

## Evaluation of Antifungal Properties of Leaves of *Pongamia pinnata* Linn. (Fabaceae)

Dahikar\* S.B. and Bhutada S.A.

Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopergaon, India-423603

\*[sbdahikar10@gmail.com](mailto:sbdahikar10@gmail.com)

### Abstract

In recent years, there has been gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries, because plant derived drugs have been reported to be safe without any side-effects. The Petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extracts of leaves of *Pongamia pinnata* Linn. were prepared and antifungal activity were studied by disc diffusion method against certain pathogenic fungi such as *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Microsporum gypseum* and *Trichophyton rubrum*. The Methanol extracts had wide range of antifungal activity on *Aspergillus niger* and *Aspergillus fumigatus*. Ethyl acetate extract has slightly higher antifungal activity than chloroform extract. Antifungal activity of various extract of leaves of *Pongamia pinnata* was carried out in attempt to support the use of these plants for developing plant-derived antimicrobial drugs.

**Keywords:** Antifungal activity, solvent extracts, *Pongamia pinnata*, *Aspergillus niger*, *Trichophyton rubrum*.

## Introduction

The use of plants as medicines is as old as human civilization itself. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been utilizing plants as effective medicines to cure many harmful diseases. India has been using crude plants as medicine since Vedic period [1, 2]. Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field [3].

*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 an eight of round 18 m and is habitat in the littoral regions of South East Asia, Australia and Fiji [1, 4]. Traditionally, its bark is used in pile; leaves are effective as medicated bath and rheumatic pains; seeds are used in hypertension, bronchitis, whooping cough, skin diseases and rheumatic arthritis [5, 6, 7]. 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 [8, 9].

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 [10, 11]. 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; the leaves, anthelmintic, digestive, and laxative, for inflammations, piles and wounds; the fruit and seed for keratitis, piles, urinary discharges, and diseases of the brain, eye, head, and skin [3]. Unani use the ash to strengthen the teeth, the seed, carminative and depurative, for chest complaints, chronic fevers, earache, hydrocele, and lumbago [12, 13].

Today there is wide spread interest in drugs derived from plants for their potential antimicrobial activity. Efforts are directed to identify plant product used in the treatment of various disease, which have broad spectrum antifungal properties [14, 15]. Therefore the study revealed that the leaves of *Pongamia pinnata* (L.), were used in various metabolic disorder, but far their antifungal properties were not demonstrated. Hence attempt was made to find out the antifungal properties of leaves of *Pongamia pinnata* (L.), against some pathogenic fungi.

## Materials and Methods

**Plant materials:** Fresh plant or plant parts of *Pongamia pinnata* were collected in September 2007 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 number being BRD1. Fresh plant materials were washed under running tap water, air dried for two week and then homogenized to fine powder and stored in airtight bottles.

**Preparation of extracts:** A 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 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, flavonoids glycosides, steroids, proteins and alkaloids, were detected by simple qualitative methods [16].

**Fungal cultures:** The standard pathogenic fungal cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The fungal culture rejuvenated in Sabouraud dextrose media (Hi-media laboratories, Mumbai, India) at 37°C for 18h and then stocked at 4°C in SDA. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 ml of sterile Potato Dextrose broth and incubated at 37°C for 24h. Turbidity of the culture was standardized to 10<sup>5</sup> CFU with the help of SPC and turbidometer.

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

Fungal Strain	MTCC Number
<i>Candida albicans</i>	183
<i>Aspergillus niger</i>	478
<i>Aspergillus fumigatus</i>	870
<i>Microsporum gypseum</i>	7675
<i>Trichophyton rubrum</i>	296

**Antifungal activity using disc diffusion method:** The modified paper disc diffusion [17] was employed to determine the antifungal activity of solvent extract of

leaves of *Pongamia pinnata* (L.). For antifungal properties, 0.1 ml fungal suspension of  $10^5$  CFU ml<sup>-1</sup> was uniformly spread on PDA 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 10mg, 8mg, 6mg, 4mg and 2mg. The blotting paper discs (10mm diameter) were soaked in various diluted extract, dried in oven at 60°C to remove excess of solvent and tested for their antifungal activity against fungal pathogens by disc diffusion technique. After incubation of 24 h at 37°C, zone of inhibition of growth was measured in mm. The antifungal activity was classified according to the zone of inhibition such as strong (19-22mm), moderate (15-18mm) and mild (11-14mm). Griseofulvin 10mcg (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 the medicinal plants, which have folklore reputation in a more intensified way.

The phytochemical investigation (Table 2) of the various solvent extracts such as petroleum ether, chloroform and ethyl acetate extracts of leaves of *Pongamia pinnata* contained only alkaloids and steroids in higher concentration, but did not contain any carbohydrates, flavonoids, glycosides, saponins. Methanol extract of leaves of *Pongamia pinnata* contained alkaloids, steroids, flavonoids, glycosides, saponins and tannins, but did not contain any proteins.

According to antifungal profile shown (Table 3), the petroleum ether extract exhibited strong inhibitory activity against *Candida albicans* and *Aspergillus niger*, but had a moderate antifungal activity against *Aspergillus fumigatus*, *Microsporum gypseum* and mild antifungal activity against *Trichophyton rubrum*. Chloroform extract showed moderate antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and mild antifungal activity against *Aspergillus niger*, *Microsporum gypseum*, *Trichophyton rubrum*. Ethyl acetate extract showed moderate antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, and mild antifungal activity against *Aspergillus niger*, *Microsporum gypseum* and *Trichophyton rubrum*. Methanol extract showed strong antifungal activity against *Candida albicans* and *Aspergillus niger* but had moderate antifungal activity against *Aspergillus*

*fumigatus* and mild antifungal activity against *Microsporum gypseum* and *Trichophyton rubrum*.

### CONCLUSION

The result of the antifungal assay showed promising evidence for the antimicrobial effect of leaves of *Pongamia pinnata*. From the above evidence; it is clear that plant extracts of *Pongamia pinnata* have great potential as antifungal compounds and that can be used in the treatment of fungal infections. This plant can be used to discover bioactive natural products that may be 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.

### ACKNOWLEDGEMENTS

The authors are very much thankful to Honorable Shri Amit Dada Kolhe, Managing Trustee, Sanjivani Rural Education Society's for providing all the facilities and support for this research work.

### References

1. Chopra, R.N., Nayar, S.L., Chopra, I.C., Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, CSIR Publications, New Delhi. C.S.I.R. (Council of Scientific and Industrial Research). 1948–1976. The wealth India 11 vols. New Delhi, 1986.
2. Rastogi, R.P. and Malhotra, B.N., Compendium on Medicinal Plants, Central drug Research Institute Lucknow and National Institute of Science Communication, New Delhi, India, 2001, 522 – 523.
3. Brown, D., Encyclopedia of Herbs and their Uses. Dorling Kindersley, D K Publishers, London, New York, 1995.
4. Simin, K., Ali, Z., Khaliq-Uz-Zaman, S. M. and Ahmad, V. U., Structure and biological activity of a new rotenoid from *Pongamia pinnata*. Nat. Prod. Lett. 2002, 16, 351-357.

5. Ballal, M., Screening of medicinal plants used in rural folk medicine for treatment of diarrhea, 2005. Internet: Http: // www.Pharmoinfo.net.
6. Tanaka, T., Iinuma, M., Yuki, K., Fujii, Y. and Mizuno M., Flavonoids in root bark of *Pongamia pinnata*. *Phytochemistry*, 1992, 31:993-998
7. Carcache Blanco, E.J., Kang, Y.H., Park, E.J., Su, B.N., Kardono, L.B.S., Riswan, S., Fong, H.H.S., Pezzuto, J.M. and Kinghorn, A.D., Constituents of the stem bark of *Pongamia pinnata* with the potential to induce quinine reductase. *J. Nat. Prods.* 2003, 66:1197-1202.
8. Burkill, J.H., Dictionary of economic products of the Malay peninsula. Art Printing Works, Kuala Lumpur. 2 Vol. medicinal plants. *Indian J. Hosp. Pharm.* 1996, 15(6):166-168.
9. N.A.S., Firewood crops. Shrubs and tree species for energy production. National Academy of science, Washington, DC 1980.
10. Kirtikar, K.R. and Basu B.D., *Indian Medicinal Plants*. Vol. 1, International book distributors, Dehardun, India, 1995, 830-832
11. Chauhan, D. and Chauhan, J.S., Flavonoid glycosides from *Pongamia pinnata*. *Pharm. Biology* 2002, 40:171-174.
12. Ahmad, G., Yadav, P.P. and Maurya, R., Furanoflavonoid glycosides from *Pongamia pinnata* fruits. *Phytochemistry* 2004, 65:921-924.
13. Yadav, P.P., Ahmad, G. and Maurya, R., Furanoflavonoids from *Pongamia pinnata* fruits. *Phytochemistry* 2004, 65:439-443.
14. Pathak, V.P., Saini, T.R. and Khanna, R.N., Isopongachromene, a chromenoflavone from *Pongamia glabra* seeds. *Phytochemistry* 1983, 22:308-309.
15. Parekh, J., Nair, R., Chanda, S., Preliminary screening of some folkloric plants from Western India for potential antimicrobial activity. *Indian J. Pharmacol.* 2005, 37: 408-409.
16. Khandelwal, K. R., Preliminary photochemical screening, in: *Practical Pharmacognosy Techniques and Experiments*. Nirali Publication, Pune 2001, 8<sup>th</sup> edn. 149-156.
17. NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for antimicrobial susceptibility testing. 8<sup>th</sup> Informational Supplement. M100 S12. National Committee for Clinical Laboratory Standards, Villanova, Pa, 2002.

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

Sr. No.	Phytochemical Constitutes	Solvent extract			
		Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract
1	Alkaloid	+	+	+	+
2	Flavonoids	-	-	-	+
3	Carbohydrates	-	-	-	+
4	Glycosides	-	-	-	+
5	Saponins	-	-	-	+
6	Proteins	-	-	-	-
7	Steroids	+	+	+	+
8	Tannins	-	-	-	+

+ =the presence of constitute, - = the absence of constitutes

**Table 3:** Antifungal activity of *Pongamia pinnata* leaves extracts against fungal pathogens (Zone of inhibition in mm, average of 3 readings)

Fungal pathogens	Petroleum ether extract					Chloroform extract					Ethyl acetate extract					Methanol extract					Negative controls				Griseofulvin (10mcg)
	10mg/disc	8mg/disc	6mg/disc	4mg/disc	2mg/disc	10mg/disc	8mg/disc	6mg/disc	4mg/disc	2mg/disc	10mg/disc	8mg/disc	6mg/disc	4mg/disc	2mg/disc	10mg/disc	8mg/disc	6mg/disc	4mg/disc	2mg/disc	Petroleum ether	Chloroform	Ethyl acetate	Methanol	
<i>C. albicans</i>	21	19	17	16	15	17	16	15	14	13	18	17	15	13	12	22	20	18	17	16	-	-	-	-	30
<i>A. niger</i>	22	19	18	16	14	14	13	12	11	-	14	13	12	-	-	20	18	17	16	15	-	-	-	-	25
<i>A. fumigatus</i>	18	17	16	14	12	15	14	13	12	-	15	14	13	12	11	18	17	15	14	12	-	-	-	-	22
<i>M. gypseum</i>	15	14	13	12	11	13	12	-	-	-	14	13	12	-	-	14	13	12	11	-	-	-	-	-	18
<i>T. rubrum</i>	14	13	12	-	-	13	12	-	-	-	13	12	11	-	-	14	13	12	-	-	-	-	-	-	20