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Pharmaceutical Properties of Marine Macroalgal Communities from Gulf of Mannar against Human Fungal Pathogens

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ABSTRACT

Objective: To evaluate the antifungal activity of seaweed extracts against human fungal pathogens. **Methods:** Antifungal activity of six species of marine macro algae *Codium decorticatum*, *Caulerpa scalpelliformis*, *Gracilaria crassa*, *Acanthophora spicifera*, *Sargassum wightii* and *Turbinaria conoides* using different solvents acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous were evaluated against *Fusarium oxysporum*, *Fusarium udum*, *Fusarium solani*, *Rhizoctonia solani*, *Alternaria alternat*, *Botrytis cinerea*, *Candida albicans*, *Candida krusei*, *Aspergillus niger and Aspergillus flavus*. **Results:** From the investigation, the maximum activity was recorded from Phaeophyceae, Chlorophyceae and Rhodophyceae respectively. The maximum inhibition zone was noted in acetone extract of *T. conoides* against *F. udum*. **Conclusions:** From these findings, it is concluded that brown seaweed *Turbinaria conoides* is more effective than the green and red seaweeds.

1. Introduction

Fungal infections causes high rate of mortality in human population and aquaculture organisms. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems [1]. Marine macro algae have rich sources of secondary metabolites. Approximately 2500 new metabolites were reported from a variety of marine organisms during the years from 1977 to 1987 [2]. There is an increasing demand of selecting therapeutic drugs from natural products, especially the seaweeds having a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumorals, anti inflammatory and antioxidants. Numerous substances were identified as antimicrobial agents from algae such as Chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocylic compounds, phenolic inhibitors *etc.*[3]. The production of antimicrobial activities was considered to be an indicator of the seaweeds to synthesize bioactive secondary metabolites [4, 5]. So in this context, the study has been made to reveal the biological and medical properties of the following marine flora such as *Chlorophyceae (C. decorticatum, C. scalpelliformis); Rhodophyceae (G. crassa, A. spicifera)* and *Phacophyceae (S. wightii, T. conoides).*

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Materials and Methods

Chemicals

Solvents- acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane.

Composition of Potato Dextrose broth

Ingredients	: Gms / Liter
Potato starch	: 4.0 g
Dextrose	: 20.0 g
pH	: 5.1± 0.2

Composition Zapex Dox agar composition

Ingredients	: Gms / Liter
Sucrose	: 30.000

Sodium nitrate	: 2.000
Dipotassium phosphate	: 1.000
Magnesium sulphate	: 0.500
Potassium chloride	: 0.500
Ferrous sulphate	: 0.010
Agar	: 15.000
Final pH	: 7.3± 0.2

Sample collection and preparation

Fresh seaweeds, *C. decorticatum*, *C. scalpelliformis*, *G. crassa*, *A. spicifera*, *S. wightii* and *T. conoides* were collected from the intertidal regions of the Mandapam coast (Lat. 09° 17.417′N; Long. 079° 08.558′E) of Gulf of Mannar and was immediately brought to the laboratory in plastic bags containing water in order to prevent evaporation. Then these algae were washed thoroughly with tap water to remove extraneous materials. The samples were shade dried until constant weight obtained and ground in an electric mixer. The powdered samples were stored in refrigerator for future use.

Preparation of extracts

Seaweed powder were soaked in the organic solvents with the increasing order of polarity viz., acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous (1:4 w/v) and kept for two weeks at room temperature and the extracts were collected and concentrated. The concentrates were reconstituted with their respective extracts (5 mg mL^{-1}).

Pathogens used for the assay

The fungal species such as *Fusarium oxysporum*, F*usarium udum*, *Fusarium solani*,, *Rhizoctonia solani Alternaria alternat*, *Botrytis cinerea*, *Candida albicans*, *Candida krusei*, *Aspergillus niger* and *Aspergillus flavus* were obtained from Department of Microbiology, Raja Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. The pathogens were maintained on Potato Dextrose broth (PD) (Hi Media, India).

Antifungal assay

Antifungal activity was evaluated using the disc diffusion technique in Petri dishes [6]. Briefly, sterile filter paper discs 6 mm in diameters (Whatman No. 1) were loaded with different extracts and air-dried. Discs containing solvents alone were used as controls. The discs were placed on Zapex Dox agar (Hi Media, India). Plates were swab inoculated using sterile cotton buds with each of the previously mentioned fungal pathogens. Plates were incubated for 72 h at 27℃. Each treatment was done in triplicates. Zone of inhibition was recorded in millimeters.

Results

The antifungal activity of seaweeds (*C. decorticatum*, *C. scalpelliformis*, *G. crassa*, *A. spicifera*, *S. wightii* and *T. conoides*) using seven different solvents (acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous) were tested against 10 fungal pathogens, *F. oxysporum*, *F. udum*, *F. solani*,, *R. solani*, *A. alternat*, *B. cinerea*, *C. albicans*, *C. krusei*, *A. niger* and *A. flavus*. The antifungal activities in different extracts of six seaweeds are in the Table. 1.

Codium decorticatum

The highest activities were recorded in ethyl acetate extract against *F. udum* (10 mm) and methanol extract against *C. krusei* (10 mm) and chloroform extract against C. albicans (10 mm). The minimum activities (2 mm) were observed in acetone extract against *F. oxysporum* methanol extract against *F. solani*, *C. albicans*, *A. flavus*, diethyl ether extract against *R. solani*, *C. krusei* chloroform extract against *F. udum*, *A. alternat*, *C. krusei*, ethyl acetate extract against *A. flavus*. No activity in hexane extract against all tested pathogens.

Caulerpa scalpelliformis

The highest activities were recorded in acetone extract against *F. udum* (14 mm) followed by R. solani (8 mm) chloroform extract (8 mm) and water extract (6 mm) against B. cinerea . The minimum (2 mm) activities were observed in acetone extract against *C. krusei*, methanol extract against *R. solani* and *C. albicans*, diethyl ether extract against A. flavus. No activities in hexane and ethyl acetate extract against all tested pathogens.

Gracilaria crassa

The maximum inhibition zones (4 mm) were noted in acetone extract against *F. udum*, *C. albicans* diethyl ether extract against A. niger, *A. flavus* chloroform extract against *A. alternat*, *R. solani* water extract against *R. solani*, *B. cinerea*. The minimum activities (2 mm) were measured in acetone extract against *R. solani*, *C. krusei*, in methanol extract against *C. albicans* in diethyl ether extract against *C. krusei* in chloroform extract against *F. solani*. Hexane and ethyl acetate extract no activity against all tested pathogens.

Acanthophora spicifera

The highest inhibition zones (8 mm) were recorded in acetone extract against *F. udum* followed by methanol extract against C. albicans (6 mm) and ethyl acetate extract against *A. alternat* (6 mm). The lowest inhibition zones (2 mm) were measured in acetone extract of A. alternat, *R. solani*, *C. albicans*, *A. Niger*, *A. flavus* in chloroform extract against *C. krusei*, *A. flavus*. Ethyl acetate extract against *R. solani*. No activity in hexane extract against all tested pathogens.

Sargassum wightii

The maximum inhibition zones (10 mm) were recorded in methanol extract against *R. solani*, *A. niger* and *C. albicans* (8 mm) followed by diethyl ether extract against C. albicans (8

Table 1

Antifungal activity of seaweeds

Pathogens	Seaweeds in different solvent extracts																				
	Codium decorticatum							*Caulerpa scalpelliformis*							*Gracilaria crassa*						
	A	M	D	C	H	EA	W	A	M	D	C	H	EA	W	A	M	D	C	H	EA	W
F. oxysporum	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–
F. udum	–	*	–	*	–	**	–	***	*	–	–	–	–	–	*	–	–	–	–	–	–
F. solani	–	*	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	*	–	–	–
A. alternat	*	–	–	*	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–
R. solani	**	–	*	–	–	*	*	**	*	*	–	–	–	*	*	–	–	*	–	–	*
B. cinerea	–	–	–	–	–	–	–	–	–	–	**	–	–	**	–	–	–	–	–	–	*
C. albicans	*	*	*	**	–	–	–	–	*	*	–	–	–	–	*	*	–	–	–	–	–
C. krusei	–	**	*	*	–	*	–	*	*	–	–	–	–	–	*	–	*	–	–	–	–
A. niger	*	–	*	–	–	–	–	–	*	–	–	–	–	–	–	–	*	–	–	–	–
A. flavus	–	*	–	*	–	*	–	–	–	*	–	–	–	–	–	–	*	–	–	–	–

Pathogens	Seaweeds in different solvent extracts																				
	Acanthophora spicifera							*Sargassum wightii*							*Turbinaria conoides*						
	A	M	D	C	H	EA	W	A	M	D	C	H	EA	W	A	M	D	C	H	EA	W
F.oxysporum	*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F. udum	**	–	–	–	–	–	–	*	*	–	–	–	–	*	***	–	–	–	–	*	*
F. solani	*	–	–	–	–	–	–	*	–	–	–	–	–	–	*	–	–	–	–	–	–
A. alternat	*	–	–	–	–	**	–	–	*	–	–	–	–	–	–	*	–	–	–	–	–
R. solani	*	–	–	–	–	*	*	–	**	–	–	–	–	**	**	–	–	–	–	**	*
B.cinerea	*	–	–	–	–	–	–	*	*	–	–	–	–	–	**	–	–	–	–	–	–
C. albicans	*	**	*	*	–	–	–	*	**	**	*	–	–	–	–	**	–	**	–	–	*
C. krusei	*	–	–	*	–	–	–	–	–	*	*	–	–	*	–	**	*	–	*	–	–
A. niger	*	*	–	–	–	–	–	*	**	–	*	–	–	*	–	–	**	*	–	–	*
A. flavus	*	–	–	*	–	–	–	–	–	*	*	*	–	–	–	–	–	**	–	–	*

No activity (–); 1–4mm (*); 5–10mm (**); 11–16mm (***)

mm) and water extract against R. solani (8 mm). The minimum inhibition zones (2 mm) observed in acetone extract against *F. udum*, *C. albicans*, methanol extract against *B. cinerea* chloroform extract against C. krusei, A. niger, hexane extract against *A. flavus* water extract against *F. udum*, *C. krusei*. No activity in ethyl acetate extract against all tested pathogens.

Turbinaria conoides

Highest activity (16 mm) observed in acetone extract against *F. udum* followed by *B. cinerea* (12 mm) and metnanol extract against *C. krusei* (10 mm) ethyle acetate extract against *R. solani* (10 mm). The minimum activities (2 mm) were observed in methanol extract against *A. alternat* water extract against *F. udum*, *R. solani*, *C. albicans*, *A. niger*.

Discussion

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities, compounds with cytostatic, antiviral, anthelmenthic, antifungal and antibacterial and antifungal activities have been detected in green, brown and red algae[7, 8]. The production of antifungal activities was considered to be an indicator of the capability of the seaweed to synthesize bioactive secondary metabolites. The search for new fungal antibiotics is important because there are few existing therapies for life-threatening infections, and they are often toxic and of limited efficacy. In this context different species of marine algae were collected and analyzed for their antifungal activity from different part of the world. Most of the algal extracts used in the present study failed to exhibit significant antifungal activity, though many earlier reports have shown the antifungal potential of seaweeds [9].

The chloroform extract of Sargassum marginatum showed antifungal activity against (Trichophyton mentagrophytes, Aspergillus flavus, Candida albicans, Aspergillus niger and Candida parapsilopsis) [9], but in the present study chloroform extract of *S. wightii* least activity against all the tested fungal pathogens.

The maximum activity was recorded from 200 mg of aqueous extract of Ulva lactuca against Aspergillus flavus. Contrast of this study aqueous extract of *C. decorticatum* and *C. scalpelliformis* showed no activity against *A. flavus* and methanolic extract showed maximum activity was recorded from 200 mg of *U. lactuca* against Aspergillus niger, also reported study among the seaweeds the high antifungal activity was noticed in the green algae *U. lactuca* followed by brown algae *S. wightii* and red algae *K. alvarezii* [10].

The aqueous extract of *T. conoides* exhibit the highest activity against Candida albicans. Similarly findings aqueous extract of *T. conoides* showed moderate activity against *C. albicans* [11]. The dichloromethane and ethanol extract of Sargassum dentifolium, Laurentia papillosa and Janio corniculata no activity against Aspergillus flavus and also reported dichloromethane and ethanol extract of Janio corniculata

showed high activity against Candida albicans [12]. Contrast of this work methanol extract of *A. spicifera* and *S. wightii* showed in high activity against *C. albicans* and chloroform extract of *T. conoides* showed high activity against *A. niger*.

The ethanol extract of red algae Gelidium acerosa showed the greatest inhibition activity against Candida albicans, Candida tropicals and Aspergillus niger [13]. Similar findings of methanol extract of red algae *A. spicifera* showed higher activity against *C. albicans* and *A. niger*.

In Acetone, methanol and chloroform extract of green algae Chlorococcum humicola showed the no activity against Candida albicans and activity in ethyl acetate and hexane extracts [14]. But contrast our work moderate activities were absorbed in acetone, methanol and chloroform extract against Candida albicans and no activity was recorded in ethyl acetate and hexane extract against Candida albicans. Also reported methanol, chloroform, ethyl acetate and hexane extract of Chlorococcum humicola is high activity against Aspergillus niger and acetone diethyl ether and hexane extract these green algae against Aspergillus flavus. But distinguish our study methanol, chloroform, ethyl acetate and hexane against Aspergillus niger and acetone, diethyl ether and hexane against Aspergillus flavus no activity was found.

The methanol and aqueous extract of green seaweed Ulva fasciata no activity was recorded against Candida albicans, Aspergillus niger and Aspergillus flavus [9]. Similarly in our study aqueous extract of green seaweeds (*C. decorticatum, C. scalpelliformis)* no activity was recorded. In contrast, methanol extract of both green seaweeds show moderate activity against Candida albicans. In the same way the methanol extract of red seaweeds Asparagopsis taxiformis, Laurencia brandenii, Laurencia ceylanica and Hypnea valentiae were high activity against Candida albicans and Candida krusei [18]. Similar our study methanol extract of red seaweeds Gracilaria crass and *Acanthophora spicifera* showed moderate activity against Candida albicans. But contrast our study no activity was recorded in above extract against Candida krusei.

Marine algae are rich sources of structurally new and biologically active metabolites. In the present investigation clearly demonstrate the antifungal activities and the greatest inhibition zone was recorded from Phaeophyceae followed by Chlorophyceae and Rhodophyceae. These results suggest that the possibility of using marine algae extracts in therapy as natural antibiotics enhanced synthetic antibiotics. Available seaweeds in Gulf of Mannar have wealthy source of biologically active compounds against fungal pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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