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FUNGITOXIC PROPERTIES OF *Pongamia pinnata* (L) PIERRE EXTRACTS AGAINST PATHOGENIC FUNGI

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ABSTRACT:

Pongamia pinnata was investigated for its antifungal properties using different plant parts –leaves, bark, roots and seeds. The powdered plant parts were extracted in three different solvents namely distilled water, ethyl alcohol and ethyl acetate. Three different dilutions as 10, 5.0 and 2.5 were used for testing the activity. The plant parts were assessed for antifungal activity against two human pathogens: *Epidermophyton floccosum* and *Candida albicans* and two plant pathogens: *Alternaria solani* and *Helminthosporium turcicum.* The seeds of plants possessed highest antifungal activity followed by roots, bark and least activity was observed in the leaves. *Epidermophyton floccosum* was most susceptible to all the extracts closely followed by *Candida albicans* indicating the susceptibility to the components of the extracts. *Helminthosporium turcicum* was most resistant to all the extracts closely followed by *Alternaria solani* indicating the resistance to plant originated chemicals being familiar to such chemicals.

Keywords: *Pongamia pinnata, antifungal, Epidermophyton, Candida, Alternaria, Helminthosporium*

[I] INTRODUCTION

In the recent years, interest in novel, safe and effective antifungal agents has grown with increased incidences of fungal infections in immunocompromised patients. In the field of natural products with antimycotic activity, higher plants remain largely unexpected in comparison with microorganisms and marine invertebrates. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

Fungi cause important human and plant diseases especially in tropical regions. Despite the existence of potent antifungal agents, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of antifungal compounds. In an effort to discover new lead compounds screening of plant extracts to detect secondary metabolites with relevant biological activities was done.

Pongamia pinnata extract of leaves, barks, root and seeds possessed antifeedant activity and insecticides [1] anti-inflammatory activity [2]

antiplasmodial properties [3]. Simin *et al.,* (2001) reported methanolic extracts of the seeds of *Pongamia pinnata* possessed antibacterial and phytotoxicity properties [4]. Muruganandan *et al.,* (2000) reported the extracts of *Pongamia pinnata* seeds were used for anti-inflammatory activity [5]. Akhtar (1999) studied the hypoglycaemic effect of *Pongamia pinnata* (Sukhchain) flowers [6]. Das and Baghel (2000) made attempt to overcome the problem of recurrence of eczema by using the therapy like Panchatikta kashaya (for internal use) and Karanja taila (for local application) [7].

Shoba and Thomas (2001) evaluated the effect of aqueous and methanolic plant extracts of *Pongamia glabra* leaves for antidiarrhoeal potential against castor oil induced diarrhoea in mice [8]. Kurkure *et al.,* (2001) studied *Pongamia pinnata* stem bark therapeutically as an antiseptic in skin diseases and for its wound healing properties [9].

The aim of this study was to screen for selected extracts that could be useful for the development of new tools for the control of diseases caused by fungi to human and plants. Therefore, a systematic evaluation of extracts from the plant species - *Pongamia pinnata* against two human pathogenic fungi (*Candida albicans* and *Epidermophyton floccusum*) and two plant pathogenic fungal strains (*Alternaria solani* and *Helminthosporium turcicum*) was undertaken.

[II] MATERIALS AND METHODS

2.1 Collection of plants

The plants were collected from different regions of Maharshtra and were identified on the spot in the field by using flora of Marathwada [10].

2.2 Cultures

The fungal human pathogen cultures of *Candida albicans* and *Epidermophyton floccosum* were bought from Department of Pathology, SC Govt. Medical College, Nanded. The cultures of plant pathogens - *Alternaria solani* and *Helminthosporium turcicum* were bought from Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded. The cultures were preserved on Sabouraud Agar (*Candida albicans, Epidermophyton floccosum*) and Malt Extract Agar (*Alternaria solani, Helminthosporium turcicum*) and subculturing was done fortnightly.

2.3 Inoculum

Spore/cell suspension was prepared by adding sterile water to 8 day old for fungal on MEA/SA slant culture and 0.5ml of it was used as inoculum in all experiments. In every case spore suspension was standardized to contain 1 X 10^5 spore/ml by using haemocytometer. All the treatments /samplings were done in triplicates and results have been presented after repeating the experiment.

2.4 Preparation of Powder

The plant materials collected were processed and used in this study soon after collection. The plant parts collected were shredded and dried completely at 50ºC for 72 h. The dried materials were then ground into fine powder and stored in airtight containers at room temperature till extraction. Crude extracts were prepared from the same plants by extracting 2g dried material with 20ml distilled water, ethanol and ethyl acetate for 30 minute separately. Extracts were filtered through Whatman No 1 and dried under vacuum. The samples were then air dried and redissolved to 10 ml water for antifungal testing.

2.5 Extraction of Plant Tissue

The plant part powder was added to distilled water/ ethyl alcohol/ ethyl acetate and was allowed to boil for further 4-5 minutes on a water-bath under hood. 10ml of ethanol was used for every gram of powder. The extract was cooled and contents were homogenized thoroughly in a mortar and pestle. The extract was filtered by passing through several layers of muslin cloth. The residual ground powder was re-extracted by boiling in solvent used earlier for 3minute to ensure the complete removal of contents. The extracts were pooled, centrifuged at 5000 rpm

and the volume was adjusted to represent 10 ml/gram of fresh weight of tissue (ml/gfw).

2.6 Plant Extract for Spore Germination The extract was used to study the effect of content on spore germination. 5 ml of alcohol extract was evaporated on water bath under hood and slowly sterile distilled water was added to make up the volume to 5ml. This water extract was used to study the spore germination in host extract.

2.7 Spore Germination

The spore suspension for germination studies was prepared by adding 5 ml of sterile distilled water to a heavily sporulating 8 days old slant culture of fungus. The suspension was filtered through several layers of muslin cloth to reduce the mycelial fragments. Spores were washed with sterile distilled water several times by centrifugation to remove nutrients from original medium. The spores finally obtained were diluted in water and adjusted to the desired concentration. The spore concentration was measured by Haemocytometer with Naubauer Counting Chamber [11]. Hanging drop slides were prepared for observation from the suspension and incubated in petriplates lined with moist blotters. Germination percentage was calculated by observing a minimum of 200 spores from different microscopic fields.

The spore germination in plant extract was compared with systemic fungicide Carbendazim. 1gm of technical grade Carbendazim obtained form BASF, Mumbai was dissolved in 1000ml of sterile distilled water. This stock solution was used in spore germination studies.

2.8 Plant Extract for Antifungal Properties

Antifungal activity of the plant extracts (free from alcohol/ ethyl acetate and converted into aqueous) was evaluated by well-diffusion method expressed by zone of inhibition mm in diameter for *Candida albicans, Epidermophyton floccosum*.

The bioassay was carried out by using 1ml of inoculum ($1X10^6$ colony forming units) prepared from an overnight culture for given test fungi. 1ml of the resultant spore /cell suspension was poured in the petriplate and the plates were poured with respective medium to seed each prepared plate. The medium was allowed to solidify. Using a sterilized cork borer, wells of 5mm diameter were made in the solidified inoculated medium and the plate area uniformly. The wells were filled with 0.5ml of extract. Plates were then incubated aerobically at 37± 2 ºC for 72 h for fungi.

Similarly, wells containing standard concentration of Amphotericin B were used to compare the antifungal property of the plant extract. 1gm of Amphotericin B (Hi-media, Mumbai) was dissolved separately in sterile distilled water and 0.5ml was used to fill the wells.

[III] RESULTS

Pongamia pinnata (L.) Pierre (Verna. karanj) belongs to family- Fabaceae Lindl. It is a medium-sized semi-evergreen glabrous tree. The root is branched tap root system. The stem is greyish green or brown, smooth or covered with tubercles. The leaves are imparipinnate, 10-20 cm long, petioles 3-5 cm long, stipules small, oblong, leaflets opposite.

The root, bark, leaves, flower and seeds of *Pongamia pinnata* (L.) Pierre possesses medicinal properties and used as medicine by the india folks. The plant parts are used as medicine for the treatment of tumors, piles, skin diseases, wounds and ulcers [12]. In Ayurveda and Unani, the plant is used for anti-inflammatory, anti-plasmodial, anti-nonciceptive, anti-hyperglycamic, anti-lipidperoxidative, anti-diarrhoeal, anti-ulcer, anti-hyperammonic and antioxidant activity [13]. Literature available on the antifungal activity of this plant is very meagre. This work reports the antifungal properties against human as well as plant pathogens.

3.1 Antifungal activity of aqueous extracts of *P. pinnata* (L) Pierre against human pathogenic fungi

Antifungal activity of *P. pinnata* (L) Pierre aqueous, alcoholic and ethyl acetate extracts of various plant parts were tested against two human fungal pathogens *C. albicans* and *E. floccosum* by zone of inhibition method. The results obtained against various dilutions of aqueous extract and Amphotericin – B used as standard antifungal agents for comparison and as control, are presented in table 1.

It is obvious from the results presented in Table 1 that all the extracts of *P. pinnata* (L) Pierre exhibited antifungal activity. It was found that 10% aqueous, alcoholic and ethyl acetate extract were more inhibitory to both human pathogenic fungi and with increase in dilution, zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The seed extract was more effective in both the test organisms compared to control. The 10% seed alcoholic extract gave a 19 mm zone of inhibition in case of *E. floccosum* which was more than control. The seed extract was more effective in both the test organism compared to control.

3.2 Antifungal activity of aqueous extracts of *P. pinnata* (L) Pierre against plant pathogenic fungi

Antifungal activity of *P. pinnata* (L) Pierre aqueous, alcoholic and ethyl acetate extracts were tested against two plant pathogen *A. solani* and *H. turcicum* by inhibition of spore germination method after 60 min. The results obtained against various dilutions of aqueous extracts and Carbendazim used as standard antifungal agents for comparison and as control, are shown in table 2.

As showed in Table 2 that aqueous, alcoholic and ethyl acetate extracts of *P. pinnata* (L) Pierre exhibited antifungal activity. Antifungal activity in terms of percent inhibition of spore germination of test fungi namely *A. solani* and *H. turcicum* shows that 10% aqueous extracts have shown maximum inhibition of spore germination and in 2.5% aqueous extracts minimum inhibition of spore germination was recorded. However, it was slightly lesser than synthetic fungicide i.e. Carbendazim. The ethnolic extract also shows slightly less inhibition than control while 10% ethyl acetate was almost equally effective as control i.e. Carbendazim.

		Aqueous				Alcoholic				Ethyl Acetate			
Plant parts		Leaves	Bark	Root	Seeds	Leaves	Bark	Root	Seeds	Leaves	Bark	Root	Seeds
C. albicans	10%	4	6	9	11	6	8	10	13	16	18	20	23
	5%	3	5	8	8	5	6	8	11	14	15	17	19
	2.50%	2	4	7	8	4	4	6	8	12	13	15	18
	C	14	14	14	14	14	14	14	14	14	14	14	14
E. floccosum	10%	9	11	12	14	13	14	17	19	20	22	24	26
	5%	6	9	11	12	11	12	16	17	18	20	22	25
	2.50%	4	7	9	10	12	9	12	14	16	18	20	23
	C	12	12	12	12	12	12	12	12	12	12	12	12

Table 1: Effect of *P. pinnata* (L) Pierre aqueous extracts on growth of test fungi

		Aqueous				Alcoholic				Ethyl Acetate			
		Leaves	Bark	Root	Seeds	Leaves	Bark	Root	Seeds	Leaves	Bark	Root	Seeds
A. solani	10%	77	73	78	67	77	76	74	70	81	78	74	73
	5%	75	72	77	65	75	74	72	68	78	77	75	72
	2.50%	74	69	74	63	74	72	70	67	76	75	74	71
	C	81	81	81	81	81	81	81	81	81	81	81	81
H. turcicum	10%	75	72	73	65	75	73	71	67	78	75	73	71
	5%	74	70	72	63	74	71	70	65	76	74	72	70
	2.50%	72	68	71	61	72	70	68	64	74	72	71	68
	C	83	83	83	83	83	83	83	83	83	83	83	83

Table 2: Effect of *P. pinnata* (L) Pierre aqueous extracts on spore germination of test fungi

[IV] DISCUSSION

The aim of study was with two goals (a) to find out efficient pesticides against important plant pathogens (b) to discover efficient antidermatophytes against human pathogens. Both the aims are important form the point of view of agriculture and public health sectors. In agriculture use of synthetic fungicides is creating numerous problems of pollution and disturbance in ecosystem. As regarding the human pathogens the aim was evolve a chief source of antidermatophytes for the use of common people. All these goals have been partially achieved in the present study.

Many antifungal screening studies use a relatively large number of microorganisms for testing. From the data it could be seen that if the results of only four test organisms (*Candida albicans, Epidermophyton floccosum, Alternaria solani* and *Helminthosporium turcicum*) are considered, the number of extracts not showing activity would be higher from 14 to 19. Hence, reducing the number of test organisms would have resulted in the failure to retrieve valuable informations. However, the number of organisms used in a screening often must be restricted due to resource limitations [14]. In our study 4 extracts show activity against all the test organisms. This means that the plant part used and the type of extraction might have resulted in activity in the test performed for this study. Earlier workers tested plant extracts at higher doses in initial screens than reported here. Preliminary antifungal activities have been detected under test conditions with crude extract concentrations as high as 20 000 mg:ml using an agar well diffusion assay [15].

It was clear that *Pongamia pinnata* leaf extracts exhibited maximum activity against the selected test fungi. The test system to asses the antimicrobial properties of the extract was extended to the fungal pathogen of plants as well as human beings.

It is clear that the leaf extract of all the plants showed activity against all the test microorganisms. However, the activity of leaf was relatively less than the activity of bark of the plants. Similarly, the activity of bark was comparatively less than the root extracts while the seed extracts possess the highest activity in all the four plants studied. These results confirm the observations made by various workers with different plant and plant parts and test systems [16, 17, 18].

In this study, the powders of various plant parts were initially extracted in distilled water, the aqueous extract showed considerable activity against all the test microorganisms. But these powders were subjected to extraction using two mostly used solvent in phytochemistry viz.

ethanol and ethyl acetate. The effect of extraction using two different solvent lead to enhance activity of these extracts. The activity in ethanol extract was higher than aqueous extraction, while ethyl acetate extraction gave greater activity than that of alcoholic extracts. This was true for all the plant parts used for all the four plants.

Initial screenings of plants for possible antimicrobial activities typically begin by using crude aqueous extractions and then followed by alcohol and then by various organic extraction methods. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides (e.g., starch) and polypeptides, including fabatin [19] and various lectins, are commonly more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by the treatment with less polar solvents.

Recently, Eloff (1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants, as well as other factors such as their relative ranking as biohazards and the ease of removal of solvent from the fraction. The study records similar observations made by different workers [18, 20, 21, 22, 23, 24].

These extracts showed better activity against the human pathogenic fungi than the plants pathogenic fungi. This may be due to the reason that plant pathogen are often exposed to the plant originated antifungal compounds like phenols, flavonoids, coumarins, alkaloids etc. whereas human pathogen are new to such compounds. The results support the observation that human pathogen fungi are susceptible to plant extracts whereas plant pathogenic fungi are relatively more resistant [25, 26, 27, 28, 29, 30, 31,32].

The broad spectrum-antifungal activity of some of the plants used for the treatment of various skin diseases appears to have justified this belief. The antifungal activity of the plant extracts was found mainly against the *C. albicans* and *E. floccosum* while *A. solani* and *H. turcicum* appear to be resistant to the plant extracts. Similar observations have been reported by several workers [33, 34, 35] and are supported by this study. These observations justify their usage for the treatment of skin infections by the indigenous people.

The main challenge nowadays is the search for plants with promising antifungal activities and the isolation of active principles. There are many difficulties which seriously impede this type of analysis. First, a broad range of structurally diverse compounds contribute to the by and large pharmacological activity of a plant extracts and synergistic effects between those active principles may exist [36]. Secondly, there is an urgent need for more appropriate pharmacological models. Existing assays are quite often not reliably predictive for clinical efficacy. For a number of common diseases with unknown or multifactorial origin, no suitable pharmacological models have as yet been developed.

[V] CONCLUSION

The active principles of the plants are not known, as no suitable pharmacological model exists. Medicinal plant research requires a multidisciplinary approach. The quality of research can only be as good as the co-operation between botanists, phytochemists and pharmacologists. A good collaboration between botanists and phytochemists has developed over the years, as numerous joint publications testify. The collaboration with pharmacologists is in general of more recent date and definitely needs to be intensified. There is an urgent need for more appropriate plant screening [37, 38].

Because of this drug resistance, the search for new antibiotics continues unabated. In this connection plants continue to be a rich source of therapeutic drugs. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industries was possible, because of the large number of the phytochemical and biological studies all over the world. The plants demonstrating broad spectra of activity may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

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