

Characterisation of the Bioactive Fungal Metabolite Isolated from the Mangrove Bark

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Received: 14.02.2016 | Revised: 22.02.2016 | Accepted: 25.02.2016

ABSTRACT

*Fungi are plant-like organisms that lack chlorophyll. Mangrove associated fungi are the second most diverse organisms that remain less explored. Secondary metabolites of fungi hold important applications in the drug discovery arena. The present study involves isolation of a fungus *Aspergillus ornatus* from the degraded mangrove bark. The fungal extract was further studied for its antimicrobial potential and chemical characteristics. The extract showed promising antimicrobial activity against the test organisms.*

Key words: Fungi, mangrove, secondary metabolites, antimicrobial, antibacterial, TLC, FT-IR

INTRODUCTION

In terms of the estimated number of species, Kingdom Fungi is second only to that of Kingdom Animalia. Conservative estimates suggest that there are more than 1.5 million fungal species, of which only approximately 5% have been identified by taxonomists (~72, 000 known species). Fungi are abundant, biologically diverse, and are known to play beneficial as well as harmful roles¹.

Perhaps more importantly, fungi have long been recognized as possessing the ability to produce novel bioactive secondary metabolites, some of which have enabled significant advances in human health and agriculture, with vast impacts on societal and economic growth^{2, 3}. Although fungi have proven to be prolific producers of diverse bioactive secondary metabolites, a great many fungi remain to be chemically explored⁴. These observations argue strongly for continued exploration of fungal chemistry in order to help meet the increasing demand for new medicinally and agriculturally beneficial agents.

Fungal extracts of mangroves are now being researched owing to their multipurpose applications in the field of pharmacology. Therefore an attempt has been made in the present study to first isolate and characterise a fungus from degrading mangrove bark and then study the chemical and antimicrobial properties of the secondary metabolites of this culture.

MATERIALS AND METHODS

Isolation of fungi

The fungi were isolated from degraded mangrove wood using a simple technique i.e. washing the sample 2-3times in sterile sea water; it is then cut into small pieces. Four small pieces of the wood were placed in a petriplate and then the plates are poured with suitable Potato Dextrose Agar.

Cite this article: Magare, V.N., Characterisation of the Bioactive Fungal Metabolite Isolated from the Mangrove Bark, *Int. J. Pure App. Biosci.* 4(1): 248-252 (2016). doi: <http://dx.doi.org/10.18782/2320-7051.2220>

This PDA was prepared in 75:25 distilled water with Benzyl penicillin salt to inhibit the growth of bacteria and incubated at 30±1°C for a week. Cultures subtending in the medium were subcultured repeatedly to obtain pure cultures. In this way total 10 cultures were purified. Out of which one culture (*Aspergillus ornatus*) was selected for further processing.

Preparation of seed Culture

About 10 ml (2%) of Potato Dextrose Broth was prepared, autoclaved and 100 ml of this media was inoculated with a loopful of *A. ornatus* culture. The inoculated broth was kept for shaking at 100 RPM at 30°C, overnight.

Submerged fermentation

The seed culture was then transferred to 1000ml PDB in a conical flask. The inoculated broth was kept for shaking at 100 RPM at 30°C for 7 days.

Extraction of fungal culture

At the end of the fermentation, the cells were centrifuged at 10,000 rpm for 10 mins aseptically in sterile falcon tubes. The cell pellet was then washed twice with sterile distilled water and freeze dried. The dried fungal mat was soaked in methanol and sonicated for 3 hours. The solvent was separated by using separating funnel and further concentrated by using rotary evaporator. The final extract was stored in a vial in a refrigerator for further use.

Antimicrobial activity of extract

The concentrated extract was screened by well diffusion bio-assay technique. Muller Hilton media (MH) with 2% agar (basal layer) and 1% agar (seed layer) was used. The plates were poured with 2% MHA medium (basal layer) and allowed to solidify. The extract was tested against four bacterial pathogens and two fungal pathogens. The selected test pathogens (Table 1) were sub cultured in tubes each containing 5ml of Nutrient broth, incubated and stored in refrigerator until use. From this stock 25µl of each culture were added to 25ml of 1% MHA (seed layer) medium and were poured onto the basal MHA medium. Wells having a diameter of 3mm were bored using a cork-borer. The extracts were diluted to a concentration of 10mg/ml in sterile water. 10µl of extracts were loaded in the wells. The plates were incubated overnight and the zones of inhibition were measured in millimetres.

Table 1 Microbial pathogens used for antimicrobial assays

S. No.	Test microbial cultures
1	<i>Staphylococcus aureus</i>
2	<i>Pseudomonas aeruginosa</i>
3	<i>Salmonella typhi</i>
4	<i>Klebsiella sp</i>
5	<i>Candida albicans</i>
6	<i>Aspergillus niger</i>

Thin Layer Chromatography

The crude extract was spotted on commercial Thin Layer Chromatography plates using alumina, backed sheets (Silica gel 60 F254, 0.25mm thick (Merck, Darmstadt, Germany). The extract was dissolved in methanol and the dissolved extract was taken in a capillary and was placed at one end of the plate 1.0 cm from the edge. BAW was used in the fixed ratio of 5:1:4. Once the solvent had reached the solvent front, it is removed from the developing chamber and air-dried. The separated components were visualized by placing the plate in the UV chamber and observed at 254nm and 366nm. The plates were sprayed with Ninhydrin and 5% Methanolic sulphuric acid and by heating in an oven at 120°C.

Fourier-transformed infrared spectroscopy (FTIR)

The major functional groups of the white solid hygroscopic powder (sample) were detected using Fourier-transformed infrared (FTIR) spectroscopy. Sample was mixed with moisture free KBr. and the FTIR spectra of the mixture were recorded in a Diffused Reflectance Spectroscopy (DRS) assembly on a Shimadzu FTIR spectrophotometer (Shimadzu, Japan) in the region of 4000–400cm⁻¹.

RESULTS AND DISCUSSION

Isolation of Fungi cultures

There were total ten cultures which were isolated from the degraded mangrove wood. After preliminary screening of all the cultures, *Aspergillus ornatus* was taken further for mass culture and antimicrobial activity. The picture of fungal culture *A. ornatus* is shown in Fig. 1.

Fig. 1: *Aspergillus ornatus* culture grown on the PDA agar plate



Fermentation and extract preparation

The fungus *Aspergillus ornatus* showed very good sporulation in PDB medium. From 1000 ml culture broth, 8 grams of free dried fungal biomass was obtained. From this fungal biomass 585 mg methanol extract was obtained in the crude form.

Evaluation of Antimicrobial activity

The antimicrobial activity results of crude extract are presented in table 2. The crude extract showed very strong growth inhibition of *S. aureus* and *P. aeruginosa*. Against both the pathogens, the inhibition zone was more than 10 mm. The crude extract showed very weak activity against *A. niger* (test fungal pathogen). The solvent control did not show any inhibition. The picture of antimicrobial activity is given below as Fig 2.

Fig. 2: Antimicrobial activity of fungal crude extract against *S. Aureus* (left side well with extract and right side well with solvent control)



Table 2 Antimicrobial activity of crude extract obtained from fungal culture

Test culture	Zone of inhibition
<i>Staphylococcus aureus</i>	13mm
<i>Pseudomonas aeruginosa</i>	11mm
<i>Salmonella typhi</i>	5mm
<i>Klebsiella sp</i>	-
<i>Candida albicans</i>	-
<i>Aspergillus niger</i>	2mm

TLC analysis

After developing in Ninhydrin, TLC plate of Crude fungal extract showed five major compounds (fig. 3). This information will be useful in further chromatographic studies and the isolation of active substance.

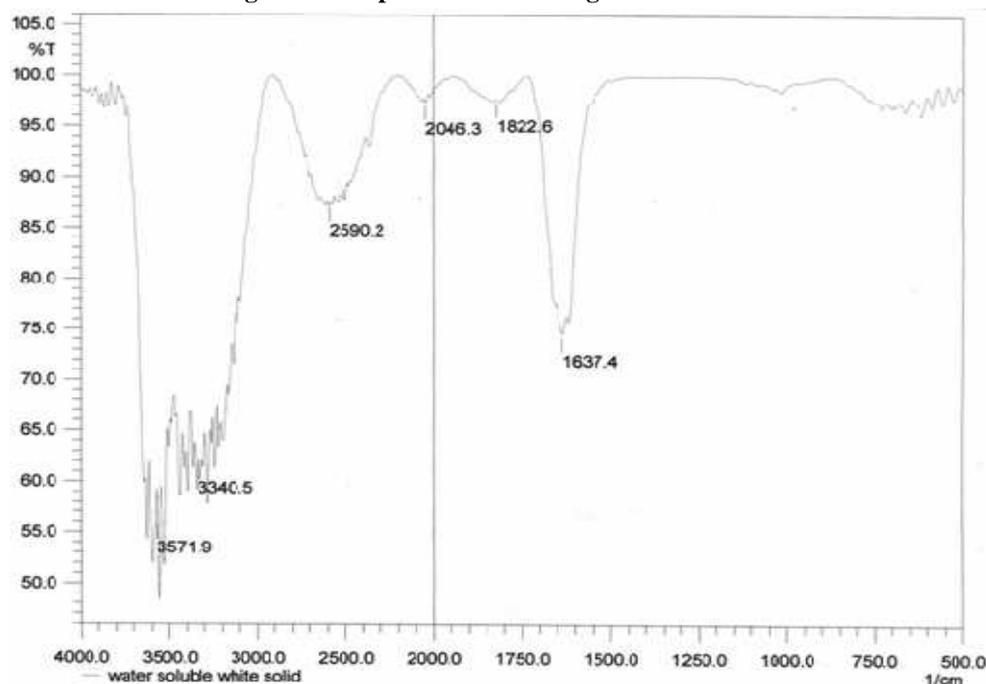
Fig. 3: TLC of Crude extract obtained from *A. ornatus*



FTIR analysis

The FTIR spectrum of the C-water (white powdery substance) of *A. ornatus* extract showed functional groups like broad stretching hydroxyl group at $3571.9-3340.5\text{ cm}^{-1}$. Further, an asymmetrical stretching peak was noticed at 1637.4 cm^{-1} , which corresponds to carboxyl groups. The FTIR spectra of the sample evidenced the presence of carboxylate anion, which may be associated with a counter cation like Na^+ . It was confirmed that the bioactive compounds present in this crude extracts are purely organic, as there was absence of peaks in the fingerprint region ($1600- 500\text{ cm}^{-1}$). The spectrum is given in Fig. 4

Fig. 4: FTIR spectrum of the fungal crude extract



Mangrove associated fungi are known to provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthenes, and others⁵. In recent times, the antimicrobial, anticancer and antioxidant properties of mangrove fungi are being increasingly reported from various parts of the world^{6,7}. The results in the present study clearly support the importance of mangrove fungi in discovering new therapeutic agents.

CONCLUSION

The fungus culture *Aspergillus ornatus*, obtained from the degraded mangrove wood showed strong antimicrobial activity against *S. aureus* and *P. aeruginosa*. Its chemical analysis showed five major organic molecules with hydroxyl and carboxyl groups. The present study therefore confirms that the mangrove fungi have great potential as antimicrobial compounds. Further studies are warranted to explore its true potential.

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