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Evaluating the *In Vitro* Antagonism of Secondary Metabolites Fractionated from the Brown Algae, *Sargassum swartzii* against Human *Candida* spp.

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Abstract

Objective: To inspect the anticandidal potency of brown algae *S. swartzii* and GC-MS analysis to delineate its bioactive principles.

Methods: The marine brown algae *S. swartzii*, was extracted and fractionated in organic solvents and quantitatively analyzed for its *in vitro* anticandidal potency against a battery of five clinically relevant species of *Candida*.

Results: The fractionation of the crude algal extract yielded a bioactive algal fraction that exhibited broadest spectra of activity. It impeded the growth of all the evaluated Yeast pathogens in variable degrees. The maximal activity was recorded against the *Candida albicans.* The GC-MS studies of active algal fraction evinced the presence of three chemical constituents. Thence, the potent broad spectra of activity against the human *Candida* could be due the presence of major principle 1,2-Benzenedicarboxylic acid, diisooctyl ester, or could be pertained to the synergistic activity all the components.

Conclusion: The overall results of this study implicates that the bioactive principles found in this algal fraction could be utilized as a lead molecule to develop natural antifungal drug to combat pathogenic *Candida* species.

Keywords: Algal fraction; Anticandidal activity; Secondary metabolites; Antimicrobials

Introduction

Fungi are the diverse group of ubiquitous eukaryotes which intercedes vital ecological processes. Nevertheless, many of them are primary or opportunistic pathogens capable of inflicting wide spectra of ailments in humans [1]. Amid the 1.5 million estimated species of fungi, around 317 species are known to smite diseases in human [2,3]. The fungal scourges have broad and variable clinical manifestations ranging from superficial skin and nails infections to disseminated life threatening infections [1]. In the developing countries, opportunistic fungi are oftentimes causing infections in immuno-compromised patients which therefore need to control promptly. Of the different infective species of fungi, Yeasts of the *Candida* genus inflict fatal systemic infections which increased substantially over the last decade. For instance, it is noted that the *Candida* spp. are the most common opportunistic pathogen in AIDS patients [4]. In addition, infections due to non-albicans species have also egressed over the past two decades, and a switch from *C. albicans* to species such as *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis* has alarmingly increasing [5].

During the last decades, pharmaceutical industries have produced limited number of new therapeutic drugs for the management of *Candida*. However, the prolonged/inadequate usage of exciting antifungal drugs has precipitated the emergence of drug resistant *Candida* spp. and poses an extra concern [6]. With the increased incidence of *Candidal* infections coupled with the setback associated with the overuse and misuse of antibiotics demonstrates the insistent need of finding safe, novel and effective antifungal agents. Therefore, novel antifungal drugs with increased potency are required in lieu of conventional antibiotics for the management of fungal diseases.

In comparison to the chemicals and drugs used for synthetic treatments, allelo-chemicals from marine origin are less associated with negative effects and have enormous therapeutic potentials to heal many infectious maladies in human [7]. Currently, marine organisms are an enormous reserve of novel drugs and drug leads for the pharmaceutical industry. Marine natural products have been discovered from a wide array of organisms including sponges, algae, bryozoans, mollusks, cnidarians, tunicates, echinoderms, sea worms and microorganisms. It is a confirmed fact that, the marine algae

are prime candidate in producing antimicrobial metabolites to thwart the invaders in their natural habitat. Pluralities of algal species have been reported to possess multitude of bioactivities and thence, potential for elaborating antimicrobial agents. For instance, literature addressed that species of genus *Sargassum* presented diverse activities such as anti-Herpetic [8], antiretroviral [9], antifungal [10], antibacterial [11], and anticancer [12].

Retrospective examination evidenced that antifungal actions of marine algae were exposited as earlier as 1915s [13]. Various marine algae from the Southwest littoral of India has been recently corroborated for exerting diverse bioactivities such as antibacterial [14], antifungal [15], antiviral [16], anticoagulant [17] and cytotoxicity [18]. In so far, there is no precedence of research being conducted to inspect the antagonistic potential of brown algae, *Sargassum swartzii* against human *Candida*. In our preliminary experiments, the crude methanolic extract of *S. swartzii* evinced pronounced antibacterial activity against clinical and biofilm forming bacteria. In this regard, the brown algae *S. swartzii* was preferred to explore its anticandidal potency and GC-MS analysis to delineate its bioactive principles.

Materials and Methods

Collection of algal specimens

Live and healthy thalli of marine algae, *S. swartzii* were handpicked at ebb tide from the rugged intertidal zone of Kollam coast, South India (08°54' N and 76°38' E). Garnered specimens were then successively rinsed with seawater to remove dirt and transferred to laboratory in plastic bags containing seawater to prevent evaporation. The rinsed thalli were air dried under a stream of air flow for one week at room temperature to prevent photolysis and thermal degradation of metabolites. Dried fronds of algae were powdered in a coffee grinder, packed in polyethylene bags and stored in moisture free place until extraction

Extraction of algae

Algal bioactives were extracted from dried algal powder according to the parameters previously optimized [14]. Briefly, a definite quantity (200 g) of dried algal powder was submerged in conical flasks (2000 mL) containing 1000 mL of methanol (MeOH) and placed at 35°C on a shaker at 120 rpm for two weeks to permit full extraction of the bioactive components. After two weeks, algal material was filtered using Whatman filter paper No 1. The filter residue was collected in a round-bottom flask and the solvent was concentrated in a rotary vacuum evaporator at 45°C for the elimination of MeOH. The resultant gummy dark coloured extract was collected in airtight plastic vials and stored in the refrigerator for further studies.

Fractionation of *Sargassum swartzii*

A known morsel of dried extract of the algae (crude solid residue collected after vacuum evaporation) was adsorbed to silica gel and applied in a column developed with petroleum ether and eluted step-wise with petroleum ether and ethyl acetate (9:1 to 1:9 and 100% ethyl acetate) followed by ethyl acetate and methanol (9:1 to 1:9 and 100% methanol) [15]. Column elute was collected in 10 mL screw cap bottles. Preliminary experiments confirmed that the fraction eluted using ethyl acetate: methanol (4:6) retained antibacterial activity (data not shown). Other fractions that displayed meagre activity were not more considered in the present study. Hence, the same fraction (4:6) was used to investigate anticandidal activity and GC-MS analysis.

Gas chromatographic and mass spectroscopic (GC-MS) analysis

The active fraction was chemically analysed through GC-MS method as described elsewhere [14].

Antifungal assay

To determine anticandidal activity, the pre-selected algal fraction was evaluated against a battery of clinically relevant five species of *Candida* those previously used and continuously maintained in our laboratory [15]. The anticandidal assay was performed as described elsewhere [15]. The Sabouraud dextrose agar (Himedia) was used for bioactivity screening and routine propagation of human fungus respectively. Cell suspensions containing 10^7 CFU/ml cells for yeasts, were prepared and aseptically besmeared onto the surface of the agar plates of Sabouraud dextrose medium using sterile swab sticks. Thereafter, wells with five millimeter of diameter were prepared using a sterile cork borer. The resultant wells were carefully filled with 100 µl (15 mg/ml) of algal fraction. The well with binary solvents used for fractionation was considered as negative control. The assay was performed in triplicates of individual Petri dishes. The clear zones of inhibition formed around wells after 48 h at 30°C were considered to be an indicative of anticandidal activity. The inhibitory activity was recorded by calculating the area of clear zone and anti-biogram was statistically analyzed.

Statistical analysis

The results are expressed as means ± SE of three experiments. Mean values were assessed using One-way analysis of variance using SPSS for Windows version 20.0 (Statistical Package for Social Services, Chicago, IL, USA).

Results

Our preliminary study posited that, the methanolic extract of *S. swartzii* was excellent for subduing the growth of clinical and biofilm forming bacteria *in vitro* (data not shown). And there is an extreme paucity of studies pertains to the anticandidal efficacy of *S. swartzii* from the Indian littoral.

Thence, in the present study, anticandidal activity of *S. swartzii* was explored against the panel of five species of *Candida*. The overall results emphasize the potentiality of using *S. swartzii* for the development of chemotherapeutic agents against *Candida* sp. **Figure 1** illustrates the inhibitory spectra produced by the *S. swartzii* against *Candida* spp. It is evident that the *S. swartzii* exerted broad spectra of activity against all the tested species of *Candida* in varying degrees. The active algal fraction produced mean zones of inhibition ranged between 102.17 ± 10.03 mm^2 to 117.51 ± 22.28 mm^2 against the *Candida* sp. The anticandidal action was very high against *C. albicans* to the extent of 117.51 ± 22.28 mm^2. The nearly active range was displayed against *C. tropicalis* (110.37 ± 17.7) and *C. glabrata* (107.03 ± 23.45). The activity range of *C. krusie* and *C. parapsilosis* accounted for 100.86 ± 20.5 and 102.17 ± 10.03 mm^2 respectively. The overall results exposited that potent anticandidal constituent can be isolated from *S. swartzii*.

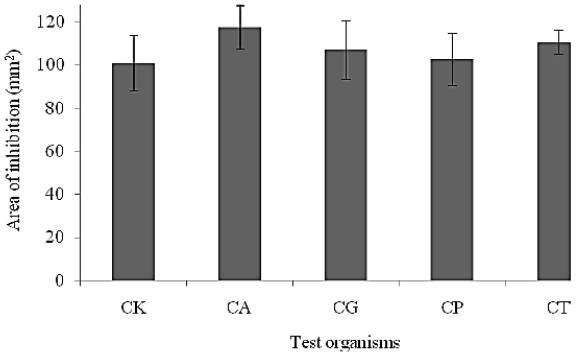

Figure 1 Anticandidal activity of active fraction of *S. swartzii*. The activity index was calculated as mm^2 area based on the diameter halo displayed [CK-*C. krusie*; CA-*C. albicans*; CG-*C. glabrata*; CP-*C. parapsilosis*; CT-*C. tropicalis*]

GC-MS analysis of active fraction of *S. swartzii*

The active fraction was subjected to GC-MS analysis to explicate its bioactive chemical constituents. The spectral data has brought a single prominent peak to fore with the retention time and molecular weight of 25.73 and 390 respectively (**Table 1**). MS data has perfectly matched a compound of molecular formula $C_{24}H_{38}O_4$ that is analogous to 1,2-Benzenedicarboxylic acid, diisooctyl ester in the NIST library.

Discussion

Globally, diseases caused by the species of genus *Candida* are leading health problems with high morbidity in immuno-competent and immuno-compromised patients [19]. The application of chemotherapeutics for the management of clinically relevant *Candida* spp. is currently restricted by the development of drug resistance. In this viewpoint, an antifungal agent with broad efficacy and minimal side effect is needed to mitigate the plights of vast masses of immunocompetent and immuno-compromised patients.

Bioactive molecules of marine algal origin have high potentiality to subjugate the growth of many infectious organisms. In fact, several *in vitro* studies are demonstrated the anticandidal activity of many marine algae [15,20-22]. Albeit, diverse species of *Sargassum* from the Indian coast has been recognized as a potential source of antibacterial agents, the anticandidal activity has seldom been reported [23]. Therefore, in the present study, secondary metabolites fractionated from *S. swartzii* were quantitatively examined anticandidal efficacy. The algal fraction exerted wider spectrum of activity, since it impeded the growth of all the evaluated Yeast pathogens in variable degrees. Anticandidal activity was found to be positively skewed toward *C. albicans* as compared to other Yeast spp. This type of variation in the efficacy was analogous to that observed for red algae, *A. taxiformis* against human *Candida* spp. [15]. In accordance with the present study, the crude extract of other sp. of *Sargassum* exhibited antifungal activity against *C. albicans* [24]. The demonstration of anticandidal efficiency is an indication that the *S. swartzii* is a potential source for bioactive-compounds with broad spectra of activity. The secondary metabolites extracted from the algae can incapacitate the growth of Yeast by mechanisms that are unlike those of antifungal agents currently available. Thence, it is posited to have significant clinical value in the management of resistant yeast strains. Howbeit, further detailed studies are necessary to verify the *in vivo* efficacy and mechanism of action.

The antimicrobial compounds responsible for the anticandidal efficacy are not elucidated in this study. Howbeit, GC-MS analysis of active fraction evinced the presence of three compounds such as, 1,2-Benzenedicarboxylic acid, diisooctyl ester (390 g/mol), n-Hexadecanoic acid (256 g/mol) and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (296 g/mol) Thence, it is envisaged that the growth inhibition of *Candida* spp. displayed by the active algal fraction could be due to the presence of major principle 1,2-Benzenedicarboxylic acid, diisooctyl ester, or could be related with synergistic activity of all components, since antimicrobial activities are pertained to the presence of secondary metabolites. The bioactive phyto-chemicals of diverse species of genus *Sargassum* were well reviewed by Liu et al. [25]. The same author noted the presence of 1,2-Benzenedicarboxylic acid; Dioctyl ester in *S. wightii*. Similarly, the GC-MS results are in line with recent studies that annotated the similar bioactive chemical constituents in other plant specimens [26-29].

Conclusion

Hitherto, there is no report on the anticandidal activity of *S. swartzii* against the five species of clinically relevant *Candida*. Hence, this report is the first to explore the anticandidal activity of *S. swartzii* from the South Indian littoral. The present findings disclose that the brown algae, *S. swartzii* is potential to oppress the growth of all tested fungal pathogens *in vitro*. The prevalence of anticandidal activity of *S. swartzii* reflects the credible evidence that algae hold effective

anticandidal chemical defences. In this context, more studies pertaining to mode of action of algal bioactives and interaction with pathogenic fungi may bring forth new drug leads for the control of fungal pathogens.

Table 1 Components identified in the active fraction of *S. swartzii* by GC-MS study

No	RT	Name of the compound	MF	MW	PA (%)
1	14.44	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	6.10
2	16.24	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.36
3	25.73	1, 2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	77.54
					100 %

RT-Retention time; MF-Molecular formula; MW-Molecular Weight; PA-Peak Area

References

1. Köhler JR, Casadevall A, Perfect J (2014) The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med 3: 5.
2. Garcia-Solache MA, Casadevall A (2010) Global warming will bring new fungal diseases for mammals. mBio 1: 1.
3. Hawksworth DL (2001) The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res 105:1422-32.
4. Sanchez-Vargas LO, Ortiz-Lopez NG, Villar M, Moragues MD, Aguirre JM, et al. (2005) Point prevalence, microbiology and antifungal susceptibility patterns of oral *Candida* isolates colonizing or infecting Mexican HIV/AIDS patients and healthy persons. Rev Iberoam Micol 22: 83-92.
5. van Asbeck EC, Clemons KV, Stevens DA (2009) *Candida parapsilosis*: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. Crit Rev Microbiol 35: 283-309.
6. Morace G, Perdoni F, Borghi E (2014)Antifungal drug resistance in Candida species. J Glob Antimicrob Resist 2: 254-259.
7. Manilal A, Sujith S, Selvin J, Shakir C, Gandhimathi R, et al. (2010) Antimicrobial potential of marine organisms collected from southwest coast of India against multiresistant human and shrimp pathogens. Sci Mar 74: 287-296.
8. Zhu W, Chiu LC, Ooi VE, Chan PK, Ang PO Jr (2006) Antiviral property and mechanisms of a sulphated polysaccharide from the brown alga *Sargassum* patens against Herpes simplex virus type 1. Phytomed 13: 695-701.
9. Paskaleva EE, Lin X, Duus K, McSharry JJ, Vei Ile JL, et al. (2008) *Sargassum fusiforme* fraction is a potent and specific inhibitor of HIV-1 fusion and reverse transcriptase. Virol J 5: 8.
10. Oranday MA, Verde MJ, Martinez-Lozano SJ, Waksman NH (2004) Active fractions from four species of marine algae. Phyton Int J Exp Bot 165-170.
11. Chiao-Wei C, Siew-Ling H, Ching-Lee W (2011) Antibacterial activity of *Sargassum polycystum C.* Agardh and Padina australis Hauck (Phaeophyceae). Afr J Biotechnol 10: 14125-14131.
12. Yamaguchi M, Matsumoto T (2005) Marine algae *Sargassum horneri* bioactive factor suppresses proliferation and stimulates apoptotic cell death in human breast cancer MDA-MB-231 cells in vitro. Integr Mol Med.
13. Pratt R, Mautner R, Gardener GM, Sha Y, Dufrenoy J (1951) Report on antibiotic activity of seaweed extracts. J Amer Pharm Asst Sci 40: 579-579.
14. Manilal A, Sujith S, Selvin J, Kiran GS (2010) Antibacterial activity of Falkenbergia hillebrandii (Born) from the Indian coast against human pathogens. Phyton Int J Exp Bot 78: 161-166.
15. Manilal A, Sujith A, Kiran GS, Selvin J, Shakir C, et al. (2009) Antimicrobial potential and seasonality of red algae collected from southwest coast of India tested against shrimp, human and phytopathogens. Ann Microbiol 59: 207-219.
16. Manilal A, Sujith A, Kiran GS, Selvin J, Shakir C (2009) *In vivo* Antiviral Activity of Polysaccharide from the Indian Green Alga, Acrosiphonia orientalis (J. Agardh): Potential Implication in Shrimp Disease Management. World J Fis Mar Sci 1: 278-282.
17. Manilal A, Sujith S, Selvin J, Panikkar MVN, George S (2012) Anticoagulant potential of polysaccharide isolated from Indian red alga, Asparagopsis taxiformis (Delile) Trevisan. Thalassas Inter J Mar Sci 28: 9-15.
18. Manilal A, Sujith A, Kiran GS, Selvin J, Shakir C (2009) Cytotoxic potentials of Laurencia brandenii collected from the Indian Coast. Global J Pharmacol 3: 90-94.
19. Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, et al. (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 48: 503-35.
20. Ismail A, Bel Hadj Salah K, Ahmed M, Mastouri M, Bouraoui M, et al. (2014) Antibacterial and antifungal activities of brown alga Zonaria tournefortii (J.V. Lamouroux). Allelopath J 34: 143-154.
21. Indira K, Balakrishnan S, Srinivasan M, Bragadeeswaran S, Balasubramanian T (2013) Evaluation of *in vitro* antimicrobial property of seaweed (Halimeda tuna) from Tuticorin coast, Tamil Nadu, Southeast coast of India. Afr J Biotechnol 12: 284-289.
22. Elnabris KJ, Elmanama AA, Chihadeh WN (2013) Antibacterial activity of four marine seaweeds collected from the coast of Gaza Strip, Palestine. Mesopot J Mar Sci 28: 81-92.
23. Prabhakar K, Sathish Kumar L, Rajendran S, Chandrasekaran M, Bhaskar K, et al. (2008) Antifungal Activity of Plant Extracts against *Candida* Species from Oral Lesions Indian. J Pharm Sci 70: 801-803.
24. El-Sheekh MM, Gharieb MM, El-Sabbagh SM, Hamza WT (2014) Antimicrobial Efficacy of Some Marine Macroalgae of Red Sea. Int J Microbiol Immunol Res 3: 021-028.

25. Liu L, Heinrich M, Myers SP, Dworjanyn SA (2012) Towards a better understanding of medicinal uses of the brown seaweed genus *Sargassum* in traditional Chinese medicine: a phytochemical and pharmacological review. J Ethnopharmacol 142: 591-619.

26. Krishnamoorthy K, Subramaniam P (2014) Phytochemical Profiling of Leaf, Stem, and Tuber Parts of Solena amplexicaulis (Lam.) Gandhi Using GC-MS. Int Sch Res Notices.

27. Padmalochana K, Dhana Rajan MS, Lalitha R, Sivasankari H (2013) Evaluation of the Antioxidant and Hepatoprotective Activity of Cryptolepis Buchanani. J App Pharm Sci 3: 99-104.

28. Al-Shammaria LA, Hassanab WHB, Al-Youssefa HM (2012) Chemical composition and antimicrobial activity of the essential oil and lipid content of Carduus pycnocephalus L. growing in Saudi Arabia. J Chem Pharm Res 4: 1281-1287.

29. Rajamurugan R, Selvaganabathy N, Kumaravel S, Ramamurthy CH, Sujatha V, et al. (2011) Identification, quantification of bioactive constituents, evaluation of antioxidant and *in vivo* acute toxicity property from the methanol extract of Vernonia cinerea leaf extract. Pharm Biol 49: 1311-1320.