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# *IN VITRO* ANTIBACTERIAL ACTIVITY OF PEPPERMINT

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**Abstract**

Antibacterial activities of different forms of peppermint (*Mentha piperita*) viz., aqueous infusion, decoction, juice and essential oil were investigated against 100 isolates belonging to 11 different species of Gram –ve bacilli viz., *Escherichia coli* (30), *Klebsiella pneumoniae* (25), *Pseudomonas aeruginosa* (15), *Salmonella typhi* (5), *S. paratyphi A* (1), *S. paratyphi B* (1), *Proteus mirabilis* (10), *P. vulgaris* (2), *Shigella dysentriae* (5), *Yersinia enterocolitica* (1), and *Enterobacter aerogenes* (5). The screening was performed by standard disc diffusion method. Essential oil of peppermint exhibited highest antibacterial activity with 11.78 mm mean zone of inhibition. The juice of peppermint also possessed antibacterial activity with 10.41 mm mean zone of inhibition, while all isolates were totally resistant to aqueous infusion and decoction of peppermint.

## Introduction

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases (Service, 1995; Iwu *et al.*, 1999). In addition to this, antibiotics are sometimes associated with adverse effects. Therefore, there is a need to develop alternative antimicrobial medicines for the treatment of infectious diseases from other sources such as plants (Cordell, 2000). Natural products of higher plants may be a new source of antimicrobial agents possibly with novel mechanisms of action (Barbour *et al*., 2004).

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 (Adam *et al.*, 1998). 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 (Sartoratto *et al.*, 2004).

Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Zaika, 1975). From ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that medicines drived fron plant are safe and dependable, compared with costly synthetic drugs that have adverse effects (Iwu *et al.*, 1999; Gordon & David, 2001). Peppermint is very beneficial and important plant. It is widely used in food, cosmetics and medicines (Scavroni *et al.*, 2005). It is chemopreventive and antimutagenic (Samarth *et al.*, 2006). It has been proven helpful in symptomatic relief of the common cold. It also decreases symptoms of irritable bowel symdrome and decrease digestive symptoms such as dyspepsia and nausea. It is also used topically as an analgestic and to treat headache (Gardiner, 2000). It is mosquito repellent (Tunon *et al*., 1994) and has antinematodal (Walker & Melin, 1996), antiviral (Herrmann & Kucera, 1967; Kerman & Kucera, 1967), antifungal (Janssen *et al.*, 1986;

El-Naghy *et al.,* 1992; El-Kady *et al.,* 1993; Pattnaik *et al.,* 1996; Pattnaik *et al.,* 1997) and antibacterial properties (Moleyar & Narasimham, 1992; El-Kady *et al.,* 1993; Pattnaik *et al.,* 1996; Pattnaik *et al.,* 1997; Saeed & Tariq, 2005). In view of this *in vitro* antibacterial activities of aqueous infusion, decoction, juice and essential oil of peppermint (*Mentha piperita*) were examined against different species of Gram –ve bacilli.

## Materials and Methods

**Maintenance of isolates:** A total of 100 isolates belonging to 11 different species of Gram –ve bacilli (Table 1) isolated from different clinical specimens of stool, urine, blood and pus from wound were maintained on tryptone soy agar (TSA) (Oxoid).

**Preparation of infusion:** The aqueous infusion was prepared by taking 10 g peppermint in 100 ml distilled water and left for 48 hours at room temperature with ocasional shaking and filtered to obtain clear infusion.

**Preparation of decoction:** The aqueous decoction was prepared by boiling 10 g peppermint in 100 ml distilled water in a flask for 20 minutes. The flask was removed from heat and allowed to cool. The content of flask was filtered to obtain clear decoction.

**Preparation of juice:** Leaves and stems of peppermint were washed with tap water followed by sterile distilled water. The juice was prepared by juicer machine (Moulinex Juice Extractor, Model No. 864).

**Essential oil:** Essential oil of peppermint (Iwan) was purchased from a local market of Karachi, Pakistan.

**Screening of antibacterial activity:** Screening of antibacterial activity was performed by standard disc diffusion method (Chaudhry & Tariq, 2006). Hundred sterilized discs of filter paper (6 mm diameter) were soaked in 1 ml of infusion, decoction, juice and oil, seperately, for 1-2 minutes and then used for screening. Thus potency of each disc was 10 µl. Mueller-Hinton agar (MHA) (Merck) was used as base medium and Mueller-Hinton broth (MHB) was used for the preparation of inoculum. Four to five isolated colonies of tested organisms were picked by sterile inoculating loop and inoculated in tubes of MHB (5 ml in each). The inoculated tubes were incubated at 35-37$^{o}$ C for 24 hours and matched with 0.5 McFarland nephelometer turbidity standard (Saeed & Tariq, 2005). A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate entire surface of a MHA plate. Discs of infusion, decoction, juice and oil were placed on the surface of inoculated plates with the help of sterile forcep. The inoculated plates were incubated at 35-37$^{o}$ C for 24 hours. After incubation inhibition zone diameters were measured to the nearest millimeter (mm).

## Results and Discussion

A total of 100 isolates belonging to 11 different species of Gram –ve bacteria were used, in the present study, to determine the antibacterial acitivities of aqueous infusion, decoction, juice, and oil of peppermint.

**Table 1. Antibacterial activities of infusion, decoction, juice and oil of peppermint (*Mentha piperita*).**

| S.No. | Name of organisms | No. of isolates | Mean zone of inhibition in mm | | | |
|---|---|---|---|---|---|---|
| | | | Infusion | Decoction | Juice | Oil |
| 1. | *E. coli* | 30 | 0 | 0 | 12.26 | 13.00 |
| 2. | *K. pneumoniae* | 25 | 0 | 0 | 10.82 | 12.67 |
| 3. | *P. aeruginosa* | 15 | 0 | 0 | 11.56 | 12.00 |
| 4. | *S. typhi* | 05 | 0 | 0 | 09.50 | 10.33 |
| 5. | *S. paratyphi* A | 01 | 0 | 0 | 08.00 | 10.00 |
| 6. | *S. paratyphi* B | 01 | 0 | 0 | 08.00 | 11.00 |
| 7. | *P. mirabilis* | 10 | 0 | 0 | 10.60 | 10.53 |
| 8. | *P. vulgaris* | 02 | 0 | 0 | 09.00 | 12.00 |
| 9. | *S. dysentriae* | 05 | 0 | 0 | 11.00 | 12.50 |
| 10. | *Y. enterocolitica* | 01 | 0 | 0 | 13.00 | 13.00 |
| 11. | *E. aerogenes* | 05 | 0 | 0 | 10.80 | 12.50 |
| | | 100 | 0 | 0 | 10.41 | 11.78 |

The results showed that essential oil of peppermint exhibited the highest antibacterial activity with 11.78 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 (Moleyar & Narasimham, 1992; El-Kady *et al.*, 1993; Pattnaik *et al.*, 1996; Pattnaik *et al.*, 1997). Similarly in another study peppermint oil was found to be strongly effective against *Enterococcus faecium* ATCC 10541, *Salmonella choleraesuis*, *Staphylococcus aureus* and *Bacillus subtilis* (Sartoratto *et al.*, 2004).

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 (Pattnaik *et al.*, 1997).

The antibacterial activity of juice of peppermint was found next to oil with 10.41 mm mean zone of inhibition (Table 1). Theses 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 (Saeed & Tariq, 2005).

In the present study, aqueous infusion and decoction of peppermint did not show antibacterial activity against all tested organisms (Table 1). Our results are in fair correlation with a study in which aqueous decoction of peppermint did not inhibit the growth of Gram –ve and Gram +ve bacteria (Woodward, 1999).

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