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In Vitro Antifungal Activity of Certain Seaweeds Collected From Mandapam Coast, Tamilnadu, India

KEYWORDS: seaweeds, antifungal activity, pathogens

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ABSTRACT *Treatment of infectious diseases towards microbial pathogens develops resistance to a particular antibiotic after repeated administration. Usage of commercial antibiotics for treatment of diseases produces undesirable side effects. Marine macroalgae (seaweeds) are rich in bioactive compounds, potentially exploited as functional ingredients for both human and animal health applications. This preliminary research work was carried out to find out the antifungal activity of seaweeds (Ulva lactuca, Padina gymnospora, Hypnea valentiae, and Gracilaria corticata) extracts collected from Mandapam coastal regions of Tamilnadu. The extracts were tested against Aspergillus flavus, Aspergillus niger and Candida albicans. Acetone and ethyl acetate extracts of four different seaweeds exhibited maximum antifungal activity against all the tested fungal pathogens. Maximum zone of inhibition was observed in acetone extract of Hypnes valentiae against Aspergillus niger, followed by Gracilaria corticata against Aspergillus flavus and Padina gymnospora against Candida albicans at 20 µg concentration.*

INTRODUCTION

Seaweeds are promising natural resources found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available, which is used for food and drug discoveries. These benthic marine algae available all over the world and many of these macro algae have been utilized since ancient times for food (vegetables), fodder, fertilizer and the source of medicines (Krishnamurthy, 2005). Seaweeds are classified into three types; green (Chlorophyta), red (Rhodophyta) and brown (Heterokontophyta), based upon the type of pigment they possess.

More than ten thousand species of marine algae have been reported all over the world. In India, about 220 genera and 740 species of marine algae were recorded of which 60 species are economically important. In Tamilnadu around Mandapam coastal areas 180 species of seaweeds are growing, of which about 40 species are economically important.

Since last decade, Indian seaweeds were more commonly utilized for industrial production of agar/alginate and as fertilizers; their applications in other contemporary area are still unexploited. In recent years the marine macroalgae have been extensively exploited for their perceived health benefits including anticoagulant, antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-herpes and anti-hyperlipidemic activity (Wang *et al.,* 2011). The recent finding on seaweeds application in clinical and nutraceutical area has awakened researcher to explore the unexploited domain of seaweed to develop novel therapeutic compounds and antioxidant potential from Indian water (Sachindra *et al.,* 2010). Seaweeds constitutes of various primary and secondary bioactive compounds, which are able to produce great variety of secondary metabolites characterized by a broad spectrum of biological activities with antiviral, antibacterial, antifungal, cytostatic and antihelminthic activities especially in green, brown and red seaweeds of Indian origin (Del Val *et al.,*2001; Newman *et al*., 2003). The bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides (Kolanjinathan *et al.,* 2009). The production of antimicrobial components was considered to be an important indicator of the capacity of the seaweeds to synthesize primary and secondary bioactive metabolites (Nair *et al*., 2007).

MATERIALS AND METHODS

Collection and Extraction of seaweeds

Live and healthy samples of *Ulva lactuca* (green), *Hypnea valentiae, Padina gymnospora* (brown) and *Gracilaria corticata* (red) collected from the Mandapam coastal regions during low tides and the macroalgae collected was brought to the laboratory in polythene bags, washed several times with seawater to remove sand and other debris, dried in shade condition at room temperature for a week. After drying the algal materials were homogenized to fine powder and used for extraction.

The powder (40 g) of dried algae was extracted in Soxhlet apparatus using 200 ml of various solvents (chloroform, ethyl acetate, acetone and hexane) and crude extracts were concentrated to dryness in rotary evaporator under reduced pressure and the resulting paste form was stored in refrigerator at 4°c for further use (Sreenivasa-Rao and Parekh, 1981).

Collection and maintenance of fungal pathogens

Three test fungal strains *viz., Aspergillus flavus, Aspergillus niger and Candida albicans* were collected from Rajah Muthiah medical college and hospital, Annamalainagar. The antifungal assay of seaweed extracts were determined by disc diffusion method (Bauer *et al.,* 1966). Fluconazole was used as the positive control and DMSO serves as negative control.

RESULTS

The antifungal activity of various solvent extracts of four seaweed species were resulted in table 1. All the data were examined as mean ±SD.

Ulva lactuca

The Acetone extract showed maximum activity against ***Aspergillus niger*** of 12.0mm zone of inhibition, followed by

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Aspergillus flavus (11.0mm) and minimum zone of inhibition was observed in *Candida albicans* (10.0 mm). The Ethyl acetate extracts showed maximum activity against *Aspergillus niger* and *Aspergillus flavus* of 10.0mm zone of inhibition, minimum activity was observed in *Candida albicans* (08.0 mm). The chloroform and hexane extracts showed minimum activity of 08.0mm against *Aspergillus niger* and no zone of inhibition was observed against *Aspergillus flavus.*

Padina gymnospora

The Acetone extract showed maximum activity against *Candida albicans* of 12.0mm zone of inhibition, followed by *Aspergillus niger* (11.0mm) and minimum zone of inhibition was observed in *Aspergillus flavus* (09.0 mm). The Ethyl acetate extracts showed maximum activity against *Candida albicans* and *Aspergillus flavus* of 10.0mm zone of inhibition, minimum activity was observed in *Aspergillus niger* (08.0 mm). The chloroform and hexane extracts showed minimum activity of 08.0 mm and less than or no zone of inhibition.

Hypnea valentiae

The Acetone extract showed maximum activity against *Aspergillus niger* of 15.0mm zone of inhibition, followed by *Candida albicans* (11.0mm) and minimum zone of inhibition was observed in *Aspergillus flavus* (10.0 mm). The Ethyl acetate extracts showed maximum activity against *Aspergillus flavus*(10.0 mm) followed by *Candida albicans* of 09.0mm zone of inhibition, minimum activity was observed in *Aspergillus niger* (08.0 mm). In chloroform extract *Candida albicans* showed maximum zone of inhibition (10.0mm) and hexane extracts showed minimum activity of 08.0mm against *Aspergillus flavus,* followed by *Candida albicans* and no zone of inhibition was observed against *Aspergillus niger.*

Gracilaria corticata

The Acetone extract showed maximum activity against *Aspergillus flavus* of 12.0mm zone of inhibition, followed by *Aspergillus niger* (12.0mm) and minimum zone of inhibition was observed in *Candida albicans* (09.0 mm). The Ethyl acetate extracts showed maximum activity against *Aspergillus niger* of 10.0mm zone of inhibition, followed by *Candida albicans* and *Aspergillus flavus* (08.0 mm). The chloroform and hexane extracts showed minimum activity of 08.0 mm and less than or no zone of inhibition.

Table: 1. *In vitro* Antifungal activity of the seaweed extracts

Seaweeds	Solvents used	Fungal strains (zone of inhibition in mm)		
		Aspergillus flavus	Aspergillus niger	Candida albicans
Ulva lactuca	Acetone	111.0	121.5	100.5
	Ethyl acetate	101.0	101.5	081.2
	Chloroform	081.0	-	081.5
	Hexane	080.5	-	-
Padina gymnospora	Acetone	092.0	111.0	121.5
	Ethyl acetate	101.0	082.0	100.5
	Chloroform	081.5	-	080.5
	Hexane	-	081.0	080.5
Hypnea valentiae	Acetone	102.0	151.0	111.5
	Ethyl acetate	102.0	081.0	091.0
	Chloroform	-	080.5	101.0
	Hexane	081.5	-	080.5
Gracilaria corticata	Acetone	121.0	110.5	100.5
	Ethyl acetate	110.5	121.0	111.5
	Chloroform	080.5	091.0	-
	Hexane	091.0	-	080.5
Positive control Fluconazole		102.0	132.0	111.0

Each value representing mean SD of 3 replicates, zone of inhibition referred as mm in dtiameter.

DISCUSSION

Aim of the present study was to evaluate the antifungal activity of different macroalgae for its potential bioactive potentials. Our results showed that acetone and ethyl acetate extracts of the collected seaweeds extracts exhibited maximum antifungal activity when compared to other solvent extracts. The hexane and chloroform extracts obtained from several *Laurencia* species possess antifungal properties (Stein *et al.,* 2011). Oranday *et al*. (2004) screened polar and non polar extracts of four species of marine seaweeds for antifungal properties against seven microorganisms by the disc diffusion method. The polar extracts of *Gracilaria tikvahiae* inhibited the growth of more than four microorganisms. Protein fraction of *Hypnea musciformis* inhibited the growth of *C.Guilliermondii*; against *C.albicans* it showed low activity. Similarly in the present study, the macroalgal extract of *Hypnea valentiae* shows low activity against *C.albicans* (Rossana *et al*., 2006). *U.fasciata* exhibited broad spectrum antimicrobial activity whereas *Hypnea musciformis* showed narrow spectrum antimicrobial activity (Selvin and Lipton, 2006), similarly in the present study both the green and red algae showed broad spectrum antifungal activity. Pandurangan *et al*. (2010) reported that aqueous and methanolic extracts of *Ulva lactuca* shows highest antifungal activity against *A.flavus* and *A.niger.* In the present study acetone and ethyl acetate extracts of *Ulva lactuca* possess highest antifungal activity against all

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the tested pathogens. Manivannan *et al.* (2011) evaluated the antimicrobial activity of *Turbinaria conoides (T. conoides), Padina gymnospora (P. gymnospora)* and *Sargassum tenerrimum* against human bacterial and fungal pathogens in which acetone extract of *T. conoides* had mild inhibition against *Aspergillus niger* (3.00±0.89), contrastingly this study reveals the highest activity of the acetone extracts of all the tested seaweeds against the fungal pathogens. From this preliminary research work, we demonstrated that solvent extracts of seaweeds showed promising activity against the tested pathogens. Among the tested marine algae, some appeared to exhibit specific antifungal activity against the tested fungal pathogens. This helps in development of specific antibiotics against fungal infections and further work is needed to identify the principle compound responsible for antifungal activity against pathogenic fungi especially those causing the human diseases.

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