14 ± 0.16	2.50 ± 0.17
Fusarium oxyporum (MNHN 963917)	33.44 ± 0.06	1.50 ± 0.20	25.04 ± 0.01	1.50 ± 0.26
Aspergillus variecolor	17.23 ± 0.23	10.0 ± 0.07	16.04 ± 0.02	1.50 ± 0.17
Fusarium acuminatum	22.50 ± 0.012	2.50 ± 0.16	18.24 ± 0.04	1.50 ± 0.02
Fusarium solani	10.22 ± 0.05	10.0 ± 0.03	25.30 ± 0.03	1.25 ± 0.01
Fusarium tabacinum	35.24 ± 0.03	1.50 ± 0.02	23.14 ± 0.02	1.35 ± 0.24
Moliniana fructicola	16.32 ± 0.03	5.50 ± 0.08	25.03 ± 0.06	3.50 ± 0.21
Penicillium spp.	34.10 ± 0.02	1.50 ± 0.01	27.25 ± 0.01	2.50 ± 0.08
Rhizoctomia saloni	28.16 ± 0.18	1.50 ± 0.10	22.04 ± 0.16	2.50 ± 0.01
Sclorotinia minor	10.22 ± 0.17	10.0 ± 0.12	28.65 ± 0.13	1.50 ± 0.04
Sclorotinia selerotiorum	15.58 ± 0.06	10.0 ± 0.14	20.03 ± 0.24	2.50 ± 0.03
Trichophyton mentagrophytes	11.55 ± 0.06	10.0 ± 0.13	12.22 ± 0.18	5.0 ± 0.05
Trichophyton rubrum	15.34 ± 0.03	5.0 ± 0.01	12.65 ± 0.05	5.0 ± 0.01

[a] Essential oil impregnated with 1.0 μL/disc.
[b] Amphotericin B impregnated with (10 μg/disc).
[c] Disc diameter (mm) included.
[d] Minimal inhibition concentrations in (μg/mL).

The results from the present study compared the oils from Iran (Yadegarinia et al., 2006), finding that the principle components are totally different, but other results from Iran (Moghaddam et al., 2013; Saharkhiz et al., 2012; Mahboubi and Kazempour, 2014) shows similar results with present study.

The oils from Taiwan (Tsai et al., 2013), menthol (30.35%), menthone (21.12%), trans-carveol (10.99%), and 1, 8-cineole are the major components, showing 100% of the total oil. Essential oil from (Bassolé et al., 2010) shows menthol (39.3%), menthone (25.2%), menthofuran (6.8%), and menthyl acetate (6.7%) as the major constituents comprising (93.4%) of the oil. Oils representing (97.60%) of the essential oil shows menthol (37.40%), menthyl acetate (17.37%), menthone (12.70%), and menthofuran (6.82%) as the principle components (Soković et al., 2007). The oils from (Park et al., 2016) show (98.27%) of the total oil, with its major constituents comprising eucalyptol (62.16%), caryophyllene (5.50%), and menthol (4.30%).

Results are totally different from the present study compared with essential oil from Colombia (Roldán et al., 2010) represents (99.43%) of the oil shows pulegone (44.54%), isomenthol (7.23%), isomenthone (26.15%) and chrysanthenone (8.07%) major components and Essential oil from Brazil (Sartoratto et al., 2004) oil representing (98.0%) of the oil shows linalool (51.0%), carvone (23.42%), 3-octanol (10.1%) and terpin-4-ol (8.00%) as major components.

Other research from Libya (Singh et al., 2015a) did not find the chemical composition of the *Mentha* × *Piperita* L. essential oil. The present study, in accordance with the previous studies, shows that the chemical constitution is similar, except for the oils collected from Iran (Yadegarinia et al., 2006) and also from Brazil (Sartoratto et al., 2004). The percentages of the principle components are different due to the geographical conditions, climate, and affect of sunlight. Comparative chemical composition results are shown in the Table 2.

The antimicrobial activity of the essential oils was tested with disc diffusion method. The results of the antimicrobial activity of the essential oils are given Tables 3 and 4. The essential oil shows the highest antibacterial activity against microorganisms. Also, *Staphylococcus aureus* (42.44 ± 0.10 mm) *Micrococcus flavus* (40.01 ± 0.10 mm), *Bacillus subtilis* (38.18 ± 0.11 mm), *Staphylococcus epidermidis* (35.14 ± 0.08 mm), and *Salmonella enteritides* (30.12 ± 0.12 mm) show good inhibition zones against *Mentha* × *Piperita* L., according to the Disc-diffusion method. Moreover, the essential oils show less inhibition zones against *Listeria monocytogenes* (17.20 ± 0.04 mm), *Enterobacter cloacae* (16.14 ± 0.13 mm), *Clavibacter mmichiganense* (15.05 ± 0.08 mm), *Klebsiella pneumonia* (14.24 ± 0.07 mm), *Streptococcus pyogenes* (13.26 ± 0.03 mm), *Acinetobacter baumannii* (12.08 ± 0.18 mm), *Proteus mirabilis* (12.13 ± 0.12 mm), *Enterobacter aerogenes* (12.17 ± 0.07 mm), *Bacillus megaterium* (10.03 ± 0.05 mm), *Bukholdria cepacia* (8.15 ± 0.10 mm), *Citrobacter freundi* (6.12 ± 0.02 mm), *Proteus vulgaris* (4.02 ± 0.05 mm), *Xanthomonas campestris* (3.18 ± 0.07 mm), and *Pseudomonas syringae* (3.12 ± 0.02 mm). The disc-diffusion method and other microorganisms show moderate antibacterial activity against essential oils. The results are given in Table 3.

The results regarding the antifungal activity of the essential oils are given Table 4. The essential oil shows strong antifungal activity against yeast and fungi strains. *Alternaria alternaria* (38.16 ± 0.10 mm), *Fusarium tabacinum* (35.24 ± 0.03 mm), *Penicillium* spp. (34.10 ± 0.02 mm), *Fusarium oxyporum* (33.44 ± 0.06 mm), and *Aspergillus fumigates* (30.08 ± 0.08 mm) all show strong antifungal activity against essential oils. Furthermore, *Aspergillus variecolor* (17.23 ± 0.23), *Candida albicans* (16.34 ± 0.26), *Moliniana fructicola* (16.32 ± 0.03), *Sclorotinia selerotiorum* (15.58 ± 0.06), *Trichophyton rubrum* (15.34 ± 0.03), *Trichophyton mentagrophytes* (11.55 ± 0.06), *Sclorotinia minor* (10.22 ± 0.17), and *Fusarium solani* (10.22 ± 0.05) show less antifungal activity against the essential oils, while *Rhizoctomia saloni, Fusarium acuminatum, Cladosporium herbarum,* and *Aspergillus flavus* show a moderate antifungal activity. The maximal and minimal inhibition concentration values are in the range of 10.22 ± 0.17 to 38.16 ± 0.10 and 0.50 ± 0.03 to 10.0 ± 0.14 μg/ml, for yeast and fungi respectively.

According to the literature, (Delaquis et al., 2002; Dorman and Deans, 2000; Reddy and Al-Rajab, 2016) state that the percentage of chemical compounds and the major constituents of the essential oil determined the antibacterial activity. The researchers go on to explain the maximum antibacterial activity and antifungal activity (Dorman and Deans, 2000; Lambert et al., 2001; Ben-Bnina et al., 2010; Proestos et al., 2006; Belghazi et al., 2002) are caused by chemical constituents containing hetero atoms such as oxygen. The present study reveals that the volatile oils extracted from the aerial parts of *Mentha* × *piperita* L. have the potential to be an

antibacterial and antifungal agent with a better performance against a wide range of microorganisms when compared to synthetic drugs.

4. Conclusions

Mentha × *piperita* L. essential oils show significant antibacterial and antifungal activity against gram positive and gram negative bacteria, as well as yeast and fungi, mostly because menthol and menthone are main chemical constituents. This work will be extended to fully analyze the potential of essential oils for their antioxidant activity. Further work is necessary to explore suitable concentrations of the components, which would extend their shelf life, their suitability as neutral cuticles, and their role in natural therapy and as pharmaceuticals for human management.

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