

## ESSENTIAL OIL COMPOSITION OF *MENTHA PIPERITA* L. AND ITS ANTIMICROBIAL EFFECTS AGAINST COMMON HUMAN PATHOGENIC BACTERIAL AND FUNGAL STRAINS

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### Abstract

The current study investigated the chemical constituents, antibacterial, antifungal and antioxidant activities of essential oil of *Mentha piperita* L. The oil was extracted by hydro-steam distillation process and the chemical composition was characterized by using a hyphenated gas chromatography-mass spectrometry (GC-MS) analytical technique. The agar well diffusion method was used for the determination of antimicrobial activities. The antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Different chemical constituents were scrutinized from the essential oil of *M. piperita* leaves, which include *p*-mentha-6,8-dien-2-one (46.434%), *p*-menthan-3-ol (25.749%), borneol (8.865%), d-limonene (5.516%), 2-isopropylidencyclohexanone (4.838%), 7-oxabicyclo [4.1.0] heptan-2-one-6-methyl-3-(1-methylethyl) (2.039%). The screened peppermint oil revealed significant antibacterial and antifungal activities against different pathogenic microorganisms. The essential oil of *M. piperita* leaves showed highest zone of inhibition against *Staphylococcus aureus* ( $17 \pm 0.61$  mm), *Escherichia coli* ( $17 \pm 0.87$  mm) and *Aspergillus niger* ( $16 \pm 0.37$  mm). The efficient scavenging of free radicals ( $83 \pm 0.43\%$  at  $50 \text{ gL}^{-1}$ ) revealed the antioxidant propensity of the isolated essential oil. These results concluded that the essential oil of *M. piperita* has beneficial chemical constituents and possess prominent antibacterial, antifungal and antioxidant activities.

**Key words:** Peppermint, Gas chromatography-Mass spectrometry, antibacterial, antifungal, antioxidant.

## Introduction

Medicinal plants have been used for centuries to treat human diseases as they contain chemical ingredients of therapeutic value. *Mentha piperita* L. (Lamiaceae) has found widespread use in medicine, fragrance, pharmaceuticals and flavors all over the world, even though it is a native genus of the Mediterranean region. Peppermint oil is one of the most extensively consumed and engendered essential oil (1-2). Lamiaceae family contains extensive variety of aromatic plants, mostly in temperate regions. The genus *Mentha* is among the rich collection of plants producing essential oil and is distributed throughout the world. *Mentha piperita* (pennyroyal) possesses antispasmodic and carminative properties and is used traditionally for healing intestinal colic and flatulent dyspepsia (3-4). It is utilized in herbal tea preparations, food and confectioneries. The therapeutic applications of mint, which date back to archaic period, include emmenagogue, carminative, antiemetic, anti-inflammatory, stimulant, anticatarrhal, antispasmodic, diaphoretic and as an analgesic. It has therapeutic applications against flatulence, nausea, colitis, anorexia, liver, bronchitis and ulcerative conditions. Mint essential oil is usually used topically as antimicrobial, antipruritic, antiseptic, rubefacient and astringent as well as for healing headaches, neuralgia, migraines and myalgia (5-8). Essential oil has been used as raw materials in several fields which include aromatherapy, perfumes, spices, nutrition, cosmetics and phototherapy (9). The peppermint oil (*Mentha piperita*) is used frequently because of its focal components, menthone and menthol, and is one of the most trendy and broadly used essential oil (10). To increase the shelf life of products and in food industry as preservatives, essential oil with antibacterial effects have widespread potential. Therefore, to prevent food spoilage and to triumph over microorganism resistance to antibiotics, the essential oil's antimicrobial properties might be helpful. The chemical composition of aromatic plants depends mostly on the individual genetic changeability and diversity among plant parts (11-12). Previous studies have shown antibacterial (13-14), antifungal (15-17), antiviral (18), antibiofilm formation (19-21), radio-protective (22), antioxidant, analgesic (23) and anti-edema (24) activities of methanolic extract and essential oil of callus cultures and herbal parts of *M. piperita*. The essential oil of *M. piperita* has shown inhibitory effects against aflatoxin production and fungal growth (25).

As an outcome of consecutive bio-solid applications, *M. piperita* grown in soil with rising cadmium (Cd) concentrations (0.12-6.1 mg/kg) did not produce any alterations in its biomass and essential oil composition (26). The antioxidant activity of essential oil is of great interest due to the conservation of food from toxic impacts of oxidants (27). The present study was designed to investigate the chemical composition of essential oil of *Mentha piperita* L. by hyphenated gas chromatography-mass spectrometry (GC-MS) and antibacterial, antifungal and antioxidant activities using well known testing paradigms.

## Methods

### **Plant collection and isolation of essential oil**

Fresh mature plant leaves of *Mentha piperita* L. were collected from the Botanical Garden of Medicinal Botanic Center, Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex, Peshawar, Pakistan and were subjected to distillation under steam. For the isolation of *M. piperita* essential oil from leaves, a modified Clevenger-type apparatus was used. Leaves were washed, cut into small pieces and were then placed in a distillation flask. It was heated for about 4 h. The upper layer of volatile oil separated out being lighter and immiscible in water. After separation from water, oil was then collected in small 50 mL flask and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. It was preserved, in light-resistant vials, labeled and stored at 4-6°C in a refrigerator for further work.

### **Characterization of essential oil by GC-MS analysis**

The chemical composition of leaves was scrutinized by hyphenated gas chromatography-mass spectrometry (GC-MS) [QP 2010 plus, Tokyo, Japan, equipped with an auto injector (AOC-20i) and sampler (AOC-20S)]. Helium was used as a carrier gas. All chromatographic separations were performed on a capillary column TRB-FFAP (Technokroma). Column specifications were; length; 30 m, thickness; 0.250 µm and internal diameter was 0.35 mm. The GC-MS conditions were: ion source (EI) and interface temperatures, 250°C and 240°C, respectively at pressure of 100 kPa. The total time of elution was 43 min and scanning was performed from *m/z* 380 to *m/z* 85. To manage the system and to attain the data, an integrated GC-MS software was used. From NIST library, recognition of chemical compounds was carried out by comparing the acquired mass spectra with mass spectra of standards (28).

### Maintenance of microorganisms

The bacterial species of *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumonia* were collected from the PCSIR Laboratories, Peshawar, Pakistan and were first subcultured at 37°C for 24 h in nutrient broth. The fungal specimens of *Candida albicans*, *Aspergillus niger*, *Aspergillus parasiticus* and *Aspergillus fumigatus* were used in the bioassay. For three to five days, the tested fungal strains were cultured on SDA (Sabouraud's dextrose agar) plates maintained at 28°C.

### Antibacterial activity

The agar well diffusion technique was used for the antibacterial bioassay of *M. piperita* essential oil. The media was poured in petri dishes and placed for solidification in an incubator for 37°C. Six millimeter wells were made in the solidified media by sterile borer and labeled precisely. One hundred microliters (100 µL) of standardized inoculum ( $10^6$  CFU/mL; 0.5 MacFarland) of each bacterium was spread on a sterile Muller-Hinton Agar (MHA) plate. Subsequently 50 µL of oil was poured in wells of the agar plates and then incubated for 24 h at 37°C and the experiment was performed in triplicate. The zone of inhibition was measured in millimeters (29).

### Antifungal activity

The well diffusion method was used for the evaluation of antifungal activities. In each flask, fungal strains were independently inoculated and kept for 3 days in an incubator at 30°C. Wells were made in the solidified media plates by a sterilized borer (6 mm). Essential oil (50 µL) was poured into each well aseptically in the agar plates. Plates were incubated at 28°C for 72 h and the experiment was performed in triplicate (30).

### Antioxidant activity

The antioxidant activity of *M. piperita* essential oil was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Essential oil of various concentrations (50, 20, 10 and 5 g/L) were used for the activity. Each solution was placed in a spectrophotometer cuvette and 2 mL DPPH methanolic solution ( $6 \times 10^{-5}$  mol/L) was added. The solutions were then incubated for 30 min in dark, after which their absorbance was measured at 517 nm with a spectrophotometer (Perkin Elmer). The control consists of blank solution without essential oil, while ascorbic acid was used as positive control. The percent DPPH free radical scavenging activity

was calculated by using the following equation (31).  
Percent scavenging activity =  $100 \times (A_{\text{control}} - A_{\text{sample}}/A_{\text{control}})$

## Results

### Chemical composition of essential oil

In the present study, essential oil was isolated from the leaves extract of *M. piperita* by using different solvents which include petroleum ether and *n*-hexane. The essential oil was subjected to hyphenated gas chromatography-mass spectrometry analytical technique for the determination of chemical composition. The primary constituents present in the essential oils were: *p*-mentha-6,8-dien-2-one (46.434%), *p*-menthan-3-ol (25.749%), borneol (8.865%), d-limonene (5.516%), 2-isopropylidene-cyclohexanone (4.838%) and 7-oxabicyclo [4.1.0] heptan-2-one, 6-methyl-3-(1-methylethyl) (2.039%) (Figure 1). The contents of eucalyptol (1.996%), o-cymene (0.835%), 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene (0.491%), *p*-menth-1-en-8-ol (0.220%), 2-isopropenyl-5-methylhex-4-enal (0.192%), alpha-phellandrene (0.168%) among others were found in small quantities (Table 1).

### Essential oil suppressed pathogenic bacteria

The obtained peppermint oil was evaluated for antibacterial activity against seven bacterial strains. Due to the presence of primary constituents like *p*-mentha-6,8-dien-2-one, *p*-menthan-3-ol and borneol in the essential oil, maximum zone of inhibition was observed against *S. aureus* ( $17 \pm 0.61$  mm), *E. coli* ( $17 \pm 0.87$  mm), *E. faecalis* ( $16 \pm 0.23$  mm), *B. cereus* ( $15 \pm 0.12$  mm), *P. aeruginosa* ( $15 \pm 0.53$  mm), *S. typhi* ( $14 \pm 0.21$  mm) and *K. pneumoniae* ( $13 \pm 0.31$  mm). The standards, ciprofloxacin and azithromycin showed a robust antibacterial activity (Table 2).

### Essential oil possessed antifungal activity

The antifungal activity of the essential oil was investigated against four fungal strains. The peppermint oil showed maximum zone of inhibition against *C. albicans* ( $15 \pm 0.52$  mm), *A. niger* ( $16 \pm 0.37$  mm), *A. fumigatus* ( $13 \pm 0.32$  mm) and *A. parasiticus* ( $15 \pm 0.16$  mm) and therefore revealed considerable antifungal activity. The positive control, clotrimazole showed a robust antifungal activity against the tested fungal strains (Table 3).

### Essential oil efficiently scavenged DPPH free radicals

The antioxidant activity of *M. piperita* essential oil was assessed by the DPPH free radical scavenging

assay. Figure 2 shows prospective free radical scavenging capacity and therefore antioxidant activity of essential oil of *M. piperita* and that of the positive control, ascorbic acid. The essential oil showed concentration dependant free radical scavenging activity ( $61 \pm 0.32\%$ ,  $69 \pm 0.59\%$ ,  $77 \pm 0.12\%$ ,  $83 \pm 0.43\%$  at 5, 10, 20 & 50  $\text{gL}^{-1}$ , respectively). Similarly, the positive control, ascorbic acid also revealed excellent DPPH free radical scavenging activity ( $68 \pm 0.37\%$ ,  $74 \pm 0.21\%$ ,  $82 \pm 0.67\%$ , and  $90 \pm 0.51\%$  at the tested concentrations of 5, 10, 20 & 50  $\text{gL}^{-1}$  respectively).

### Discussion

The escalating emergence of antibiotic resistance have deviated the attention of researchers towards the medicinal plants in search of new, less toxic and effective drugs. In this regard, the present study has been carried out to determine the phytochemical composition and evaluation of antibacterial, antifungal and antioxidant activities of essential oil isolated from *Mentha piperita*.

The chemical composition revealed that the essential oil of *M. piperita* has abundant quantities of menthone (12.7%), menthol (37.4%) and menthyl acetate (17.4%) as previously reported in Serbia (32). In Pakistan, the major components of *M. piperita* reported are menthone and menthol (33). The dominant constituents of *M. piperita* essential oil reported in India are menthol (30-55%), menthofuran and menthyl acetate (1.0-9.2%) (34). A study in Iran shows that the essential oil of *M. piperita* contains menthanol (36.24%) and menthone (32.42%) as main constituents (17). In Turkey, the reported chemical constituents of peppermint oil are menthon (44.1%), menthol (29.5%), menthyl-acetate (3.8%) and mentho-furon (0.9%) (35). However, in Korea, *M. piperita* leaves essential oil has different composition and include limonene (64.5 and 94.2%), 1,8-cineole (46.1%), p-menth-2-en-ol (34.5%), menthol (33.4%) and linalyl-acetate (28.2%) as main components (24).

Recently, there is an alarming emergence of bacterial pathogens that show resistance to the commonly used antibiotics (multidrug resistance, MDR). It has been reported that *E. coli* and *P. vulgaris* are 78.6% MDR while *S. typhi* and *V. cholera* are 71.4% MDR. *P. aeruginosa* and *S. aureus* are 50% MDR, whereas *Shigella* shows 35.8% MDR against all the tested antibiotics (30). *M. piperita* essential oil possessed strong antibacterial and antifungal activities, which are evident from the zones of inhibition, compared to respective positive controls. Essential oils are potential sources of novel

antimicrobial compounds. Hydrophobicity is one of the major distinctiveness of essential oils which enables their assimilation into the cell membrane. The isolated essential oil is rich in menthol and compounds similar to menthol show that the hydroxyl group and the presence of a system of delocalized electrons are important for the antimicrobial activity. These similar compounds destabilize the cytoplasmic membrane and, in addition, act as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death (36).

The major components of *M. piperita* essential oils reported were menthol (71.40%), piperitenone oxide (17.10%), trans-carveol (19.48%), alpha-terpene (20.11%), p-menthone (8.04%), neomenthol (3.18%) and iso-menthone (5.42%) in Morocco (1). In Iran, *M. piperita* essential oil contains  $\alpha$ -terpinene (19.7%), isomenthone (10.3%),  $\beta$ -caryophyllene (7.6%), pipertinone oxide (19.3%), trans-carveol (14.5%) as foremost compounds (37). Iranian *Myrtus communis* and *M. piperita* essential oils were analyzed by GC-MS and hydro-distillation extraction technique which lead to the identification of thirty two and twenty six compounds, respectively. Essential oil of *M. piperita* revealed significant antibacterial and antifungal activities against *C. albicans*, *E. coli* and *S. aureus*, which suggests their potential of healing infections triggered by these pathogens. The antioxidant activities also revealed that *M. piperita* possess prospective free radical scavenging property and is able to mitigate pathological conditions associated with oxidative stress (37). These results are well corroborated with our study. Essential oils are in escalating demand from manufacturers of pharmaceuticals, cosmetics and foods. The chemical contents of essential oils are associated with fastidious bioactive properties (38). In this regard, the present work has been carried out to determine the chemical constituents of *M. piperita* essential oil and its potential biological activities. Among other important bioactive constituents, *M. piperita* contains abundant quantities of p-mentha-6,8-dien-2-one (46.434%), p-menthan-3-ol (25.749%), borneol (8.865%), d-limonene (5.516%), 2-isopropylidencyclohexanone (4.838%) and 7-oxabicyclo [4.1.0] heptan-2-one, 6-methyl-3-(1-methylethyl) (2.039%). These bioactive constituents are reported to have important applications in pharmaceutical, cosmetic and food industries, as they possess important biological properties, including antioxidant, antibacterial, antifungal,

antispasmodic, analgesic and antiseptic activities. Essential oils are beneficial for depression, sinus congestion, apathy, nervous stress, mental fatigue, respiratory disorders, cholera and bronchitis etc (39-40). It is recommended that *M. piperita* essential oil is effective from therapeutic perspective.

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**Table 1.** Phytochemical composition of *Mentha piperita* L. essential oil.

Chemical constituent	Retention time (min)	Area	Concentration (%)
Alpha-Phellandrene	9.380	4384	0.168
Alpha-Pinene	9.704	6405	0.245
Camphene	10.550	5313	0.203
Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)	12.097	14861	0.569
Beta-Pinene	13.009	12591	0.480
o-Cymene	15.094	21815	0.835
D-Limonene	15.333	144117	5.516
Eucalyptol	15.472	52155	1.996
Beta-Linalool	20.359	7391	0.283
Borneol	25.091	234994	8.865
p-Menthan-3-ol	25.739	691415	25.749
p-Cymen-8-ol	26.403	5728	0.219
p-menth-1-en-8-ol	26.718	5907	0.220
2-Isopropenyl-5-methylhex-4-enal	29.014	5011	0.192
p-Mentha-6,8-dien-2-one, (R)-(-)-	29.740	1252626	46.434
7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)	29.973	53262	2.039
Alpha-Santalol	33.922	8485	0.325
Santalol	33.922	8485	0.325
2-isopropylidenecyclohexanone	35.734	126406	4.838
1H-cycloprop[e]azulen-7-ol,decahydro--1,1,7-trimethyl-4-methylene	45.198	12824	0.491
Caryophyllene oxide	45.199	10627	0.407
Epiglobulol	45.198	12824	0.491

**Table 2.** Antibacterial activity of *Mentha piperita* L. essential oil.

Microorganism	Zone of inhibition (mm)	Zone of inhibition of positive control (mm)
<i>Bacillus cereus</i>	15 ± 0.12	19 ± 0.25**
<i>Escherichia coli</i>	17 ± 0.87	20 ± 0.34*
<i>Enterococcus faecalis</i>	16 ± 0.23	19 ± 0.65*
<i>Klebsiella pneumoniae</i>	13 ± 0.31	17 ± 0.39*
<i>Pseudomonas aeruginosa</i>	15 ± 0.53	20 ± 0.81*
<i>Staphylococcus aureus</i>	17 ± 0.61	23 ± 0.37**
<i>Salmonella typhi</i>	14 ± 0.21	17 ± 0.36*

Data expressed as mean ± standard deviation of three separate experiments.

\*Ciprofloxacin

\*\*Azithromycin

**Table 3.** Antifungal activity of *Mentha piperita* L. essential oil

Microorganism	Zone of inhibition (mm)	Zone of inhibition of positive control (mm)
<i>Candida albicans</i>	15 ± 0.52	21 ± 0.19*
<i>Aspergillus niger</i>	16 ± 0.37	19 ± 0.41*
<i>Aspergillus parasiticus</i>	15 ± 0.16	17 ± 0.20*
<i>Aspergillus fumigatus</i>	13 ± 0.32	18 ± 0.18*

Data expressed as mean ± standard deviation of three separate experiments.

\* Clotrimazole

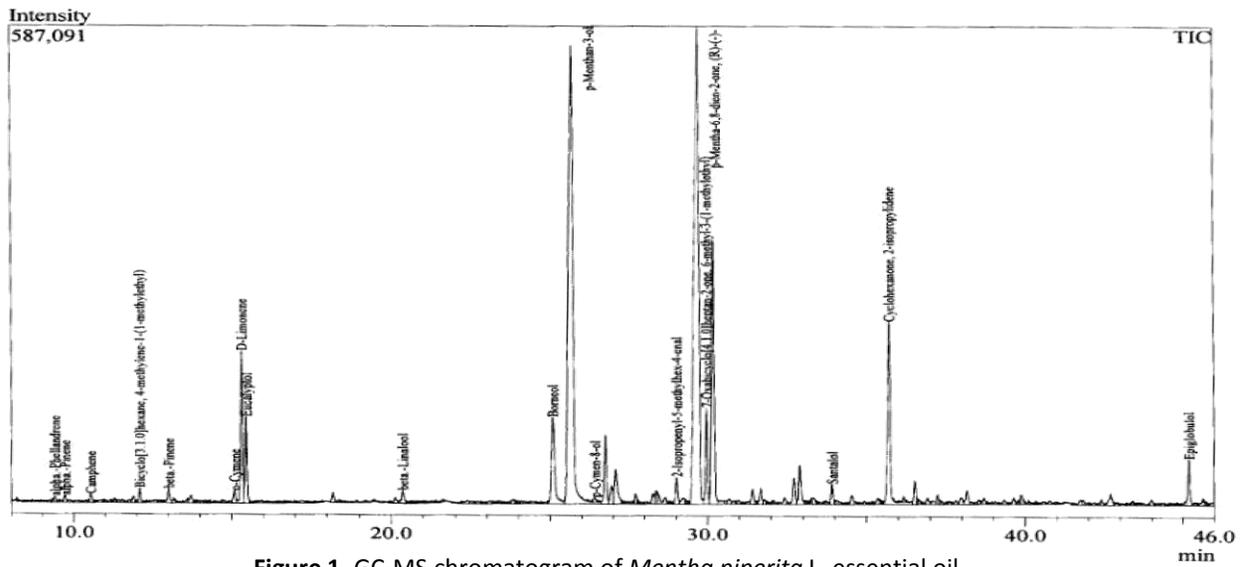


Figure 1. GC-MS chromatogram of *Mentha piperita* L. essential oil.

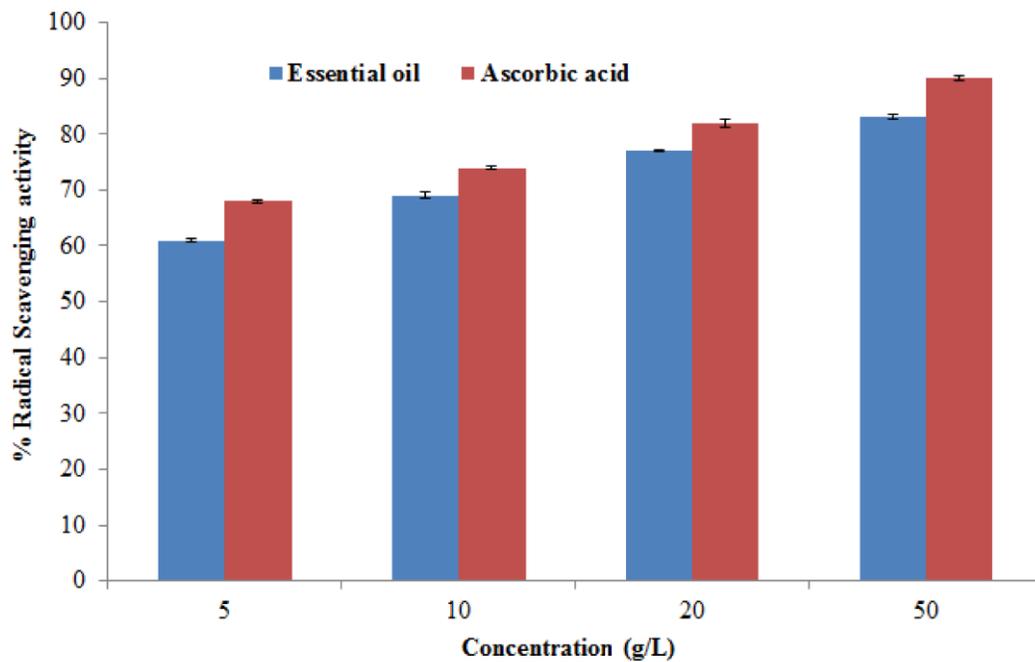


Figure 2. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay of *Mentha piperita* L. essential oil and ascorbic acid (positive control).