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Short Communication

Antibacterial potential of extracts of leaves of *Parrotia persica*

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Ethanol, methanol, chloroform, petroleum ether and aqueous extracts of leaves of *Parrotia persica* were evaluated for antibacterial activity. The zone of inhibition varied from 13 to 22 mm. The highest inhibition was obtained with methanol and ethanol. Chloroform and petroleum ether extracts did not show any activity. The minimal inhibitory concentration (MIC) value of the methanol extract for the test bacteria ranged between 3.12 and 6.25 mg/ml and that of ethanol extract ranged between 6.25 and 12.5 mg/ml. The results scientifically validate the use of this plant in the traditional medicine of Iran.

Key words: *Parrotia persica*, antibacterial activity, methanol extract, traditional medicine.

INTRODUCTION

Parrotia persica (DC) CA. Mey (Persian Ironwood) is a deciduous tree of the family Hamamelidaceae, the sole species in the genus *Parrotia*. It is native to northern Iran, and endemic to the Alborz Mountains. Many herbalists use this plant in the treatment various fevers and respiratory infections. It is also used for food coloring and food flavoring (Rechinger, 1999). There are no scientific reports on the antibacterial properties of the plant. Such lack of scientific knowledge has been major constraint in the use of traditional herbal remedies (Patwardhan et al., 2004). Hence, the aqueous and solvent extracts of leaves of *P. persica* were quantitatively screened for antibacterial activity against five important human pathogenic bacteria, obtained from Microbial Type Culture Collection and Gene bank (MTCC) India.

MATERIALS AND METHODS

Plant material

Apparently healthy leaves of *P. persica* were collected from the mountainous region of Mazandaran province of I.R. Iran during November 2005. The voucher specimen is deposited in the herbarium of Department of Studies in Botany and Microbiology, University of Mysore, Mysore, India.

Extraction of plant materials

The leaves of *P. persica* were washed thoroughly three times with running water and once with distilled water. The materials were air dried under shade and powdered. The shade dried leaves and powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Aqueous extraction

Samples (20 g) of thoroughly dried leaves were macerated with 40 ml sterile water in a waring blender for 10 min. The macerate was first filtered through double-layered muslin cloth, and then centrifuged at 4000 rpm for 10 min. The supernatant was filtered through Whatman No.1 filter paper. The extract was preserved aseptically in a brown bottle at 5ºC until further use.

Solvent extraction

Sample (20 g) dried powder was filled in the thimble and extracted successively with chloroform, ethanol, methanol and petroleum ether in Soxhlet extractor until the extract was clear.

Phytochemical screening

The methanol extract, which was antibacterially active, was selec-

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Table 1. Antibacterial activity of leaves of *Parrotia persica* extracts at 100 µl.

Bacteria	Zone of Inhibition (mm)					
	Aqueous extract	Chloroform extract	Ethanol extract	Methanol extract	Petroleum ether extract	Gentamycin 10 (µg)
E. coli	13.0±04	0.0	14±0.5	15±0.4	0.0	34±0.
K. pneumoniae	16±0.3	0.0	15±0.4	22±0.2	0.0	30±0.4
P. aeruginosa	15±0.4	0.0	18±0.3	17±053	0.0	31±0.5
S. typhi	15±0.6	0.0	22±0.2	21±0.6	0.0	30±0.3
S. aureus	16±0.5	0.0	16±0.5	20±0.5	0.0	33±0.5

Values presented are means of six replicates ± standard error. When subjected to analysis of variance (ANOVA), $p < 0.05$.

Table 2. Minimal inhibitory concentration (MIC) of methanol extract of *P. persica* against the test bacteria.

Bacteria	Zone of Inhibition in mm							
	MIC of methanol extract (mg/ml)							Gentamycin (10 µg)
	50	25	12.5	6.25	3.12	1.56	0.78	
E. coli	9±0.3	5±0.4	3±0.3	2±0.2	0.0	0.0	0.0	34±0.7
K. neumoniae	17±0.5	14±0.4	11±0.2	8±0.4	4±0.4	0.0	0.0	30±0.9
P. aeruginosa	13±0.3	8±0.6	5±0.3	3±0.3	2±0.5	0.0	0.0	31±0.3
S. typhi	15±0.3	11±0.7	6±0.3	4±0.4	2±0.3	0.0	0.0	30±0.3
S. aureus	9±0.2	6±0.2	4±0.7	3±0.2	2±0.4	0.0	0.0	33±0.2

Values presented are means of six replicates ± Standard error. When subjected to analysis of variance (ANOVA), $p < 0.05$.

ted for phytochemical analysis. The dried extract was first reconstituted in methanol and subjected to standard phytochemical analysis for the presence of flavonoids, tannins, saponins, carbohydrates, terpenoids and protein (Harborne, 1988).

Test bacterial strains

Standard type cultures of *Escherichia coli* (MTCC 443), *Klebseilla pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 737) were obtained from MTCC Chandigrah, India. All the test strains were subcultured on Muller Hinton agar (MHA).

Antibacterial activity assay

The extracts were spot checked for antibacterial activity employing the agar well diffusion technique (Okeke et al., 2001). Standardized inoculum (5×10^5 cfu/ml) of each test bacterium was spread on to sterile MHA plates so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. The dried extracts were reconstituted in methanol to a concentration of 50 mg/ml. Subsequently, a 100 µl volume of the extracts were introduced in triplicate wells into the MHA cultures. The plates were allowed to stand for 1 h. for diffusion to take place and then incubated at 37ºC for 24 h. The zone of inhibition was recorded to the nearest mm. Only the extracts exhibiting apparent zone of inhibition were chosen for further evaluation. For comparative evaluation the antibiotic Gentamycin (10 µg) was used.

Determination of minimal inhibitory concentration (MIC)

The MIC was determined only for the methanol extract by agar well diffusion method as it is showed a wider spectrum of antibacterial activity compared to others. A two-fold serial dilution of the extract was prepared by first reconstituting in methanol then diluting in sterile distilled water to achieve a decreasing concentration range of 50 to 0.781 mg/ml. A 100 µl volume of each dilution was introduced in triplicate wells into MHA plates already seeded with the standardized inoculums (5×10^5 cfu/ml) of the test bacterial cells. All test plates were incubated at 37ºC for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

RESULTS

The results of the antibacterial activity of the extracts are recorded in Table 1. The aqueous extract, ethanol extract and methanol extract of leaves of *P. persica* showed good activity against all the test bacteria. The chloroform and petroleum ether extracts did not show any activity against the test bacterial strains. The methanol extracts of the leaves recorded activity to all standard cultures with highest mean zone of inhibition. The MIC value of methanol extract was 3.12 mg/ml for *K. pneumoniae*, *P. aeruginosa, S. typhi* and *S. aureus* and 6.25 mg/ml for *E. coli* (Table 2).

The MIC values of ethanol extract for *K. pneumoniae*

Table 3. MIC of ethanol extract of *P. persica* against the test bacteria.

Bacteria	Zone of Inhibition in mm							
	MIC of ethanol extract (mg/ml)							Gentamycin (10 µg)
	50	25	12.5	6.25	3.12	1.56	0.78	
E. coli	8.0±0.3	4.5±0.4	3±0.3	0.0	0.0	0.0	0.0	33±0.7
K. neumoniae	11±0.5	8±0.4	4±0.2	2.0±03	0.0	0.0	0.0	29±0.9
P. aeruginosa	12±0.3	7±0.6	4±0.3	2.0±02	0.0	0.0	0.0	30±0.3
S. typhi	13±0.3	8±0.7	4±0.3	2.0±03	0.0	0.0	0.0	30±0.3
S. aureus	7±0.2	4±0.2	2±02	0.0	0.0	0.0	0.0	30±0.2

Values presented are means of six replicates ± Standard error. When subjected to analysis of variance (ANOVA), $p < 0.05$.

Table 4. Phytochemical analysis of methanol extract of *P. persica.*

Constituent	Methanol extract
Alkaloids	-
Carbohydrates	+
Flavonoids	+
Gum	+
Phenolics	+
Proteins	+
Saponins	+
Tannins	+
Terpenoids	+
Waxes	-

+ Present, - absent.

was 6.25 and 12.5 mg/ml for *E. coli* and *S. aureus* (Table 3). The methanol extract of leaves of *P. persica* was antibacterially active to *K. pneuminiae* with highest mean zone of inhibition (22 mm). The phytochemical analysis showed that the methanol extract of *P. persica* contained tannin, flavonoid, protein, saponin and carbohydrate (Table 4).

DISCUSSION

Review of literature reveals lack of information on the antibacterial potential of *P. persica*. Aqueous and different solvent extracts of leaves of *P. persica* evaluated for antibacterial potential, revealed highly significant activity of methanol extract against all the test bacteria followed by ethanol extract. None of the earlier reports have demonstrated the antibacterial potential of this plant (Okoli et al., 2002; Khan et al., 2002; Ahmet et al., 2005). In the present investigation the antibacterial activity of this plant has been demonstrated for the first time.

It is also evident from the results that methanol extract has significantly high degree of antibacterial activity, suggesting that methanol is the appropriate solvent for extraction of the antibacterial principle (Nkere and Iroegbu, 2005). Thus methanol is recommended for the large-scale extraction of the active principle (Taous et al., 2005).

Saponins and tannins are reported to possess antibacterial activity (Newman et al., 2000). Preliminary phytochemical analysis of the methanol extract of leaves of *P. persica* revealed the presence of saponin, tannin and flavonoid, which could be the active principle. Further investigations are in progress to isolate and characterize the active principle. This study scientifically validates the use of this plant for treatment of fever and other respiratory infection in traditional medicine of Iran.

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