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Anticandidal and Antidermatophytic Activities of *Caulerpa* Species from the Gulf of Mannar Coast, Mandapam Tamilnadu India

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Abstract. The present investigation was carried out to evaluate the antifungal activity of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Caulerpa chemnitzia*, (Epser) J.V. Lamououx *C. racemosa* (Forsk), and *C. scalpelliformis* (R.Br.) Weber-van-Bosse, (Chlorophyceae) against *Candida albicans*, *C. krusei*, *C. guilliermondii*, *C. parapsilosis*, *C. tropicalis, C. glabrata*, four dermatophytes *viz., Trichophyton rubrum*, *T. mentagrophytes, Microsporum gypseum* and *Epidermophyton flocossum*. The antifungal activity was evaluated by agar disc diffusion method, determination of MIC and MFC. The mean zones of inhibition produced by the extracts in disc diffusion assay against the tested fungal strains were ranged from 7.1 to 15.1 mm. The lowest MIC (250 µg/ml) and MFC (500 µg/ml) values were observed in the ethyl acetate extract of *C. racemosa* against *C. parapsilosis, C. albicans*, *C. krusei*, *C. glabrata, C. guilliermondii, T. rubrum, M. gypseum* and *T. mentagrophytes.* The finding suggests that ethyl acetate extract of *C. racemosa* showed the higher antifungal activity against *C. parapsilosis* and *C. albicans*.

Introduction

Fungal infections are an increasing threat to human health. In the developed world, these infections predominantly occur in the context of increasingly aggressive immunosuppressive therapies. The overall mortality for invasive diseases caused by *Candida* spp. and *Aspergillus* spp. is 30–50%, despite the advent of new diagnostic and therapeutic strategies. In the developing world, there are 1 million cases of cryptococcal disease per year, results in 675 000 deaths [1]. Allergic fungal syndromes are increasingly recognised. Continued efforts are required to improve the often suboptimal therapeutic outcomes associated with fungal infections.

Dermal mycoses or superficial fungal infections are among the most common infections worldwide [2]. They are believed to affect 20–25% of the world's population and the incidence of these infections have been increased significantly over last 15–20 years which may be ascribed to changing patterns of migration, growth in tourism, and changes in socioeconomic conditions. These infections are mainly caused by dermatophytes belonging to genera *Epidermophyton, Microsporum* and *Trichophyton*; and the causative species vary with geographic regions. Skin, hair, nail and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses [3]. The most common strains of pathogenic fungus in humans is *C. albicans*, filamentous fungi that is found in normal human skin flora. *C. albicans* is best known among the general populace as the fungus that cases thrush and topical Candidiasis. *C. albicans* is the most common causative agent of oral candidiasis, but non-*albicans Candida* spp., such as *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. guilliermondii*, have also emerged as significant pathogens [4].

The difficulty in diagnosis of fungal infections and delays in initiation of treatments are important factors, drugs for effective treatment of these emerging infections are in short supply, thereby contributing to a high mortality rate. Available drugs are essentially limited to the polyene natural product Amphotericin-B and various newer lipid formulations [5], the azole compounds such as fluconazole, itraconazole and flucytosine (5-fluorocytosine). Controlled release systems can address both issues. Consequently, there has been increasing interest, in both research and clinical

practice, in providing localized treatments of infectious processes, e.g. delivery of gentamicin using various materials are delivery systems [6].

In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti inflammatory, antitumoric, cytotoxic and antimitotic activities [7,8]. *Caulerpa* has been novel compounds of di-, sesqui- and mono-terpenes, 1,4-diacetoxybutadiene moiety, nitrogen-containing compounds bisindole alkaloids and caulerpicin [9]. The secondary metabolites of plants belongs to the genus *Caulerpa* have been extensively investigated and are best known for bisindole alkaloids and a suite of unusual di-, sesqui- and mono-terpenes with the terminal 1,4- diacetoxybutadiene moiety [9].

Hence, the present study was aimed to evaluvate the antifungal activity of different extracts of *C. chemnitzia*, *C. racemosa* and *C. scalpelliformis* against six human pathogenic yeast and four dermatophytes in order to discover new antifungal metabolites.

Materials and Methods

Sample Collection

Caulerpa chemnitzia, *C. racemosa* and *C. scalpelliformis* (Chlorophyceae) were collected from the rocky shores of Mandapam (Lat. 09° 17.417′N; Long. 079° 08.558′E) of Rameshwaram district, the Gulf of Mannar Marine Biosphere Reserve, Tamilnadu, India. The collections were made during the month of December, 2012. The algae were 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, Annamalainagar.

Preparation of Extracts

The algal samples were handpicked during low tide and manually cleaned to remove sand, epiphytes and animal waste. Then the samples were rinsed with seawater to remove associated debris, planktons and loosely attached microorganisms. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in an icebox containing slush ice and transported to laboratory. Further, the materials were washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the algae and spread on the blotting paper to remove the excess water. The shade dried samples were again cleaned with distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvents during the extraction process. The algal samples were shade dried followed by oven drying at 50°C for half an hour and milled in an electrical blender. Five hundred gram of powdered samples were individually packed in Soxhlet apparatus and extracted with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 h. The extracts were pooled and the solvents were evaporated under vacuum in rotary evaporator (Heidolph, Germany) under reduced pressure at <40°C and the crude extracts were kept at 4°C for antifungal assays.

Fungi Strains and Culture Conditions

The fungal strains *viz.,* Yeasts: *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.

Preparation of Inocula

Twenty-four hour old cultures of selected *Candida* strains were mixed with physiological saline and turbidity was adjusted by adding sterile physiological saline until a McFarland turbidity

standard of 0.5-2.5 × 10^3 cells/mL. The filamentous fungal strains were subcultured on SDA and incubated at 30°C for 4-7 days for dermatophytes. The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water and inoculum of fungal strains were obtained according to reported procedures and adjusted to 0.4-5 × 10^4 cells/mL.

Antifungal Assay

Disc-Diffusion Assay

The *in vitro* antifungal activities of different algal crude extracts were screened using agar diffusion method [10]. For that assay, petriplates were prepared by pouring 20 ml of sabouraud dextrose agar and allowed to solidify for 20 minutes. The standardized inoculum suspension were swabbed on the top of the solidified media and allowed to dry for 10 minutes. Discs with different concentrations of extracts (1000, 500 and 250µg/disc) were prepared and aseptically applied on the surface of the petriplates. 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. After that, the plates were incubated at 28 °C for 24 hours for yeast and 30 °C for 3-5 days with dermatophytes. The zone of the inhibitions was measured in millimeter. The experiments were carried out in triplicates.

Minimum Inhibitory Concentration (MIC) for Fungi

The MIC of the crude extracts of *Caulerpa* species were determined by using broth micro dilution technique as recommended by CLSI [11] and [12] 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 propane sulfonic acid (MOPS). Fifty milligram of crude extracts were dissolved in 1 mL of 10% DMSO and stock solution was obtained for the determination of MIC. For crude extracts, 20 µL of each plant extracts, were dissolved with 980 µL of RPMI-1640 medium (2 mg/mL). From that, two fold serial dilutions in the range from 1000 to 15.7 µg/mL were prepared. 200 µL of solution was poured into first well of 96 well microtitre plates and then, 100 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 cfu/mL for yeast 0.4-5×10^4 cfu/mL for dermatophytes were transfered to each well. The control well contained only sterile water and devoid of inoculum. The microtitre tray plates were incubated without agitation at 28°C for 24 h for yeast and 30°C for 4-7 days for dermatophytes. The MIC of the extracts were recorded as the lowest concentration of extracts were inhibited the growth of the *Candida* and dermatophytic strains when compared to that of control.

Minimum Fungicidal Concentration (MFC)

MFC of the extracts were determined by plating 100 µl of samples from each MIC assay well with growth inhibition. The wells were transferred to freshly prepared sabouraud dextrose agar plates and incubated in incubator at 28 °C for 24 hours for yeasts and 30 °C for 3-5 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 different solvents of *viz.,* hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia*, *C. racemosa* and *C. scalpelliformis* were screened against fungal strains. Among the tested extracts, the ethyl acetate and chloroform extracts showed the antifungal activity against fungal strains tested. The mean zones of inhibition of the extracts, assayed against the test organisms ranged between 7.1 and 15.1 mm. The ethylacetate extract of *C. racemosa* showed promising activity against *C. parapsilosis* (15.1 mm), followed by *C. albicans* (14.3 mm) and *T. rubrum* (14.1 mm). The chloroform extracts showed activity against *C. parapsilosis* (13.1 mm), followed by *T. rubrum* (13.3 mm), *C. albicans* (12.6 mm) and *T. rubrum* (12.3 mm) are presented in Tables 1, 2 & 3. 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 *C. chemnitzia*, *C. racemosa* and *C. scalpelliformis* 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 *Caulerpa chemnitzia.*

Plant extracts/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	1000	500	250	AmphotercinB (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	-	-	-	11.5 ± 0.50	-	-
Chloroform	10.0 ± 0.57	9.1 ± 0.15	7.1 ± 0.11	12.6 ± 0.76	500	1000
Ethyl acetate	10.6 ± 0.76	9.4 ± 0.40	7.3 ± 0.20	11.8 ± 0.57	500	1000
Acetone	-	-	-	11.0 ± 0.50	-	-
Methanol	-	-	-	13.5 ± 0.50	-	-
Candida krusei						
Hexane	-	-	-	11.3 ± 0.57	-	-
Chloroform	9.5 ± 0.25	8.6 ± 0.17	7.0 ± 0.50	12.6 ± 0.76	500	1000
Ethyl acetate	10.2 ± 0.25	9.5 ± 0.21	7.3 ± 0.34	12.5± 0.50	500	1000
Acetone	-	-	-	14.3 ± 0.57	-	-
Methanol	-	-	-	15.6 ± 0.76	-	-
Candida guilliermondii						
Hexane	-	-	-	14.0 ± 0.50	-	-
Chloroform	10.1 ± 0.15	9.0 ± 0.50	7.1 ± 0.11	12.1 ± 0.28	500	1000
Ethyl acetate	10.5 ± 0.50	9.1 ± 0.15	7.3 ± 0.20	13.5 ± 0.50	500	1000
Acetone	-	-	-	14.0 ± 0.57	-	-
Methanol	-	-	-	13.6 ± 0.76	-	-
Candida glabrata						
Hexane	-	-	-	7.1 ± 0.28	-	-
Chloroform	10.1 ± 0.50	9.1 ± 0.15	7.1 ± 0.11	7.3 ± 0.57	500	1000
Ethyl acetate	10.5 ± 0.50	9.3 ± 0.20	7.3 ± 0.20	8.3 ± 0.57	500	1000
Acetone	-	-	-	7.5 ± 0.50	-	-
Methanol	-	-	-	7.8 ± 0.76	-	-
Candida parapsilosis						
Hexane	-	-	-	11.3 ± 0.57	-	-
Chloroform	10.1 ± 0.15	9.1 ± 0.28	7.0 ± 0.50	12.1 ± 0.28	500	1000
Ethyl acetate	11.1 ± 0.15	10.1 ± 0.15	7.3 ± 0.20	13.5 ± 0.50	500	1000
Acetone	-	-	-	12.0 ± 0.28	-	-
Methanol	-	-	-	13.3 ± 0.57	-	-

[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 1: Continued

Plant extracts/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc) 1000	500	250	AmphotercinB (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida tropicalis						
Hexane	-	-	-	12.6 ± 0.76	-	-
Chloroform	9.3 ± 0.40	8.2 ± 0.34	7.0 ± 0.15	11.8 ± 0.57	500	1000
Ethyl acetate	10.2 ± 0.40	9.4 ± 0.40	7.2 ± 0.25	15.0 ± 0.50	500	1000
Acetone	-	-	-	13.5 ± 0.50	-	-
Methanol	-	-	-	12.8 ± 0.57	-	-
T. rubrum						
Hexane	-	-	-	18.0 ± 0.50	-	-
Chloroform	10.2 ± 0.25	9.3 ± 0.20	7.3 ± 0.20	17.1 ± 0.28	500	1000
Ethyl acetate	11.2 ± 0.26	9.4 ± 0.40	7.4 ± 0.15	17.6 ± 0.76	500	1000
Acetone	-	-	-	15.6 ± 0.76	-	-
Methanol	-	-	-	18.5 ± 0.50	-	-
T.mentagrophytes						
Hexane	-	-	-	17.5 ± 0.50	-	-
Chloroform	10.1 ± 0.15	9.1 ± 0.15	7.2 ± 0.34	17.3 ± 0.57	500	1000
Ethyl acetate	11.2 ± 0.26	9.4 ± 0.40	7.4 ± 0.15	18.5 ± 0.50	500	1000
Acetone	-	-	-	15.1 ± 0.28	-	-
Methanol	-	-	-	16.6 ± 0.76	-	-
Epidermophyton flocossum						
Hexane	-	-	-	18.1 ± 0.28	-	-
Chloroform	9.5 ± 0.50	8.0 ± 0.57	7.0 ± 0.57	14.1 ± 0.28	500	1000
Ethyl acetate	10.1 ± 0.36	9.4 ± 0.15	7.1 ± 0.15	18.3 ± 0.57	500	1000
Acetone	-	-	-	18.5 ± 0.50	-	-
Methanol	-	-	-	17.8 ± 0.76	-	-
Microsporum gypseum						
Hexane	-	-	-	16.3 ± 0.57	-	-
Chloroform	9.6 ± 0.76	8.1 ± 0.17	7.3 ± 0.20	17.3 ± 0.28	500	1000
Ethyl acetate	10.3 ± 0.15	8.3 ± 0.25	7.0 ± 0.50	15.1 ± 0.28	500	1000
Acetone	-	-	-	16.8 ± 0.28	-	-
Methanol	-	-	-	15.0 ± 0.50	-	-

[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 Testeed

Table 2. Antifungal activity of different extracts of *Caulerpa racemose.*

Plant extracts/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	1000	500	250	AmphotercinB (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	12.0 ± 0.50	9.2 ± 0.25	7.6 ± 0.76	12.5 ± 0.50	500	1000
Chloroform	12.6 ± 0.17	10.1 ± 0.25	8.0 ± 0.57	11.2 ± 0.25	500	1000
Ethyl acetate	14.5 ± 0.50	11.2 ± 0.25	8.1 ± 0.28	13.8 ± 0.57	250	500
Acetone	11.2 ± 0.25	9.2 ± 0.28	7.3 ± 0.57	10.0 ± 0.50	500	1000
Methanol	11.1 ± 0.15	8.5 ± 0.50	7.0 ± 0.57	12.5 ± 0.50	500	1000
Candida krusei						
Hexane	10.7 ± 0.25	9.3 ± 0.20	7.3 ± 0.32	9.3 ± 0.20	500	1000
Chloroform	11.2 ± 0.25	9.1 ± 0.15	7.6 ± 0.76	13.6 ± 0.76	500	1000
Ethyl acetate	13.3 ± 0.20	10.3 ± 0.20	8.1 ± 0.28	14.5 ± 0.50	250	500
Acetone	10.1 ± 0.15	9.1 ± 0.15	7.1 ± 0.11	10.3 ± 0.30	500	1000
Methanol	-	-	-	11.6 ± 0.76	-	-
Candida guilliermondii						
Hexane	11.1 ± 0.15	9.1 ± 0.15	7.1 ± 0.15	12.2 ± 0.20	500	1000
Chloroform	12.2 ± 0.20	10.1 ± 0.15	7.4 ± 0.26	11.1 ± 0.17	500	1000
Ethyl acetate	13.4 ± 0.36	10.5 ± 0.15	8.1 ± 0.15	10.1 ± 0.15	250	500
Acetone	10.3 ± 0.30	9.3 ± 0.20	7.3 ± 0.20	10.3 ± 0.30	500	1000
Methanol	-	-	-	11.6 ± 0.76	-	-
Candida glabrata						
Hexane	11.3 ± 0.20	9.1 ± 0.50	7.1 ± 0.15	11.3 ± 0.20	500	1000
Chloroform	12.1 ± 0.15	10.1 ± 0.15	7.3 ± 0.20	12.1 ± 0.15	500	1000
Ethyl acetate	14.1 ± 0.15	10.3 ± 0.20	8.2 ± 0.25	14.1 ± 0.15	250	500
Acetone	11.1 ± 0.15	9.1 ± 0.15	7.3 ± 0.20	11.1 ± 0.15	500	1000
Methanol	-	-	-	11.8 ± 0.76	-	-
Candida parapsilosis						
Hexane	12.2 ± 0.25	10.0 ± 0.20	8.0 ± 0.57	11.3 ± 0.57	500	1000
Chloroform	13.1 ± 0.15	10.3 ± 0.57	8.2 ± 0.25	13.1 ± 0.15	500	1000
Ethyl acetate	15.1 ± 0.17	11.6 ± 0.52	9.3 ± 0.32	11.0 ± 0.50	250	500
Acetone	11.2 ± 0.32	9.5 ± 0.50	7.3 ± 0.20	9.5 ± 0.50	500	1000
Methanol	11.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.15	11.3 ± 0.57	500	1000
Candida tropicalis						
Hexane	-	-	-	13.6 ± 0.76	-	-
Chloroform	10.1 ± 0.15	8.1 ± 0.28	7.1 ± 0.15	12.8 ± 0.57	500	500
Ethyl acetate	11.2 ± 0.25	9.1 ± 0.15	7.3 ± 0.20	11.0 ± 0.50	500	1000
Acetone	-	-	-	11.5 ± 0.50	-	-
Methanol	-	-	-	11.8 ± 0.57	-	-
T. rubrum						
Hexane	11.2 ± 0.26	10.0 ± 0.57	7.2 ± 0.25	18.0 ± 0.50	500	1000
Chloroform	12.3 ± 0.11	10.4 ± 0.10	8.3 ± 0.32	17.3 ± 0.57	500	1000
Ethyl acetate	14.3 ± 0.32	10.6 ± 0.76	8.9 ± 0.57	15.6 ± 0.76	250	500
Acetone	11.0 ± 0.50	9.5 ± 0.50	7.3 ± 0.20	14 .6 ± 0.76	500	1000
Methanol	10.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.11	18.5 ± 0.50	500	1000
T.mentagrophytes						
Hexane	9.0 ± 0.50	8.0 ± 0.50	7.0 ± 0.50	17.5 ± 0.50	500	1000
Chloroform	11.1 ± 0.15	9.1 ± 0.28	7.2 ± 0.34	17.3 ± 0.57	500	1000
Ethyl acetate	13.2 ± 0.26	10.1 ± 0.15	8.0 ± 0.11	18.5 ± 0.50	250	500
Acetone	-	-	-	16.1 ± 0.28	-	-
Methanol	-	-	-	17.6 ± 0.76	-	-
Epidermophyton flocossum						
Hexane	10.4 ± 0.40	9.0 ± 0.57	7.3 ± 0.20	15.1 ± 0.28	500	1000
Chloroform	11.1 ± 0.15	9.1 ± 0.28	7.2 ± 0.25	16.5 ± 0.28	500	1000
Ethyl acetate	12.1 ± 0.15	10.1 ± 0.15	7.3 ± 0.32	18.3 ± 0.57	500	1000
Acetone	10.1 ± 0.28	9.0 ± 0.50	7.0 ± 0.11	14.5 ± 0.50	500	500
Methanol	-	-	-	17.8 ± 0.76	-	-
Microsporum gypseum						
Hexane	10.1 ± 0.15	9.0 ± 0.57	7.3 ± 0.32	14.3 ± 0.57	500	500
Chloroform	10.5 ± 0.50	9.3 ± 0.20	7.5 ± 0.50	16.3 ± 0.28	500	500
Ethyl acetate	13.3 ± 0.15	10.2 ± 0.10	8.0 ± 0.50	17.5 ± 0.50	500	1000
Acetone	-	-	-	19.0 ± 0.28	-	-
Methanol	-	-	-	18.3 ± 0.57	-	-

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm
[b]-mean of three assays; ± - standard deviation; ** significant at $p < 0.05$

Table 3. Antifungal activity of different extracts of *Caulerpa scapelliformis.*

Plant extracts/ solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	1000	500	250	AmphotercinB (100 units/disc) Ketoconazole (10µg/disc)	MIC (µg/ml)	MFC (µg/ml)
Candida albicans						
Hexane	10.1 ± 0.15	9.1 ± 0.28	7.1 ± 0.11	11.5 ± 0.50	500	1000
Chloroform	10.5 ± 0.50	9.5 ± 0.50	7.3 ± 0.57	13.6 ± 0.76	500	1000
Ethyl acetate	11.5 ± 0.50	10.1 ± 0.15	8.0 ± 0.50	11.3 ± 0.57	500	1000
Acetone	-	-	-	11.4 ± 0.50	-	-
Methanol	-	-	-	12.5 ± 0.50	-	-
Candida krusei						
Hexane	-	-	-	11.3 ± 0.57	-	-
Chloroform	9.8 ± 0.76	8.3 ± 0.57	7.1 ± 0.11	11.6 ± 0.76	500	1000
Ethyl acetate	10.8 ± 0.76	9.5 ± 0.50	7.6 ± 0.76	14.5± 0.50	500	1000
Acetone	-	-	-	13.3 ± 0.57	-	-
Methanol	-	-	-	14.6 ± 0.76	-	-
Candida guilliermondii						
Hexane	-	-	-	15.0 ± 0.50	-	-
Chloroform	10.3 ± 0.20	9.3 ± 0.20	7.3 ± 0.57	12.5 ± 0.50	500	1000
Ethyl acetate	10.5 ± 0.50	9.5 ± 0.50	7.8 ± 0.76	9.5 ± 0.50	500	1000
Acetone	-	-	-	11.3 ± 0.57	-	-
Methanol	-	-	-	12.6 ± 0.76	-	-
Candida glabrata						
Hexane	9.5 ± 0.50	8.6 ± 0.50	7.1 ± 0.11	13.1 ± 0.28	500	1000
Chloroform	10.8 ± 0.76	9.3 ± 0.20	7.3 ± 0.32	12.3 ± 0.57	500	1000
Ethyl acetate	11.1 ± 0.15	9.5 ± 0.50	7.6 ± 0.76	12.1 ± 0.57	500	1000
Acetone	-	-	-	13.5 ± 0.50	-	-
Methanol	-	-	-	14.8 ± 0.76	-	-
Candida parapsilosis						
Hexane	10.5 ± 0.50	9.0 ± 0.20	7.1 ± 0.11	13.3 ± 0.57	500	1000
Chloroform	11.1 ± 0.15	9.5 ± 0.50	7.3 ± 0.20	14.1 ± 0.28	500	1000
Ethyl acetate	12.1 ± 0.15	10.0 ± 0.50	8.1 ± 0.28	11.3 ± 0.57	500	1000
Acetone	-	-	-	9.0 ± 0.50	-	-
Methanol	-	-	-	10.3 ± 0.57	-	-
Candida tropicalis						
Hexane	-	-	-	15.5 ± 0.50	-	-
Chloroform	10.0 ± 0.50	8.5 ± 0.50	7.1 ± 0.11	13.8 ± 0.57	500	1000
Ethyl acetate	10.5 ± 0.50	9.0 ± 0.20	7.3 ± 0.32	14.0 ± 0.50	500	1000
Acetone	-	-	-	14.5 ± 0.50	-	-
Methanol	-	-	-	12.8 ± 0.76	-	-
T. rubrum						
Hexane	10.0 ± 0.50	9.1 ± 0.15	7.1 ± 0.11	18.0 ± 0.50	500	1000
Chloroform	10.3 ± 0.20	9.5 ± 0.50	7.3 ± 0.32	17.1 ± 0.28	500	1000
Ethyl acetate	11.6 ± 0.32	10.1 ± 0.15	8.9 ± 0.57	17.6 ± 0.76	500	1000
Acetone	-	-	-	15.6 ± 0.76	-	-
Methanol	-	-	-	18.3 ± 0.57	-	-
T.mentagrophytes						
Hexane	-	-	-	17.5 ± 0.50	-	-
Chloroform	10.6 ± 0.76	9.3 ± 0.20	7.1 ± 0.11	17.8 ± 0.57	500	1000
Ethyl acetate	11.0 ± 0.50	9.8 ± 0.76	7.8 ± 0.76	18.5 ± 0.50	500	1000
Acetone	-	-	-	18.1 ± 0.28	-	-
Methanol	-	-	-	16.6 ± 0.76	-	-
Epidermophyton flocossum						
Hexane	-	-	-	18.1 ± 0.28	-	-
Chloroform	10.1 ± 0.15	9.5 ± 0.50	7.1 ± 0.11	17.1 ± 0.28	500	1000
Ethyl acetate	10.8 ± 0.76	9.8 ± 0.15	7.5 ± 0.50	18.3 ± 0.57	500	1000
Acetone	-	-	-	14.1 ± 0.50	-	-
Methanol	-	-	-	15.1 ± 0.76	-	-
Microsporum gypseum						
Hexane	-	-	-	14.3 ± 0.57	-	-
Chloroform	9.5 ± 0.50	8.5 ± 0.20	7.3 ± 0.20	18.3 ± 0.28	500	1000
Ethyl acetate	10.0 ± 0.50	9.3 ± 0.20	7.6 ± 0.76	14.1 ± 0.28	500	1000
Acetone	-	-	-	15.8 ± 0.28	-	-
Methanol	-	-	-	16.0 ± 0.50	-	-

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm

[b]-mean of three assays; ± - standard deviation; ** significant at $p < 0.05$

Discussion

Nowadays, many biological activities have been evaluated for numerous species of plants. This demonstrates that compounds from plants are indeed useful as alternative therapy, either directly or as models for new synthetic substances [13]. In the present investigation, new antifungal plant derivatives could be useful alternatives for the treatment of *Candida* where a topical therapy is required. The advantage of using these marine natural compounds may be a reduced risk of side-effects and lower cost. Seaweeds have been traditionally been used as food and folk medicine for curing helminthes infectious, gout and eczema, particularly by coastal peoples in several countries.

In this study, the antifungal activity of chloroform and ethyl acetate extracts of *Caulerpa chemnitzia*, *C. racemosa* and *C. scalpelliformis* against the selected five yeast type fungi and dermatophytic strains tested. The ethyl acetate extracts of *C. racemosa,* showed the highest antifungal activity against *C. parapsilosis*, *C. albicans* and *T. rubrum* with the lowest MIC values of 250 –500 µg/ml. Salem et al. [14] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *C. racemosa*, *Sargassum dentifolium, Padina gymnospora*, methanolic extracts of *Sargassum hystrix*, *C. racemosa*, *C. fragile*, *S. dentifolium* and *Cystoseria myrica* against *E. coli*, *S. aureus*, *E. faecalis*, *Salmonella* sp., *B. cereus* and *P. aeruginosa*. Zovko *et al.* [15] obtained the same results against fungal strains with a high activity of algal extracts against *C. albicans*. This difference in results may be firstly due to difference in species used, time and place of sample collection, secondly; there may also be differences in the capability of the extraction protocols to recover the active metabolites and finally, differences in the assay methods that would results in different susceptibilities of the target strains.

In this study, ethyl acetate extracts of *C. racemosa* showed the highest zone of inhibition (15.1 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.* Karthick et al. [16] determined the chloroform, benzene, acetone, diethyl ether and methanol extracts of *Caulerpa scalpelliformis* against *Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger, A. terreus* and *A. flavus*. 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. Mao et al. [17] reported that isolated from ethyl acetate extracts of *C. racemosa,* the discovery of one new polyacetylenic fatty acid, (8E, 12 Z, 15Z)-10-hydroxy-8, 12, 15- octadecatrien-4, 6-diynoic acid. *Caulerpa* has attracted the attention of researchers due to its important secondary metabolite caulerpenyne (CYN) that is reported to exhibit the antineoplastic, antibacterial and antiproliferative activities [18]. Lee et al. [19] reported that seven sesquiterterpenes sulphates isolated from the tropical sponge *Dysidea* sp., and investigated their inhibitory activitie against isocitrate lyase from *C. albicans*. Most of the compounds were found to be strong isocitrate lyase inhibitors and also showed the potent antibacterial effect against *Bacillus subtilis* and *Proteus vulgaris*.

In this study, ethyl acetate extract of *C. racemosa* demonstrated the highest antifungal activity than the other extracts against yeast and filamentous fungi.

Oh et al. [20] reported that *Odonthalia corymbifera*, crude extracts were exhibited antimicrobial activity against various microorganisms and bioassay-guided separation of the crude extract afforded several bromophenol compounds. Among the isolated natural products, 2,2,3,3'-tetrabromo-4,4',5,5'-tetrahydroxydiphenylmethane was found to be the most active derivative against *C. albicans*, *A. fumigatus*, *T. rubrum* and *T. mentagrophytes*. 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* [21].

In the present study, the ethyl acetate extracts of *C. chemnitzia*, *C. racemosa* and *C. scalpelliformis* the antifungal activity may be due to the presence of phytochemicals, terpenoid, tannin and phenolic compounds. 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, antifeedant, antiviral, anticancer and vasodilatory actions [22]. 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 [23]. Zodape et al. [24] reported that the eighteen components, were β–sitosterol (28.57%), *n*-hexadecanoic acid (15.57%), phytol (13.65%), stigmastan-3-ol, 5-chloroacetate (8.48%), 3,7,11,15-tetramethyl-2-hexadecane-1-ol (7.57%) and stigmastan-6,22–dien,3,5-dedihydro (6.35) from the *Caulerpa racemosa.* In the present study ethyl acetate extract showed highest antibacterial activity than other studied extracts. This may be due to the existence of secondary metabolites with antimicrobial properties.

In the present study we have used two control drugs namely Amphotericin B and ketoconazole. Amphotericin B (Amp B) and the azoles are mainly used in common clinical situations. Furthermore, Amp B is considered as the drug of choice for the treatment of fungal infections [25]. However, dose-limiting nephrotoxicity associated with amphotericin B, rapid development of resistance with flucytosine, drug–drug interactions, fungistatic mode of action and resistance development with the azoles. In case of Amp B, due to its poor permeability across the membrane an increased amount of Amp B must be administered to patients, which can result in severe side effects such as renal damage [26]. 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 [27]. The utilization of various latex in combination with an antifungal drug such as amphotericin-B or ketoconazole may involve a reduction of the dose of drugs used in treatment of mycoses and therefore in a reduction of their side-effects.

Since natural products have been proven to be an excellent source of novel chemical entities, we have employed screening of microbial extracts in our search for new novel compounds.

Conclusion

Finally, it can be concluded that the ethyl acetate extracts of *C. chemnitzia*, *C. racemosa* and *C. scalpelliformis* showed the best antifungal activity against *C. parapsilosis* and *C. albicans.* Among the types of fungi tested, the *Candida* species were more susceptible than dermatophytes. Further, the isolation of antifungal compound from the ethyl acetate extract of *C. racemosa* is in progress.

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References

[1] B.J. Park, Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS, Aids. 23 (2009) 525–530.

[2] E.J. Bottonae, Dermatophytes, chromoblastomycosis and mycetoma. An Atlas of the Clinical Microbiology of the Infectious Diseases, Viral, Fungal and Parasitic Agents, Taylor and Francis, UK. 2. (2006) 41–50.

[3] S. Amer, M.M. Aly, S. Sabbagh, Biocontrol of dermatophytes using some plant extracts and actinomycetes filtrates, Egyptian J. Biotechnol. (2006) 330-315.

[4] F. Barchiesi et al., Emergence of oropharyngeal candidiasis caused by non-*albicans* species of *Candida* in HIV-infected patients, European J. Epidemiology. 9(4) (1993) 455–456.

[5] J. Hiemenz, Lister, Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis, Clin. Infect. Dis. 29 (1999) 1402–1407.

[6] M. Zilberman, J.J. Elsner, Antibiotic-eluting medical devices for various applications, J Control Release. 130(3) (2008) 202–215.

[7] G. Adaikala Raj et al., Antibacterial activity of different solvent extracts of *Caulerpa chemnitzia* (Esper) J.V. Lamououx, from Mandapam, Gulf of Mannar Southeast Coast, Tamil Nadu, India, J. Med. Herbs Ethnomed. 1 (2015) 24-31.

[8] H. Harada, T. Naro, Y. Kamei, Selective antitumor activity *in vitro* from marine algae from Japan coasts, Biol. Pharm. Bull. 20 (1997) 541-546.

[9] S.C. Mao, Y.W. Guo, X. Shen, Two novel aromatic valerenane-type sesquiterpenes from the Chinese green alga *Caulerpa taxifolia*, Bioorg. Med. Chem. Lett. 16 (2006) 2947–2950.

[10] A.W. Bauer et al., Antibiotic susceptibility testing by a standardized single disk method, American J. Clinical Pathol. 45 (1966) 493-496.

[11] Clinical and Laboratory Standard Institute (CLSI), Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved Standard M38-A, Wayne, PA. 2002.

[12] Clinical and Laboratory Standards Institute (CLSI), Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard, 3rd ed. Clinical and Laboratory Standards Institute M27-A3, Wayne, Pa. 2008.

[13] P.J. Houghton, Chemistry and biological activity of natural and semi-synthetic chromone alkaloids, Studies in Natural Products Chemistry. 21 (2000) 123–155.

[14] W.M. Salem, H. Galal, F. Nasr El-deen, Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt), Afr. J. Microbiol. Res. 5(15) (2011) 2160-2167.

[15] A. Zovko et al., Antifungal and antibacterial activity of three-alkyl-pyridinium polymeric analogs of marine toxins, Int. Biodeterior. Bio. Degra. 68 (2012) 71-77.

[16] N. Karthick et al., Screening of phytochemicals and antimicrobial activity of *Caulerpa scalpelliformis* collected from Manapad Coast, Tuticorin district, Tamilnadu, South India, J. Coast. Life Med. 2 (2014) 107-111.

[17] S.C. Mao et al., A new polyacetylenic fatty acid and other secondary metabolites from the Chinese green alga *Caulerpa racemosa* (Caulerpaceae) and their chemotaxonomic significance, Biochemical Systematics and Ecology. 39(4) (2011) 253–257.

[18] L. Cavas et al., Antiproliferative and newlyattributed apoptotic activities from a marina alga: *Caulerpa racemosa* var. cylindracea, Journal of Experimental Marine Biology and Ecology. 339(1) (2006) 111–119.

[19] D. Lee et al., Inhibition of *Candida albicans* isocitrate lyase activity by sesterterpene sulfates from the tropical sponge *Dysidea,* Bioorg. Med. Chem. Lett. 18 (2008) 5377-5380.

[20] K.B. Oh et al., Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives, Bioorg. Med. Chem. Lett. 18(1) (2008) 104-108.

[21] M. Chandrasekaran, V. Venkatesalu, Antibacterial activity and antifungal activity of *Syzygium jambolanum* seeds, J. Ethanopharmacol. 91 (2004) 105-108.

[22] C. Rievere et al., Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*, Phytochem. 70(1) (2009) 86-94.

[23] D.E. Okwu, Evaluation of the chemical composition of indigenous spices and flavouring agents, Global J. Pure Appl. Sci. 7(3) (2011) 455–459.

[24] S.T. Zodape et al., Effect of *Kappaphycus alvarezii* extract on grain quality, yield and some yield components of wheat (*Triticum aestivum* L.), Int. J. Plant Prod. 3 (2009) 97-101.

[25] S.C. Hartsel, T.R. Weiland, Amphotericin B binds to amyloid fibrils and delays their formation: a therapeutic mechanism, Biochem. 42 (2003) 6228–6233.

[26] J. Mayer et al., Reduced nephrotoxicity of conventional amphotericin B therapy after minimal nephroprotective measures: animal experiments and clinical study, J. Infect. Dis. 186 (2002) 79–388.

[27] A.M. Sugar et al., Pharmacology and toxicity of high-dose ketoconazole, Antimicrob. Agents Chemother. 31 (1987) 1874–1878.