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Phytochemical Analysis and Antifungal activity of *Ulva* Species from the Kanniyakumari Gulf of Mannar, South Coast India

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Abstract

To investigate the in vitro antifungal activity of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Ulva lactuca* Linn. *U. fasciata* Delile. and *U. reticulata* Forsk., against *Candida albicans, C. krusei, C. guilliermondi, C. parapsilosis, C. tropicalis, C. glabarata,* four dermatophytes viz., *Trichophyton rubrum, T. mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum*. The antifungal activity was evaluated by agar disc diffusion method, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). The mean zones of inhibition produced by the tested extracts in disc diffusion assays against fungal strains ranged from 7.1 mm to 14.0 mm. The MIC values were between 250 µg/ml and 500 µg/ml, while the MFC values were between 500 µg/ml and 1000 µg/ml. The highest mean zones of inhibition (14.0 mm ± 0.50 mm) was observed with ethyl acetate extract of *U. lactuca* against *C. parapsilosis*. The ethyl acetate extracts of *U. lactuca*, *U. fasciata* and *U. reticulata* showed the presence of phytochemicals such as terpenoids, tannins and phenolic compounds strongly than the other extracts. The finding suggested that ethyl acetate extracts of *U. lactuca, U. fasciata* and *U. reticulata* exhibited an antifungal substance for the treatment of fungal infections.

Keywords: ***Ulva species; Phytochemical analyses; Antifungal activity; MIC***

Introduction

Marine macro algae 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 [1] with antiviral, antibacterial and antifungal activities [2] which acts as potential bioactive compounds of interest for pharmaceutical applications [3]. Most of these bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides [4].

The genus Ulva (Phylum Chlorophyta, Class Ulvophyceae, Order Ulvales, Family Ulvaceae) was first identified by Linnaeus [5]. Since then many taxonomists and phycologists have been involved in the identification of *Ulva* species [6]. The Ulva are a group of edible algae that are widely distributed along the coasts of the world's oceans [6] and they have an interesting

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chemical composition that makes their commercial exploitation attractive to produce functional or health promoting food [7]. The macroalgae of the order Ulvales are already used in Asia as a food condiment and as a nutritional supplement in Japan, China and other Southeast Asian countries as well as in North and South America and Oceania. For instance, they are consumed as part of the traditional Hawaiian cuisine [8], in Japan, they are included in a variety of dishes such as salads, soups, cookies, meals and condiments as well as a mixed product with other green seaweeds [9]. Interestingly, the interest in these algae as a novelty food is expanding in the West [6] and especially in France where they were authorized for human consumption as vegetables [10].

It is interesting to note that only few species of Ulva have been studied for their application in food industry. These include *U. lactuca*, *U. pertusa*, *U. compressa* and *U. clathrata.* These macroalgae exhibited a broad spectrum of nutritional composition which makes them excellent candidates for a healthy food for human nutrition [11]. With high levels of protein (between 10% and 25% of dry mass), dietary fiber, low total lipid contents and relatively high levels of essential amino acids, they constitute a good alternative source of amino acids and of some essential polyunsaturated fatty acids such as oleic, linoleic and linolenic acids, vitamins and minerals, especially iron [11], while *U. lactuca* is used in salads, cookies and soups [12].

The incidence of fungal infections has drastically increased over the past three decades and has become a major cause of morbidity and mortality [13,14]. Candidiasis is the most frequent infection by opportunistic fungi, where the species commonly associated with infections are *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata and C. krusei*. The spectrum of candidiasis is very extensive, going from mild manifestations, such as a colonization of mucosal tissues, up to systemic pictures, with the invasion of various organs [15]. These yeasts are part of the normal microbiota, may become pathogenic in some cases resulting in congenital or acquired immunodeficiency and immunosuppression induced by severe stress [16]. Dermatophytes are pathogenic fungi, which can affect billions of individuals worldwide. Trichophyton, Microsporum and Epidermophyton are the major dermatophytes, which have the capacity to invade keratinized tissues such as the skin, nail and hair follicles to cause infections in human beings such as *Tinea captis, Tinea corporis, Tinea inguinalis, Tinea manus, Tinea unguinum and Tinea pedis* [17].

Fungi cause a range of illnesses (mycoses) ranging from the chronic to the serious. The treatment options for invasive fungal infections are limited since there are relatively few chemical classes and targets represented by existing antifungal drugs. Current drugs target cell wall and membrane components [18,19]. The most commonly used antifungal drugs are broadly classified into those of systemic and topical based on their route of administration. Systemic administration antifungal drugs are griseofulvin, amphotericin B, flucytosine and azole compounds [20]. Fluconazole exhibits linear pharmacokinetics (i.e. the dose is directly proportional to the area under the concentration-time curve) [21], is water-soluble and is very well tolerated. Despite its frequently reduced against *C. glabrata* and the inherent resistance of *C. krusei*, along with the absence of activity against *Aspergillus* spp. and other medically important moulds, fluconazole is extensively used for the prevention and treatment of superficial and invasive Candida infections [19]. Fluconazole is also used for the treatment of cryptococcal infections and has demonstrated efficacy against dermatophytes [22]. Flucytosine (5-fluorocytosine), an oral antifungal compound has side effects and drug resistance developed by certain fungal species. Innate resistance in some fungal pathogens against the triazoles, viz., fluconazole and itraconazole is a concern in their use. Also, many of the currently

available antifungal compounds are proved for their toxicity against mammalian cells. For example, severe toxicity like impairment of renal function limits the use of Amphotericin-B [23].

In the present study, antifungal activity of different organic solvents extracts of green algae *U. lactuca, U. fasciata* and *U. reticulata* was examined against *Candida albicans, C. krusei, C. guilliermondi, C. parapsilosis, C. tropicalis, C. glabarata, T. rubrum, T. mentagrophytes, M. gypseum* and *E. flocossum*.

Materials and Methods

Collection of algal sample and preparation of crude extracts

Fresh green seaweed samples of *Ulva lactuca Linn. Ulva fasciata Delile*. and *Ulva reticulata Forsk*., (Chlorophyceae) were collected from the rocky shores of Kanniyakumari (Lat. 9°11′N; Long. 79°24′E), Kanniyakumari district and Tuticorin (Lat. 8°45′N; Long. 78°10′E) Tuticorin district, the Gulf of Mannar Biosphere Reserve, Tamilnadu, India, respectively. The collections were made from the months of November to December 2011 during the low tide. The algae was identified by Dr. R. Selvaraj, Former Professor, Department of Botany, Annamalai University and the museum specimens are deposited in the Department of Botany, Annamalai University, Annamalai Nagar. The algal sample species were handpicked during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal castings and adhering sand particles etc, morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in an ice box containing slush ice and transported to the laboratory. Then, the samples were blot dried using sterile tissue paper. Then the seaweeds were shade dried under room temperature and kept in a hot air oven for 50°C for half an h. After that the material was ground by using electric blender. The powdered materials were stored in air tight container. Five hundred gram of seaweed materials was packed inside a Soxhlet apparatus and successive extraction was carried out using solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 h. The solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried extracts were stored at 4°C until further assay.

Phytochemical screening

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. lactuca, U. fasciata* and *U. reticulata* were used for qualitative phytochemical studies. The phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, phenolic compounds and coumarins were carried analyzed according to the method described by Harborne [24] and Trease and Evans [25].

Fungal strains used: The ten human fungal pathogenic microorganisms such as Yeast viz., *Candida albicans* (MTCC 3017), *Candida krusei* (MTCC 9215), *Candida guilliermondi* (NCIM 3216), *Candida parapsilosis* (MTCC 2509), *Candida tropicalis* (MTCC 184) and *Candida glabarata* (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. [26] with modifications. Petri 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 µg/disc, 500 µg/disc and 250 µg/disc) were prepared and aseptically applied on the surface of the petri plates. The agar plates were inoculated and incubated for the plates were incubated at 28°C for 24 h for yeast and 30°C for 4-7 days for dermatophytes. Amphotercin-B (100 units/disc) for Yeast and Ketoconazole (5 µg/disc) for dermatophytes were used as positive controls and 10% 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 *Ulva* species was determined by using broth micro dilution technique as recommended by CLSI M27-A3 [27] and M38-A2 [28] 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 (1 mg/ml). From that, the two fold serial dilutions in the range from 1000 µg/ml to 15.7 µg/ml were prepared. 100 µl of solution was poured into first well of 96 well microtitre plates and then, 50 µl were transformed to the next well containing 100 µl of RPMI-1640. The same procedure was performed for all wells. 10 µl of fungal standardized inoculum suspensions containing 0.5-2.5 × 10^3 for yeast 0.4-5 × 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 h 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 h 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 vivo* 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 *U. lactuca, U. fasciata and U. reticulata* showed the presence of, terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds and coumarins. The ethyl acetate extracts of U. *lactuca, U. fasciata* and *U. reticulata* showed the presence of phytochemicals like terpenoids, tannins and phenolic compounds strongly than the other solvents extracts and the results are presented in Tables 1-3. Among the phytochemicals, cardiac glycosides were present in all the extracts except acetone and methanol extracts. Steroids were absent in all extracts of *U. lactuca, U. fasciata and U. reticulata. Alkaloids* and *coumarins* were absent in all the extracts of *U. lactuca, U. fasciata* and *U. reticulata*.

TABLE 1. **Phytochemical analysis of *Ulva lactuca*.**

S. No.	Secondary metabolites	Hexane	Chloroform	Ethylacetate	Acetone	Methanol
1	Terpenoids	++	++	+++	-	-
2	Tannins	-	-	+++	+	+
3	Cardic glycosides	+	+	+	_	_
4	Steroids	-	_	_	_	_
5	Alkaloids	-	_	_	_	_
6	Phenolic compounds	+	++	+++	+	+
7	Coumarins	_	-	_	_	_
- = Absence, + = weak, ++ = medium, +++ = strong						

TABLE 2. **Phytochemical analysis of *Ulva fasciata*.**

S.no	Secondary metabolites	Hexane	Chloroform	Ethylacetate	Acetone	Methanol
1	Terpenoids	++	++	+++	-	-
2	Tannins	-	-	+++	++	++
3	Cardic glycosides	+	+	+	_	_
4	Steroids	-	_	_	_	_
5	Alkaloids	-	_	_	_	_
6	Phenolic compounds	+	+	+++	++	+
7	Coumarins	-	_	_	_	_
- = Absence, + = weak, ++ = medium, +++ = strong						

TABLE 3. **Phytochemical analysis of *Ulva reticulate*.**

S.no	Secondary metabolites	Hexane	Chloroform	Ethylacetate	Acetone	Methanol
1	Terpenoids	+	++	+++	+	-
2	Tannins	-	+	+++	+	+
3	Cardic glycosides	+	++	+	–	–
4	Steroids	-	–	–	–	–
5	Alkaloids	-	–	–	–	–
6	Phenolic compounds	-	++	+++	+	+
7	Coumarins	-	–	–	–	–
- = Absence, + = weak, ++ = medium, +++ = strong						

In present study, the antifungal activities of chloroform and ethyl acetate extracts of *U. lactuca*, *U. fasciata* and *U. reticulata* against the selected five yeast type fungi and three dermatophytic strains were evaluated and the activity of ethyl acetate extract were found to be highest activity as compared to the other extracts. The ethyl acetate extract of *U. lactuca* showed promising activity against *C. parapsilosis* (14.0 mm), followed by *C. albicans* (13.8 mm) and *T. rubrum* (13.6 mm). The chloroform extracts showed activity against *C. parapsilosis* (12.8 mm), followed by *T. rubrum* (12.5 mm) and *C. albicans* (12.5 mm) and the ethyl acetate extract of *U. fasciata* showed the activity against *C. parapsilosis* (13.5 mm), followed by *C. albicans* (13.1 mm) and *T. rubrum* (13.1 mm). The chloroform extracts showed activity against *C. parapsilosis* (12.8 mm), followed by *C. albicans* (12.6 mm) and *T. rubrum* (12.5 mm) and the results are presented in TABLES 4-6. The mean zones of inhibition of the extracts, assayed against the test organisms ranged between 7.1 mm and 14.0 mm. The Amphotericin-B (100 units/disc), anticandidal positive control produced zones of inhibition were from 9.0 mm to 14.5 mm. Ketoconazole (10 µg/disc), anti dermatophytic positive control produced zones of inhibition ranged from 14.1 mm to 19.5 mm. The negative control (10% DMSO) did not produce any zone of inhibition for all the fungal strains tested. The results of MIC values of the different extracts of *U. lactuca*, *U. fasciata* and *U. reticulata* ranged between 250 µg/ml and 500 µg/ml. while the MFC values were between 500 µg/ml and 1000 µg/ml.

TABLE 4. **Antifungal activity of different extracts of *Ulva lactuca*.**

Fungal Strains/ Seaweed extracts prepared with different solvents	**Mean zone of inhibition[a] (mm)[b]**					
	1000 (µg/disc)	**500 (µg/disc)**	**250 (µg/disc)**	**Amphotericin-B (100 units/disc)**	**MIC (µg/ml)**	**MFC (µg/ml)**
Candida albicans						
Hexane	12.0 ± 0.50	9.5 ± 0.50	7.6 ± 0.76	11.5 ± 0.50	500	1000
Chloroform	12.5 ± 0.50	10.0 ± 0.50	7.8 ± 0.76	10.1 ± 0.15	500	1000
Ethyl acetate	13.8 ± 0.76	11.0 ± 0.15	8.0 ± 0.50	12.8 ± 0.57	250	500
Acetone	10.5 ± 0.50	9.3 ± 0.20	7.5 ± 0.50	10.5 ± 0.50	500	1000

Methanol	10.1 ± 0.15	9.1 ± 0.15	7.1 ± 0.11	12.1 ± 0.15	500	1000
Candida krusei						
Hexane	0.8 ± 0.76	9.0 ± 0.50	7.3 ± 0.20	9.3 ± 0.20	500	1000
Chloroform	11.0 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	11.6 ± 0.76	500	1000
Ethyl acetate	13.3 ± 0.57	10.0 ± 0.50	7.8 ± 0.76	13.1 ± 0.15	250	500
Acetone	10.0 ± 0.50	8.5 ± 0.50	7.1 ± 0.11	11.5 ± 0.50	500	1000
Methanol	NA	NA	NA	9.6 ± 0.76	NT	NT
Candida guilliermondi						
Hexane	11.0 ± 0.50	11.0 ± 0.50	7.3 ± 0.20	10.2 ± 0.20	500	1000
Chloroform	12.0 ± 0.50	9.8 ± 0.76	7.6 ± 0.76	11.5 ± 0.50	500	1000
Ethyl acetate	13.1 ± 0.15	10.0 ± 0.50	7.8 ± 0.76	9.3 ± 0.20	250	500
Acetone	10.1 ± 0.15	9.1 ± 0.15	7.1 ± 0.11	10.5 ± 0.50	500	1000
Methanol	NA	NA	NA	10.6 ± 0.76	NT	NT
Candida glabarata						
Hexane	11.0 ± 0.50	9.5 ± 0.50	7.3 ± 0.20	9.3 ± 0.20	500	1000
Chloroform	12.3 ± 0.57	10.0 ± 0.50	7.6 ± 0.76	14.1 ± 0.15	500	1000
Ethyl acetate	13.5 ± 0.50	10.5 ± 0.50	8.0 ± 0.50	12.5 ± 0.50	250	500
Acetone	10.6 ± 0.76	9.1 ± 0.15	7.1 ± 0.11	12.1 ± 0.28	500	1000
Methanol	NA	NA	NA	14 .5 ± 0.57	NT	NT
Candida parapsilosis						
Hexane	12.3 ± 0.57	9.5 ± 0.50	7.8 ± 0.76	9.3 ± 0.57	500	1000
Chloroform	12.8 ± 0.76	10.1 ± 0.15	8.0 ± 0.50	13.1 ± 0.15	500	1000
Ethyl acetate	14.0 ± 0.50**	11.0 ± 0.50	9.1 ± 0.15	13.0 ± 0.50	250	500
Acetone	11.5 ± 0.50	9.3 ± 0.20	7.5 ± 0.50	9.5 ± 0.50	500	1000
Methanol	11.0 ± 0.50	9.0 ± 0.50	7.3 ± 0.20	11.5 ± 0.50	500	1000
Candida tropicalis						
Hexane	NA	NA	NA	11.5 ± 0.50	NT	NT
Chloroform	10.1 ± 0.15	8.0 ± 0.50	7.1 ± 0.11	12.8 ± 0.57	500	500
Ethyl acetate	10.5 ± 0.50	9.1 ± 0.15	7.5 ± 0.50	9.0 ± 0.50	500	1000
Acetone	NA	NA	NA	10.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	10.1 ± 0.15	NT	NT
Tricophyton rubrum						
Hexane	11.8 ± 0.76	9.8 ± 0.76	7.8 ± 0.76	17.0 ± 0.50	500	1000
Chloroform	12.5 ± 0.50	10.1 ± 0.15	8.0 ± 0.50	17.3 ± 0.57	500	1000
Ethyl acetate	13.6 ± 0.76**	10.0 ± 0.50	8.5 ± 0.50	14.6 ± 0.76	250	500

Acetone	10.3 ± 0.57	9.1 ± 0.20	7.3 ± 0.20	16 .6 ± 0.76	500	1000
Methanol	10.0 ± 0.50	8.0 ± 0.50	7.0 ± 0.50	16.5 ± 0.50	500	1000
T. mentagrophytes						
Hexane	NA	NA	NA	17.1 ± 0.28	NT	NT
Chloroform	10.8 ± 0.76	9.5 ± 0.50	7.3 ± 0.20	17.5 ± 0.50	500	1000
Ethyl acetate	12.5 ± 0.50	10.1 ± 0.15	7.8 ± 0.76	17.5 ± 0.50	500	1000
Acetone	NA	NA	NA	18.1 ± 0.28	NT	NT
Methanol	NA	NA	NA	16.5 ± 0.50	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	16.1 ± 0.28	NT	NT
Chloroform	11.0 ± 0.50	9.1 ± 0.20	7.3 ± 0.20	17.5 ± 0.50	500	1000
Ethyl acetate	12.5 ± 0.50	10.1 ± 0.15	7.6 ± 0.76	18.1 ± 0.28	500	1000
Acetone	NA	NA	NA	15.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	16 .8 ± 0.76	NT	NT
Microsporum gypseum						
Hexane	NA	NA	NA	15.3 ± 0.57	NT	NT
Chloroform	10.1 ± 0.15	9.3 ± 0.20	7.3 ± 0.20	14.5 ± 0.50	500	1000
Ethyl acetate	13.0 ± 0.50	10.3 ± 0.57	7.8 ± 0.76	16.5 ± 0.50	500	1000
Acetone	NA	NA	NA	19.0 ± 0.50	NT	NT
Methanol	NA	NA	NA	18.1 ± 0.28	NT	NT

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

TABLE 5**. Antifungal activity of different extracts of *Ulva fasciata.***

Fungal Strains/Seaweed extracts prepared with different solvents	**Mean zone of inhibition[a] (mm)[b]**					
	1000 (µg/disc)	**500 (µg/disc)**	**250 (µg/disc)**	**Amphotericin-B (100 units/disc)**	**MIC (µg/mL)**	**MFC (µg/mL)**
Candida albicans						
Hexane	11.6 ± 0.76	9.5 ± 0.50	7.3 ± 0.20	9.5 ± 0.50	500	1000
Chloroform	12.6 ± 0.76	10.1 ± 0.15	7.6 ± 0.76	13.2 ± 0.25	500	1000
Ethyl acetate	13.1 ± 0.28	10.5 ± 0.50	7.8 ± 0.76	13.5 ± 0.50	250	500
Acetone	10.1 ± 0.15	9.3 ± 0.20	7.3 ± 0.20	10.6 ± 0.76	500	1000
Methanol	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.11	10.1 ± 0.15	500	1000
Candida krusei						
Hexane	10.3 ± 0.57	9.1 ± 0.20	7.5 ± 0.50	11.3 ± 0.20	500	1000
Chloroform	10.6 ± 0.76	9.3 ± 0.20	7.6 ± 0.76	13.6 ± 0.76	500	1000

Ethyl acetate	12.8 ± 0.76	9.5 ± 0.50	7.8 ± 0.76	13.5 ± 0.50	500	1000
Acetone	10.1 ± 0.15	9.0 ± 0.50	7.0 ± 0.50	9.3 ± 0.57	500	1000
Methanol	NA	NA	NA	11.5 ± 0.50	NT	NT
Candida guilliermondi						
Hexane	10.8 ± 0.76	9.1 ± 0.20	7.1 ± 0.11	9.2 ± 0.20	500	1000
Chloroform	11.8 ± 0.76	9.5 ± 0.50	7.5 ± 0.50	13.5 ± 0.50	500	1000
Ethyl acetate	13.0 ± 0.50	9.8 ± 0.76	8.0 ± 0.50	10.5 ± 0.50	250	500
Acetone	10.0 ± 0.50	9.0 ± 0.50	7.0 ± 0.50	10.1 ± 0.15	500	1000
Methanol	NA	NA	NA	11.5 ± 0.50	NT	NT
Candida glabarata						
Hexane	10.0 ± 0.50	9.8 ± 0.76	7.3 ± 0.20	10.3 ± 0.20	500	1000
Chloroform	11.3 ± 0.57	10.1 ± 0.15	7.8 ± 0.76	14.1 ± 0.15	500	1000
Ethyl acetate	12.8 ± 0.76	10.3 ± 0.20	8.0 ± 0.50	12.5 ± 0.50	500	1000
Acetone	NA	NA	NA	11.0 ± 0.50	500	1000
Methanol	NA	NA	NA	13 .8 ± 0.76	NT	NT
Candida parapsilosis						
NT Hexane	12.0 ± 0.50	9.3 ± 0.20	7.6 ± 0.76	11.5 ± 0.50	500	1000
Chloroform	12.8 ± 0.76	9.8 ± 0.76	8.1 ± 0.28	13.1 ± 0.15	500	1000
Ethyl acetate	13.5 ± 0.50**	10.8 ± 0.76	8.5 ± 0.50	9.5 ± 0.50	250	500
Acetone	11.1 ± 0.28	9.0 ± 0.50	7.3 ± 0.57	10.5 ± 0.50	500	1000
Methanol	10.5 ± 0.50	8.5 ± 0.50	7.3 ± 0.20	12.3 ± 0.57	500	1000
Candida tropicalis						
Hexane	NA	NA	NA	11.6 ± 0.76	NT	NT
Chloroform	9.8 ± 0.76	8.1 ± 0.28	7.0 ± 0.50	13.8 ± 0.57	500	500
Ethyl acetate	10.3 ± 0.20	8.5 ± 0.50	7.3 ± 0.57	10.0 ± 0.50	500	1000
Acetone	NA	NA	NA	12.1 ± 0.15	NT	NT
Methanol	NA	NA	NA	12.5 ± 0.50	NT	NT
T. rubrum						
Hexane	11.3 ± 0.57	9.5 ± 0.50	7.6 ± 0.76	19.0 ± 0.50	500	1000
Chloroform	12.5 ± 0.50	9.8 ± 0.76	7.8 ± 0.76	18.3 ± 0.57	500	1000
Ethyl acetate	13.1 ± 0.28**	10.0 ± 0.50	8.0 ± 0.50	17.6 ± 0.76	250	500
Acetone	10.5 ± 0.50	9.1 ± 0.20	7.3 ± 0.20	15 .5 ± 0.50	500	1000
Methanol	10.1 ± 0.15	8.8 ± 0.76	7.1 ± 0.11	19.5 ± 0.50	500	1000
T. mentagrophytes						
Hexane	NA	NA	NA	16.5 ± 0.50	500	1000

Chloroform	10.3 ± 0.15	9.3 ± 0.20	7.1 ± 0.11	18.3 ± 0.57	500	1000
Ethyl acetate	12.5 ± 0.50	9.8 ± 0.76	7.5 ± 0.50	18.1 ± 0.28	500	1000
Acetone	NA	NA	NA	18.8 ± 0.50	NT	NT
Methanol	NA	NA	NA	15.6 ± 0.76	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	14.1 ± 0.28	NT	NT
Chloroform	10.8 ± 0.76	9.5 ± 0.50	7.1 ± 0.28	19.5 ± 0.50	500	1000
Ethyl acetate	11.5 ± 0.50	9.8 ± 0.76	7.5 ± 0.50	14.5 ± 0.50	500	1000
Acetone	NA	NA	NA	15.3 ± 0.57	NT	NT
Methanol	NA	NA	NA	16 .8 ± 0.76	NT	NT
Microsporum gypseum						
Hexane	NA	NA	NA	15.5 ± 0.50	NT	NT
Chloroform	10.1 ± 0.15	9.1 ± 0.20	7.1 ± 0.11	18.3 ± 0.28	500	1000
Ethyl acetate	12.5 ± 0.50	9.5 ± 0.50	7.6 ± 0.76	16.5 ± 0.50	500	1000
Acetone	NA	NA	NA	19.0 ± 0.28	NT	NT
Methanol	NA	NA	NA	15.3 ± 0.57	NT	NT

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

TABLE 6. **Antifungal activity of different extracts of *Ulva reticulate*.**

Fungal Strains/Seaweed extracts prepared with different solvents	**Mean zone of inhibition[a] (mm)[b]**					
	1000 (µg/disc)	**500 (µg/disc)**	**250 (µg/disc)**	**Amphotericin -B (100 units/disc)**	**MIC (µg/mL)**	**MFC (µg/mL)**
Candida albicans						
Hexane	10.1 ± 0.15	9.3 ± 0.20	7.3 ± 0.20	10.5 ± 0.50	500	1000
Chloroform	11.0 ± 0.50	9.6 ± 0.76	7.5 ± 0.50	11.6 ± 0.76	500	1000
Ethyl acetate	12.5 ± 0.50	10.1 ± 0.15	7.8 ± 0.76	11.8 ± 0.76	500	1000
Acetone	NA	NA	NA	10.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	12.1 ± 0.15	NT	NT
Candida krusei						
Hexane	9.8 ± 0.76	8.5± 0.50	7.0 ± 0.50	11.3 ± 0.57	500	1000
Chloroform	10.3 ± 0.20	9.6 ± 0.76	7.3 ± 0.20	12.5 ± 0.50	500	1000
Ethyl acetate	11.5 ± 0.50	10.0 ± 0.50	7.5 ± 0.50	12.1 ± 0.15	500	1000
Acetone	NA	NA	NA	14.1 ± 0.28	NT	NT
Methanol	NA	NA	NA	13.6 ± 0.76	NT	NT

Candida guilliermondi						
Hexane	NA	NA	NA	13.5 ± 0.50	NT	NT
Chloroform	10.5 ± 0.50	9.1 ± 0.15	7.3 ± 0.20	14.1 ± 0.28	500	1000
Ethyl acetate	12.0 ± 0.50	9.5 ± 0.50	7.6 ± 0.76	13.1 ± 0.15	500	1000
Acetone	NA	NA	NA	14.1 ± 0.28	NT	NT
Methanol	NA	NA	NA	12.6 ± 0.76	NT	NT
Candida glabarata						
Hexane	NA	NA	NA	13.1 ± 0.28	NT	NT
Chloroform	10.6 ± 0.76	9.3 ± 0.20	7.5 ± 0.50	12.3 ± 0.57	500	1000
Ethyl acetate	11.5 ± 0.50	10.1 ± 0.15	8.0 ± 0.50	13.3 ± 0.57	500	1000
Acetone	NA	NA	NA	13 .5 ± 0.50	NT	NT
Methanol	NA	NA	NA	11 .8 ± 0.76	NT	NT
Candida parapsilosis						
Hexane	11.5 ± 0.50	9.6 ± 0.76	7.5 ± 0.50	11.5 ± 0.50	500	1000
Chloroform	12.3 ± 0.57	9.8 ± 0.76	7.8 ± 0.76	12.3 ± 0.57	500	1000
Ethyl acetate	13.0 ± 0.50**	10.1 ± 0.15	8.0 ± 0.50	13.0 ± 0.50	250	1000
Acetone	10.8 ± 0.76	9.0 ± 0.50	7.3 ± 0.50	11.0 ± 0.50	500	1000
Methanol	10.0 ± 0.50	8.5 ± 0.50	7.0 ± 0.50	13.3 ± 0.57	500	1000
Candida tropicalis						
Hexane	NA	NA	NA	11.6 ± 0.76	NT	NT
Chloroform	9.5 ± 0.50	8.2 ± 0.34	7.1 ± 0.11	12.8 ± 0.57	500	1000
Ethyl acetate	10.8 ± 0.76	9.1 ± 0.15	7.3 ± 0.20	11.0 ± 0.50	500	1000
Acetone	NA	NA	NA	13.1 ± 0.15	NT	NT
Methanol	NA	NA	NA	11.8 ± 0.76	NT	NT
T. rubrum						
Hexane	11.0 ± 0.50	9.3 ± 0.20	7.5 ± 0.50	16.0 ± 0.50	500	1000
Chloroform	12.3 ± 0.57	9.6 ± 0.76	7.8 ± 0.76	19.1 ± 0.28	500	1000
Ethyl acetate	12.8 ± 0.76**	10.0 ± 0.50	8.0 ± 0.50	18.6 ± 0.76	500	1000
Acetone	10.8 ± 0.76	9.3 ± 0.20	7.3 ± 0.20	17 .6 ± 0.76	500	1000
Methanol	9.5 ± 0.50	9.0 ± 0.50	7.1± 0.11	16.5 ± 0.50	500	1000
T. mentagrophytes						
Hexane	NA	NA	NA	16.5 ± 0.50	NT	NT
Chloroform	10.3 ± 0.57	9.3 ± 0.57	7.1 ± 0.11	16.3 ± 0.57	500	1000
Ethyl acetate	12.0 ± 0.50	9.6 ± 0.76	7.5 ± 0.50	15.5 ± 0.50	500	1000

Acetone	NA	NA	NA	14.1 ± 0.28	NT	NT
Methanol	NA	NA	NA	16.5 ± 0.50	NT	NT
Epidermophyton flocossum						
Hexane	NA	NA	NA	17.1 ± 0.28	NT	NT
Chloroform	10.1 ± 0.15	9.1 ± 0.20	7.3 ± 0.20	15.1 ± 0.28	500	1000
Ethyl acetate	11.1 ± 0.36	9.5 ± 0.50	7.8 ± 0.76	17.5 ± 0.50	500	1000
Acetone	NA	NA	NA	19.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	16 .8 ± 0.76	NT	NT
Microsporum gypseum						
Hexane	NA	NA	NA	19.3 ± 0.57	NT	NT
Chloroform	9.8 ± 0.76	8.3 ± 0.57	7.1 ± 0.11	15.3 ± 0.57	500	1000
Ethyl acetate	12.0 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	15.1 ± 0.28	500	1000
Acetone	NA	NA	NA	16.5 ± 0.50	NT	NT
Methanol	NA	NA	NA	16.0 ± 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; Amphotericin-B for yeast; Ketoconazole for dermatophytes; ** significant at $p<0.05$; NA-No activity; NT-Not Tested

Discussion

Antifungal drug resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases [29]. Recently considerable research activity has been focused on seaweeds for isolating and developing newer antimicrobial agents. During the past four decades many novel bioactive compounds have been isolated from marine organisms [30]. The characteristic green colour of green algae is mainly due to the presence of chlorophyll a and b in the same proportion like higher plants [31]. There are a few reports of novel secondary metabolites and the most important natural product isolated from the green algae and their biological activities. The present studies phytochemical analysis and antifungal activity from the *U. lactuca*, *U. fasciata* and *U. reticulata*. The phytochemical analysis of different extracts of *U. lactuca*, *U. fasciata* and *U. reticulata* showed the presence of phytochemicals, terpenoids, tannins and phenolic compounds. A wide range of compounds, particularly terpenes, polyphenolic compounds and steroids have been reported from various marine green algae [32]. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [33]. Many tannins containing drugs are used in medicine as astringents. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medicinally used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidotes. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, anti-inflammatory, antiulcer and antioxidant properties for possible therapeutic applications [34,35]. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and atrial arrhytmias and many glycoside compounds, belonging to other structural groups, show cytotoxic, antimicrobial, hypocholesterolemic and other biological activities [35].

In this study, the antifungal activity of chloroform and ethyl acetate extracts of *U. lactuca*, *U. fasciata* and *U. reticulata* against the selected six yeast type fungi and four dermatophytic strains were tested.

Kolanjinathan and Stella [36] reported the antifungal activity of marine seaweeds extracts of *U. reticulata* and *U. lactuca against Aspergillus niger, A. flavus, A. fumigatus, Saccharomyces cerevisiae, C. albicans* and *C. glabrata. Thirumaran* and *Anantharaman* [37] reported the antimicrobial activity of *Enteromorpha compressa* using petroleum ether, chloroform, diethyl ether, acetone, ethanol and methanol extracts against *Shigella sonii* and *Mucor* sp. Among the ethanol and methanol extracts showed the highest antimicrobial activity than the other extracts. Vallinayagam et al. [38] reported that *U. lactuca* and *Gracilaria edulis* against human bacterial pathogens *Staphylococcus aureus*, *Vibrio chlorae, Shigella dysentriae, S. boydii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The activity and inactivity of marine algae against microorganisms could due to the reproductive state and seasonality [10]. The extraction protocol and the harvest period are other important factors [39].

In this study, ethyl acetate extract of *U. lactuca* showed the highest zone of inhibition (14.0 mm) and the lowest MIC values (250 µg/ml) against *C. parapsilosis*. The antimicrobial activity of the ethyl acetate, hexane and water extracts of *U. lactuca* were studied against *Bacillus subtilis, S. aureus, Micrococcus luteus*, three MRSA, *Escherichia coli, K. pneumoniae, Salmonella typhimurium, Vibrio parahaemolytics, E. tard* and *C. albicans* [40].

Abdel-Khaliq et al. [41] reported that the antibacterial and antifungal activities of marine seaweed ethanol extracts of *U. lactuca, U. fasciata* and *U. intestinalis against S. aureus, S. epidermidis, S. saprophyticus, Streptococcus pyogenes, S. pneumonia, S. mutans, B. subtilis, B. cereus, Enterococcus faecalis, Corynebacterium diphtheria, Geotricum candidum, C. albicans, Aspergillus clavatus, A. fumigatus, Rhizopus oryzae, Mucor circinelloides, Penicillium marneffei, Syncephalastrum racemosum, Absidia corymbifera and Stachybotrys chartarum.* Chandrasekaran et al. [42] reported that marine green alga hexane, chloroform, ethyl acetate, acetone and methanol extracts of *U. fasciata* against multi-drug resistant standard and clinical bacterial strains viz., *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, Shigella flexneri, Proteus mirabilis* and *P. vulgaris*.

Chakraborty and Paulraj [43] reported isolated of five sesquiterpens viz., 2,5,5-trimethyl-4-(4/-methyl-3/-pentenyl)-2-cyclohexen-1-ol, 4-isopentyl-3,4,5,5-tetramethyl-2-cyclohexen- 1-ol, two diastereoisomeric compounds), 6-isopentyl-1,5,5,6- tetramethyl-1-cyclohexene and 3,4,5,5-tetramethyl-4-(3/-oxopentyl)-2-cyclohexen-1-one from the methanol extracts of *U. fasciata.*

In this study, ethyl acetate extract of *U. lactuca* demonstrated the highest antifungal activity than that of other extracts against yeast and filamentous fungi. Thillairajasekar et al. [44] reported that large number of algal extracts were found to be antimicrobial properties of fatty acid, hydroxyl unsaturated fatty acid, glycolipids and steroids [44]. The methanol extracts of *Padina pavonica, Rhodomela confervoides* and *U. lactuca* against *A. niger, Mucor ramaniannus* and *C. albicans* [45]. Bhagavathy et al. [46] reported the different organic extracts of *Chlorococcum humicola* against *B. subtilis, S. aureus, E. coli, P. aeruginosa, S. typhimurium, K. Pneumoniae, V. chlorae, A. flavus, A. niger* and *C. albicans*.

In the present study two standard antibiotics like Amphotericin-B and Ketoconazole were used. Amphotericin-B is considered as the drug of choice for the treatment of fungal infections [47]. However, toxicity and resistance to these

antifungal drugs are a major problem [48]. In case of Amphotericin-B, due to its poor permeability across the membrane [49], an increased amount of Ampotercin-B must be administered to patients, which can result in severe side effects such as renal damage [50]. Amphotericin-B also has effects of oxidative pathways that may enhance antifungal activity. The possible effects of amphotericin-B and its lipid formulations on the immune system have been recently reviewed [51]. Ketoconazole is an imidazole antifungal agent currently used in the treatment of a broad range of fungal infections. Ketoconazole has a boxed warning regarding serious hepatotoxicity, which may potentially result in liver transplantation or death. Some patients had no obvious risk factors for liver disease. The drug inhibits cytochrome P450 14a-demthylase, an enzyme involved in the synthesis of ergosterol, a crucial component the fungal cell wall. However, the unpleasant side effects of this drug include with acute or chronic liver disease [52].

In this context, new antifungal plant derivatives could be useful alternatives for the treatment of Candida and dermatophytoses where a topical therapy is required. The advantage of using these natural compounds may be a reduced risk of side-effects and lower cost. It is thus not surprising that, in recent years, there has been growing interest in the use of marine plants to cure fungal diseases.

Conclusion

Finally it can be concluded that different extracts *of U. lactuca*, *U. fasciata* and *U. reticulata* were screened in the present study. Among the two types of fungal strains like yeast and dermatophytic strains. Candida are more susceptible compared to dermatophytes. Based on these results of the present work, the ethyl acetate extract of *U. lactuca* can be used to treat diseases caused by *C. parapsilosis, C. albicans* and *T. rubrum.*

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