

ANTIBACTERIAL ACTIVITIES OF *MENTHA PIPERITA*, *PISUM SATIVUM* AND *MOMORDICA CHARANTIA*

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Abstract

The juices of leaves and stem of *Mentha piperita* (peppermint), skin and seeds of *Pisum sativum* (garden pea), skin and pulp of *Momordica charantia* (bitter melon) were screened for antibacterial activities against 56 isolates belonging to 11 different species of Gram-negative bacilli: *Escherichia coli* (19), *Klebsiella pneumoniae* (11), *Pseudomonas aeruginosa* (9), *Salmonella typhi* (3), *Salmonella paratyphi A* (1), *Salmonella paratyphi B* (1), *Proteus mirabilis* (5), *Proteus vulgaris* (1), *Enterobacter aerogenes* (4), *Shigella dysenteriae* (1), and *Yersinia enterocolitica* (1). The screening was performed by well diffusion method. Leaves of *M. piperita* exhibited highest antibacterial activity (average zone of inhibition 17.24 mm \pm 0.87 SD) while stem of *M. piperita* exhibited least antibacterial activity (average zone of inhibition 15.82 mm \pm 3.56 SD). The skin and seeds of *P. sativum*, skin and pulp of *M. charantia* exhibited good antibacterial activity with average zone of inhibition of 16.30 mm \pm 2.02 SD, 16.39 mm \pm 3.16 SD, 16.16 mm \pm 2.17 SD and 15.88 mm \pm 2.24 SD respectively.

Introduction

Gram -ve bacilli are ubiquitous. They are found in 10-15% of the indigenous bacterial flora. Temperature, moisture and reduction of the normal Gram +ve flora favour a rapid establishment of Gram -ve bacilli and development of clinical infections (Wassilew, 1989). Their association with urinary tract infections (Bouza *et al.*, 2001; Khan & Musharraf, 2004), wound infections (Wassilew, 1989) and brain abscess (Rau *et al.*, 2002) is well documented. They have also been reported to be associated with nosocomial bloodstream infections (Kang *et al.*, 2005; Blot *et al.*, 2005).

A wide variety of antibiotics are commonly used for the treatment of serious infections caused by aerobic Gram -ve bacteria (Tumah, 2005). The increased use of antibiotics has resulted in the development of resistant bacteria (Jacobs, 1998). In recent years, misuse of antibiotics resulting in multi-drug resistance among bacteria has accelerated the search for drugs and dietary supplements effective against such multi-drug resistant bacteria. It has been reported that in 1996, sales of botanical medicines increased by 37% over 1995 (Klink, 1997). In this connection, different parts of plants, herbs and spices have been used for many years for prevention of infections. These are easily available and can be used in domestic setting for self-medication. The present report gives an account of the antibacterial effect of different parts of plants viz., stem and leaves of *Mentha piperita*, skin and seeds of *Pisum sativum*, and skin and pulp of *Momordica charantia* against Gram -ve bacilli isolated from different clinical specimens of stool, urine, blood and pus from wound.

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Materials and Methods

Maintenance of isolates: A total of 56 isolates belonging to 11 different species of Gram -ve bacilli (Table 1), isolated from clinical specimens of stool, urine, blood and pus from wound were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of juices: The vegetables were purchased from a local market of Karachi, Pakistan. The parts of vegetables viz., skin and pulp of *M. charantia* (bitter melon), skin and seeds of *P. sativum* (garden pea), and stem and leaves of *M. piperita* (peppermint) were washed separately with tap water followed by sterile distilled water. Juices were prepared separately by juicer machine (Moulinex Juice Extractor, Model No. 864).

Screening of antibacterial activity

Media: Mueller-Hinton agar (MHA) (Merck) was used as base medium for screening of antibacterial activity and Mueller-Hinton broth (MHB) (Merck) for preparation of inoculum.

Preparation of McFarland Nephelometer standard: McFarland tube number 0.5 was prepared by mixing 9.95 ml 1% Sulphuric acid in MHB and 0.05 ml 1% Barium chloride in distilled water in order to estimate bacterial density (Baron *et al.*, 1994). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Preparation and standardization of inoculum: Four to five colonies from pure growth of each test organism were transferred to 5 ml of MHB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was compared to 0.5 McFarland Nephelometer standard to get 150×10^6 CFU/ml. The standardized inoculum suspension was inoculated within 15-20 minutes.

Well diffusion technique: Screening of antibacterial activity was performed by well diffusion technique (Kivanc & Kunduhoglu, 1997). The MHA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 100 µl of each fresh vegetable juice was introduced in the well.

Incubation: The inoculated plates were incubated at 35-37°C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm).

Statistical analysis: Mean zone of inhibition and standard deviations were calculated.

Results and Discussion

Different plants and their parts (flowers, buds, leaves, stem, skin, pulp) have been used for thousands of years to enhance the flavour and aroma of food. In addition, plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoides which have been found *in vitro* to have antimicrobial properties (Cowan, 1999). In this connection, the present study was conducted to evaluate the antibacterial activity of juices of stem and leaves of *M. piperita*, skin and pulp of *M. charantia* and skin and seeds of *P. sativum*.

Table 1. Antibacterial activity of juices of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*.

S. No.	Name of organisms	No. of isolates	Mean zone of inhibition in mm and standard deviation (SD)											
			A		B		C		D		E		F	
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1.	<i>E. coli</i>	19	16.84 ± 2.11	16.43 ± 3.41	16.25 ± 3.26	16.37 ± 3.07	16.14 ± 3.70	16.38 ± 3.56						
2.	<i>K. pneumoniae</i>	11	16.82 ± 2.33	16.91 ± 2.87	15.91 ± 2.15	15.64 ± 2.46	16.64 ± 2.01	17.00 ± 2.41						
3.	<i>P. aeruginosa</i>	09	15.67 ± 1.05	17.11 ± 1.45	15.89 ± 1.45	15.22 ± 1.87	15.00 ± 1.70	18.11 ± 0.99						
4.	<i>S. typhi</i>	03	16.00 ± 0.82	16.00 ± 0.82	15.67 ± 1.25	16.33 ± 0.47	16.33 ± 0.47	16.67 ± 0.94						
5.	<i>S. paratyphi A</i>	01	16.00 --	18.00 --	14.00 --	19.00 --	17.00 --	19.00 --						
6.	<i>S. paratyphi B</i>	01	15.00 --	16.00 --	15.00 --	17.00 --	18.00 --	18.00 --						
7.	<i>P. mirabilis</i>	05	14.00 ± 1.10	14.60 ± 1.50	16.00 ± 2.19	15.40 ± 2.33	16.20 ± 0.75	17.00 ± 1.55						
8.	<i>P. vulgaris</i>	01	20.00 --	18.00 --	14.00 --	17.00 --	16.00 --	16.00 --						
9.	<i>S. dysenteriae</i>	01	16.00 --	19.00 --	17.00 --	17.00 --	15.00 --	17.00 --						
10.	<i>Y. enterocolitica</i>	01	15.00 --	17.00 --	14.00 --	17.00 --	16.00 --	18.00 --						
11.	<i>E. aerogenes</i>	04	16.75 ± 1.09	16.25 ± 0.83	17.25 ± 0.83	16.00 ± 0.71	17.25 ± 0.83	16.50 ± 1.12						
Total		56	16.30 ± 2.02	16.39 ± 3.16	15.88 ± 2.24	16.16 ± 2.17	15.82 ± 3.56	17.24 ± 0.87						

Key: A = Juice of skin of *P. sativum* C = Juice of skin of *M. charantia* E = Juice of stem of *M. piperita*
 B = Juice of seeds of *P. sativum* D = Juice of pulp of *M. charantia* F = Juice of leaves of *M. piperita*

The juice of leaves of *M. piperita* exhibited highest antibacterial activity ($17.24 \text{ mm} \pm 0.87 \text{ SD}$) while juice of stem exhibited least antibacterial activity ($15.82 \text{ mm} \pm 3.56 \text{ SD}$) (Table 1). It has been documented in the literature that *Mentha piperita* is used internally as a tea, tincture, oil or extracts, and applied externally as a rub or liniment. Herbalists consider it as an astringent, antiseptic, antipuritic, antipasmodic, anticatarrhal, antimicrobial, rubefacient, stimulant and emmenagogue (Gardiner, 2000). The principal active constituents of *Mentha piperita* 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 peripheral blood vessels and inhibit bacteria. Its oils especially menthol have a broad spectrum antibacterial activity since Gram +ve and Gram -ve bacteria were found susceptible to the oils (Pattnaik *et al.*, 1997).

Both pulp and skin of *M. charantia* were found effective against tested organisms with $16.16 \text{ mm} \pm 2.17 \text{ SD}$, and $15.88 \text{ mm} \pm 2.24 \text{ SD}$ average zone of inhibition respectively (Table 1). Its active constituents are 5-a-stigmasta-7, 25-dien-3-b-ol, elasterol and lanosterol which may be responsible for its antibacterial activity. Leaf extracts of *M. charantia* showed broad spectrum antimicrobial activity since various water, ethanol and methanol extracts of the leaves have exhibited antibacterial activities against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella*, *Streptobacillus*. Besides, extract of the entire plant has shown antiprotozoal activity against *Entamoeba histolytica* and its fruit extract has demonstrated antibacterial properties against *Helicobacter pylori*, the bacteria causing stomach ulcer. In addition to these properties, it has also been used as appetite stimulant, a treatment for gastrointestinal infection and to lower blood sugar in diabetics. Its use for the treatment of certain types of cancer and viral infections has also been reported (Derrida, 2003).

Juices of skin and seeds of *P. sativum* were found effective against tested organisms with $16.30 \text{ mm} \pm 2.02 \text{ SD}$ and $16.39 \text{ mm} \pm 3.16 \text{ SD}$ average zone of inhibition respectively (Table 1). Pea is a major vegetable found in all temperate zone countries. Beside nutrient components, legume seeds are rich in phenolic antioxidants. Proanthocyanidine (condensed tannins) are the predominant phenolic compound. Legume seeds also contain phenolic acids (hydroxybenzoic and hydroxycinnamic acid) (Troszynska & Ciska, 2002) and phenolic phytochemicals, phytoalexins (Ho *et al.*, 2003). In this connection, Ho *et al.*, (2003) also reported that Phytoalexins in phenolics extract of sprouted pea (*Pisum sativum*) contributed to the antimicrobial activity against *Helicobacter pylori*. *P. sativum* has value in the treatment of acne. For this purpose, face masks made from crushed fresh fruits are used in cases of acne and faded, wrinkled skin (Chiej, 1988). However, substantial research about its antibacterial properties against other bacteria is lacking in the literature.

As the work for the development of herbal medicines is in progress worldwide, the present report will help in the isolation of new products/drugs. Besides, the same may also be used for self medication in domestic settings.

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