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ASPERGILLOSIDAL EFFECT OF *PONGAMIA GLABRA* AND *PEDALIUM MUREX* - A COMPARATIVE STUDY

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The aspergillosidal properties of aqueous and organic solvent extracts (benzene, butanol and dimethylformamide) from the leaves of *Pongamia glabra* and *Pedalium murex* were tested against three *Aspergillus* species like *A. flavus*, *A. fumigatus* and *A. niger* by conducting disc diffusion assay. The aqueous extract contributes some activity in tested fungal strains. The organic solvent extracts were found to have moderate to least activity against most of the organisms tested. This study supports the notion that the aqueous and solvent extraction of these plants may prove/provide better antifungal properties and it may be suggested to be used to cure diseases caused by *Aspergillus* groups.

Keywords: Aqueous and organic solvents, *Aspergillus* species; Aspergillosidal activity; *Pedalium murex*; *Pongamia glabra.*

Introduction

Aspergillus is a cosmopolitan genus containing 900 species which are commonly found in soil and on decaying organic matter[1]. Several species of *Aspergillus* are known to cause many important animal diseases such as mycotic abortion of sheep and cattle, pulmonary infection in birds and otomycosis, mycotic keratitis, allergy and rarely mycetoma commonly known as Aspergillosis in human beings[2]. According to Schubert[3] there are three invasive (acute necrotizing, chronic invasive and granulomatus invasive) and other non invasive (fungal ball allergic) forms of fungal rhinosinustis. *Aspergillus* species can cause allergic bronchopulmonary, meningitis and meningoencephalitis, ocular infections, endocartitis and sinusitis.

Aflatoxins (a toxin produced by mold that can damage the liver and may lead to liver cancer) are chemical substances produced by the members of *Aspergillus* and are common and widespread in nature. Aflatoxin are bifuranocumarin mycotoxin produced mainly by *Aspergillus flavus*, with aflatoxin B_1 (AFB_1) being the most hepatotoxic showing mutagenic, carcinogenic and probably teratogenic properties in animals[4,5]. According to the International Agency for Research on Cancer, aflatoxins are classified under human carcinogens class I type[6]. The biosynthesis of aflatoxin B_1 can be inhibited by a number of compounds[7]. The extracts of certain plants are toxic to fungal growth and mycotoxin production[8,9].

The use of chemical preparations and commercial antibiotic drugs can effectively control the disease causing pathogenic microbes. However, the use of chemical substances to minimize the growth is changing rapidly, because of their cost, side effects and increasing resistance to the microbes. In the present situation, natural plant products could effectively replace some of these chemicals to control the human diseases caused by microbial populations.

Plants may offer a new source of antifungal agents[10] because they produce great deal of secondary metabolites that have antifungal activity[11,12]. The antifungal action of plant extracts has got great potential as they can be handled easily and their residual effects tend to be systematic in their activity, easily biodegradable and stimulate host metabolism among other characteristics. In recent times, the antimicrobial properties of some plant constituents are being exploited in protecting man from moulds and myco-toxicosis[13]. The powder and crude extracts of different medicinal plants[14,15] are reported to be effective antimicrobial natural sources/ agents.

Considering the above views, the aim of this current investigation was to study the comparative analysis of the aqueous and organic solvents (benzene, ethanol, butanol and dimethyl formamide) extracts of the leaves from *Pongamia glabra* and *Pedalium murex* against *Aspergillus flavus*, *A. fumigatus* and *A. niger*

Table 1. Antifungal properties of two medicinal plant extracts against *Aspergillus* species.

Name of plants	Solvents used	Name of the Organisms (Diameter of growth inhibition in mm)		
		Aspergillus niger	*Aspergillus flavus*	*Aspergilus fumigatus*
Pongamia glabra	Ethanol	0	0	0
	Aqueous	8	12	14
	Benzene	0	0	7
	Butanol	0	8	8
	Dimethyl formamide	0	8	10
Pedalium murex	Ethanol	9	0	0
	Aqueous	15	7	0
	Benzene	0	0	0
	Butanol	0	12	12
	Dimethyl formamide	10	7	14

through *in vitro* approach.

Material and Methods

Plants collection-The leaves of two medicinal plants *i.e. Pongamia glabra* and *Pedalium murex* were collected from the roadside thickets in and around Tiruchirappalli, Tamil Nadu.

Sterilization of plant materials-The disease free and fresh plants were selected for this investigation. About 2 g of fresh and healthy leaves were taken for each solvent including aqueous. These were washed with tap and distilled water three times. Then, surface sterilized with 0.1% mercuric chloride or alcohol for few seconds. Again the plant material was washed thoroughly with distilled water (three times).

Preparation of plant extracts- Two grams of sterilized plant parts (leaves) were kept in the 10 ml aqueous and organic solvents such as benzene, butanol and dimethylformamide. Then these were ground with the help of mortar and pestle. The ground plant material was subjected to centrifugation for 10-15 minutes (at 10000 rpm). Again, it was filtered through Whatmann no.1 filter paper. The supernatant was collected and made to known volume, by adding sterile distilled water and stored for further antimicrobial screening purposes.

Fungal inoculum preparation- Three human pathogenic fungal strains were used throughout this investigation (*Aspergillus flavus, A. fumigatus* and *A. niger*). The fungal strains were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh, India and maintained in the laboratory. The young microbial inoculum/culture was prepared and used in the entire research periods. The sabouraud dextrose broth (SDB) were prepared and poured into several tubes and sterilized. The pure microbial cultures were collected from the institute (either solid or liquid medium) and inoculated in the tubes using inoculation needles or loops. These tubes were incubated at different temperatures and time duration (at 27°C for 24-48 hours).

Screening for antifungal assay - The antifungal activity was performed based on disc diffusion method[16]. Each plant leaf extract was tested against the selected fungal strains. The sterilized sabouraud dextrose agar medium was poured into each sterile petriplate and allowed to solidify. Using a sterile cotton swab, the test fungal cultures were evenly spread over the appropriate media. The sterile discs (5 mm diameter) were individually soaked with aqueous and organic solvent extract from the leaves (10 µl / disc). These discs were kept in undisturbed place for the evaporation of solvents. Then the discs were placed on the top layer of the Petri dishes pertaining to the test cultures. All the plates were incubated at 27°C for 24-72 hours. After the incubation period the results were observed and the diameter of inhibition zone around each disc/organism was measured.

Results and Discussion

The results of different extracts from the fresh leaves of *Pongamia glabra* and *Pedalium murex* tested, against *Aspergillus* species *i.e. A. flavus, A. fumigatus* and *A. niger* were tabulated (Table 1).

Antifungal effect of Pongamia glabra leaf extracts.The aqueous and various solvents such as benzene, butanol, ethanol and dimethyl formamide extracts from *P. glabra*

expressed broad spectrum of antifungal activity. The aqueous extracts of this plant showed significant activity against *A. fumigatus* (14 mm) and *A. flavus* (12 mm) and least activity in *A. niger* (8 mm). The butanol, benzene and dimethyl formamide extracts reflected least activity in tested fungal species, growth inhibition between the range of 7-9mm. The dimethyl formamide extracts provide moderate activity against *A. fumigatus* (10 mm) and least against *A. flavus* (8 mm) while, *A. niger* were found to be inactive. Jain and Agarwal[17] and Jambotkar *et al.*[18] have reported the antifungal properties of *Pongamia glabra* against *A. niger.*

Antifungal effect of Pedalium murex leaf extracts - The antifungal screening of leaf extracts of aqueous and organic solvents (ethanol, benzene, butanol and dimethyl formamide) from *Pedalium murex* exhibited moderate activity against the pathogens tested. The ethanolic leaf extract had medium level of activity against *A. niger*. The aqueous extract has expressed significant activity in *A. niger* (15 mm) followed by others. The benzene extract did not show any activity, whereas the butanol extracts gave similar inhibitory action against the growth of *A. fumigatus* and *A. niger*. The dimethyl formamide extracts showed good to better activity against *A. fumigatus* (14 mm) followed by *A. niger* (10 mm). *A. flavus* showed very little activity (7 mm).

In the early 19th century, when methods of chemical analysis first became available, scientists began extracting and modifying the active ingredients from plants. Recently, the World Health Organization (WHO) estimated that 80% of the people worldwide rely on herbal medicines. This is the back bone of the present investigation, which is carried out to test the aspergillosidal properties of the aqueous and organic solvents extracts of *Pongamia glabra* and *Pedalium murex*. These extracts were screened for their antifungal activity against three most common and disease causing *Aspergillus* species. Among them, *A. niger* showed great inhibitory effect against aqueous extracts of the plants tested. The ethanol and dimethyl formamide extracts from *Pedalium murex* showed moderate activity against *A. niger*. The aqueous and organic solvent extracts of *Pongamia glabra* contribute better against *Aspergillus fumigatus*, while, *P. murex* extracts exhibited some activity against the butanol and dimethyl formamide extracts, respectively. The aqueous, butanol and dimethyl formamide extracts from both plants were active against *A. flavus* which exhibited the growth inhibitory effect against the both plant's extracts tested.

The overall performance of this study, described that the extracts from *Pongamia glabra* and *Pedalium murex* exhibited significant activity against the tested *Aspergillus* species. The water extracts were also found to have remarkable antifungal activity. This study was supported by Singh and Singh[19] who reported 50 plants and their crude extracts effective against *A. flavus* and *A. niger.* Similarly, Bohra and Purohit[20] investigated the effect of aqueous extracts from some plants tested against toxigenic strain of *A. flavus*. Alex Ramani *et al.*[21] reported antimicrobial activity of aqueous and ethanol extracts from *Anisochillus carnosus* which expressed better activity against *A. flavus* and *A. fumigatus*. The antimicrobial properties of the crude ethanol and water extracts of leaves and barks from *Cassia alata*[22] were tested against many microorganisms including *A. fumigatus*. This study will be open for further active phytochemical constituents and pharmacological studies of such medicinal plants against the human disease causing pathogens.

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