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Research Article

Screening of Anticandidal and Antidermatophytic activities of *Gracilaria edulis* and *Gracilaria verrucosa* (Rhodophyceae) from the Gulf of Mannar, Manappad Coast, Tamilnadu

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ABSTRACT

The present studies five different solvents of *viz*., hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Gracilaria edulis* (S.G. Gemelin) P .C. Silva and *Gracilaria verrucosa* (Huds.) Papenfuss, were collected from Manappad, Gulf of mannar Biosphere, Reserve, Tamilnadu, India. Their crude extracts were tested against six yeast *viz., Candida albicans*, *C. krusei*, *C. guilliermondi*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Four dermatophytes *viz., Trichophyton rubrum*, *T. mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum* were determined. Among the highest antifungal activity was recorded in the ethyl acetate extract of *G. edulis* than the other extracts. The mean zone of inhibition produced by the extracts in disc diffusion assays against the tested fungal strains ranged from 7.0 to 14.5 mm and the lowest MIC (250 µg/ml) and MFC (500 µg/ml). The ethyl acetate extracts of the seaweeds showed the strong phytochemicals, terpenoids, tannins, phenolic compounds and steroids in *G. edulis* and *G. verrucosa* than the other solvents extracts. The results obtained in the present study suggest that ethyl acetate extracts of *G. edulis* and *G. verrucosa* can be used in treating disease caused by *C. parapsilosis*, *C. albicans* and *T. rubrum*.

Key words: *Gracilaria edulis, Gracilaria verrucosa,* Antifungal activity and phytochemical analyses

Introduction

Marine macro algae refer to any large marine benthic algae that are multicellular, macrothallic, and thus differentiated from most algae that are of microscopic size. These plants form an important renewable resource in the marine environment and have been a part of human civilization from time immemorial. The long history of seaweed utilization for avariety of purposes has led to the gradual realization that some of their constituents are more superior and valuable in comparison to their counterparts on land. In India, seaweeds are mainly exploited as a source of phycocolloids such as agar-agar, alginate and carrageenan and not for their beneficial aspect with respect to food and medicine [1]. Over the last decades, marine algae have received a lot of attention as functional food ingredients because of their richness in protein, vitamins, and minerals. One hundred grams of seaweeds provides more than the daily requirement of vitamins A, B2, B12 and C [2]. In addition, seaweeds have traditionally been used in several countries in folk medicine for curing infectious diseases, gout and eczema.

Gacilaria edulis (S.G. Gmelin) (Gracilaria, Rhodophyta) is a red marine alga, which is widely distributed in the Indian Ocean and in the Pacific Ocean. *Gracilaria edulis* belongs to the family Rhodophyceae plants in can grow up to 27cm full. It is brownish – red and arises from a discoid hold fast the branching is dense and fastigiated, extremely divergent, dichotomous to trichotomous, in up to 7 orders and with branches at long intervals. The branches are 1-1.5 mm in diameter, earlilaginuous, flexuous and cyclindrical and ends in pointed apices. *Gracilaria verrucosa* (Papenfuss) Huds (*Gracilaria*, Rhodophyta) is a red marine alga, which is widely distributed in the Indian Ocean and in the Pacific Ocean. *Gracilaria edulis* belongs to the family Rhodophyceae plants thalli are usually highly branched, flattened, long filamentous, more dichotomously branch, *Gracilaria verrucosa* height 15 cm, the assimilator of the plant 3.2 cm and holdfast large disc shape.

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Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide [3]. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/AIDS patients. This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs has been the reason for an extended search for newer drugs to treat opportunistic fungal infections [4].

The yeast fungus, *Cryptococcus neoformans*, has been identified as the fourth most common cause of life-threatening infection in AIDS patients. Potentially fatal infections with *Candida albicans* and other species of *Candida* are also known [5]. Candidiasis caused by Candida species has increased substantially in the past 20 years. Candida species are now at fourth ranks among microbes. These organisms are opportunistic pathogens that can cause local and systemic infections in predisposed persons, commonly affecting immunologically compromised patients and those undergoing prolonged antibiotic treatment. Among various Candida species, *C. albicans* is the organism most often associated with serious fungal infection and it is showing increased resistance to traditional antifungal agents [6]. Recently, non-*Candida albicans* species such as *C. glabrata, C. guilliermondii, C. parapsilosis* and others species also showed dramatic increase in fungal infections and antifungal resistances [7].

Dermatophyes are the most common agents of fungal infections worldwide [8]. Dermatophytic infections have been considered to be a major public health problem in many parts of the world. The infections are common in the developing countries, and are of particular concern in the tropics, especially in infants. The infections are caused by 40 species of fungi which are grouped into three genera; *Trichophyton, Microsporum and Epidermophyton*. Trichophyton is a fungal species that causes superficial mycoses commonly known as tinea infections in humans and other animals [9].

Several works have demonstrated in laboratory trials that plants tissues, such as roots, leaves, seeds and flowers posses inhibitory properties against bacteria, fungi and insects [10, 11, 12]. The search for new effective medicines remains a challenge for scientists. Therefore around the world, many researchers have focused on natural sources for new molecules with marine macro algae among the targets of these studies.

In the present study antifungal activity of different organic solvents extracts of *G. edulis* and *G. verrucosa* was examined against *Candida albicans*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *Trichophyton rubrum*, *T. mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum.*

Materials and Methods

Sample collection

Gracilaria edulis (S.G. Gemelin) P .C. Silva, and *Gracilaria verrucosa* (Huds.) Papenfuss (Rhodophyceae) were collected from Manappad (Lat. 8° 30′N; Long. 78 °8′E), Tuticorin district, the Gulf of Mannar Marine Biosphere Reserve, Tamil Nadu, India. The collections were made from the month of January, 2012. The algae were identified by Prof. R. Panneerselvam, Head and the museum specimens are deposited in the Department of Botany, Annamalai University.

Preparation of extracts

The collected algal species were handpicked rocks and shells submerged under water during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal casting, and adhering sand particles etc.,. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in a ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. The seaweed materials dry in one week in room temperature. After getting from all the samples were grounds in to a fine powder.

Six hundred grams of powdered samples were packed in Soxhlet apparatus and extracted with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 hours. The extracts were pooled and the solvents were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 40°C and the dried extracts were stored at 4°C for antifungal assay.

Phytochemical screening

The qualitative phytochemical analyses studies hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. edulis* and *G. verrucosa*. Phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, phenolic compounds, Coumarins and diterpenoids were carried out according to the method described by Harborne [13]; Trease and Evans [14].

Fungal strains used

The ten human fungal pathogenic microorganisms such as Yeast *viz., Candida albicans* (MTCC 3017), *Candida krusei* (MTCC 9215), *Candida guilliermondii* (NCIM 3216), *Candida parapsilosis* (MTCC 2509), *Candida tropicalis* (MTCC 184) and *Candida glabrata* (MTCC 3019), four dermatophytes *viz., Trichophyton rubrum* (MTCC 296), *Trichophyton mentagrophytes* (MTCC 8476), *Microsporum gypseum* (MTCC 2819) and *Epidermophyton flocossum* (MTCC 7880) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Biochemical Sciences Division, National Chemical Laboratory, Pune, India.

In vitro antifungal activity was determined by using Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) (for mycelial fungi), Yeast Nitrogen Base (YNB) (for yeast) and Roswell Park Memorial Institute Medium (RPMI) and they were obtained from Himedia Ltd., Mumbai.

Antifungal assays

Disc diffusion method

Antifungal activity tests were performed by using the agar disc diffusion method according to Bauer et al. [15] with modifications. Perti plates were prepared by pouring 20 ml of sterile SDA. The standardized fungal suspension was applied on the solidified culture medium by using sterile cotton swabs and allowed to dry for 5 min. The standard inoculum using yeast suspensions containing 10^6 CFU/mL and mould fungal suspensions containing 10^4 spores/mL were swabbed on the top of the solidified respective media and allowed to dry for 10 minutes. The disks with different concentrations of extracts (1000, 500 and 250 µg/disc) were prepared and aseptically applied on the surface of the petriplates. The agar plates were inoculated and incubated for the plates were incubated at 28 °C for 24 hours for yeast and 30 °C for 4-7 days for dermatophytes. Amphotercin-B (100 units/disc) for Yeast and Ketoconazole (5µg/disc) for dermatophytes were used as positive controls and 10 per cent DMSO was used as blind controls in all the assays. The zone of inhibitions was observed and measured in millimeters. All assays were performed in triplicate.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of the different extracts from the red algae species were determined by using broth micro dilution technique as recommended by CLSI M27-A3 [16] and M38-A2 [17] for yeast and filamentous fungi, respectively. The MIC values were determined in RPMI-1640 (Himedia, Mumbai) with L –glutamine without sodium bicarbonate, pH 7.0 with Morpholine – sulfonicacid (MOPS). 20 µl of a stock solution (2 mg/ml) of each algae extracts in 10 % DMSO was dissolved with 980 µl of RPMI-1640 made a solution 1000 µl (1mg/ml). From that, the two fold serial dilutions in the range from 1000 to 15.7 µg/ml were prepared. 100µl of solution was poured into first well of 96 well microtitre plates and then, 50µl were transformed to the next well containing 100 µl of RPMI-1640. The same procedure was performed for all wells. 10µl of fungal standardized inoculum suspensions containing 0.5-2.5 $\times 10^3$ for yeast 0.4-5 $\times 10^4$ for dermatophytes CFU/mL was transferred to each well. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated at 28 °C for 24 hours for yeast and 30 °C for 4-7 days for dermatophytes. The MIC of the extracts was recorded as the lowest concentration of inhibited the growth of the *Candida* and dermatophytic strains as compared to that of control.

Determination of the Minimum Fungicidal Concentration (MFC)

The MFC was determined by plating a loopful of samples from each MIC assay well with growth inhibition in to freshly prepared SDA plates. The plates were incubated at 28 °C for 24 hours for yeast and 30 °C for 4-7 days for dermatophytes. The MFC was recorded as the lowest concentration of

the extracts that did not permit any visible fungal growth after the period of incubation.

Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between different extracts for *in vitro* antifungal assays. Comparison of means for *in vitro* antifungal assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. *P* value < 0.05 was considered statistically significant.

Results

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. edulis* and *G. verrucosa* were used to analyses the phytochemicals such as terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds, coumarins and diterpenoids. The ethyl acetate extracts of *G. edulis* and *G. verrucosa* contained presence of phytochemicals terpenoids, tannins, phenolic compounds and steroids than the other solvent extracts. Among the tested phytochemicals, diterpenoids and cardiac glycosides were absent in all the extracts of *G. edulis* and *G. verrucosa*. Alkaloids were present only in the chloroform and ethyl acetate extracts of *G. edulis* and *G. verrucosa*. Coumarins were absent in all the extracts tested.

The different solvents of *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. edulis* and *G. verrucosa* were screened against fungal strains. Among the tested extracts, the ethyl acetate and chloroform extracts showed antifungal activity against fungal strains tested. The mean zones of inhibition of the extracts, assayed against the test organisms ranged between 7.0 and 14.5 mm. The ethylacetate extract of *G. edulis* showed the promising activity against *C. parapsilosis* (14.5 mm), followed by *C. albicans* (14.3 mm) and *T. rubrum* (14.1 mm). The chloroform extract showed the activity against *C. parapsilosis* (13.1 mm), followed by *T. rubrum* (12.8 mm), *C. albicans* (12.8 mm) and *T. rubrum* (12.6 mm) and the table are presented in (Tables 1 & 2). The Amphotericin-B (100 units/disc), anticandidal positive control produced zones of inhibition were from 9.0 to14.5 mm. Ketoconazole (10 µg/disc), antidermatophytic positive control produced zones of inhibition ranged from 14 to 19 mm. The negative control (10% DMSO) did not produce any zone of inhibition for all the fungal strains tested. The result of MIC values of the different extracts of *G. edulis* and *G. verrucosa* ranged between 250 and 500 µg/ml. While the MFC values were between 500 and 1000 µg/ml.

Table 1: Antifungal activity of different extracts of *Gracilaria edulis*

Fungal strains/ Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000 (µg/disc)	500 (µg/disc)	250 (µg/disc)	Amphotercin-B (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	12.3 ± 0.57	9.8 ± 0.76	7.6 ± 0.76	11.0 ± 0.50	500	1000
Chloroform	12.8 ± 0.76	10.1 ± 0.15	7.8 ± 0.76	10.3 ± 0.15	500	1000
Ethyl acetate	14.3 ± 0.57	11.1 ± 0.15	8.3 ± 0.57	12.1 ± 0.15	250	500
Acetone	10.8 ± 0.76	9.5 ± 0.50	7.3 ± 0.20	10.8 ± 0.76	500	1000
Methanol	10.3 ± 0.57	9.1 ± 0.15	7.0 ± 0.11	12.5 ± 0.50	500	1000
Candida krusei						
Hexane	10.8 ± 0.76	9.1 ± 0.15	7.3 ± 0.20	9.1 ± 0.20	500	1000
Chloroform	11.8 ± 0.76	9.8 ± 0.76	7.5 ± 0.50	11.5 ± 0.50	500	1000
Ethyl acetate	13.5 ± 0.50	10.3 ± 0.57	7.8 ± 0.76	13.3 ± 0.57	250	500
Acetone	10.1 ± 0.15	8.8 ± 0.76	7.1 ± 0.11	11.0 ± 0.50	500	1000
Methanol	NA	NA	NA	9.8 ± 0.76	NT	NT
Candida guilliermondii						
Hexane	10.5 ± 0.50	9.3 ± 0.20	7.3 ± 0.20	10.0 ± 0.50	500	1000
Chloroform	11.5 ± 0.50	9.6 ± 0.76	7.6 ± 0.76	11.1 ± 0.15	500	1000
Ethyl acetate	13.3 ± 0.15	10.1 ± 0.15	7.8 ± 0.76	9.5 ± 0.50	250	500
Acetone	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.11	10.1 ± 0.15	500	1000
Methanol	NA	NA	NA	9.5 ± 0.50	NT	NT
Candida glabrata						
Hexane	10.1 ± 0.15	9.0 ± 0.50	7.3 ± 0.20	12.3 ± 0.57	500	1000
Chloroform	11.1 ± 0.15	9.3 ± 0.57	7.5 ± 0.50	13.5 ± 0.50	500	1000
Ethyl acetate	13.0 ± 0.50	10.3 ± 0.57	7.8 ± 0.76	12.1 ± 0.15	250	500
Acetone	9.5 ± 0.50	8.1 ± 0.15	7.1 ± 0.11	11.1 ± 0.15	500	1000
Methanol	NA	NA	NA	12 .3 ± 0.57	NT	NT
Candida parapsilosis						
Hexane	12.3 ± 0.57	9.8 ± 0.76	7.6 ± 0.76	9.5 ± 0.50	500	1000
Chloroform	12.8 ± 0.76	10.3 ± 0.57	7.8 ± 0.96	13.3 ± 0.57	500	1000
Ethyl acetate	14.5 ± 0.50	11.5 ± 0.50	9.1 ± 0.15	10.6 ± 0.76	250	500
Acetone	11.8 ± 0.76	9.5 ± 0.50	7.5 ± 0.50	11.1 ± 0.15	500	1000
Methanol	11.0 ± 0.50	9.1 ± 0.15	7.3 ± 0.20	11.0 ± 0.50	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
[b]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$; NA-No activity; NT-Not Testet

Table 1: Continued

Fungal strains/ Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000 (µg/disc)	500 (µg/disc)	250 (µg/disc)	Amphotercin-B (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida tropicalis						
Hexane	NA	NA	NA	9.5 ± 0.50	NT	NT
Chloroform	10.1 ± 0.15	8.5 ± 0.50	7.1 ± 0.11	12.1 ± 0.15	500	500
Ethyl acetate	10.8 ± 0.76	9.3 ± 0.57	7.6 ± 0.76	10.3 ± 0.57	500	1000
Acetone	NA	NA	NA	11.8 ± 0.76	NT	NT
Methanol	NA	NA	NA	10.5 ± 0.50	NT	NT
T. rubrum						
Hexane	12.0 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	15.1 ± 0.15	500	1000
Chloroform	12.6 ± 0.76	10.1 ± 0.15	7.8 ± 0.76	16.3 ± 0.57	500	1000
Ethyl acetate	14.1 ± 0.15	10.8 ± 0.76	8.1 ± 0.15	14.5 ± 0.50	250	500
Acetone	10.5 ± 0.50	9.3 ± 0.57	7.3 ± 0.57	15 .6 ± 0.76	500	1000
Methanol	10.1 ± 0.15	9.1 ± 0.11	7.0 ± 0.50	18.5 ± 0.50	500	1000
T.mentagrophytes						
Hexane	NA	NA	NA	18.0 ± 0.50	NT	NT
Chloroform	11.8 ± 0.76	9.3 ± 0.57	7.1 ± 0.11	19.1 ± 0.15	500	1000
Ethyl acetate	12.5 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	17.0 ± 0.50	500	1000
Acetone	NA	NA	NA	18.8 ± 0.76	NT	NT
Methanol	NA	NA	NA	16.0 ± 0.50	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	18.1 ± 0.28	NT	NT
Chloroform	10.5 ± 0.50	9.0 ± 0.50	7.1 ± 0.11	17.0 ± 0.50	500	1000
Ethyl acetate	12.1 ± 0.15	10.0 ± 0.50	7.5 ± 0.50	17.3 ± 0.57	500	1000
Acetone	NA	NA	NA	15.1 ± 0.15	250	500
Methanol	NA	NA	NA	15 .5 ± 0.50	NT	NT
Microsporum gypseum						
Hexane	9.8 ± 0.76	8.1 ± 0.15	7.1 ± 0.11	14.3 ± 0.57	NT	NT
Chloroform	10.0 ± 0.50	9.1 ± 0.15	7.5 ± 0.50	14.0 ± 0.50	250	500
Ethyl acetate	12.8 ± 0.76	10.1 ± 0.15	7.6 ± 0.76	18.3 ± 0.57	500	1000
Acetone	NA	NA	NA	19.1 ± 0.15	NT	NT
Methanol	NA	NA	NA	18.0 ± 0.50	NT	NT

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation; ** significant at $p < 0.05$; NA-No activity; NT-Not Tested.

Table 2: Antifungal activity of different extracts of *Gracilaria verrucosa*

Fungal strains/ Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	500 (µg/disc)	250 (µg/disc)	125 (µg/disc)	Amphotercin-B (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	11.5 ± 0.50	9.8 ± 0.76	7.5 ± 0.50	11.8 ± 0.76	500	1000
Chloroform	12.8 ± 0.76	10.3 ± 0.57	7.8 ± 0.76	13.5 ± 0.50	500	1000
Ethyl acetate	13.6 ± 0.76	10.8 ± 0.76	8.1 ± 0.15	13.1 ± 0.15	250	500
Acetone	10.5 ± 0.50	9.8 ± 0.76	7.3 ± 0.20	12.6 ± 0.76	500	1000
Methanol	10.1 ± 0.15	9.0 ± 0.50	7.1 ± 0.11	13.0 ± 0.50	500	1000
Candida krusei						
Hexane	10.1 ± 0.15	9.3 ± 0.57	7.3 ± 0.11	10.8 ± 0.76	500	1000
Chloroform	11.3 ± 0.57	9.5 ± 0.50	7.6 ± 0.76	9.6 ± 0.76	500	1000
Ethyl acetate	13.1 ± 0.15	9.8 ± 0.76	7.8 ± 0.76	10.0 ± 0.50	500	1000
Acetone	9.5 ± 0.50	8.1 ± 0.15	7.0 ± 0.50	9.8 ± 0.76	500	1000
Methanol	NA	NA	NA	12.5 ± 0.50	NT	NT
Candida guilliermondii						
Hexane	10.5 ± 0.50	9.0 ± 0.50	7.0 ± 0.50	13.6 ± 0.76	500	1000
Chloroform	11.3 ± 0.57	9.5 ± 0.50	7.3 ± 0.20	11.5 ± 0.50	500	1000
Ethyl acetate	13.0 ± 0.50	10.1 ± 0.15	7.8 ± 0.76	9.5 ± 0.50	250	500
Acetone	10.0 ± 0.50	8.1 ± 0.15	7.0 ± 0.50	14.1 ± 0.15	500	1000
Methanol	NA	NA	NA	10.8 ± 0.76	NT	NT
Candida glabrata						
Hexane	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.11	11.1 ± 0.15	500	1000
Chloroform	10.5 ± 0.50	9.1 ± 0.15	7.5 ± 0.50	13.5 ± 0.50	500	1000
Ethyl acetate	12.8 ± 0.76	10.1 ± 0.15	7.8 ± 0.76	12.1 ± 0.15	500	1000
Acetone	9.5 ± 0.50	8.3 ± 0.57	7.0 ± 0.50	10.8 ± 0.76	500	1000
Methanol	NA	NA	NA	11.5 ± 0.50	NT	NT
Candida parapsilosis						
Hexane	12.1 ± 0.15	9.5 ± 0.50	7.6 ± 0.76	9.8 ± 0.76	500	1000
Chloroform	12.5 ± 0.50	10.0 ± 0.50	7.8 ± 0.76	11.0 ± 0.50	500	1000
Ethyl acetate	14.0 ± 0.50	11.0 ± 0.50	8.6 ± 0.76	9.1 ± 0.15	250	500
Acetone	11.5 ± 0.50	9.3 ± 0.57	7.3 ± 0.20	13.8 ± 0.76	500	1000
Methanol	10.6 ± 0.76	8.5 ± 0.50	7.1 ± 0.11	12.1 ± 0.15	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
[b]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$
NA-No activity; NT-Not Tested

Table 2: Continued

Fungal strains/ Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	1000 (µg/disc)	500 (µg/disc)	250 (µg/disc)	Amphotercin-B (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida tropicalis						
Hexane	NA	NA	NA	10.3 ± 0.57	NT	NT
Chloroform	10.0 ± 0.50	8.3 ± 0.57	7.0 ± 0.50	11.5 ± 0.50	500	500
Ethyl acetate	10.5 ± 0.50	9.0 ± 0.50	7.6 ± 0.76	11.1 ± 0.15	500	1000
Acetone	NA	NA	NA	12.0 ± 0.05	NT	NT
Methanol	NA	NA	NA	10.3 ± 0.57	NT	NT
T. rubrum						
Hexane	11.8 ± 0.76	9.6 ± 0.76	7.6 ± 0.76	18.0 ± 0.50	500	1000
Chloroform	12.3 ± 0.57	10.0 ± 0.50	7.8 ± 0.76	19.3 ± 0.57	500	1000
Ethyl acetate	13.8 ± 0.76	10.5 ± 0.50	8.0 ± 0.50	16.0 ± 0.50	250	500
Acetone	10.3 ± 0.57	9.1 ± 0.15	7.3 ± 0.20	15 .1 ± 0.15	500	1000
Methanol	10.0 ± 0.50	8.8 ± 0.76	7.0 ± 0.50	18.5 ± 0.50	500	1000
T.mentagrophytes						
Hexane	NA	NA	NA	17.0 ± 0.50	500	1000
Chloroform	10.1 ± 0.15	9.1 ± 0.11	7.0 ± 0.11	18.1 ± 0.15	500	1000
Ethyl acetate	12.0 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	17.3 ± 0.57	500	1000
Acetone	NA	NA	NA	17.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	18.6 ± 0.76	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	15.0 ± 0.50	NT	NT
Chloroform	10.3 ± 0.57	9.1 ± 0.15	7.0 ± 0.50	14.3 ± 0.57	500	1000
Ethyl acetate	11.6 ± 0.76	9.5 ± 0.50	7.3 ± 0.57	16.5 ± 0.50	500	1000
Acetone	NA	NA	NA	17.3 ± 0.57	NT	NT
Methanol	NA	NA	NA	15 .1 ± 0.15	NT	NT
Microsporum gypseum						
Hexane	NA	NA	NA	16.3 ± 0.57	NT	NT
Chloroform	9.5 ± 0.50	8.1 ± 0.20	7.1 ± 0.11	17.1 ± 0.28	250	500
Ethyl acetate	12.3 ± 0.57	9.8 ± 0.76	7.5 ± 0.50	16.0 ± 0.50	500	1000
Acetone	NA	NA	NA	16.3 ± 0.57	NT	NT
Methanol	NA	NA	NA	18.5 ± 0.50	NT	NT

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
[b]-mean of three assays; ± - standard deviation; ** significant at $p < 0.05$; NA-No activity; NT-Not Tested.

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Discussion

In this study, the antifungal activities of chloroform and ethyl acetate extracts of *G. edulis* and *G. verrucosa* against the selected five yeast type fungi and dermatophytic strains tested. the ethyl acetate extracts of *G. edulis,* showed the highest antifungal activity against *C. parapsilosis*, *C. albicans* and *T. rubrum* with the lowest MIC values of 250 – 500 µg/ml. Hebsibah elsie et al. [18] reported that ethanolic and acetone extracts of *Gelidium acerosa* against human pathogens like *Staphylococcus aureus, Bacillus aureus, Micrococcus leutus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and Fungi like *Aspergillus flavus, A. niger, A. fumigatus, C. albicans* and *C. tropicalis*. Tuney et al. [19] reported found that ethanolic extract of *Padina pavonica* were active against *C. albicans.* Abirami and Kowsalya [20] reported the methanolic extracts of *Acanthophora spicifera*, *G. edulis*, *Padina gymnospora*, *Ulva fasciata* and *Enteromorpha flexuosa* against *S. aureus, Streptococci, E. coli, K. aerogenes, P.vulgaris, A.niger* and *C. albicans***.**

In this study, ethyl acetate extracts of *G. edulis* showed the highest zone of inhibition (14.5 mm) against *C. parapsilosis* and the lowest MIC values (250 µg/ml) were observed in *C. albicans*, *C. krusei*, *C. glabrata, C. guilliermondi, T. rubrum, M. gypseum* and *T. mentagrophytes.* The highest activities were observed with species of the genus of *Cystoseira.* With regards to the components responsible for the antifungal activity, both chloroform and ethyl acetate extracts from *C. crinita* and *C. sedoides* showed remarkable activity strongly suggesting that several compounds of distinct nature were actives as antifungal agents [21]. Adaikala Raj et al. [22] reported that methanol and aqueous extracts of *G. verrucosa, Gracilaria ferugusoni, G. verrucosa* var *Hypnea musciformis, Enatiocladia prolifera* and *Gelidium* species against *B. subtilis, E. coli, P.aeruginosa, S. typhi, Streptococcus aureus* and *Candida albicans.* Vallinayagam et al. [23] reported the ethanol extracts from *G. debilis, G. domingensis* and *G. sjoestedii* were active against *Candida albicans.*

In this study, ethyl acetate extract of *G. edulis* demonstrated the highest antifungal activity than the other extracts against yeast and filamentous fungi. Chandrasekaran and Venkatesalu [24] reported the methanol extracts of S*. jambolanum* seeds showed antifungal activity against *C. albicans*, *Cryptococcus neoformans*, *A. flavus*, *A. fumigatus*, *A.niger*, *Rhizopus* sp., *T. mentagrophytes*, *T. rubrum* and *M. gypseum.* Mansuya et al. [25] reported the aqueous and methanolic extract of *Ulva lactuca, Ulva reticulata, Cladophora glomerata*, *Gracilaria corticata, Kappaphycus alvarezii* and *Sargassum wightii* against *E. coli, P. aeruginosa, S. typhi, Staphylococcus epidermis* and *Streptococcus pyogenes.* Albuquerque et al. [26] showed the antibacterial activity from chloroform extract of *G. edulis* was tested against bacterial strains of *Vibrio cholerae*, *S. aureus, Shigella dysenteriae, S. bodii, Salmonella paratyphi, P. aeruginosa* and *K. pneumoniae.* Salvador et al. [27] studied the antimicrobial activities of 82 marine algae in fresh and lyophilized forms and according to a seasonal variation; they reported that red algae had both the highest values and the broadest spectrum of bioactivity. The highest percentages of active extracts of *Phaeophyceae* and *Rhodophyceae* were found in the autumn, whereas, they were found in the summer for *Chlorophyceae*.

In the present work, the ethyl acetate extract of *G. edulis* and *G. verrucosa* showed the antibacterial activity due to the presence of phytochemicals, terpenoids, tannins, phenolic compound, and steroids. Seaweeds extracts are considered to be a rich source of phenolic compounds [28]. Chandrasekaran et al. [29] reported that of *G. verrucosa*, phytochemical analyses of sterols, terpenoids and alkaloids. In general, phenolic compounds possess specific physical, chemical and biological activities that make them useful as drugs. Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-

feedant, anti-viral, anticancer and vasodilatory actions [30]. Steroids of plant origin are known to be important for insecticidal, antimicrobial, antiparasitic and cardiotonic properties. Steroids also play an important role in nutrition, herbal medicine and cosmetics [31]. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent [32].

In the present study we have used two control drugs namely Amphotericin B and ketoconazole. Amphotericin-B is a polyene macrolide that has been used for over 40 years as an antifungal drug. Although it has undesirable toxic side effects towards mammalian cells, this antibiotic remains the cornerstone for therapy in immunocompromised patients with severe systemic fungal infections. Its fungicide action is believed to be related to the presence of ergosterol, the main sterol in fungal membranes. The amphotericin B toxicity towards humans, such as nephrotoxicity, is thus generally attributed to the great similitude of structure between the two involved sterols, namely ergosterol and cholesterol the latter being the main sterol in mammalian cells. Some studies have suggested that the role of sterols, namely cholesterol and ergosterol, is to modulate the formation and expression of the channels [33] by affecting membrane properties such as the lipid packing [34]. Ketoconazole is one of the commonly used antifungal drugs administered orally for the treatment of both superficial and deep infections caused by *Trichophyton*. However, the unpleasant side effects of this drug include nausea, abdominal pain and itching, and its toxicity limits its therapeutic use in many cases [35].

The search for new effective medicines remains a challenge for scientists. Therefore around the world, many researchers have focused on natural sources for new molecules with marine macro algae among the targets of these studies.

Finally it can be concluded that the provided a scientific basis for use of ethyl acetate extracts from *G. edulis* and *G. verrucosa* for the treatment of *C. parapsilosis*, *C. albicans* and *T. rubrum*. Based on the results ethyl acetate extracts could serve as useful source of new antifungal agent.

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