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Antimicrobial Activity of Mangrove Wood Fungi

T. Ushadevi* and A. Panneerselvam

*Department of Biotechnology, Prist University, Thanjavur, Tamil Nadu.
Department of Botany and Microbiology, A.V.V.M. Sri. Pushpam College,
Poondi 613 503, Thanjavur, Tamil Nadu, India.

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A total number of fifty fungi were isolated from wood samples of mangrove environment. Crude extracts of these fungi were prepared and tested against a gram positive bacterium, a gram negative bacterium and one fungus. Among the fifty fungi tested twenty-two exhibited antibacterial and eleven of them showed both antibacterial and antifungal activities. Out of the 22 fungal isolates 19 showed inhibition zone against Gram positive bacterium, 20 isolates were active against Gram negative bacterium and 17 isolates inhibited both Gram positive and Gram negative bacteria.

Key words: Mangrove, Antimicrobial, Antibacterial, Antifungal, Gram positive and Gram negative.

Mangroves are dominant along Indian coastline and provide niches and habitats for many marine and estuarine organisms. However, very few attempts have been made to investigate the fungi associated with decaying substrata of mangrove plants. This is especially true with mangroves of the east coast of India, which accounts for approx. 33% of the total Indian mangroves[1].

Recently, investigation on marine fungi as a source of new bioactive compounds has contributed significantly to natural products chemistry. Marine-derived fungi have been shown to produce interesting bioactive metabolites[2,] including some potential antibiotics[3, 4] .Therefore it is an imperative need to record and quantify the abundance of marine fungi in the mangrove ecosystem and to culture them to ensure their conservation for future biochemical, genetic and molecular studies. Thus the present study has been framed on one of the major mangrove forests of Tamil Nadu, the Muthupet Mangrove.

Muthupet is located at the southern end of the Cauvery delta on the East coast of India (Lat.10^{o} 20$^{'}$ N; Long. 79^{o} 35$^{'}$ N). It is one of the largest mangrove forests in Tamil Nadu and it covers a total area of 120 sq km in three districts viz. Nagapattinam, Thiruvarur and Thanjavur.

* To whom all correspondence should be addressed.
E-mail: ushadevi29@gmail.com

MATERIAL AND METHODS

Collection of litter samples

The litter (wood) samples were collected randomly from the Muthupet mangrove environment during low tide period of December 2005. The samples after collection were kept in new polythene bags, tied with a string and transported to the laboratory. The litter samples were washed with sterile seawater, cut in to small pieces and placed in a sterile polythene bags containing few drops of sterile seawater.

Isolation of fungi

Pieces of 100 wood samples were randomly selected and a portion of the surfaces were cut into 0.5cm length[5]. The pieces were washed with sterile water for 10 seconds and placed onto the Seawater Cornmeal Agar medium (SWCMA). The plates were incubated at 28 ± 2° C for 7 days. After incubation the development of colonies on the medium was observed. The fungal cultures were then transferred, subcultured and the pure cultures were maintained on 50% SWCMA medium. The microscopic features were then observed using Nickon microscope. All isolates were identified, based on the morphology of the sporulating structures as well as the morphology and pigmentation and also referring the standard manuals such as The manual of soil Fungi[6], Hyphomycetes[7] Dematiaceous Hyphomycetes[8], More Dematiaceous hyphomycetes[9] and In Marine Mycology - The Higher Fungi[10].

Screening of fungi for antimicrobial activity

Antimicrobial activities of the fungi were screened by agar diffusion well method[11]. All the fungal species that could be cultured were screened for antimicrobial activity. The fungi were inoculated in corn meal broth preparations 50% v/v seawater and cultured incubating for 7 days at 28 ± 2° C. After incubation, each culture broth was filtrated through Whatman No 1 filter paper followed by Millipore filter (0.2µm) and the filtrates were used for the assay of antimicrobial activity. The assay was conducted against a Gram positive bacterium *Bacillus subtilis,* a Gram negative bacterium, *Escherichia coli,* and one fungus, *Candida albicans.* Sterile Nutrient Agar (NA) medium and Potato Dextrose Agar (PDA) medium were used as basal media for growing these pathogenic bacteria and fungus respectively. Inoculums of the pathogen for the assay were prepared in liquid media of the respective composition. One ml of the broth inoculum was mixed with medium poured into the Petri plates and allowed for solidification. After solidification 6mm diameter duplicate well was made with the help of a sterile cork borer in the medium. In each well 100µl of the filtrate was poured. All the plates were incubated at room temperature and the zone of inhibition was recorded. For bacteria, the plates were incubated for 24 hours and fungi 48 hours.

RESULTS AND DISCUSSION

From the 100 litter samples subjected to plating technique for mycological examination a total number of morphologically distinct fifty micro fungi including six sterile mycelium were isolated. All of them were the members of Deuteromycetes except one species.

Culture filtrates of all the fifty isolates raised in culture were screened for their antimicrobial activity against pathogenic bacteria and fungi by agar diffusion well method. Among the fifty isolates twenty-two including *Aspergillus* genus comprising 12 species, *Penicillium* and *Trichoderma* shares 3 species each, *Cephalosporium* and *Cladosporium* shares one

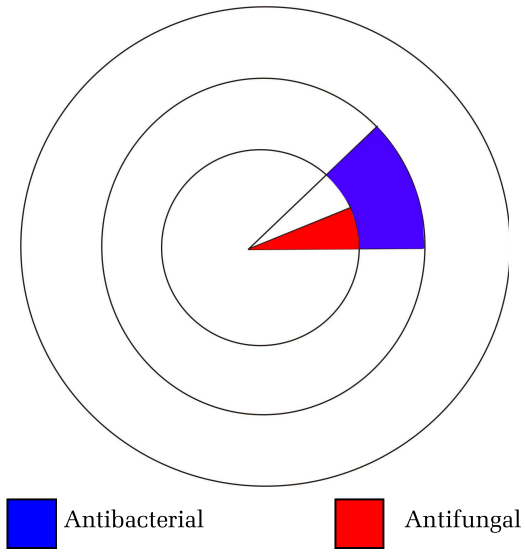

Fig. 1. Percentage contribution of antimicrobial potential of fungi from the total isolates

species each and two different kinds of white sterile mycelium exhibited antimicrobial activity. All the twenty-two isolates showed antibacterial while eleven fungi among them showed both antibacterial and antifungal activity (Table 1). Cuomo *et al.*[11] reported that a similar prescreening of 1500 marine fungal isolates yielded 364 antimicrobial extracts and concluded that more antimicrobially active strains were found among marine than terrestrial fungi. In general, marine fungal metabolites are proved to be biologically more effective than those derived from terrestrial strains. Christophersen *et al*.[12] also tested 227 marine isolates belonged to *Penicillium, Aspergillus, Eurotium, Fusarium, Emericella, Alternaria* and *Gliocladium*. Among them 61 isolates (27%), representing 18 different species, exhibited activity against at least one species of bacteria. Similarly, antagonistic antimicrobial activity of marine fungi and bacteria isolated from marine biofilm and seawaters was screened[13].

Moreover, organisms were mainly collected in lagoons and mangroves presenting ecological niche systems expected to furnish optimal conditions for the discovery of new metabolites[14]. The present investigation has also proved that the mangrove fungi possess antimicrobial activity by inhibiting the growth of pathogenic bacteria and fungus. This may be due to the presence of secondary metabolites in mangrove fungi.

Among the 22 fungal isolates, which exhibited antibacterial activity, 19 inhibited the Gram positive bacterial growth, 20 isolates were active against the growth of Gram negative bacteria and 17 isolates inhibited both Gram positive and Gram negative bacteria. The percentage contribution of antimicrobial fungi from the total culturable isolates was 44. Among them 22 % showed both antibacterial and antifungal activity (Fig: 1).

Table 1. List of fungi screened and their antimicrobial activity against *B. subtilis, E. coli* and *C. albicans*

S. No.	Fungal species	Zone of inhibition (diameter in mm)		
		B. subtilis	*E. coli*	*C. albicans*
1.	*Aspergillus candidus*	3	2	-
2.	*A. clavatus*	15	14	12
3.	*A. flavus*	7	3	-
4.	*A. giganteus*	4	-	-
5.	*A. humicola*	3	6	-
6.	*A. japonicus*	-	5	-
7.	*A. sulphureus*	9	6	-
8.	*A. terricola*	8	6	7
9.	*A. terreus*	10	9	4
10.	*A. unguis*	8	7	-
11.	*A. ustus*	6	3	-
12.	*A. versicolor*	17	15	13
13.	*Cephalosporium* sp.	9	-	-
14.	*Cladosporium* sp.	8	11	3
15.	*Penicillium brefeldianum*	-	8	-
16.	*P. citrinum*	11	6	4
17.	*P. lividum*	15	16	12
18.	*Trichoderma lignorum*	13	14	11
19.	*T. glaucum*	10	7	5
20.	*T. harzianum*	9	11	7
21.	White sterile mycelium-1	-	7	-
22.	White sterile mycelium-2 (reddish brown pigment)	14	16	10

Discovery of endophytic fungi in plant tissues opened up new possibilities in the search for metabolically active compounds. However, reports on mangrove endophytic fungal metabolites are scarce. Marine derived fungi have developed unique metabolic and physiological capabilities that offer the potential for the production of bioactive secondary metabolites. Particularly the endophytic fungi of mangroves can produce many kinds of metabolites with great potential for anti-microbial and anti-tumor medicinal use[15]. In the present investigation, 44% of the fungi tested showed anti-microbial activity, which emphasis that the litter fungi on mangroves could potentiality be the producers of novel metabolites.

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