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Anti-fungal activities of extracts of some species of Mangrove plants towards some selected strains

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ABSTRACT

The bio-materials of four marine mangrove medicinal plants viz., Aegiceras Corniculatum (AGC), Excoecaria agallocha (EA) Rahizophora Mucronata (RM) and Xylocarpus Granatum (XG), are extracted with hexane, methanol and dichloromethane. These extracts are submitted to the antifungal activity towards the strains: C.albicans NCIM 3471, C.albicans NCIM 3557, C.neoformans, NCIM 3452, C.glabrata, NCYC 388 and C.tropicalis, NCIM 3118 adopting Disc Diffusion method. It is found that XG MeOH extract is effective towards C.albicans NCIM 3471 strain while EA MeOH extract is effective towards the strains of C.albicans NCIM 3471, C.neoformans, NCIM 3452 and C.glabrata, NCYC 388. The AGC (MeOH) extract is found to be effective towards the strains: C.albicans NCIM 3557, C.albicans, NCIM 3471, C.neoformans, NCIM 3452, C.glabrata, NCYC 388 and C.tropicalis, NCIM 3118. With C.albicans, NCIM 3471 strain, the order of effectiveness of the extracts is: XG MeOH (2)> EA MeOH extract (16) = AGC (MeOH) extract (16) while with C.glabrata NCYC 388 strain the order is: XG MeOH (4) > AGC (MeOH) extract (32) > EA MeOH extract (64). With C.glabrata, NCYC 388 strain, the order of effectiveness is found to be: XG MeOH extract (4)> AGC (MeOH) extract (32) > EA MeOH extract (64) while with C.tropicalis, NCIM 3118 strain, only AGC (MeOH) extract (64) is found to be effective.

Key words: Mangrove plants, extracts, antifungal activity on different strains

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INTRODUCTION

The recent investigations are concentrating on the exploring of antiviral, antimicrobial and ant insecticidal activities of different plants extracts [1-4]. As the substitute for synthetic antibiotics, the extracts of the plant kingdom are being probed [5-11]. In this context, some species of mangrove have been investigated and their extracts have been screened for their various bacteriological activities [12-15]. These mangroves and mangrove associates are turning to be the potential source of compounds possessing good combating abilities towards bacteriological diseases.

In the present investigation, the different biological parts of four mangrove species namely, *Excoecaria agallocha, Rhizophora mucronata, Xylocarpus granatum and Aegiceras corniculatum,* have been extracted with different solvents, methanol, hexane and dichloromethane. These extracted have been screened for antifungal activity towards the strains *C.albicans NCIM* 3471, *C.albicans NCIM* 3557, *C.neoformans*, *NCIM* 3452, *C.glabrata , NCYC 388 and C.tropicalis, NCIM 3118.* The results are encouraging and are presented comprehensively in this article.

MATERIALS AND METHODS

Collection of Mangrove Medicinal Plants

The different species of Mangrove plants viz., *Excoecaria agallocha and Xylocarpus Granatum,* were collected from Corangi Mangrove forest near Bhiravapalem in Godavary Estuary (Latitude 16^0 15' N and Longitude 82^0 15' E) and further, *Aegiceras Corniculatum* and *Raziphora mucronata* (Latitude 8^0 99' N and Longitude 76^0 87' E) were collected from Kollam mangrove forest near Krishnapatnam Port, Nellore.

Fungal Cultures strains:
C.albicans (*NCIM* 3557, *NCIM* 3471), *C.neoformans* (*NCIM* 3542), *C.glabrata*(*NCYC*388), *C.tropicalis*(*NCIM* 3118) and *A.niger*, *A.fumigatus* produced in National Chemical Laboratary (NCL) ,Pune, India, were used in this investigation.

Disc Preparation
Six mm (6 mm) diameter discs sterile Whatsman No 1 filter papers were used in this investigation. The Mangrove medicinal plants extract (300 mg/ml) using solvents methanol, hexane and dichloromethane was collected. To these extracts, 1ml of 5% Dimethyl sulfoxide (DMSO) was added. The discs were saturated with 20µl of these solvent extracts of mangrove plants to test their antifungal activity. The Triazole compound (300 mg/ml) was used as positive control and 5% DMSO was used as a blind control.

Antifungal Assay Protocol
Antifungal activities of the extracts (in terms of Minimum Inhibitory Concentration; MIC) against *C. albicans* ATCC 24433, *C. albicans* ATCC 10231, *C. glabrata* NCYC 388, *C. neoformans* ATCC 34664, (CLSI - Clinical Laboratory Standards Institute document M27-A3) and *A. fumigatus* NCIM 902, *A.niger* ATCC 10578 (CLSI M38-A2), were determined by CLSI broth micro-dilution assay method. For the assay, the growth medium used was YPG. Appropriate amounts of compounds were dissolved in dimethyl sulfoxide to get 100X final strength. The stock was then diluted 1:50 in YPG medium and 200 µL was added to the first row of a 96-well microtitre plate. The compounds were diluted two fold in successive wells to get a range of 1-128 µg/mL. Yeast cells (~2x10^3 cfu/mL), freshly grown in YPG broth in logarithmic phase, were drooping in the medium and inoculated (100 µL) in the wells of the plate. For filamentous fungi, 2x104 spores/mL were added. The micro-titre plate was incubated for 24 h and 48 h for yeasts and filamentous fungi, respectively. The absorbance was measured at 600 nm by using micro-titre plate reader (xMark™ Micro-plate Absorbance Spectrophotometer, Bio-Rad, CA, USA) to assess cell growth. The MIC was defined as the lowest concentration exhibiting >90% inhibition of visible growth as compared to the growth of the control [16].

Table 1: Abbreviation of Mangrove Medicinal Plant Extracts

Name of the Plant Species	Parts used	Extractions of Solvent	Abbreviation
Aegiceras Corniculatum	Fruits	Hexane Methanol	DS2 DS9
Excoecaria Agallocha	Roots	Hexane Methanol	DS3 DS8
Razhiphora Mucronata	Fruits	Hexane Methanol	DS1 DS6
Xylocarpus Granatum	Roots	Hexane Methanol Dichloro Methane	DS4 DS5 DS7

Table 2: Results of antifungal assay mangrove medicinal plants

	Minimum Inhibitory Concentration (MIC_{90})						
S.NO	*C. albicans* NCIM 3557	*C. albicans* NCIM 3471	*C. neoformans* NCIM 3542	*C. glabrata* NCYC388	*C. tropicalis* NCIM 3118	*A. niger*	*A. fumigatus*
Plant extracts							
DS1	>256	>256	>256	>256	>256	>256	>256
DS3	>256	>256	>256	>256	>256	>256	>256
DS4	>256	>256	>256	>256	>256	>256	>256
DS5	>256	2	4	4	>256	>256	>256
DS7	>256	>256	>256	>256	>256	>256	>256
DS8	>256	16	32	64	>256	>256	>256
DS9	32	16	64	32	64	256	256
Triazole compounds							

DS1 – RM hexane extract ;DS3 – EA hexane extract ;DS4 – XG hexane extract DS5 – XG MeOH extract;DS7 – XG dichloromethane extract ;DS8 – EA MeOH extract ;DS9 – Agc (MeOH) extract;
DS2 – Agc hexane extract and DS6 – RM (MeOH) extract did not dissolve in 100% DMSO or water.

Minimum inhibitory concentration for fungi
The Minimum inhibitory concentration (MIC) of the selected mangrove medicinal plants extracts across fungal confine was tested in sabouraud's dextrose broth by Broth macro dilution manner (Ericsson and sherri, 1971). The mangrove plant extracts were soluble in 5% DMSO to obtain 128µg/ml stock solutions. 0.5 ml of stock solution was integrated into 0.5 ml of sabouraud's dextrose fluid for fungi to receive absorption of 20, 40, 80,160,320 and 640mg/ml for mangrove plants extracts and 50 µl of regulated suspension of the test organism was shifted on to each tube. The control tube involved only organisms and infrequent of mangrove plant extracts. The culture tubes were incubated at 28°C for 48 hours (yeasts) and 36 hours (moulds). The lowest of these concentrations, which did not

display any growth of tested organism after macroscopic estimation, was resolved as minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The Minimum Inhibitory Concentrations of different plant extracts towards different strains have been presented in Table 2. The following observations are significant:

- Of all the extracts tested, DS5, DS 8 and DS 9 have shown some remarkable antifuntgal behaviour.
- With DS 5 extract, the antifungal activity for strains: *C.albicans NCIM 3471, C.neoformans, NCIM* 3452, and C.glabrata, *NCYC 388* is maximum with the MIC_{90} values, 2, 4 and 4 respectively.
- With DS 8, the antifungal activity for strains: *C.albicans NCIM 3471, C.neoformans, NCIM* 3452, and *C.glabrata, NCYC 388* are maximum with the MIC_{90} values, 16, 32 and 64 respectively.
- With *DS 9*, the antifungal activity for strains: *C.albicans NCIM 3557, C.albicans, NCIM 3471, C.neoformans NCIM* 3452, and *C.glabrata , NCYC 388, C.tropicalis, NCIM 3118* are maximum with the MIC_{90} values, 32, 16, 64, 32, and 64 respectively.
- With *C.albicans, NCIM 3471*strain, DS 9 extract only shows the maximum antifungal nature with MIC_{90} value 32 while the other extracts have only marginal effect.
- With *C.albicans, NCIM 3471* strain, *DS 5 , DS 8 and DS 9* extracts have been effective and the order is *: DS 5 (2)> DS 8 (16) = DS 9 (16)*
- With *C.neoformans, NCIM* 3452 strain, DS 5, DS 8 and DS 9 extracts have been found to have antifungal nature in the order: DS 5(4)>DS 8 (32) > DS 9 (64)
- With *C.glabrata , NCYC 388* strain, *DS 5 (4), DS 8 (64) and DS 9 (32)* extracts have found to be active in the order: *DS 5 (4)> DS 9 (32) > DS 8 (64)*
- With *C.tropicalis, NCIM 3118* strain, only DS 9 (64) is found to be effective.

CONCLUSION

The extracts of parts of different species of Magrove Plants have been tested for their anti-fungal activity towards the strains *C.albicans NCIM 3471, C.albicans NCIM 3557, C.neoformans, NCIM* 3452, C.glabrata *, NCYC 388 and C.tropicalis, NCIM 3118.* It is found that XG MeOH extract *(DS 5) is effective towards C.albicans NCIM 3471 strain;* EA MeOH extract *(DS8) towards the strains of C.albicans NCIM 3471 , C.neoformans*, NCIM 3452, and C.glabrata, *NCYC 388 and* AGC (MeOH) extract *(DS9) towards the strains: C.albicans NCIM 3557, C.albicans, NCIM 3471, C.neoformans*, NCIM 3452, and *C.glabrata , NCYC 388, C.tropicalis, NCIM 3118.* With *C.albicans, NCIM 3471* strain, the order of effectiveness of the extracts is: *DS 5 (2)> DS 8 (16) = DS 9 (16)* while with *C.glabrata NCYC 388* strain the order is: *DS 5 (4)> DS 9 (32) > DS 8 (64).* With *C.glabrata, NCYC 388* strain, the order of effectiveness is found to be: *DS 5 (4)> DS 9 (32) > DS 8 (64)* while with *C.tropicalis, NCIM 3118* strain, only *DS 9 (64)* is found to be effective.

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