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Research Article

Screening the *in-vitro* antifungal activity of certain seaweeds collected from Mandapam coast, Tamil Nadu, India

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Abstract

In the present study, three algal samples (*Gracilaria corticata, Gracilaria edulis* and *Hypnea musciformis*) were collected from the Mandapam coast was screened for its antifungal activity against human pathogenic fungi. Crude algal samples were prepared using various solvents such as methanol, acetone, ethyl acetate and hexane. Disc diffusion method was used for the evaluation of antifungal activity at 20 µl concentration. The sol vent extracts of *Gracilaria corticata* inhibited all the tested pathogens, maximum zone was observed in methanol extract against *Aspergillus flavus*. Methanol and acetone extracts of *Gracilaria edulis* and *Hypnea musciformis* also exhibited moderate activity and ethyl acetate and hexane extracts of all the seaweed samples showed low antifungal activity. Flucanozole served as positive control and DMSO served as negative control.

Keywords: Human pathogen, antifungal activity, Flucanozole, positive and negative control.

Introduction

Indian sub-continent is developing in all aspects, as well as to improve our status in the field of pharmaceuticals against all types of infection, natural bioremedy is required. Hence the bioactive components from all types of sources needed to be isolated to assess their activity in all aspects, marine algae are greater resource of bioactive compounds and needed to be isolated. This present study emphasises on the antifungal activity on some human pathogenic fungi using some selected seaweeds collected from the Mandapam coast, Tamilnadu. Seaweeds are one of most commercial important living marine resources belongs to the primitive groups of non-flowering plants. Marine algae grow abundantly along the coastal regions of Tamilnadu; about 700 species of marine algae have been reported from different parts of Indian coast (Hebsibah Elsie and Dhana Rajan 2010). Seaweeds are of immense interest in screening of therapeutic drugs from natural products, since they consists a broad range of biological activities such as antiviral, antibiotic, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic (Bansemir *et al.*, 2006).

A great variety of secondary metabolites were characterized and identified from the marine algae by a broad spectrum of biological activities, compounds with cytostatic, antioxidant, antiviral, antihelmenthic, antifungal and antibacterial and antifungal activities have been detected in green, brown and red algae (Yuan *et al.*, 2005; Chew *et al.*, 2008).

Materials and Methods

Seaweeds collection and extraction

Three algal samples (*Gracilaria corticata, Gracilaria edulis* and *Hypnea musciformis*) were collected from the Mandapam coastal areas of Tamilnadu, southeast coast of India. Collected samples were cleaned of epiphytes and transported to the laboratory in plastic

bags with water to prevent evaporation. Collected seaweeds were washed with tap water to remove any associated debris and shade dried at room temperature (28±2°C). After complete drying, seaweeds were finely powdered using electrical blender. 50 g of powdered sea weeds were extracted successively with 250 ml of solvents (Methanol, Acetone, chloroform, ethyl acetate and Hexane) in Soxhlet extractor. The liquid extract was then cooled and evaporated using rotary evaporator. The extracts were labelled and stored at 4^{o}C for future use.

Fungal pathogens tested

Fungal pathogens *Aspergillus flavus, A.niger, Candida albicans* and *C.glabrata* were obtained from the Department of Microbiology, Faculty of Science, Rajah Muthaiah Medical College, Annamalainagar, Tamil Nadu, India.

Screening of antifungal activity

The antifungal activities of Seaweeds extracts were determined by Disc diffusion method proposed by Bauer *et al*. (1966). 20µl of extracts were applied to the sterile filter paper discs (Whatmann no.1) of 6mm in diameter. The test organisms were seeded onto the sterile Potato Dextrose agar (PDA) medium and the filter paper discs with algal extracts were impregnated onto the petriplates containing test organisms. Flucanozole (5mg/ml) was used as positive control and DMSO serves as negative control. The clear zone of inhibition was measured in terms of mm in diameter. The experiment was performed in triplicates to observe mean average.

Minimum Inhibitory Concentration

MIC of the seaweed extracts was tested using Sabouraud's dextrose broth for fungi by Broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth to get a concentration of 1, 2, 4, 8, 16, 32 and 64 mg/ml. 50 µl of standardized suspension of the test organism was inoculated and incubated at 28°C for 2 days (yeasts) and 3 days (moulds). The lowest concentration without any growth of tested organism after macroscopic valuation was determined as the Minimum inhibitory concentration.

Results

The antifungal activity of marine seaweed crude extracts of *Gracilaria corticata* was investigated against fungal pathogens (*Aspergillus niger Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-16. The methanol crude extract of *Gracilaria corticata* (5 mg/ml) showed highest mean zone of inhibition (16 ± 0.6 mm) against *Aspergillus flavus*. No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol crude extract was recorded against *Candida albicans.*

The antifungal activity of marine seaweed crude extracts of *Gracilaria edulis* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-17. The *Gracilaria edulis* methanol extract (10 mg/ml) showed highest mean zone of inhibition (15 ± 0.6 mm) against *Aspergillus niger.* No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0. 6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol extract was recorded against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida glabrata*.

The antifungal activity of marine seaweed crude extracts of *Hypnea musciformis* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-14. The methanol extract of *Hypnea musciformis* (5 mg/ml) showed highest mean zone of inhibition (13 ± 0.5 mm) against *Candida albicans.* No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc)

Table-1: Antifungal activity of crude extracts of *Gracilaria corticata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positivecontrol	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positivecontrol
Aspergillus flavus	16±0.6	15±0.5	12±0.6	11±0.7	13±0.4	17±0.6	8	16	32	3 2	16	8
Aspergillus niger	15±0.7	15±0.4	13±0.7	12±0.8	14±0.6	15±0.4	4	8	16	16	8	8
Aspergillus fumigatus	15±0.5	14±0.6	14±0.6	13±0.6	14±0.7	16±0.7	8	8	16	32	16	8
Candida albicans	14±0.8	14±0.4	12±0.7	11±0.7	10±0.4	14±0.7	2	4	8	16	8	4
Candida glabrata	14±0.5	13±0.6	10±0.3	10±0.4	11±0.6	15±0.6	4	8	16	32	8	4

* Flucanozole- positive control, Mean± SD.

Table-2: Antifungal activity of crude extracts of *Gracilaria edulis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positivecontrol	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol
Aspergillus flavus	14±0.3	13±0.6	12±0.6	10±0.6	10±0.7	17±0.6	2	4	8	16	4	8
Aspergillus niger	15±0.6	12±0.3	11±0.3	10±0.6	10±0.5	15±0.4	2	4	8	16	8	8
Aspergillus fumigatus	11±0.7	10±0.4	11±0.4	9±0.3	9±0.6	16±0.7	4	8	16	32	1 6	8
Candida albicans	14±0.4	11±0.6	11±07	9±0.7	10±0.7	14±0.7	2	4	8	16	8	4
Candida glabrata	13±0.5	12±0.7	10±0.6	8±0.4	11±0.6	15±0.6	2	4	8	16	8	4

* Flucanozole- positive control, Mean± SD.

showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Candida albicans.*

Discussion

The present study was aimed to screen the antifungal activity of various solvents extracts of selected seaweeds collected from the Mandapam coastal areas of Tamilnadu. Our results revealed that methanol and acetone extracts of all the selected seaweeds contributed maximum antifungal activity. More commonly *Gracilaria corticata* exhibited maximum antifungal activity against all the tested pathogens followed by *Gracilaria edulis.* Santhanam Shanmughapriya *et al.* (2008) found methanol: toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae.

Table-3: Antifungal activity of crude extracts of *Hypnea musciformis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethylacetate	Positivecontrol	Methanol	Acetone	Chlorofor m	Hexane	Ethylacetate	Positivecontrol
Aspergillus flavus	12±0.3	10±0.7	9±0.3	8±0.5	9±0.5	17±0.6	16	16	32	64	32	8
Aspergillus niger	11±0.5	10±0.8	7±0.3	7±0.4	8±0.6	15±0.4	8	16	32	32	16	8
Aspergillus fumigatus	11±0.3	10±0.6	8±0.5	7±0.7	8±0.4	16±0.7	8	8	16	32	16	8
Candida albicans	13±0.5	11±0.3	7±0.5	8±0.3	9±0.3	14±0.7	4	8	16	16	8	4
Candida glabrata	12±0.6	10±0.3	7±0.3	7±0.4	10±0.5	15±0.6	8	8	16	32	16	4

* Flucanozole- positive control, Mean± SD.

Roberta Paulert *et al*. (2007) studied the antifungal activity of cell wall polysaccharides and crude extracts from the chlorophycian members against filamentous fungi and yeast. The methanol extracts exhibited maximum antifungal activity, similarly our present study also resulted that methanol extracts exhibited maximum activity against all the tested pathogens. Manigandan and Kolanjinathan (2014) reported that acetone extracts of all the red and green algae collected from Mandapam coastal areas exhibited maximum antifungal activity against *A.niger, A.flavus* and *C.albicans*, similarly in the present study the methanol and acetone extracts exhibited maximum antifungal activity. Subba Rangaiah *et al*. (2010) and Kolanjinathan *et al*. (2014) reported that *Sargassum myriocastum* showed low antifungal activity similarly in our present study also reveals that *S.wighitii* showed low antifungal activity. Hebsibah Elsie and Dhana Rajan (2010) studied that ethanolic extract of *G.acerosa* exhibited greater activity against both the bacterial and fungal pathogens than the methanol and acetone extracts, which is contrasting to our results that methanol and acetone extract of *Gracilaria corticata* showed maximum antifungal activity.

Conclusion

The present investigation concludes that the solvent extracts of selected seaweeds of Mandapam coasts of Tamilnadu showed maximum antifungal activity against the selected pathogens. They are potential source of bioactive compounds and should be investigated for natural antibiotics.

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