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CHEMICAL ANALYSES OF THE BIOACTIVE FUNGI ISOLATED FROM A MANGROVE WOOD

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ABSTRACT

Mangrove habitat is well known as a source of finding novel microorganims. Six fungal isolates were isolated from a degraded mangrove wood collected from the local coastal area of Sindhudurg district, Maharashtra. Out of these six isolates, the antimicrobial potential of the three fungal isolates was investigated using agar cup method against both bacteria and fungi. Results for bioassay were found to be very encouraging. Chemical characterization of the bioactive extracts was carried out using thin layer chromatography.

KEYWORDS: Mangroves, Maharashtra, bioassay, Thin Layer chromatography, Spectrometry.

INTRODUCTION

Natural products form a fundamental source of new chemicals and therefore are an integral component of today's pharmaceutical industry. Microorganisms are considered as one of the best source for isolating structurally unique and pharmacologically active natural products and are therefore have attracted considerable attention in recent years.[1]

Mangroves represent an intertidal wetland ecosystem formed by a unique association of animals and plants in the coastal areas and river estuaries throughout the low lying tropical and sub-tropical latitudes.[2] The ecological, economical and medicinal importance of flora and fauna from mangroves is well recognised and continue to being explored. India has a rich

diversity of mangroves with thirty nine species of mangrove plants.[3] An attempt has been made in the present study to isolate a few fungi from the degrading mangrove wood and to study the bioactivity and chemical analyses of these fungal isolates.

MATERIALS AND METHODS

Collection of rotting wood samples

The wood samples were obtained from the local mangrove areas of Sindhudurg, Maharashtra. The samples collected were washed with seawater and brought back to the laboratory in a sterile plastic bag.

Isolation of fungi

A piece of 2 sq.cm of the wood sample was taken and washed with sterile seawater on a vortex for 20 seconds. The seawater was decanted. The sample was treated with 70% ethanol on a vortex for 1 minute and decanted. Fresh sterile seawater was added and vortexed for 30 seconds. The sample was crushed using sterile mortar and pestle. 200µl of the solution (crushed sample) was spread plated directly on Zobell's marine agar (ZMA) plates. Along with this dilutions of 10^{-2} & 10^{-3} were spread plated on ZMA. The plates were incubated at room temperature for two weeks and checked daily for growth.

Cultures growing or extending into the medium were subcultured onto ZMA plates. This procedure was repeated till pure cultures were obtained which were maintained at 4^0C. Out of the 6 fungal cultures, only 3 fungal cultures namely WF1, WF4 and WF5 were studied further for chemical analysis and bioactivity after primary screening.

Fermentation of fungal cultures

The fungal cultures (WF1, WF4 and WF5) were picked up from the plates and inoculated into Potato Dextrose broth 500ml. After inoculation, the fungal cultures were kept for 21 days at 30^0C on a shaker (100 RPM).

Preparation of fungal extracts

After 21 days of submerged fermentation, fungal cultures were taken for extraction. The fungal matt was subjected to lyophilisation. The dehydrated matt was then crushed in a mortar and pestle along with ethyl acetate. The crushed matt (approximately 50 grams) of all three fungal species was sonicated with ethyl for 10 minutes and filtered through Whatman's

filter paper. The process of crushing, sonication and filtration was repeated twice. The filtrate (ethyl acetate extract) was then concentrated using a rotavapor.

Antibacterial and antifungal assays

The extracts were tested against five pathogenic bacteria and five fungi.

Preparation of agar plates

Muller Hinton agar was prepared in a conical flask by dissolving 11.4g of the agar powder in 300ml of distilled water. The flask containing the agar was then sterilized in an autoclave. After this the agar was pour plated into sterile petriplates under sterile conditions. The agar was then allowed to solidify. These plates were used for antibacterial assay. Potato dextrose agar plates were prepared in a similar way and used for antifungal assay.

Preparation of discs by loading extract

The crude ethyl acetate extract (5 mg) was dissolved in 1 ml ethyl acetate and appropriate amount was loaded on sterile paper discs of 6mm, in order to get 100 µg per disc concentration.

Bioassay

Muller Hinton agar plates were inoculated with pathogenic bacteria and Potato dextrose agar plates were inoculated with fungi. The extract treated discs were placed on respective plates in triplicates. The plates were inoculated up to 48 hours at 30^0C. After incubation the zone of inhibition surrounding the disc was measured.

Thin Layer Chromatography

The loading front was marked 1cm from the bottom of the sheet. The extracts were loaded on the loading front using a capillary tube. The capillary tube was rinsed with methanol before and after loading each extract.

Following two solvent systems were used to develop the plates.

1) Petroleum Ether-Ethyl Acetate solvent system (90%PE: 10%EA)
2) Chloroform-Methanol solvent system (90% Chloroform: 10% Methanol)

The silica plates were placed in a beaker containing two different solvent systems. The solvent was allowed to rise upto $3/4^{th}$ of the plates. The plates were then air dried and then visualized under UV at 255 nm and 366 nm for spots. The same plates were also placed in the iodine chamber and observed for the spots.

UV spectroscopy

The UV spectrum of the extract was taken by using Schimadzu UV Spectro-photometer.

IR spectroscopy

Fungal extract (5 mg) was taken and mixed with potassium bromide (KBR) and crushed to a fine powder using mortar and pestle. The homogenized sample was then placed in the moulds and pressed using mechanical strength for 20-30 sec using a clean alcohol sterilized spatula. The sample pellet with the mould was then placed on the sample pan and was ready for analysis. Analysis was done using Shimadzu FTIR system. The scanning was done at frequency wavelength 400-4000 cm^{-1} with resolution of 4 cm^{-1}.

RESULTS

Fungal cultures isolated from mangrove wood samples

A total of six fungal cultures were successfully isolated from the wood sample collected from the mangrove area of the Sindhudurg. The isolates were given the codes as WF1 to WF6. Isolates WF1 and WF5 showed white coloured colonies while WF3 and WF6 were greenish. WF2 was yellowish in colour whereas WF4 showed colonies greyish in colour.

Antibacterial assay

Crude extract prepared from the fungal isolateWF1 showed promising antibacterial activity against all the test bacteria except the *S. aureus*. This was followed by the activity by the extract of WF5 whereas least activity was shown by WF4 (Table 1).

Table 1: Antibacterial activity of the extracts of fungal isolates.

Crude extract	Zone of inhibition in mm (including 6mm disc)				
	Escherichia coli	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Salmonella typhii***	***Shigella Sp.***
WF1	13	7	-	8	11
WF4	-	-	-	-	8
WF5	7	10	7	12	-

Antifungal assay

Crude extract prepared from the fungal isolate WF1 showed promising antifungal activity against all the test fungi. This was followed by the activity by the extract of WF4 whereas least activity was shown by WF5 (Table 2).

Table 2: Antifungal activity of the extracts of fungal isolates.

Crude extract	Zone of inhibition in mm (including 6mm disc)				
	Aspergillus fumigatus	***Rhodotorula Sp.***	***Candida albicans***	***Cryptococcus neoformans***	***Aspergillus niger***
WF1	8	7	11	10	7
WF4	9	8	10	8	7
WF5	-	-	8	-	-

Thin Layer Chromatography

TLC of all fungi showed several spots. This indicates that there are different organic compounds present in crude extract (Fig. 1).

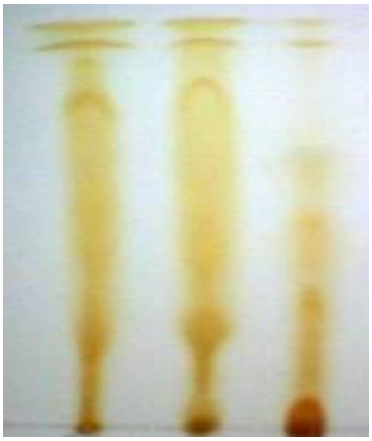

Figure 1: TLC plate showing separation of the extracts of WF1, WF4 and WF5.

UV Spectroscopy

The three extracts of chloroform, petroleum ether, butanol and ethyl acetate were analysed by UV Spectroscopy. The maximum UV absorption was seen at the wavelengths - 246 for ethyl acetate extract. From this it was concluded that there is a presence of carbonyl group which was also confirmed by infrared spectroscopy.

IR spectroscopy

Results for IR spectroscopy are shown in the Table 3 indicating the presence of different compounds with various functional groups.

Table 3: Results for the IR spectroscopy of the extracts of fungal isolate WF1.

Extract	Frequency (cm^{-1})	Range (cm^{-1})	Functional groups
Ethyl Acetate (WF1)	2927.7, 2854.5 2673.2	3300-2500	Acid OH
	1710.7	1850-1750	3-4 membered ring C=O, cyclohexanone- 1715
	1463.9, 1415.7	1600-1400	Aromatic C=C
	1242.1, 1099.3, 1035.7	1300-1000	C-O
	1377.1, 945.1, 837.0, 723.3, 607.5	1500-400 (fingerprint region)	Various

DISCUSSION

The present study indicates that fungi isolated from a rotten mangrove wood exhibit promising antibacterial as well as antifungal activity. This observation is on the similar lines with that made by many other researchers who have reported that mangrove associated fungi exhibit antimicrobial, antifungal, anti-inflammatory as well as anti-cancer activity.[4, 5, 6] Parulekar (2016) and Abraham (2016) have also carried out isolation of microorganisms from the rotten mangrove wood.[7-8] Parulekar reported production of manganese peroxidase enzyme from the fungi isolated from the degrading wood whereas Abraham reported production of lignin peroxidase enzyme from the bacteria isolated from the degrading wood. These studies clearly indicate that neglected habitats such as mangroves of Konkan area of Maharashtra state should be thoroughly screened as it could act as a hot spot of novel microbes with bioactive compounds and secondary metabolites such as enzymes having industrial applications.

CONCLUSION

WF1 fungal isolate isolated from mangrove wood sample showed broad spectrum activity against both bacteria and fungi. The extract of this fungal isolate showed the presence of Carbonyl group.

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