

Antimicrobial Activity of Different Extracts of *Pongamia pinnata*

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ABSTRACT

Plant material was collected from Western Ghats, India, shade-dried and extracted successively with different polarity solvents of petroleum ether and ethyl acetate. The extracts were tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria and against a fungal species using the disc-diffusion method. The antimicrobial activity of extracts of *Pongamia pinnata* was tested against bacterial strains such as *Bacillus subtilis* (NCIM 2117), *Escherichia coli* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2036), *Staphylococcus aureus* (NCIM 2079) and a fungal species *Candida albicans* (NCIM 3100). In both extracts of petroleum ether and ethyl acetate, the extracts from leaf, bark and seeds showed an inhibition zone on *B. subtilis* and *S. aureus* cultures. Extract from the seeds of petroleum ether and ethyl acetate showed maximum inhibition zone on *B. subtilis*, while the leaf extract of petroleum ether and ethyl acetate showed comparable activity against *S. aureus*. *E. coli* and *C. albicans* were unaffected by any of the extracts of *P. pinnata*, except for the bark extract of petroleum ether. These findings also support the use of this plant in traditional medicine for the treatment of bacterial and fungal infections.

Keywords: antimicrobial activity, crude extracts, disc-diffusion, Karanja, Leguminosae, medicinal plant

INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine in India, especially in rural areas. Thus, phytotherapy is practiced by a large proportion of the Indian population for the treatment of several physical, physiological, mental and social ailments. 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 reputation folklore in a more intensified way (Austin *et al.* 1999). Natural products of higher plants may be new sources of antimicrobial agents with possibly novel mechanisms of action (Hamil *et al.* 2003; Machado *et al.* 2003; Motsei *et al.* 2003; Barbour *et al.* 2004).

The extracts of various plants were used for the treatment of diseases, which forms the basis of all Indian systems of medicine (Krishna Kumar *et al.* 1997). Due to the lack of improper scientific proof, these fields have not been well developed. Scientific approaches in different fields of medicine have been increasing in day-to-day life. A new search to treat harmful diseases evolving from bacteria and other microorganisms is also in progress. Infectious diseases arising from microorganisms may be pathogenic harboring either the external or internal environment of the human body. Over the last 20 years a large number of plant species have been evaluated for their antimicrobial activities (Castello *et al.* 2002).

Pongamia pinnata is a common medicinal plant belonging to the family Leguminosae, grown all over India especially near the coast and extending from the Central to Eastern Himalayas to Ceylon. The seeds, leaves and oil derived from the seeds are all used in Hindu medicine as remedies for fever, skin diseases, piles, ulcers, bronchitis, whooping cough etc. Pongam oil showed inhibitory effects on few bacteria and contains bitter flavonoid constituents, pongapin and karanjin (Duke 1983). The common name of this plant is 'Karanja' and the oil derived from the seeds is known as Karanja oil. The plants have a capacity to produce a large



Fig. 1 A twig of *Pongamia pinnata* showing fruits.

number of organic chemicals of high structural diversity, the secondary metabolites (Castello *et al.* 2002). This paper reports the first attempt to study the antimicrobial activity of the various extracts of *P. pinnata* on some bacterial and fungal species.

MATERIALS AND METHODS

Plant materials

The plant materials were collected from Western Ghats of Karnataka India. The plant parts namely, leaf, bark, and seeds were thoroughly washed and shade-dried. Fifty g of each powdered plant material was extracted with different solvent fractions using a Soxhlet apparatus, and the obtained extracts were evaporated using a vacuum evaporator to get the crude dried extract.

Preparation of plant extracts

Dried plant material (leaves, bark, and seeds) were extracted with petroleum ether and ethyl acetate and 500 µg of extracts were dissolved per ml of dimethyl sulfoxide (DMSO).

Microorganisms

The following microorganisms were used as test organisms: *Bacillus subtilis* (NCIM 2117), *Escherichia coli* (NCIM 2079), *Pseudomonas aeruginosa* (NCIM 2036), *Staphylococcus aureus* (NCIM 2079) and *Candida albicans* (NCIM 3100). All cultures were obtained from the National Chemical Laboratory, Pune.

Antimicrobial assay

The disc-diffusion method (Jebanesan and Venkatachalam 2002) was used to determine the antimicrobial activity. Nutrient agar medium (peptone 5 g/l, beef extract 3 g/l, sodium chloride 8 g/l, agar-agar 20 g/l, pH 7.2) was inoculated with microbial cell suspension (200 µl in 20 ml medium) and was poured into sterilized Petri dishes (90 mm diameter). In order to identify antifungal activity of extracts against fungal pathogen, the Disc-diffusion assay was performed in Potato Dextrose Agar culture media (pH 6.5). Fungal cells were obtained by centrifugation at 1500 x g/4°C for 15 min and diluted in Phosphate Buffer Saline (PBS), pH 7.2. Cell count was taken using a Hemacytometer after loading 10 µl of the cell suspension in PBS and the number of cells/ml was calculated, the final concentration of each strain was 106 cells/ml. Sterile filter paper discs 6 mm in diameter were impregnated with 20 µl extract solution (500 µg dissolved per ml of DMSO) and after evaporation placed on the surface of the inoculated agar plates. Penicillin and streptomycin (Janssen-Cilag Pharmaceuticals, Bangalore, India) were used as positive controls. Negative controls were done using paper discs loaded with 20 µl of the solvents. Later bacterial culture inoculated plates were incubated at 34 ± 2°C for 48 hours and for fungi, 72 hours. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones.

Statistical treatments

The results of these experiments are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) and mean separations were carried out using Duncan's Multiple Range Test (DMRT, Gomez and Gomez 1984) followed by Tukey's multiple comparison tests to assess the statistical significance. P ≤ 0.05 was considered as statistically significant, using statistical software SPSS ver. 11 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

The paper describes the antimicrobial activity of *P. pinnata* against bacterial and fungal strains. The results of the antimicrobial activity of the investigated extracts are shown in **Table 1**. The antimicrobial activity of the extracts was aimed at mainly from the seeds extract of both the solvent on *B. subtilis* and *S. aureus*. None of the extracts showed

antimicrobial activity against *E. coli* and *C. albicans* except for the bark extract of petroleum ether which exhibited a zone of inhibition on *E. coli* (16.00 ± 2.65 mm). Generally, among the tested extracts the petroleum ether and ethyl acetate extracts of seeds exhibited significant antibacterial effect on both *B. subtilis* and *S. aureus* when compared to antibiotics penicillin and streptomycin and in the petroleum ether extract of seeds showed comparable activity against *P. aeruginosa*, whereas no activity was observed in ethyl acetate extract of seeds. In the bark extract of both ethyl acetate and petroleum ether solvents comparable activity against *B. subtilis* was observed. The ethyl acetate extracts showed less significant activity on *Staphylococcus aureus* compared to antibiotics. The most pronounced activity with inhibition zones of more than 70 mm was shown by the ethyl acetate extracts of seeds of *P. pinnata*. The majority of the extracts of seeds were shown strong antimicrobial activity. The leaf, seed and bark extracts showed maximum inhibition zones on Gram-positive bacteria *B. subtilis*. The petroleum ether and ethyl acetate bark extracts showed comparable activity against *S. aureus*. Generally the Gram-positive bacteria are more susceptible due to an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt 1971). In contrast, the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents. So the maximum inhibitory activity was observed in Gram-positive bacteria *S. aureus* and *B. subtilis*. In Gram-negative *P. aeruginosa* the zone of inhibitory activity was less significant because of multilayered phospholipidic membrane carrying the structural lipopolysaccharide components (Nikaido and Vaara 1985). In spite of these barriers the seed extract of *P. pinnata* was effective in controlling the growth of pathogenic strains to a considerable extent. The antibacterial activity of Karanj (*Pongamia pinnata*) and neem (*Azadirachta indica*) seed oil *in vitro* against fourteen strains of pathogenic bacteria was assessed by Baswa *et al.* (2001), and it was observed that 57.14 and 21.42% of the pathogens were inhibited at 500 µl/ml; 14.28 and 71.42% at 125 µl/ml; and 28.57 and 7.14% at 250 µl/ml of Karanj and neem oils, respectively. The report suggests that the seeds of *P. pinnata* have an oil which has strong antimicrobial activity. Consequently the seed extracts exhibited a strong zone of inhibition on bacterial strains. The fractionation procedure to characterize and isolate the antibacterial active constituents needs to be carried out and the isolation of pure compounds from seeds and identification of the active compound is in progress.

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Table 1 Antimicrobial activity of different extracts of *Pongamia pinnata*.

Test sample	Extract type	Zone of inhibition (in mm)				
		<i>Bacillus subtilis</i> NCIM 2117	<i>Escherichia coli</i> NCIM 2079	<i>Pseudomonas aeruginosa</i> NCIM 2036	<i>Staphylococcus aureus</i> NCIM 2079	<i>Candida albicans</i> NCIM 3100
Petroleum ether	Leaf extract	32.33 ± 0.88 f	--	10.16 ± 0.44 n	17.66 ± 0.66 l	--
	Bark extract	26.00 ± 0.15 hi	16.00 ± 0.57 m	9.00 ± 0.28 n	--	--
	Seeds extract	72.00 ± 0.57 a	--	25.33 ± 0.88 i	45.00 ± 0.57 c	--
Ethyl acetate	Leaf extract	27.33 ± 0.33 h	--	--	21.16 ± 0.16 k	--
	Bark extract	31.66 ± 0.33 fg	--	--	31.67 ± 0.33 fg	--
	Seeds extract	72.00 ± 0.00 a	--	--	44.33 ± 0.33 c	--
Penicillin 10 µg		69.00 ± 0.57 b	20.00 ± 0.57 k	27.00 ± 0.57 hi	39.00 ± 0.57 e	nt
Streptomycin 10 µg		46.00 ± 0.57 c	23.00 ± 0.57 j	30.00 ± 0.57 g	41.00 ± 0.57 d	nt

--: No activity

nt: not tested

The values are the mean of three experiments ± S.E. Means followed by the same letter was not significantly different by the DMRT test at 0.05 % probability level.

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