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EVALUATION OF ANTIFUNGAL ACTIVITY OF *SYRINGODIUM ISOETIFOLIUM*AGAINST SELECTED FUNGAL PATHOGENS

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Abstract

The antifungal efficiency of various solvent extracts of seagrass Syringodiumisoetifolium was observed against some fungal pathogens, which includes Aspergillus fumigatus (MTCC 4333), Aspergillus niger (MTCC 961), Rhizomucormiehei (MTCC 546), Candida glabrata (MTCC 3984), Candida albicans (MTCC 183) and Candida tropicalis(MTCC 184). Fresh seagrass were collected from kanniyakumri coast of India and crude extracts were prepared by using three different solvent namely methanol, chloroform and acetone. The antifungal activity was estimated by agar well method. In addition to that,minimum inhibitory concentration and minimum fungal concentration were determined for Syringodiumisoetifolium and it was compared with an appropriate positive control Fluconazole. Among the three solvent extracts, maximum activity (12±0.96 mm) was against C.tropicalis in methanol extract and minimum activity of (5±0.22 mm) was against A.fumigatus in acetone extract. The results of the present study reveals that the activities of methanol extracts of seagrass S.isoetifolium was higher than that of acetone and chloroform extracts, against fungal pathogens.

Keywords: Antifungal Activity, Syringodiumisoetifolium, MIC,MFC , Methanol ,Chloroform and Acetone.

INTRODUCTION

In recent years, a significant number of novel metabolites with pharmacological properties have been discovered from marine organisms. Bioactive marine natural products play an important role in chemotherapy (Umamaheswari *et al* ., 2009). Although research on marine natural products started only about 50 years ago, marine organisms have been used in traditional system of medicine much before that. The use of marine flora in the treatment of human ailments is extensive. Seagrasses, a functional group of flowering plants rooted in the world's coastal oceans, are well known for their secondary metabolites (Ponnambala *et ai* .,2013). Seagrasses are marine flowering plants that successfully grow in tidal marine environment. Seagrasses consists of about 60 species of marine flowering plants which form the most widespread and productive coastal systems in the world. Seagrass are well documented for the presence of potent diverse secondary metabolites (Puglisi *et al*.,2007). It has been realized that many of these metabolites are being biologically active and biomedical importance and could be used as potential drugs (Aswathi *et al* ., 2012). A variety of medicines and chemicals are prepared from seagrass and their associates. New trends in drug discovery from natural sources emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities for the treatment of many diseases such as cancer, inflammatory condition, arthritis, malaria and large variety of viral bacterial, fungal diseases. There are only a very few reports concerning antifungal, antiviral and antibacterial activity of crude extracts of marine plants, including seagrass and seaweeds (Bernard *et al* ., 1989). Hence, an endeavor has been made in the present study to investigate the antifungal activity of seagrass from Kanyakumari coast.

MATERIALS AND METHOD

Collection

The seagrass *S.isoetifolium* was collected in the coastal area of Kanyakumari (8°4'33" N,77°32'53"E)Tamilnadu, India during the month of July 2014.

Sample collection & Drying

Live and healthy seagrass samples were collected during the low tide period. Then they were immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. The seagrasses were washed thoroughly with tap water to remove all sand particles and epiphytes, shade dried at a room temperature (35±2°c) until a constant weight was obtained . The grasses were grinded with electric grinder till they formed fine granules and kept at room temperature in dark bottle until used.

Extraction

The grounded seagrasses powder of 100 gm dry weight was taken separately in three different air tight glass jars and required quantity of methanol, acetone and chloroform solvents were added and kept for one week at room temperature under dark conditions. After 7 days, the contents were stirred well and then filtered by using Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure using a rotary evaporator.The dry aqueous extracts were lyophilized and stored in a refrigerator until further analysis.

Strain collection

In the present study, fungal pathogens which includes *Aspergillus fumigatus* (MTCC 4333), *Aspergillus niger* (MTCC 961), *Rhizomucormiehei* (MTCC 546), *Candida glabrata* (MTCC 3984), *Candida albicans* (MTCC 183) and *Candida tropicalis*(MTCC 184) were collected from Microbial type culture collection, Chandigarh, India.

Antifungal assay

In the present study antifungal efficacy of the crude extracts of *S. isoetifolium* was determined by agar well diffusion method. For this, Sabouraud dextrose agar plates (SDA) were prepared. After solidification, 72 h cultures of selected fungal suspension were seeded individually over the surface of SDA plates and then wells of 6 mm diameter were made over agar plates. Each well was then loaded with 100 µl of sample containing 500 µg of extracts which was prepared by using DMSO. Fluconazole (50 µg) was used as positive control and DMSO was used as negative control. The plates were incubated at 37°C for 72h. The antifungal activity of both the extracts and controls were determined by measuring zone of inhibition from the edges of the discs to the clear zone in millimeter. The assay was carried out in triplicates.

Determination of minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC)

Crude extracts were further assessed for their MICs, and MFCs by the same method using 2-fold serially diluted crude extracts from 0.25 to 128 µg/ml. The lowest concentration of extract that inhibited growth was recorded as the MIC. All positive wells that showed growth inhibition were streaked onto nutrient agarfor bacteria or Sabouraud dextrose agar for yeasts and fungi and incubated under appropriate conditions. The lowest concentration of extract that exhibited no visible growth was considered to be the MFC.

Results.

Table 1. Antifungal activity of crude extracts of *S. isoetifolium* and antibiotic (fluconazole) against fungal pathogens (Zone of inhibition – mm)

Fungal pathogens	Crude extracts			Fluconazole (Positive control)	DMSO (Negative control)
	Methanol	Chloroform	Acetone		
A. fumigatus	9 ± 0.44	0 ± 0.00	5 ± 0.22	12 ± 0.88	0 ± 0.00
A. niger	11 ± 0.65	6 ± 0.38	13 ± 0.86	13 ± 0.95	0 ± 0.00
R. miehei	12 ± 0.84	8 ± 0.45	9 ± 0.72	14 ± 1.12	0 ± 0.00
C. albicans	10 ± 0.71	8 ± 0.40	9 ± 0.80	20 ± 1.54	0 ± 0.00
C. tropicalis	12 ± 0.96	9 ± 0.56	0 ± 0.00	15 ± 0.82	0 ± 0.00

Each value is the Mean ± SD of three replicates

Table 1a. One-way ANOVA for the data on antifungal activity of crude methanolic extract of *S. isoetifolium* as a function of variation due to different fungal pathogens

Source of variations	Sum of Squares	df	Mean Square	F	P-value
Variation due to fungal pathogens	20.4	4	5.1	9.281503	P < 0.001*
Error variance	5.4948	5.4948	0.54948	-	-
Total variance	25.8948	14	-	-	-

* Statistically significant

Table 2a. One-way ANOVA for the data on antifungal activity of crude chloroform extract of *S. isoetifolium* as a function of variation due to different fungal pathogens

Source of variations	Sum of Squares	df	Mean Square	F	P-value
Variation due to fungal pathogens	158.4	4	39.6	9.281503	P < 0.001*
Error variance	1.641	10	0.1641	-	-
Total variance	160.041	14	-	-	-

* Statistically more significant

Table 3a. One-way ANOVA for the data on antifungal activity of crude acetone extract of *S. isoetifolium* as a function of variation due to different fungal pathogens

Source of variations	Sum of Squares	df	Mean Square	F	P-value
Variation due to fungal pathogens	290.4	4	72.6	186.4982	P < 0.001*
Error variance	3.8928	10	0.38928	-	-
Total variance	294.2928	14	-	-	-

** Statistically more significant*

Table 4a. One-way ANOVA for the data on antifungal activity of antibiotic fluconazole as a function of variation due to different fungal pathogens

Source of variations	Sum of Squares	df	Mean Square	F	P-value
Variation due to fungal pathogens	116.4	4	29.1	24.35024	P < 0.001*
Error variance	11.9506	10	1.19506	-	-
Total variance	128.35	14	-	-	-

*Statistically significant

Table 2. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of crude extracts of *S. isoetifolium*and antibiotic fluconazole against fungal pathogens

Fungal pathogens	Crude extracts						Antibiotic	
	Methanol		Chloroform		Acetone		Fluconazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. fumigatus	50	200	0	0	200	NF	10	40
A. niger	25	50	100	NF	25	100	10	40
R. miehei	10	25	200	400	100	200	5	10
C. albicans	50	100	200	NF	200	NF	2.5	5
C. tropicalis	25	50	100	400	0	0	2.5	5

Antifungal activity

The *Syringodiumisoetifolium* extract was tested for antifungal activity against the fungal pathogens viz. *A.fumigatus* ,*A.niger* , *R. miehei* , *C.albicans* and *C.tropicalis* by agar well diffusion method. In that we used three different solvents such as methanol, chloroform and acetone for the extraction of *S.isoetifolium* and the collected crude extracts were tested against fungal pathogens.

The seagrass extract exhibited antifungal activity against all the fungal pathogens. Of the five pathogens,higher antifungal activity (zone of inhibition) was observed for methanol extract against *C.tropicalis*(12±0.96 mm), while minimum inhibition was noticed in acetone extract against *A.fumigatus* (5±0.22 mm).

MIC and MFC

Lowest MIC value was recorded with crude methanol extract for *R.miehei*(10 mg) and the highest MIC value was observed with chloroform against *R.meihei* (200 mg) and *C.albicans* (200 mg).

The same crude extracts were further determined for MFC . Of the five fungal pathogens, highest value was observed for chloroform extract against *R. miehei* (400 mg) while lowest value was recorded for methanol extract against the same *R.miehei* (25 mg).

DISCUSSION

It is mandatory that, the presence of metabolites and natural bioactive compounds and comparison of the efficiency of solvent used for extracting the chosen marine seagrass is to be evaluated before going for drug development and screening. Since, the present work has paid attention to evaluate the effectiveness of antifungal ability of seagrass by using three types of solvent extract. The results exhibited in the present paper shows that the different test pathogens are valuable tools for describing the antifungal activity of crude extracts of seagrass.

In the present study, marine seagrass collected from the kanniyakumari coast of India were screened for their antifungal activities using methanol, chloroform and acetone. The results illustrated that, the highest activity was obtained with methanol extract which is more active against all the fungal pathogens compared to other solvents. Similar kind of experiments and results has been documented previously. Manilal *et al*., 2009 concluded that the Methanol extract of Sargassum polycystum showed more activity against E.coli, P.vulgaris, E. caratovora, K. pneumonia and fungal strain of methanol extract of A.niger and R. stolonifer. Chloroform extract showed moderate activity with Sargassum tenerrimum.Ethanol extract of Sargassum tenerrimum showed highest activity against S.aureus and water extract of A.niger showed moderate activity . Preuttiporn *et al*., 2013 found in his study that more than 50% of the fungal extracts had antifungal activity either against only pathogenic yeasts or only filamentous fungi or both. CH extracts from *Fusarium* sp. PSU-ES73 showed strong fungicidal activity against both strains of *C. neoformans* with MIC and MFC difference of 2 to 4 folds. Hay M.E *et al* ., 1992 observed that most of the compounds of marine algae were reported as antibacterial in human medicine. K. Sivakumar*et al* ., 2013 observed that fungal mycelia growth was strongly inhibited by methanol and ethyl acetate extract against two plant fungal species (Pythium aphanidermatam and colletotrichum capsici) Lamia Mhadhebi *et al* ., 2012 showed that the chloroform extracts obtained from *C. crinita*and *C. sedoides*have a strong antifungal activity against Candida strains, which were slightly greater than that produced by the ethyl acetate extracts. Arun panichlert *et al*. have isolated many pure compounds from this isolate including a new beta-resorcylic macrolide (5' hydroxyzearalenone) and six known beta-resorcylic macrolides (zearalenone, 8'-hydroxyzearalenone, 7'- dehydrozearalenone, beta-zearalenol, 5'-hydroxyzearalenol and relgro). Only zearalenone showed moderate activity against *C. neoformans* (MIC 16 µg/ml).

CONCLUSION

Our investigation showed that the crude extracts of *Syringodium isoetifolium* revealed appreciable antifungal activity against fungal pathogens. It is clearly indicated that the selection of solvent play a vital role in the extraction of seagrass. It is a promising indication that the tested seagrass plant species could be used to synthesize novel antibiotics. Further research is necessary for successful separation, purification and characterization of biologically active compounds using various techniques.

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