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Full Length Research Paper

Phytochemical screening and antibacterial properties of leaves of *Pongamia pinnata* Linn. (Fabaceae) from India

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Herbal medicine is the most ancient form of health care known to man and its being used increasingly reveals it as dietary supplement to fight or prevent common diseases. Petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extracts of leaves of *Pongamia pinnata* Linn. were prepared and antibacterial activity were studied by disc diffusion method against certain enteric bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Salmonella typhi, Staphylococcus epidermidis* and *Proteus vulgaris.* The methanol extracts had wide range of antibacterial activity on these bacterial pathogens than the petroleum ether extract. Ethyl acetate extract was slightly higher antibacterial activity against bacterial pathogens than chloroform extract. Phytochemical screening of the plant leaves reveals the presence of carbohydrates, alkaloids, flavonoids glycosides, steroids, tannins and saponins. Antibacterial activity of various extract of leaves of *P. pinnata* was carried in attempt to support the use by medicinal practitioner for the treatment of enteric infection.

Key words: Antibacterial activity, *Pongamia pinnata,* enteric bacterial pathogens.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants. Herbs have always been principal forms of medicine in India and presently they are becoming popular throughout developing countries, as people strive to stay healthy in face of chronic stress and pollution and to treat illness with medicines that work in concert with body's own defense. India recognizes more than 2500 plant species which have medicinal values. However, large flora are waiting for investigation for their medicinal properties (Kirtikar and Basu, 1995).

Pongamia pinnata (L.), locally known as Karanja, is a mangrove plant belonging to the family, Fabaceae. It is a medium size glabrous tree with a short bole and attaining a height of round 18 m and its habitat is in the littoral regions of South East Asia, Australia and Fiji (Chopra et al., 1986; Simin et al., 2002). Traditionally, its bark is used in pile; leaves are effective as medicated bath and rheumatic pains; and the seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis (Ballal, 2005; Tanaka et al., 1992; Carcache et al., 2003). In primitive areas of Malaysia and India, root extracts are applied to abscesses; other plant parts, especially crushed seeds and leaves are regarded as having antiseptic properties (Burkill, 1966; NAS, 1980).

In India, seeds were used for skin ailments. Today the oil is used as a liniment for rheumatism; their juice is used for colds, coughs, diarrhea, dyspepsia, flatulence, gonorrhea, and leprosy. Roots are used for cleaning gums, teeth, and ulcers also effective in fistulous sores and gonorrhea (Rastogi and Malhotra, 2001; Chauhan et al., 2002). Ayurvedic medicine described the root and bark as alexipharmic, anthelmintic, and useful in abdominal enlargement, diseases of the eye, skin, and vagina, itch, piles, splenomegaly, tumors, ulcers, and wounds.

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The leaves are anthelmintic, digestive, and laxative, for inflammations, piles and wounds; while the fruit and seed used for keratitis, piles, urinary discharges, and diseases of the brain, eye, head, and skin (Brown,1995). Unani uses the ash to strengthen the teeth, the seed, carminative and depurative, for chest complaints, chronic fevers, earache, hydrocele, and lumbago (Ahmad et al., 2004; Yadav et al., 2004).

Enteric or diarrheal infections are major public health problems in developing countries and contribute to the death of 3.3 to 6.0 million children, annually. Enteric bacteria comprised *Salmonella* spp., *Shigella* spp*., Proteus* spp*., Klebsiella* spp., *Escherichia coli, Pseudomonas* spp*., Vibrio cholerae* and *Staphylococcus aureus* which are major etiologic agents of sporadic and epidemic diarrhea both in children and adults. WHO (1985, 1993) reported that 80% populations rely mainly on traditional therapies, involving the use of plant extracts or their active constituents (Tambekar et al*.,* 2007).

Today there is wide spread Interest in drugs derived from plants for their potential antibacterial activity. Efforts are directed to identify plant product used in the treatment of various disease, which have broad spectrum antibacterial properties (Pathak et al*.,* 1983*;* Parekh et al*.,* 2005*).* Therefore, literature reveals that the leaves of *P. pinnata* (L.), were used in various metabolic disorder, but their antibacterial properties were not demonstrated. Hence attempt was made to find out the phytochemical constitutes and antibacterial properties of leaves of *P. pinnata* (L.), against enteric bacterial pathogens.

MATERIALS AND METHODS

Plant materials

Fresh plant or plant parts of *P. pinnata* were collected from local region of Ahmednagar District in India. The leaves were identified by Mr P.S.N. Rao, Joint Director, Botanical Survey of India, Koregaon road, Pune by comparing morphological features (leaf arrangement, flower/inflorescence arrangement, fruit and seed morphology etc.). The herbarium of the plant specimen has been deposited at B.S.I. Pune, the voucher specimen No. being BRD1. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of extracts

1.5 kg of the plant material in each batch was exhaustively extracted by soxhlet extraction method using Petroleum ether, chloroform, ethyl acetate and methanol. The solvent used in each batch was recovered under pressure until dry extracts were obtained and then labeled and stored separately at 4°C in amber colored airtight bottles.

Phytochemical screening of plant materials

The presence of saponins, tannins, carbohydrates, alkaloids, flavornoids glycosides, steroids, proteins and alkaloids were detected by simple qualitative methods (Khandelwal, 2001).

Table 1. Bacterial cultures used in study (IMTECH, Chandigarh, India).

Bacterial Pathogens	MTCC Number
Proteus vulgaris	426
Staphylococcus epidermidis	435
Staphylococcus aureus	96
Escherichia coli	739
Pseudomonas aeruginosa	424
Bacillus subtilis	441
Klebsiella pneumoniae	109
Salmonella typhi	733
Enterobacter aerogenes	111
Salmonella typhimurium	98

Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacteria rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at 37°C for 3 h. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and turbidometer.

Antibacterial activity using disc diffusion method

The modified paper disc diffusion (NCCLS, 2000) was employed to determine the antibacterial activity of solvent extract of leaves of *P. pinnata* (L.). For antibacterial properties, 0.1 ml bacterial suspendsion of 10^5 CFU $ml^{-}1$ was uniformly spread on nutrient agar plate to form lawn cultures. The petroleum ether, chloroform, ethyl acetate and methanol extracts were prepared in their respective solvents in such a manner that ultimate amount (in dry form) in each disc came to 10, 8, 6, 4 and 2 mg. The blotting paper discs (10 mm diameter) were soaked in various diluted extract, dried in oven at 60°C to remove excess of solvent and tested for their antibacterial activity against bacterial pathogens by disc diffusion technique. After incubation of 24 h at 37°C, zone of inhibition of growth was measured in mm. Ampicillin 10 mcg (Hi-Media disc) was used as positive control while discs soaked in various organic solvents and dried were placed on lawns as negative control.

RESULTS AND DISCUSSION

Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way.

The photochemical investigation (Table 2) of the various solvent extract of *P. pinnata* showed the petroleum ether extract to contain only alkaloids and steroids in high concentrations. The chloroform extract and ethyl acetate extract contain similar photochemical constitutes,

Table 2. Phytochemical analysis of *Pongamia pinnata* leaves.

No	Phytochemical constituent	Extracting solvent			
		Petroleum ether	Chloroform	Ethyl acetate	Methanol
		+	+	+	+
2	Flavonoids	-	-	-	+
3	Carbohydrates	-	-	-	+
4	Glycosides	-	-	-	+
5	Amino acids	-	-	-	-
6	Saponins	-	-	-	+
7	Proteins	-	-	-	-
8	Steroids	+	+	+	+
9	Tannins	-	-	-	+

+ = presence, – = absence.

Table 3. Antibacterial activity of *Pongamia pinnata* leaves extracts against enteric bacterial pathogens.

Bacterial Pathogens	Zone of inhibition (mm)																								
	Petroleum ether					Chloroform					Ethyl acetate					Methanol					-ve controls				
	10*	8	6	4	2	10	8	6	4	2	10	8	6	4	2	10	8	6	4	2	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Ampicilin (10mcg)
P. vulgaris	21	19	17	16	15	17	16	15	14	13	18	17	15	13	12	22	20	18	17	16	-	-	-	-	16
S. epidermidis	22	19	18	16	14	14	13	12	11	-	14	13	12	-	-	20	18	17	16	15	-	-	-	-	25
S. aureus	25	23	22	19	17	20	19	17	15	14	15	14	13	12	11	24	22	20	18	17	-	-	-	-	24
E. coli	15	14	13	12	11	13	12	-	-	-	14	13	12	-		20	18	16	15	14	-	-	-	-	11
P. aeruginosa	14	13	12	-	-	13	12	-	-	-	13	12	11	-	-	18	17	15	14	12	-	-	-	-	16
B. subtilis	13	12	11	-	-	17	16	15	13	12	15	14	13	12	11	22	20	19	17	16	-	-	-	-	18
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	12	11	-	-	-	15	13	12	11	-	-	-	-	-	30
S. typhi	-	-		-	-	-	-	-	-	-	-	-	-	-	-	12	11	-	-	-	-	-	-	-	18
E. aerogenes	20	18	16	15	14	18	17	16	14	12	18	16	14	13	12	22	20	18	17	15	-	-	-	-	14
S. typhimurium	13	12	-	-	-	15	14	13	12	11	19	16	14	13	12	22	19	17	16	15	-	-	-	-	19

Values are average of 3 readings.
*Amount of extract in mg/disc.

alkaloids and steroids in high concentrations, but did not contain any carbohydrates, flavonoids, glycosides, saponins. Methanol extract contained alkaloids, steroids, flavonoids, glycosides, saponins and tannins, but did not contain any proteins.

According to the antibacterial profile (Table 3), the maximum inhibition of bacterial growth which the petroleum ether extracts observed was only on *Proteus vulgaris*, *Staphylococcus epidermidis*, *S. aureus*, and *Enterobacter aerogenes*, but mild inhibitory effect on *Pseudomonas aeruginosa*, *E. coli, Salmonella typhimurium* and no inhibitory effect on *Klebsiella pneumonia* and *Salmonella typhi.* Chloroform extract inhibited the growth of *S. aureus, P. vulgaris, S. epidermidis* and *S. typhimurium* but mild or negligible effect on *E. coli, P. aeruginosa, S. typhi* and *K. pneumonia.* Ethyl acetate extract shows maximum inhibitory effect on *S. aureus, P. vulgaris, S. typhimurium,* but no effect on *K. pneumonia* and *S. typhi.* Methanol extract shows maximum inhibitory effect on *S. aureus, P. vulgaris, S. epidermidis, E. coli, P. aeruginosa, E. aerogenes, S. typhimurium* but mild or negligible inhibitory effect on *S. typhi* and *K. pneumonia.*

Conclusion

The result of the antibacterial assay show promising evidence for the antibacterial effect of leaves of *P. pinnata*. Form the above evidence, it is clear that plant extracts have great potential as antibacterial compounds against enteric pathogens and that they can be used in the treatment of enteric infectious. This plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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