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PHARMACOGNOSTICAL AND ANTIFUNGAL ACTIVITY OF SELECTED SEAWEEDS FROM GULF OF MANNAR REGION

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Abstract

The antifungal activities of six important seaweeds namely the green seaweed *Cladophora glomerata*, *Ulva lactuca* and *Ulva reticulata*, the red seaweed *Gracilaria corticata* and *Kappaphycus alvarezii* and the brown seaweed *Sargassum wightii* were screened against fungal pathogens *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatous, Saccharomyces cervisiae* and *Mucor indicus.* The zone of inhibition ranged between 56 - 8mm in aqueous extract and 56 – 4mm in methanolic extract. The maximum activity (56mm) was recorded from 200mg of aqueous extract of *Ulva lactuca* against *Aspergillus flavus* and minimum (8mm) by *Gracilaria corticata* against *Mucor indicus* at 50 mg level whereas, the methanolic extract showed the maximum activity (56mm) was recorded from 200mg of *U. lactuca* against *Aspergillus niger* and minimum (4mm) by 50 mg of *Ulva reticulata* against *Aspergillus flavus.*

Keywords: Seaweeds, Antifungal activity, Methanolic extract, Aqueous extract, Pharmacognosy

Introduction

Seaweeds are macroscopic algae found attached to the bottom in relatively shallow coastal waters. They form one of the important living resources grouped under three divisions namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae). They are abundant on hard substrates and commonly extending to depths of 30-40 m. About 624 species have been reported in India with a potential of 77,000 tons (wet weight) per annum. The red seaweeds contribute 27.0%, brown 0.2 % and others 72.8 %. About 206 species of algae have been reported from the mangrove environment. Seaweeds are the only source of photochemical namely agar agar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents. They are also used for human consumption, animal feed and as manure in several countries. The seaweeds found in the mangrove environment are mostly attached to the aerial roots of the trees. The unsteady and muddy nature of the substratum, as well as the fluctuations in the salinity level makes the mangrove environment unfavorable for the growth of many types of seaweed. As they are primary producers they play a significant role in the benthic food web process. They serve as both feeding and breeding grounds for invertebrates and fishes due to the presence of trace elements, vitamins and bioactive compounds hence, are of great economic value (Abdussalam, 1990; Scheuer, 1990).

Algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients. Some important algae aspects, such as natural character, easy cultivation, their rapid growing (for many of the species) and the possibility of controlling the production of some bioactive compounds by manipulating the cultivation conditions. In this way, algae can be considered as genuine natural Reactors being (Plaza et *al*., 2008).

Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition (Fayaz *et al*., 2005). The lipids which are present in very small amounts are unsaturated and afford protection against cardiovascular pathogens. Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al*., 2003).

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Borowitzka and Borowitzka (1992) mentioned that the cell extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria. Extracted substances from seaweeds have antibacterial actions and other properties include antifungal activities and growth inhibition of plants (Rizvi and Shameel, 2003). Seaweeds are also known to aid and stimulate growth of vegetables, fruits and also protect them from different pathogens and physiological hazards either *in vivo* or storage conditions (Washington *et al.*, 1999). The present study was undertaken to investigate the antifungal activities of methanol and aqueous extract of six seaweeds from Gulf of Mannar against five pathogenic funguses.

Materials and Methods

Sample collection and preparation

Live and healthy specimens of 10 Kg the green seaweed *Cladophora glomerata* (Linn.) Kützing, *Ulva lactuca* Linn. and *Ulva reticulata* Forsskal , the red seaweed *Gracilaria corticata* J. Agardh and *Kappaphycus alvarezii* (Doty) Doty.ex. Silva and the brown seaweed *Sargassum wightii* Greville were collected along the coast of Mandapam, Gulf of Mannar region during low tides. Then the seaweeds were washed thoroughly with seawater to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried in till constant weight obtained and ground in pulverization to get coarse powder. The powdered samples subsequently stored in refrigerator.

Dried algal material is highly controversial to identify the particular species so we were tried to carry out the pharmacognostical behavior of these powder samples. The selected algal species aqueous and methanolic extract were used to screen the antifungal activity.

Preparation of the extracts

The coarse powder was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for antifungal activity and the aqueous extract was analyzed for pharmacological activity.

Pharmacognostical studies

The powder of *Cladophora glomerata, Ulva lactuca*, *Ulva reticulata, Gracilaria corticata, Kappaphycus alvarezii* and *Sargassum wightii,* were mixed with different chemical substances for their identification purposes.

S.No	Treatment
1	Seaweed powder + 50% H_2SO_4
2	Seaweed powder + concentrated H_2SO_4
3	Seaweed powder + 50% HCl
4	Seaweed powder + concentrated HCl
5	Seaweed powder + 50% HNO_3
6	Seaweed powder + concentrated HNO_3
7	Seaweed powder +10% NaOH
8	Seaweed powder +5% $FeCl_2$
9	Seaweed powder + 5% KOH
10	Seaweed powder + Ethanol
11	Seaweed powder +Acetic acid
12	Seaweed powder + 1N HCL
13	Seaweed powder + 1N NaOH +Ethanol

Media preparation

Fungal media (Potato Dextrose Agar)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were pored with 5mm dia cork borer.

Fungal strains

Antifungal activity of six different seaweed extract was investigated against five fungal strains *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatous, Saccharomyces cervisiae* and *Mucor indicus* used for the present study were received from Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India

Antifungal activity of the algae extract

The methanolic and aqueous extract of *Cladophora glomerata, Ulva lactuca*, *Ulva reticulata, Gracilaria corticata, Kappaphycus alvarezii* and *Sargassum wightii* were used throughout the study. The condensed extracts were dissolved in 4% $DMSO_4$ (Dimethysulphoxide). The methanolic and aqueous extract 50, 100 and 200 mg were tested against

different fungal pathogens for their antifungal activity. It was demonstrated by well diffusion assay.

Well diffusion method

Antifungal activity of the plant extract was tested using well diffusion method (Bauer *et al*., 1996). The prepared culture plates were inoculated with different fungal using plate method. Wells were made on the agar surface with 5mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2^{o}C for 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The aqueous and methanolic extract of the dried materials of *Cladophora glomerata, Ulva lactuca, Ulva reticulata, Gracilaria corticata, Kappaphycus alvarezii* and *Sargassum wightii* were used throughout the study. The methanolic extract was dissolved in sterile distilled water to form dilution such as 50, 100 and 200 mg. Each concentrations of the drug were tested against different fungal pathogens.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions were tabulated.

Result and Discussion

In the world, pharmaceutical industrial depending marine seaweeds due to its active principle. So in this study, we selected six seaweeds for the experimental purposes. Which were collected from Gulf of Mannar, Tamil Nadu, India.

Pharmacognostical studies

In normal visible light, different colors were observed in the reaction of different chemical substance. All the above tabulated chemical substances were mixed with fine powder of *Cladophora glomerata, Ulva lactuca, Ulva reticulata, Gracilaria corticata, Kappaphycus alvarezii* and *Sargassum wightii.* The remarkable red color in *Cladophora glomerata,* Pink and yellow in *Kappaphycus alvarezii,* dark red in *Sargassum wightii* were observed (Table 1).

Table 1: Fluorescence studies of seaweed powder

	Treatment	Name of the seaweeds					
		Cladophora glomerata	*Ulva lactuca*	*Ulva reticulata*	*Gracilaria corticata*	*Kappaphycus alvarezii*	*Sargassum wightii*
1	Powder + 50% H_2SO_4	Brown	Dark green	Dark green	Ash	Wheat color	Black
2	Powder + Conc. H_2SO_4	Brownish black	Dark brown	Brownish black	Brown	Dark brown	Brown
3	Powder + 50% HCl	Brown	Ash like brown	Pale green	Light ash	Light brown	Black
4	Powder + Conc. HCl	Greenish black	Green	Dark green	Pale green	Light brown	Black
5	Powder + 50% HNO_3	Dark brown	Light brown like green	Brown	Light brown	Light yellow	Dark red
6	Powder + Conc. HNO_3	Dark red	Light brown	Light brown	Yellowish brown	Light yellow	Dark red
7	Powder +10% NaOH	Dark green	Oil green	Dark brown	Pale green	Light brown	Brown
8	Powder +5% $FeCl_2$	Brownish black	Brown	Brownish green	Brown	Brown	Greenish black
9	Powder + 5% KOH	Black	Dark oil green	Dark green	Pale green	Light brown	Brownish black
10	Powder + Ethanol	Black	Green	Dark green	Ash	Light brown	Black
11	Powder +Acetic acid	Brown	Green	Oil green	Dark ash	Light pink	Black
12	Powder + 1N HCL	Brown	Dark brown	Dark green	Ash	Light brown	Black
13	Powder + 1N NaOH +Ethanol	Brownish black	Light green	Oil green	Brownish green	Sandle color	Dark red

Antifungal activity was the most widespread (70% of the plants), whilst the incidence of antibacterial activity was extra ordinarily low (6% of the plants) (Ballesteros *et al*., 1992). Dried algal material is highly controversial to identify the particular species so we were carried out the pharmacognostical behavior of powder samples and the selected algal species

aqueous and methanolic extract were also used to screen the antifungal activity.

The antifungal activity of aqueous and methanolic extract of six seaweeds against five fungal strains was presented in Table-2 and Table-3 respectively.

Table 2: Inhibition zone of aqueous seaweed extracts against test pathogens

Seaweeds	Concentrations (mg)	Fungal pathogens showing zone of inhibition (mm)				
		Aspergillus niger	*Aspergillus flavus*	*Aspergillus fumigatous*	*Saccharomyces cervisiae*	*Mucor indicus*
Cladophora glomerata	50	12 ± 2.0	15 ± 3.7	14 ± 4.9	20 ± 4.2	10 ± 3.7
	100	15 ± 1.7	22 ± 2.8	36 ± 7.3	26 ± 4.2	13 ± 4.2
	200	20 ± 1.7	40 ± 8.8	46 ± 5.7	42 ± 4.2	26 ± 3.7
Ulva lactuca	50	12 ± 2.8	-	-	24 ± 1.4	08 ± 3.7
	100	22 ± 2.8	26 ± 4.9	10 ± 3.7	26 ± 3.7	14 ± 2.4
	200	25 ± 6.2	56 ± 7.5	26 ± 5.1	56 ± 2.4	20 ± 2.8
Ulva reticulata	50	-	-	-	-	-
	100	-	16 ± 2.4	24 ± 3.7	-	-
	200	20 ± 1.4	28 ± 2.4	26 ± 4.9	-	24 ± 4.9
Gracilaria corticata	50	-	13 ± 4.2	-	-	08 ± 2.4
	100	-	15 ± 6.2	17 ± 2.8	10 ± 2.4	16 ± 2.8
	200	20 ± 4.2	23 ± 2.8	30 ± 6.2	20 ± 2.4	30 ± 1.4
Kappaphycus alvarezii	50	13 ± 2.4	12 ± 2.4	09 ± 2.4	12 ± 1.4	15 ± 3.7
	100	20 ± 2.4	19 ± 5.7	28 ± 3.7	22 ± 2.8	25 ± 3.7
	200	33 ± 3.7	30 ± 4.9	36 ± 2.4	29 ± 3.7	30 ± 3.7
Sargassum wightii	50	-	12 ± 2.4	-	13 ± 3.7	-
	100	-	29 ± 1.4	09 ± 2.4	25 ± 3.7	11 ± 2.4
	200	-	45 ± 3.7	14 ± 2.4	36 ± 3.7	20 ± 2.8

Table 3: Inhibition zone of methanolic extracts against test pathogens

Seaweeds	Concentrations (mg)	Fungal pathogens showing zone of inhibition (mm)				
		Aspergillus niger	*Aspergillus flavus*	*Aspergillus fumigatous*	*Saccharomyces cervisiae*	*Mucor indicus*
Cladophora glomerata	50	10 ± 2.6	10 ± 2.8	-	-	15 ± 6.2
	100	13 ± 1.0	15 ± 6.2	20 ± 7.1	23 ± 2.8	22 ± 2.8
	200	35 ± 4.4	36 ± 4.9	30 ± 7.1	30 ± 1.4	36 ± 7.3
Ulva lactuca	50	19 ± 2.4	14 ± 2.8	10 ± 2.8	-	20 ± 2.4
	100	30 ± 2.8	23 ± 4.2	22 ± 2.4	15 ± 4.9	32 ± 3.7
	200	56 ± 5.1	36 ± 5.7	36 ± 5.1	30 ± 4.9	42 ± 2.4
Ulva reticulata	50	-	-	-	-	-
	100	20 ± 5.1	04 ± 1.4	12 ± 2.4	22 ± 1.4	15 ± 2.4
	200	26 ± 7.5	27 ± 2.4	20 ± 5.1	25 ± 3.7	21 ± 2.8
Gracilaria corticata	50	10 ± 2.8	12 ± 2.4	17 ± 2.8	-	21 ± 2.8
	100	30 ± 3.7	34 ± 6.5	24 ± 5.1	-	30 ± 2.4
	200	34 ± 1.4	40 ± 6.2	28 ± 5.1	19 ± 5.1	36 ± 2.8
Kappaphycus alvarezii	50	-	23 ± 1.4	12 ± 2.8	-	11 ± 2.4
	100	25 ± 2.8	30 ± 3.7	23 ± 2.8	12 ± 2.4	28 ± 2.4
	200	40 ± 6.2	42 ± 1.4	44 ± 5.7	24 ± 4.9	34 ± 1.4
Sargassum wightii	50	10 ± 2.4	20 ± 2.4	12 ± 2.4	10 ± 3.7	11 ± 1.4
	100	17 ± 1.4	32 ± 2.8	25 ± 5.1	20 ± 5.7	15 ± 6.2
	200	35 ± 6.2	37 ± 2.8	30 ± 6.5	28 ± 4.9	35 ± 3.7

The zone of inhibition ranged between 56 - 8mm in aqueous extract and 56 – 4mm in methanolic extract. The maximum activity (56mm) was recorded from 200mg of aqueous extract of *U. lactuca* against *A. flavus* and minimum (8mm) by *G. corticata* against *Mucor indicus* at 50 mg level whereas, the methanolic extract showed the maximum activity (56mm) was recorded from 200mg of *U. lactuca* against *A. niger*

and minimum (4mm) by 50 mg of *U. reticulata* against *A. flavus.* Extracts obtained from the brown alga *Stoechospormum marginatum* and green alga *Codium iyengarii* control the growth of *Fusarium solani* in vitro when used at 6 mg-1 disc (Ara et al., 1998). Extracts of *Caulerpa filiformis*, *Ulva rigida*, *Zonaria toumefortii*, *Hypnea spicifera*, *Gelidium alottiorum* and *Osmundaria serrata* inhibited the fungal growth by more than 50%. Whereas, the extracts of red seaweeds, *Spyridia cupressian* and *Beckerella pinnatifida* showed minimum antifungal activity (Barreto et al., 1997). In present study among the seaweeds the highest antifungal activity was noticed in the green alga *U. lactuca* followed by brown alga *S. wightii*, green alga *C. glomerata* and red alga *K. alvarezii*. There was no inhibitory effect of aqueous extract from *S. wightii* against *A. niger* and *U. reticulata* against *S. cervisiae.* Aqueous extract *C. glomerata* and *K. alvarezii* was active all the tested pathogens against all the concentrations but in the case of methanolic extract of S*. wightii* was the only seaweed active against all the tested pathogens. This may be due to active components which are present in seaweed extracts. A meroditerpenoid metabolite has been isolated from the brown alga *Cystoseira tamariscifolia* and characterized as Methoxybifurcarenone. Methoxybifurcarenone posses antifungal activity against three tomato pathogenic fungi, *Botrytis cinerea*, *Fusarium oxysperium* sp. *mycopersici* under *in vitro* (Bennanmara *et al*., 1999). The present investigation concluded that the aqueous and methanolic extract showed activity against fungus. They are potential source of bioactive compounds and should be investigated for natural antibiotics.

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