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Antimicrobial Effect of Mint Essential Oils on Some Pathogenic Bacteria

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Abstract: **The increasing resistance of microorganisms to conventional chemicals and drugs is a serious and evident worldwide problem that has prompted research into the identification of new biocides with broad activity. Plants and their derivatives, such as essential oils, are often used in folk medicine. In the present study, we evaluated the antibacterial activity in the leaf Water extracts and EO of *Mentha piperita* L. against pathogenic bacteria like *Bacillus subtilis, Pseudomonas aureus, Escherichia coli, Salmonella typhi* and *Streptococcus aureus*.**

The screening was performed by standard disc diffusion method. Essential oil of peppermint exhibited highest antibacterial activity with 12.00 mm mean zone of inhibition. The Water extract of peppermint also possessed antibacterial activity with 9.00 mm mean zone of inhibition, comparing with chloremphemicol as control.

Keywords: ***Mentha piperita*** **L., antibacterial activity, leaf extracts, essential oil.**

I. INTRODUCTION

The increasing resistance of microorganisms to conventional chemicals and drugs has prompted scientists to search for novel sources of biocides with broad-spectrum activities (1). Since ancient times, plants and their derivatives, such as essential oils (EOs), have been used in folk medicine. In nature, EOs play an important role in the protection of plants. They also may attract some insects to promote the dispersion of pollens and seeds or keep away other undesirable insects. Thus, EOs can play a role in mediating the interactions of plants with the environment (2). EOs are concentrated natural products with strong smells that are produced by aromatic plants as secondary metabolites. Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Most of their properties are due to essential oils produced by their secondary metabolites. Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries and food spoilage, including Gram-negative and Gram-positive bacteria (15).

Gram-negative bacteria are more resistant to EOs than Gram-positive bacteria. Before examining the effects of EOs on bacteria, and should briefly consider the differing structures of the cell walls of Gram-positive and Gram-negative bacteria. Approximately 90%–95% of the cell wall of Gram-positive bacteria consists of peptidoglycan, to which other molecules, such as teicoic acid and proteins, are linked (16).

The antimicrobial activity of EOs, is dependent on their chemical composition and the amount of the single components. Many of the antimicrobial compounds are constitutively expressed by the plants, and others can be synthesised as mechanism of self-defence in response to pathogens. Aromatic and medical plants with high level of EOs are excellent sources of natural elements with activity against microorganisms of agricultural and health interest (*12*). These molecules can be naturally present in their active form in the plant or can be activated by specific enzymes when the vegetal organism is subjected to particular biotic or abiotic stress [*9.*]. Some EOs, such as those found in basil, sage, hyssop, rosemary, oregano and marjoram, are active against *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* spp. but are less effective against *Pseudomonas* spp. due to the formation of exopolysaccharides that increase resistance to EOs (*7,4*). The mechanism of action of EOs depends on their chemical composition, and their antimicrobial activity is not attributable to a unique mechanism but is instead a cascade of reactions involving the entire bacterial cell (*4*); together, these properties

are referred to as the "essential oils versatility". In general, EOs act to inhibit the growth of bacterial cells and also inhibit the production of toxic bacterial metabolites. Most EOs have a more powerful effect on Gram-positive bacteria than Gram-negative species, and this effect is most likely due to differences in the cell membrane compositions (*6).*

Mentha piperita L. (peppermint) is a medicinally important plant that belongs to the family Labiate (10). Peppermint is a non-native herbaceous plant, it is a perennial, which can reach 100 cm in height (40 inches) has four-sided stem. The leaves are stalked opposite and toothed. The flower are irregular in shape, they are pinkish or purplish. Peppermint leaves contains about 0.5-4 % volatile oil that is composed of 50-78 % free menthol, monoterpene, menthofurane and traces of jasmine (0.15 %) to improve the oils quality remarkably. Peppermint is largely cultivated in Indiana, Mexican and California for the production of peppermint oil. Peppermint oil or peppermint tea is often used to treat gas and indigestion; it may also increase the flow of bile from the gall bladder Mimica et al. (2003)(11) Peppermint oils relaxing action also extended to tropical use, when applied tropically it acts as counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area. Peppermint oil and menthol have moderate antibacterial effects against both gram-positive and gram negative bacteria Saeed et al. 2006). Peppermint extracts are bacteriostatic against S*treptococcus pyrogens*, S*treptococcus aureus*, *Streptococcus pyrogens*, *Serratia marcescens*, *E.coli* and *Mycobacterium avium* (*3*). Peppermint is also found to have antiviral and fungicidal activity . Menthol is virucidal against influenza, herpes and other viruses. Aqueous extracts of peppermint leaves were antiviral against influenza A, newcastle disease virus in egg and cell culture system Hirobe (1994) (8). Menthol and peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and *Dermatophytic* fungi. (*15)*

In the present study we established antimicrobial activity of *Mentha piperita* against pathogenic bacteria. To confirm that both aqueous leaf extracts possess and EO are strong antibacterial properties againstvarious pathogens.

II. MATERIALS AND METHODS

Mint Extract: Water extracts 10 gr of mint leaf \ 100 ml DW in flask, boiling for 20 minutes, cool and filtration.

Mint Oil: EO purchasing from local market Jeddah KSA.

Screening of antibacterial activity:

- Standard disc diffusion methods
- Disc 6 mm diameter soaked in 1 ml of each treatment 1-2 min
- Discs were placed on the surface of inculated plates of microorganisms
- Incubation at 35-37 ^{o}C
- Inhibition zone diameter mm

Inoculums: The test microorganisms B.s = *Bacillus substillus*, P.ar = *Pseudomonas aureus Escherichia coli*, *Salmonella typhi* and *S.a = Streptococcus aureus* were obtained from culture repository of Plant Pathology Lab, Agriculture collage. Qassim Un. KSA. The organisms were inoculated onto NB (Nutrient Broth), (0.5 % Peptone, 0.5 % Sodium Chloride, 0.15 % Yeast extract; pH 7.4) and incubated at 37 ºC for overnight.

Determination of antibacterial activity: The antibacterial activity of the leaf extracts was determined using agar well diffusion method following published procedure with slight modification (5) Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) or standard antibiotic solution (positive control) viz., Chloromphenicol (100 µg/ml) were also run parallel in the same plate (Saeed & Tariq, 2005). The plates were incubated at 37 ºC for 18 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition for the respective drug. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drugs viz., Choloramphenicol. The resultant clear zones around the discs were measured in mm. The antibacterial activity of plant extracts were indicated by clear zones of growth inhibition. Five replicates were maintained for each treatment. The data were subjected to statistical analysis.

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III. RESULTS AND DISCUSSION

A total of isolates belonging to different species of Gram –ve bacteria were used, in the present study, to determine the antibacterial activities of aqueous water extract, and oil of peppermint.

The results showed that essential oil of peppermint exhibited the highest antibacterial activity with 12.00 mm mean zone of inhibition (Table 1). Our results are in fair correlation with the studies in which peppermint oil has antibacterial activities against both Gram –ve and Gram +ve bacteria Similarly in another study peppermint oil was found to be strongly effective against *Enterococcus faecium* ATCC 10541, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Bacillus subtilis* (15).

The principle active constituents of peppermint are the essential oils, which comprise about 1% of the herb. The oils are dominated by monoterpenes, mainly menthol, menthone and their derivatives (e.g., isomenthone, neomenthol, acetylmenthol, pulegone). These essential oils dilate blood vessels and inhibit bacteria. Especially menthol has a broad spectrum antibacterial activity (*3*).

The antibacterial activity ofwater extract of peppermint was found next to oil with 8.1 mm mean zone of inhibition (Table 1). These results are in correlation with a previous study in which juices of leaves and stem of peppermint exhibited good antibacterial activity against Gram –ve bacilli (13). The antibacterial activity of the *Mentha piperita* was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated (Table. 1). The results shown in (Table 1) indicate that the EO possessed strong antibacterial activity. In EO, the highest antibacterial activity was retained in 50 µl and 100 µl concentration. It was found that both water extract and EO were successful in killing the bacteria in a dose dependent manner. The MIC (Minimal inhibitory Concentration) for the water extracts was found out to be 10 mg/ml for *Bacillus substillis* and *Pseudomonas aureus*. While *Streptococcus aureus* required about 0.25 mg/ml of the crude extract for effective killing. On the other hand *E. coli and Salmonella* was inhibited at the dose of 0.35 mg/ml of the crude extract (data not shown). The zone of inhibition assay results demonstrated that the 100 µl of crude EO was able to produce same effect as that of Cholorompheniсol. Beside the 50 µl concentration of leaf extracts, the 100 µl concentration of leaf extracts was found to possess maximum inhibition, although, overall potency was about half to that of the 50 µl concentration of leaf extracts. (Table 1). The EO had very large zones of inhibition ranging from 6-12 mm and it also showed high degree of inhibition against *Bacillus subtilis, Streptococcus aureus* than *Pseudomonas aureus*, *E. coli and Salmonella* The leaf extracts showed moderate inhibition against *Pseudomonas aureus* and *Streptococcus aureus* than *Bacillus subtilis,E.coli and Salmonella*

The result of the study reveals that both of treaments, both of the water extract and essential oil of mint. actively against the strains of the bacteria that are common cause of infections. *Mentha piperita shows* significant activity as because the leaf contains many potent compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone (Hirobe (1994), (15). It was observed that the maximum activity at 100 µl concentration against *all pathogenic strains.*The present reports indicates that increased lipophillic compounds are extracted using the petroleum ether, chloroform and methanol increased the suspended higher compounds. The results from the present We subjected the leaf extracts and EO of mint should be studied more extensively to explore its potential in the treatment of infectious diseases as well.

Table (1). The antibacterial activity of leaf extracts of menthapiperita was screened at 100 µl concentration against B.s = *Bacillus substillus*, P.ar = *Pseudomonas aureus Escherichia coli*, *Salmonella typhi* and *S.a = Streptococcus aureus.*The leaf extracts. Water e. Control – Chloromphenicol

Treatments	**Zone of inhibition in (mm) (Mean ± SD)**									
	50µl					100µl				
	E. coli	***Typhi S.***	***B.s***	***P.ar***	***S.a***	***E. coli***	***Typhi S.***	***B.s***	***P.ar***	***S.a***
Water extract	4.9±0.25	4.3±0.25	4.3±0.25	3.3±0.23	4.2±0.25	9.9±0.25	8.3±0.25	9.3±0.24	7.3±0.25	6.8±0.25
Oil	6.4±0.18	6.2±0.18	6.2±0.18	5.8±0.19	6.2±0.18	12.4±0.18	10.2±0.18	12.2±0.18	10.2±0.18	9.8±0.18
Chloromphenicol	8.6±0.23	8.2±0.23	8.2±0.23	9.2±0.23	7.2±0.23	12.6±0.23	11.2±0.23	13.9±0.23	14.2±0.23	15.2±0.23

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