

ANTIBACTERIAL PROPERTY OF FRUITS OF *PARROTIA PERSICA* AGAINST SOME HUMAN PATHOGEN

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Summary

Ethanol and methanol, extracts of fruits of *Parrotia persica* were evaluated for antibacterial activity. The zone of inhibition varied from 4.5 to 19.5 mm. The highest inhibition was obtained with methanol.

The minimal inhibitory concentration (MIC) value of the methanol extract for the test bacteria ranged between 3.12 and 6.25 mg/ml. The results scientifically validate the use of this plant in the traditional medicine of Iran.

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

Introduction

Parrotia persica (family Hamamelidaceae) is an important plant used the treatment of bone fracture and for treatment of some infections in the traditional medicine of Iran (1&2). Hence, solvent extracts of fruits of *P. persica* were quantitatively screened for antibacterial activity against five important human pathogenic bacteria,

Hot extraction is more efficient, where the plant material is boiled in a solvent for a stipulated period. Soxhlet extractor is the ideal choice for hot extraction where with minimum solvent complete extraction is effected (3). Concentrating the extracts need careful consideration. Since nearly all of the identified compounds from plants which are active against microorganisms are aromatic or saturate organic compounds, they are most often obtained through initial ethanol or methanol extraction.

Materials and Methods

The fruit is a two-parted capsule (Fig. 1) containing two seeds; one in each half.

Fig;1. *Parrotia persica* DC.C.A.Mey.

(Fruits)

Preparation of extracts

Apparently healthy fresh plant material (50g) was thoroughly washed and then dried lab temperature. Two solvents, ethanol and methanol were selected for the study. Solvent extraction was done by using Soxhlet extraction apparatus. Powdered material was placed in a porous thimble in the upper chamber. In the lower boiling flask the extracting solvent was added. The boiling flask was heated by thermostat controlled heating mantle. The temperature of the heating mantle was maintained at the boiling point of the solvent used for extraction. The solvent is heated to reflux the distillate as it drops from the condenser collects in the chamber. By coming in contact with the solid in the thimble, the liquid effects the extraction. After chamber fills to the level of the upper reach of the siphon arm, the solution empties from this chamber into the boiling flask by a siphoning action. This process is continued automatically and without attendance for as long as is necessary for effective removal of the desired component (3). The solvent thus collected in the boiling flask was removed and concentrated under reduced pressure in a rotary flash evaporator. The process was continued until all the solvent was removed and only the extract remained. This extract was collected in a brown bottle and stored at 50 C until further use. The extracts were dissolved in methanol (1:10 w/v) before subjecting to antibacterial activity assay.

Test bacteria

Standard type culture of *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 737) were obtained from Microbial Type Culture Collection (MTCC) Chandigarh India. All test strains were re-isolated three successive times on Mueller Hinton Agar.

Antibacterial activity assay

Antibacterial activity of the solvent plant extracts determined by the cup diffusion method(4) on Mueller Hinton agar medium (MHA).

One gram each of concentrated solvent extract of methanol and ethanol was dissolved in 9 ml methanol and ethanol. The sterile MHA medium (15ml) in Petri dishes was uniformly smeared with test culture of human test pathogenic bacteria.

Wells (5 mm) were made in each Petridis to which 50 μ l of solvent extracts were added. For each treatment five replicates were maintained. Methanol served as control. All the plates were incubated for 24 h at 37 $^{\circ}$ C and zone of inhibition if any around the wells was measured in mm (millimeter). The data was subjected to statistical analysis.

Antibiotic

Gentamycin, (Cartridge at 10mcg/disc obtained from Paten Teb of Iran) generally used in the management of human pathogenic bacteria was selected for antibacterial activity assay by disc diffusion method for comparative evaluation.

Results

The antibacterial effect of methanol and ethanol extract of fruits of *P. persica* is presented in Table 1.1 and 1.2 respectively. It was observed that *E. coli*, *Salm. typhi* and *Staph. aureus* were sensitive to the methanol extract at 10 μ l onwards, whereas, for *Kl. Pneumoniae* and *Ps. aeruginosa* it was 20 μ l onwards. Higher activity was observed at 50 μ l concentration against all the test bacteria. *Salm. typhi* recorded highest sensitivity and the zone of inhibition was 19.50 mm, more than 30 mm of zone of inhibition was observed in all the test bacteria treated with Gentamycin (Table 1.1).

Table 1.1. Antibacterial activity of methanol extract of fruits of *Parrotia persica* against test human pathogenic bacteria

Bacteria	Zone of Inhibition in mm							F-value
	10(μ l)	20(μ l)	30(μ l)	40(μ l)	50(μ l)	Gm*	control	
<i>E. coli</i>	5.50. \pm 0.00	8.40. \pm 0.00	11.50 \pm 0.56	14.35 \pm 0.25	16.20 \pm 0.45	31.50 \pm 0.40	0.00 \pm 0.00	232.112
<i>Kl. pneumoniae</i>	0.00. \pm 0.00	6.40. \pm 0.50	9.25. \pm 0.30	11.45 \pm 0.50	13.25 \pm 0.50	30.25 \pm 0.50	0.00 \pm 0.00	242.214
<i>Ps. aeruginosa</i>	0.00. \pm 0.00	6.20. \pm 0.50	10.20. \pm 0.50	12.50 \pm 0.45	14.25 \pm 0.32	31.25 \pm 0.44	0.00 \pm 0.00	341.422
<i>Salm. typhi</i>	10.50 \pm 0.56	13.55 \pm 0.35	15.25 \pm 0.45	17.50 \pm 0.30	19.50 \pm 0.55	33.50 \pm 0.55	0.00 \pm 0.00	320.358
<i>Staph. aureus</i>	8.40 \pm 0.50	10.30 \pm 0.40	13.45 \pm 0.65	15.25 \pm 0.24	18.50 \pm 0.25	34.45 \pm 0.53	0.00 \pm 0.00	420.631

The values are mean of five replicates \pm standard error. Note: Zone of inhibition was 0.00 in methanol control in all concentrations against all the bacteria. Gm*= Gentamycin 10 μ g/disc

Table 1.2. Antibacterial activity of ethanol extract of fruits of *Parrotia persica* against test human pathogenic bacteria

Bacteria	Zone of Inhibition in mm							F-value
	10(μ l)	20(μ l)	30(μ l)	40(μ l)	50(μ l)	Gm*	control	
<i>E. coli</i>	0.00. \pm 0.00	4.250. \pm 0.30	7.25 \pm 0.46	10.35 \pm 0.55	13.45 \pm 0.25	30.50 \pm 0.450	0.00 \pm 0.00	421.102
<i>Kl. pneumoniae</i>	0.00. \pm 0.00	4.50. \pm 0.35	7.20. \pm 0.50	10.35 \pm 0.40	14.32 \pm 0.42	31.45 \pm 0.51	0.00 \pm 0.00	435.236
<i>Ps. aeruginosa</i>	0.00. \pm 0.00	0.00. \pm 0.00	5.40. \pm 0.25	8.55 \pm 0.36	11.25 \pm 0.36	30.55 \pm 0.45	0.00 \pm 0.00	457.132
<i>Salm. typhi</i>	0.00. \pm 0.00	6.50 \pm 0.45	9.50 \pm 0.35	12.30 \pm 0.40	15.25 \pm 0.45	32.50 \pm 0.255	0.00 \pm 0.00	345.321
<i>Staph. aureus</i>	4.50 \pm 0.50	9.45 \pm 0.43	10.25 \pm 0.55	12.35 \pm 0.33	15.40 \pm 0.55	33.35 \pm 0.23	0.00 \pm 0.00	250.435

The values are mean of five replicates \pm standard error. Note: Zone of inhibition was 0.00 in ethanol control in all concentrations against all the bacteria. Gm*= Gentamycin 10 μ g/disc

It was observed that except *Staph. aureus* no activity was observed against the other test bacteria at 10 µl concentration. Higher activity was observed at 50 µl concentration against all the test bacteria. *Salm. typhi* and *Staph. aureus* were highly sensitive followed by *E. coli*, *Kl. Pneumoniae* and *Ps. aeruginosa* at 50 µl concentration. Gentamycin showed highest activity against all the test bacteria with zone of inhibition of more than 30 mm.

Minimal inhibitory concentration of the methanol extract of fruits of *P. persica* against test human pathogenic bacteria is presented in Table 1.3. *Ps. aeruginosa* was fairly resistant and the MIC value was 12.5 mg/ml, whereas, for *Kl. Pneumoniae*, *E. coli* and *Staph. aureus* it was 6.25 mg/ml. *Salm. typhi* was highly sensitive and the MIC value 3.12 mg/ml (Table 1.3). Gentamycin recorded significant antibacterial activity and the zone of inhibition against all the test bacteria was more than 30 mm.

Table 1.3. MIC of methanol extract (mg/ml) of fruits of *Parrotia persica* against test human pathogenic bacteria

Bacteria	Zone of Inhibition in mm						
	50	25	12.5	6.25	3.12	1.56	0.78
<i>E. coli</i>	16.20 ±0.30	10.54±0.55	7.34±0.50	4.20±0.50	0.00±0.00	0.00±0.00	0.00±0.00
<i>Kl. pneumoniae</i>	18.25 ±0.45	11.50±0.25	7.30±0.40	4.50±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Ps. aeruginosa</i>	14.25 ±0.46	8.55±0.25	4.55±0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
<i>Salm. typhi</i>	19.55 ±0.45	14.25±0.50	11.40±0.24	7.50±0.40	4.35±0.40	0.00±0.00	0.00±0.00
<i>Staph. aureus</i>	18.50±0.45	12.505±0.56	9.47±0.45	5.55±0.53	0.00±0.00	0.00±0.00	0.00±0.00

The values are mean of five replicates± standard error, When subjected to analysis of variance (ANOVA), p<0.05.

Discussion

Higher plants have been the source of medicinal agents since times immemorial and they have continued to play an important role in the health care of majority of the world population (5). Plant based traditional medicine systems have been inexistence for thousands of years in countries such as china (6&7) and Iran (8). The potential of medicinal plants have not been utilized properly in Iran (9). A results methanol and ethanol extract of fruits of *P. persica* observed to be antibacterially active. thus in the present investigation the antibacterial potency of fruits of *P. persica* against important human pathogenic bacteria like *E. coli*, *Kl. pneumoniae*, *Ps. aeruginosa*, *Salm. typhi* and *Staph. aureus* have been demonstrated for the first time. The findings of the present investigations are important in scientific justification of the traditional use of *P. persica* in traditional medicine of Iran.

Methanol and ethanol extracts were antibacterially active. Methanol extract of fruits showed slightly higher activity than ethanol extract against all the test bacteria. The activity increased with the increasing concentration of all the extracts against all the test bacteria and thus, it was concentration dependent.

Kl. pneumoniae was highly sensitive to the methanol extract of *P. persica*, while *Salm. typhi* was highly sensitive to the ethanol extract, the MIC values of the methanol extract of fruits of *P. persica* was as low as 3.12 mg/ml against *Kl. pneumoniae*, *Ps. aeruginosa* and *Salm. typhi*. A similar results was also observed in the ethanol extract.

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References

- 1-Rechinger, K.H. . *Flora Iranica*.No. 48, Akademische Druck-U., Verlagsanstalt, Graz. 1987: Pp : 215-216.76.
- 2- Ghorbani, A.B. *Torkman Sahra of Herbal Drugs*. Publ. Research center of Traditional Medicine, Shahid Beheshti University, Tehran, Iran.2006: Pp: 69-70
- 3-Roberts, R.M., Gilbert, J.C., Rodewald, L.B. and Wingrove, A.S. *Modern experimental organic chemistry*. 3rd edition. Saunders golden sunbuest series: Saunders college (Philadelphia), and Holt- Saunders Japan (Tokyo). 1981: pp 495-505.
- 4- Satish, S., Raveesha, K. A. and Janardhana, G. R. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Letters in Applied Microbiology* 1999; 28, 145-147.
- 5- Anon. *Drugs and Pharmaceuticals- Industry Highlights incorporating Patent Information*, Central Drug Research Institute, Lucknow. 1998; 21, 33-34.
- 6-Chang, H.M. and But, P.P.H. *Pharmacology and Applications of Chinese Materia Medica*. World Scientific Publishing, Singapore, 1986; vol 1&2.
- 7-Kapoor, A. *Neem: The wonder plant*. *Pesticides Information* 2001; 27, 33-34
- 8-Bonjar, G.H.S. Antibacterial screening of plants used in Iranian folkloric medicine. *Fitoterapia* 2004; 75, 231-235.
- 9-Koocheki, A and Nadjafi, F. The status of medicinal and aromatic plants in Iran and strategies for sustainable utilization. The 3rd congress on Medicinal and Aromatic plants for Human welfare. Chiung Mai, Thailand. 2003;