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SCREENING OF ANTIFUNGAL ACTIVITY OF SELECTED MACROALGAE AGAINST *Candida albicans* AND *Candida glabrata*

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Abstract

The marine biodiversity provides an important source of chemical compounds which have large number of therapeutic applications like antiviral, antibacterial, antifungal and anticancer activities. Sea weeds or marine algae are potentially prolific sources of highly biologically active secondary metabolites that might represent useful leads in the development of new pharmaceutical bioactive compounds. The antifungal activity of selected marine macroalgae crude extracts was studied against two pathogenic yeasts. The selected marine macroalgae crude extracts showed zone of inhibition against pathogenic yeasts *viz*., *Candida albicans* and *Candida glabrata*. The crude methanolic extract of *Padina gymnospora* showed maximum mean zone of inhibition against pathogenic yeasts. The crude methanolic extract of *Padina gymnospora* showed best against pathogenic yeasts.

Key words: Seaweeds, antifungal activity, Minimum inhibitory concentration, *Candida albicans* and *Candida glabrata*.

1. Introduction

Pharmaceutical industries are giving importance to the biologically active compounds which were derived from the traditional sources like soil & plants and less traditional sources like marine organisms which includes micro and macro species (McGee, 2006). The marine biodiversity provides an important source of chemical compounds which have large number of therapeutic applications like antiviral, antibacterial, antifungal and anticancer activities (Pereira *et al*., 2004).

Marine macroalgae are floating submerged large algae of shallow marine meadow. Macroalgae are the most nutritious plants on earth, since they are low in fats but contain vitamins and bioactive compounds like terpenoids and sulphated polysaccharides and they offer a good source of useful chemicals like alginic acid, mannitol, laminarin, fucoidin and iodine (Kim *et al*., 1997).

Many seaweeds or macroalgae are known to synthesize bioactive secondary metabolites which have antimicrobial activities. There are numerous reports of compounds derived from macroalgae with a broad range of antibacterial activities (Magallanes *et al*., 2003).

Marine macroalgae comprises of various natural source of a wide variety of beneficial drugs which plays a major role in pharmaceutical industries, food industries and cosmetics based applications which includes carotenoids, terpenoids, steroids, amino acids, phlorotannins, phenolic compounds, halogenated ketones, alkanes and cyclic polysulphides (Taskin *et al*., 2007; Guedes *et al*.,. 2011). Therefore, the use of

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algae have been increased in traditional medicine (Fitton, 2006).

2. Materials and Methods

Collection of seaweeds

Five different seaweeds *viz*., *Sargassum wightii, Caulerpa racemosa, Acanthophora spicifera, Padina gymnospora* and *Turbinaria conoids* were collected from Mandapam coast of Tamil Nadu, India, that is situated in 9°17′N latitude and 79°07′E longitude and having 9 m MSL in Tamil Nadu. The seaweeds were taxonomically identified at the Center for Advanced Studies in Marine Biology, Annamalai University, Tamil Nadu, India.

Preparation of seaweed extracts

The collected seaweeds samples were cleaned and the necrotic parts were removed. The seaweeds washed with tap water to remove any associated debris and shade dried at room temperature for 5 - 8 days or until they are brittle easily by hand. After completely drying, the seaweed materials (1.0 kg) were ground to a fine powder using electrical blender. Forty gram of powdered seaweeds was extracted successively with 200 ml of solvents (Methanol, Acetone, Chloroform, Hexane and Ethyl acetate) in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness by reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4 °C for future use (Cheessborough, 2000).

Collection of test fungal cultures

Six different fungal isolates were used in this present study. The fungal cultures were procured form Microbial Type Culture Collection (MTCC). Chandigarh.

a) *Candida albicans* (MTCC 7315)
b) *Candida glabrata* (MTCC 3983)

Cultures maintenance and inoculum preparation

Maintenance of test fungal cultures

The test fungal isolates were sub-cultured and maintained on Sabouraud's dextrose agar slants and stored in refrigerator at 4 °C.

Fungal inoculums preparation

Fungal inoculums was prepared by inoculating a loopful of test organisms in 5 ml of Saboruraud's dextrose broth and incubated at 28 °C for 2 days (yeasts) and 3 days (moulds) till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards and then used for the determination of antifungal activity.

Disc preparation

Preparation of algal disc for antifungal activity

Six mm diameter disc were prepared using sterile Whatmann No.1 filter paper. The seaweeds crud extracts (300 mg/ml) obtained using solvents (Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform) were mixed with 1 ml of 5 % Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 µl of different solvent extracts seaweeds to check their antifungal activity. The Flucanozole (100 units/disc) was used as positive control and the 5 % DMSO was used as a blind control.

Antifungal assay

Disc diffusion method

The antifungal activity of seaweed extracts were determined by Disc diffusion method proposed by Bauer *et al*. (1966). Petri plates were prepared by pouring 20 ml of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculums suspension was poured and uniformly spreaded. The excess inoculums were drained and the plates were allowed to dry for five minutes.

After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The flucanozole (100 units/disc) was used as positive control and the 5 % DMSO was used as a blind control in these assays. The plates were incubated at 28 ºC for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

Minimum inhibitory concentration for fungi

Minimum inhibitory concentration (MIC) of the seaweed extracts against fungal isolates was tested in Sabouraud's dextrose broth by Broth macro dilution method (Ericsson and Sherri, 1971). The seaweed extracts were dissolved in 5 % DMSO to obtain 128 mg/ml stock solutions. A volume of 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth for fungi to get a concentration of 1, 2, 4, 8, 16, 32, and 64 mg/ml for seaweeds extract and 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 28 ºC for 48 hours (yeasts) and 72 hours (moulds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

3. Results and discussion

Antifungal activity of marine macro algae against *Candida albicans*

The antifungal activity of selected marine macroalgae crude extracts tested against *Candida albicans* and the results were presented in Table - 1. The selected marine macroalgae crude extracts showed zone of inhibition against *Candida albicans*. The crude methanolic extract of *Padina gymnospora* showed maximum mean zone of inhibition against *Candida albicans* (15 ± 0.9 mm) followed by *Turbinaria conoides* (15 ± 0.7 mm), *Sargassum wightii* (15 ± 0.5 mm), *Caulerpa racemosa* (12 ± 0.8 mm) and *Acanthophora spicifera* (12 ± 0.6 mm) at 300 mg/ml**.** The crude hexane extract of marine macroalgae crude extracts showed minimum zone of inhibition against *Candida albicans* when compared to the other solvent extracts. No zone of inhibition was seen in DMSO negative control and the positive control Fluconazole (100 units) showed 17 ± 0.8 mm inhibition zone against *Candida albicans*. The minimum inhibitory concentration (MIC) of marine macroalgae crude extracts against *Candida albicans* was ranged between 20 mg/ml to 640 mg/ml and the results were showed in Table - 2. The crude methanolic extract of *Padina gymnospora, Turbinaria conoides* and *Sargassum wightii* showed best MIC at 20 mg/ml against *Candida albicans*. Extract of *Caulerpa racemosa* and *Acanthophora spicifera* showed best MIC at 40 mg/ml.

The findings of their study was in line with the research of Mtolera and Semesi (1996) because in this present study *Caulerpa racemosa* showed inhibitory activity against the tested fungal pathogens.

Antifungal activity of marine macro algae against *Candida glabrata*

The antifungal activity of selected marine macroalgae crude extracts was studied against *Candida glabrata* and the results were tabulated in Table - 3. The selected marine macroalgae crude extracts showed zone of inhibition against *Candida glabrata*. The crude methanolic extract of *Padina gymnospora* showed maximum mean zone of inhibition against *Candida glabrata* (16 ± 0.6 mm) followed by *Turbinaria conoides* (15 ± 0.6 mm), *Sargassum wightii* (14 ± 0.6 mm) *Caulerpa racemosa* (12 ± 0.8 mm) and *Acanthophora spicifera* (12 ± 0.6 mm)**.** The crude hexane extract of marine macroalgae crude extracts showed minimum zone of inhibition against *Candida glabrata* when compared to the other solvent extracts. No zone of inhibition was seen in DMSO negative control and the positive control Fluconazole (100 units) showed zone of

Table - 1: Antifungal activity of marine macro algae crude extracts against *Candida albicans*

Marine macro algae crude extracts (300 mg/ml) and Zone of inhibition (mm)						
Name of the marine macroalgae	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Turbinaria conoides	10±0.6	11±0.8	12±0.6	14±0.4	15±0.7	17±0.8
Sargassum wightii	10±0.3	11±0.4	11±0.8	13±0.7	15±0.5	
Padina gymnospora	12±0.3	13±0.5	13±0.8	15±0.3	15±0.9	
Caulerpa racemosa	9±0.7	11±0.3	11±0.7	12±0.2	12±0.8	
Acanthophora spicifera	9±0.7	9±0.6	10±0.5	11±0.4	12±0.6	

Mean ± SD, *Fluconazole (100 units)

Table - 2: Minimum inhibitory concentration of marine macro algae crude extracts against *Candida albicans*

Minimum inhibitory concentration of marine macro algae crude extracts (mg/ml)						
Name of the marine macroalgae	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control***
Turbinaria conoides	160	80	80	40	20	20
Sargassum wightii	160	80	80	40	20	20
Padina gymnospora	160	80	80	40	20	20
Caulerpa racemosa	160	160	80	80	40	20
Acanthophora spicifera	320	160	80	40	40	20

*Fluconazole (100 units)

Table - 3: Antifungal activity of marine macro algae crude extracts against *Candida glabrata*

Marine macro algae crude extracts (300 mg/ml) and Zone of inhibition (mm)						
Name of the marine macroalgae	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Turbinaria conoides	11±0.6	12±0.4	12±0.7	14±0.7	15±0.6	18±0.7
Sargassum wightii	11±0.3	11±0.7	12±0.5	13±0.8	14±0.6	
Padina gymnospora	12±0.4	13±0.8	14±0.2	14±0.7	16±0.6	
Caulerpa racemosa	9±0.7	10±0.4	11±0.5	1±0.7	12±0.8	
Acanthophora spicifera	9±0.5	10±0.2	10±0.8	11±0.5	12±0.6	

Mean ± SD, *Fluconazole (100 units)

Table - 4: Minimum inhibitory concentration of marine macro algae crude extracts against *Candida glabrata*

Minimum inhibitory concentration of marine macro algae crude extracts (mg/ml)						
Name of the marine macroalgae	**Hexane**	**Chloroform**	**Ethyl acetate**	**Acetone**	**Methanol**	**Positive Control ***
Turbinaria conoides	160	160	80	80	40	20
Sargassum wightii	160	160	80	80	40	20
Padina gymnospora	160	80	80	40	20	20
Caulerpa racemosa	320	160	160	80	40	20
Acanthophora spicifera	320	160	160	80	40	20

*Fluconazole (100 units)

inhibition was 18 ± 0.7 mm against the *Candida glabrata*. The minimum inhibitory concentration (MIC) of marine macroalgae crude extracts against *Candida glabrata* was ranged between 20 mg/ml to 640 mg/ml and the results were showed in Table - 4. The crude methanolic extract of *Padina gymnospora* showed best MIC at 20 mg/ml against *Candida glabrata*. Extracts of *Turbinaria conoides, Sargassum wightii, Caulerpa racemosa* and *Acanthophora spicifera* showed best MIC at 40 mg/ml.

The findings of the present study also showed that the polar solvent as an effective one for the extraction of antimicrobial compounds from the seaweed *Acanthophora spicifera* and the results of the present study was similar with the studies of Oranday *et al*. (2004).

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