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Effect of *Caulerpa scalpelliformis* Against Invasive Fungal Pathogens from Periyathalai Coastal Waters, Tuticorin District, Tamilnadu, India

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Abstract

Fungi cause diseases in a variety of marine animal hosts. The need for new antifungal agents arises from the rising prevalence of invasive fungal infections (IFI) and the ineffectiveness, severe side effects, and high mortality of the current antifungal agents. The present study aims to assess *Caulerpa scalpelliformis* seaweed extract's antifungal efficacy against human fungal infections. Using methanol solvents, the antifungal activity of marine macroalgae *C.scalpelliformis* was assessed against *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus,* and *Cryptococcus neoformans. C. scalpelliformis* showed strong fungal activity against *A. fumigatus*, *A. niger*, and *A. flavus* compared to *C. albicans* and *C. neoformans*, similar to the positive control drug Amphotericin B in the current study.This is the first report from Tuticorin's Periyathalai coast. These results point to the use of green seaweed, *C. scalpelliformis* methanol extract as a natural antibiotic in conjunction with synthetic antibiotics, which has paved the way for the development of new anti-aspergillus, anti-cryptococcal, and anti-candidal compounds.

Keywords: fungal infections, C.scalpelliformis, antifungal efficacy, drug, natural antibiotic

1. INTRODUCTION

The risk of fungal infections to human health is rising. These infections mostly arise in the developed world when patients are receiving increasingly potent immunosuppressive treatments. Invasive diseases caused by *Aspergillus* and *Candida* species still have a 30 to 50% overall death rate, even with the development of new diagnostic and treatment methods. Every year, there are one million cases of cryptococcal disease in developing nations, which leads to 6,75,000 deaths. As the physical environment is altered by human activity, the environment is capable of shifting to favor pathogens over the hosts (Saidani *et al.*, 2012).One of the most dangerous clinical invasive fungal infections is invasive aspergillosis (IA), which has a high case-fatality rate

in patients with compromised immune systems (Lin *et al*., 2001). The term "invasive fungal infections" (IFIs) refers to systemic infections brought on by the growth of molds or yeasts in deep-seated tissues. IFIs are deadly illnesses with high rates of morbidity and mortality, as opposed to superficial fungal infections. It has been found that *Candida*, *Aspergillus*, and *Cryptococcus* species are the most common causes of invasive infections (Pourakbar *et al.,* 2021). Although *Aspergillus* infections most often affect the lungs, they can also affect other organs and spread through the bloodstream. The central nervous system (CNS) is one of the most common sites of dissemination (Groll *et al*., 1996). Recognition of allergic fungal syndromes is growing. Sustained endeavors are necessary to enhance the frequently inadequate treatment results linked to fungi infections.

Antimicrobial compounds from marine macroalgae have the potential to be useful as starting points for biotechnology and pharmaceutical industries' research and development, as well as for the creation of new drugs and therapeutic agents (Perez *et al*., 2016). Compounds derived from macro algae have been reported to exhibit a wide range of biological activities in recent years, including antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumoric, cytotoxic, and antimitotic properties (Raj *et al*., 2015; Harada *et al*., 1997). New compounds found in *caulerpa* include bisindole alkaloids, caulerpicin, 1,4-diacetoxybutadiene moiety, and di-, sesqui-, and monoterpenes. Furthermore, the compound contains nitrogen. After thorough investigation, the most well-known secondary metabolite in the genus *Caulerpa* is bisindole alkaloids with the terminal 1,4-diacetoxybutadiene moiety and a group of peculiar di-, sesqui-, and monoterpenes (Mao *et al*., 2006).

Pharmaceutical companies can combat antibiotic resistance by employing antimicrobial compounds made from marine macroalgae as adjunctive or substitute therapies for various infections (Silva *et al*., 2021).Infectious diseases are a major cause of morbidity and mortality worldwide. Synthetic drugs are often expensive and prone to adulterations and unfavorable side effects. There is a growing market for prescription drugs derived from natural sources. An increasing number of marine organisms, especially algae, may contribute to the identification of new bioactive compounds.Drugs for the effective treatment of these emerging infections are in short supply, which contributes to a high mortality rate. Difficulties in diagnosing fungal infections and delays in starting treatments are significant factors. Medication options are basically restricted to the polyene Amphotericin-B, a natural product, and several more recent lipid formulations (Wingard *et al*., 1999).Finding cutting-edge methods for managing microbial infections becomes necessary as a result. The importance of compounds found in soil, plants, and other environments including marine life is being recognized by the pharmaceutical industry more and more.

Recently, there has been an increase in the selective use of marine algae to produce pharmaceutical agents. Bioactive substances present in seaweed prevent fungal pathogens. The development of new, powerful antifungal agents is thus greatly encouraged by research on marine macro algae.Studies focusing on the therapeutic qualities of seaweeds, particularly those with anti-fungal activity, have increased significantly in light of the need for treatments, specifically in immunocompromised people. The current study also marks the first experimental investigation of *C. scalpelliformis* algae with antifungal activity in the Periyathalai coastal waters of Tuticorin district in Tamilnadu. The present work is done through the *in vitro* testing of crude extracts of algae using methanol solvent on fungal strains, with the goal of expanding the options for less expensive and more efficient treatment of fungal infections.

2. MATERIALS AND METHODS

2.1. Collection of marine macroalgae

Caulerpa scalpelliformis were collected from Periyathalai coastal waters, Tuticorin District(Latitude-8°20'12.81"N, 77°58'20.77"E). The collected macroalgae were identified byDr. Ebenezer Immanuel, Assistant Professor, Department of Botany, The American College, Madurai – 625002.

Table 1: Scientific classification of *Caulerpa scalpelliformis*

Kingdom	Plantae
Phylum	Chlorophyta
Class	Ulvophyceae
Order	Bryopsidales
Family	Caulerpaceae
Genus	*Caulerpa* J.V.F.Lamouroux, 1809
Species	*scalpelliformis*

Figure 1: Fresh and Grinded form of *C.scalpelliformis*

2.2. Cleaning and drying process of the macroalgae

The samples of the fresh marine macroalgae were rinsed with running tap water to remove unwanted epiphytes, demise portion and the salt from the seawater. The cleansed samples were kept for air dried for 4 days

2.3. Extraction process of the macroalgae

The dried raw algae were grinded to the powdered forms (Figure 1). 50 grams of powered sample was taken and loaded the sample material containing the desired compound into the thimble. Thimble was placed into the main chamber of the Soxhlet extractor. Extraction process of the above algae was done with the 150 ml of Methanol solvent. Methanol solvent was added to a round bottom flask and placed onto a heating mantle. Soxhlet extractor was attached above the round bottom flask. Reflux condenser was attached above the extractor, with cold water entering at the bottom and exiting above. Now the apparatus was set up, heated the solvent to reflux and leaved the extract for the required amount of time.

2.4. *In vitro* Anti-fungal actvitiy

Principle

The anti-fungal agent present in the given sample was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

Potato dextrose agar medium, Amphotericin B, antimycotic solution, test samples, test tubes, beakers conical flask, spirit lamp, double distilled water and petri-plates.

1. AGAR WELLDIFFUSION METHOD

a. Potato Dextrose Agar Medium

The potato dextrose agar medium was prepared by dissolving 20 gm of potato influsion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

Procedure

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strains (*Candida albicans,Aspergillus fumigatus,Aspergillus niger, Aspergillus flavus,* and*Cryptococcus neoformans*) different concentration of sample SJ (*Caulerpa scalpelliformis*) (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

2.5. Statistical analysis

The mean ± standard deviation (SD) of three replicates is used to present the results. With Graph Pad Prism 6.0 software (USA), the statistical analyses were performed. A one-way analysis of variance (ANOVA) was used to statistically analyze the collected data and determine the degree of significance.P value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. Anti-fungal activity of *Caulerpa scalpelliformis* (SJ) against fungal strains

Table 2. SD± Means of the zone of inhibition obtained by sample SJ against *Candida albicans, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus* and *Cryptococcus neoformans*

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	*Candida albicans*	SJ	11.5±0.7	9.5±0.7	0	0	16.5±0.7
2.	*Aspergillus fumigatus*		12.3±0.42	6.2±0.28	0	0	15.5±0.707
3.	*Aspergillus niger*		13.4±0.56	0	0	0	15.35±0.49
4.	*Aspergillus flavus*		16.5±0.707	15.35±0.49	0	0	17.25±0.35
5.	*Cryptococcus neoformans*		14.35±0.49	11.25±0.35	0	0	19.45±0.63

SD – Standard Deviation, *Significance - p< 0.05

The agar well-diffusion method was used to evaluate the effectiveness of *C.scapelliformis'* methanol extraction against invasive fungal pathogens. As mentioned, the outcomes were also contrasted with the standard antifungal medication, amphotericin B, which inhibits the growth of invasive fungal infections. The means ± standard deviation was used to express the results of the triplicates.

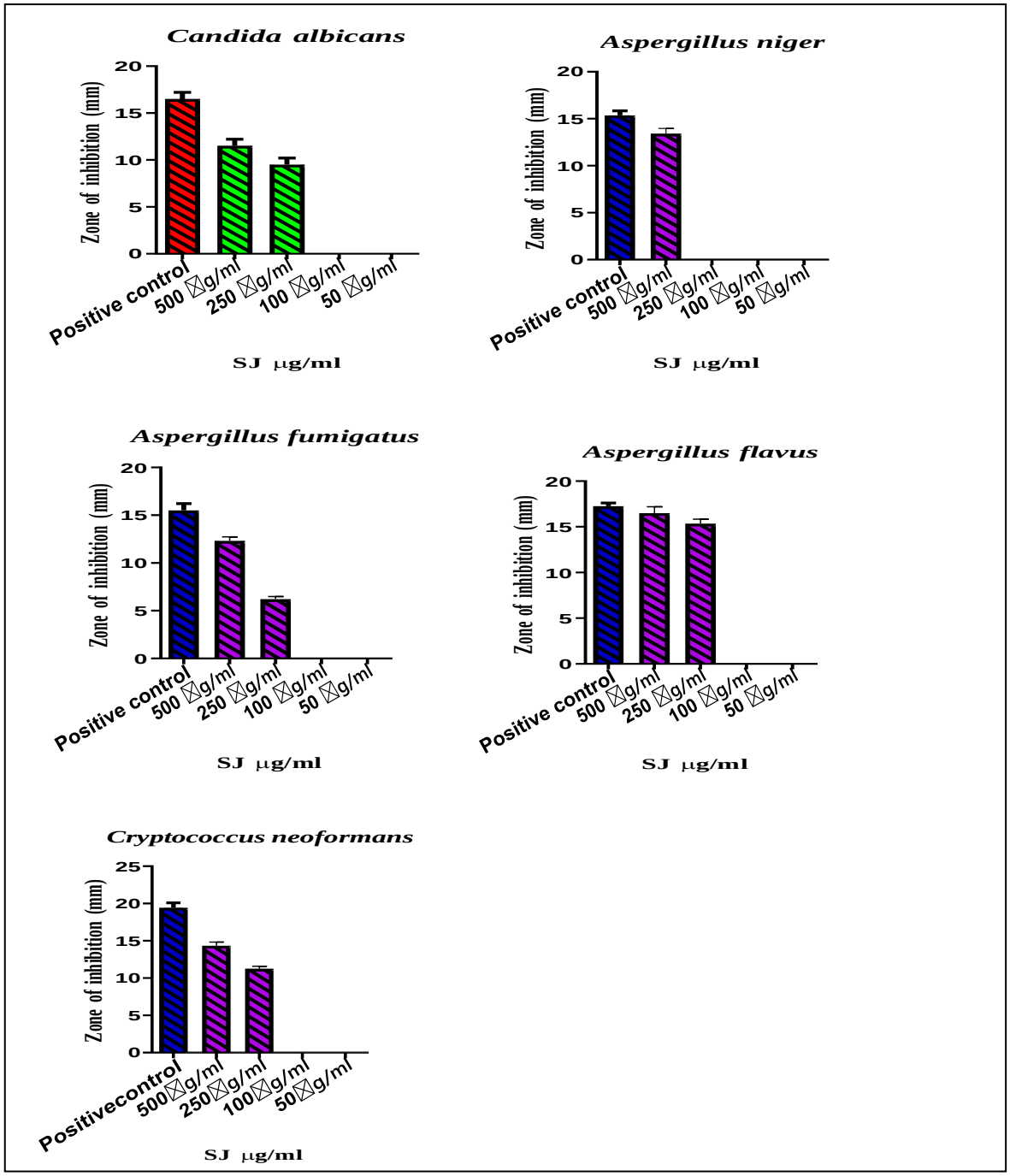

Figure 2: Graphical representation of *C.scalpelliformis* (SJ) against human fungal pathogens

C. albicans,*A. fumigates*,*A. niger*,*A. flavus* and *C. neoformans* were observed to be affected by *C. scalpelliformis*, as shown in Figure 2. The highest level of activity (11-16 mm) against all fungal pathogens was noted at 500µg/ml. *A. flavus* and *C. neoformans* measuring 11 and 15 mm were observed to be susceptible to 250µg/ml of *C. scalpelliformis*.

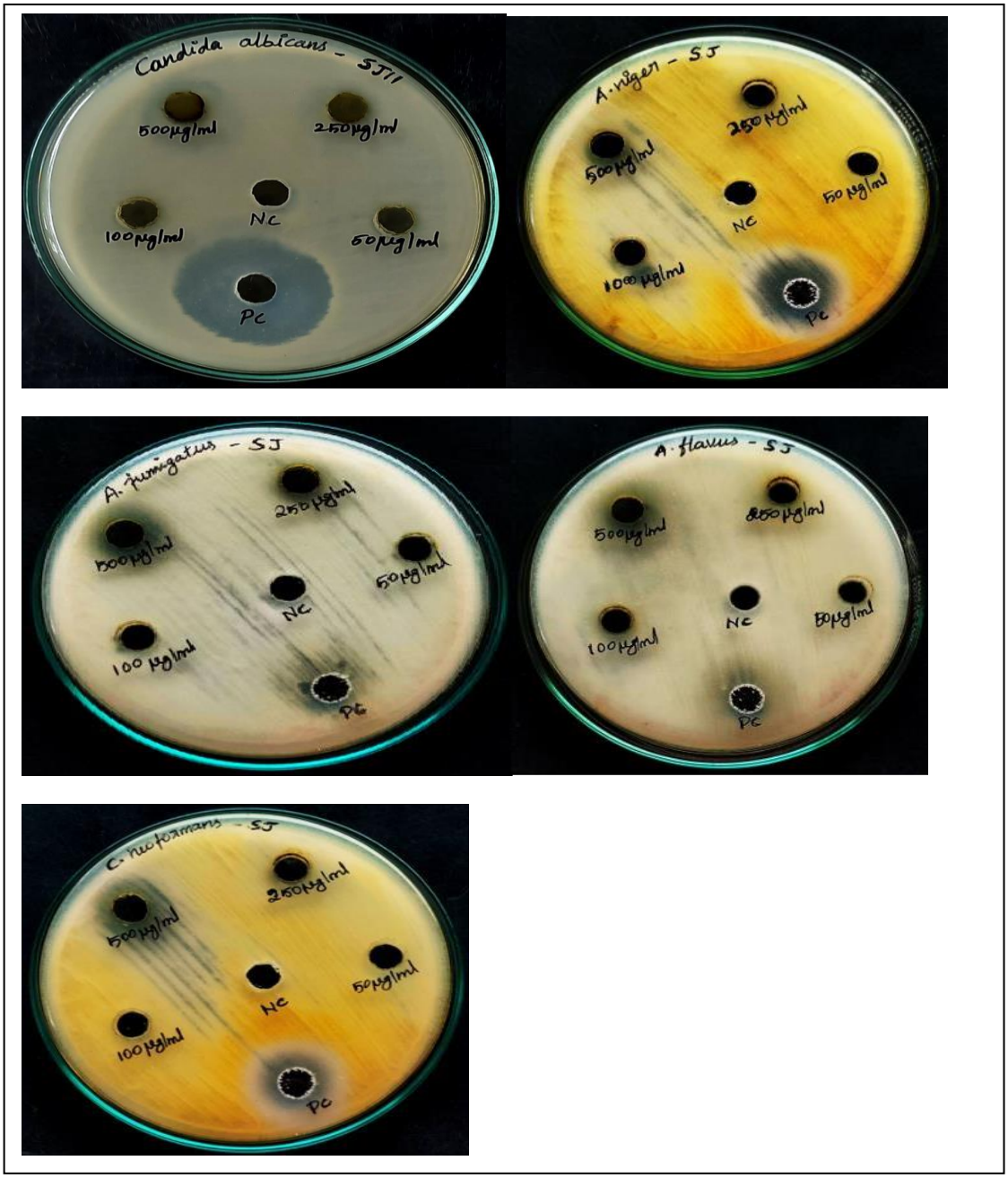

Figure 3: Agar well diffusion assay of *C.scalpelliformis* (SJ) against *C. albicans,A.fumigates,A. niger,A. flavus* and *C. neoformans*

Figure 3 illustrated the agar well diffusion method of anti-fungal activity of *C.scalpelliformis*. The zone (11.5 mm and 9.5 mm) against *C. albicans* was inhibited by the *C. scalpelliformis* methanol extract at 500µg/ml and 250µg/ml. 6.2 mm at 250 µg/ml and 12.3 mm at 500 µg/ml in *A. niger*. According to the findings, *A. fumigatus* measures 14.35 mm at 500µg/ml and 11.25mm at 250µg/ml, *A. flavus* measures 13.4 mm at 500µg/ml, and *C. neoformans* measures 16.5 mm at 500 µg/ml and 15.35 mm at 250µg/ml.

3.2. Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the means for the *in vitro* antifungal assessment. The obtained P-value ($p<0.05$) demonstrated statistical significance.

4. DISCUSSION

Caulerpa scalpelliformis's antifungal activity has been the subject of numerous studies. In South India, an investigation was conducted on the antimicrobial activity of *C.scalpelliformis* against a range of fungal pathogens. *A. fumigatus*, *C. neoformans* and *C. albicans* are among the fungi that can cause fatal systemic

infections. Nevertheless, no literature was reported at the current study location. *Candida* species actually rank fourth in the US in terms of common causes of systemic infections acquired in hospitals, with crude mortality rates reaching 50%. Humans can contract two primary forms of Gram-positive *Candida albicans* infections: superficial infections such as oral or vaginal candidiasis and potentially lethal systemic infections (Mayer *et al*., 2013). According to the current investigation, *C. albicans,A. fumigatus,A. niger, A. flavus* and *C. neoformans* were tested against the anti-fungal agent found in the *C.scalpelliformis* sample. Positive control employed in this study was amphotericin B. The methanol extract of *C. scalpelliformis* at 500µg/ml and 250µg/ml inhibited the zone (11.5mm and 9.5mm) against *C. albicans* in this current investigation. In *A.niger,* 6.2 mm at 250 µg/ml and 12.3 mm at 500 µg/ml. The results show that *A. fumigatus* has 14.35mm at 500µg/ml and 11.25µg/ml at 250µg/ml, *A. flavus* has 13.4mm at 500µg/ml, and *C. neoformans* has 16.5mm at 500µg/ml and 15.35mm at 250µg/ml (Table 2). According to Raj *et al*., 2017, *C. racemosa* ethylacetate extract demonstrated encouraging activity against *C. parapsilosis* (15.1 mm), *C. albicans* (14.3 mm), and *T. rubrum* (14.1 mm). The extracts prepared with chloroform demonstrated efficacy against *C. parapsilosis* (13.1mm). Subsequently, *T. rubrum* (13.3 mm), *C. albicans* (12.6 mm), and *T. rubrum* (12.3 mm) are displayed.The zones of inhibition produced by the Amphotericin-B (100 units/disc) anti-candidal positive control ranged from 9.0 to 14.5 mm. *Zonaria tournefortii's* aqueous extracts and fractions have antifungal properties against *C. albicans* and *C. neoformans*, according to research by Ismail *et al*., 2014. Taskin *et al*., 2010 reported that a methanolic extract from *Cystoseira mediterranea* inhibited the growth of *C. albicans*.The crude methanolic extracts of marine organisms were tested by Manilal *et al*.,2010 against human pathogens that are multiresistant. They discovered that *Asparagopsistaxiformis's* was extremely active and that oleic acid and n-hexadecanoic acid were the most prevalent metabolites.Oleic acid found in *C. scalpelliformis* may be the cause of the inhibitory activity observed in this study against fungal strains. The antifungal potency of the *Sphaerococcus coronopifolius* were found to possess the strongest antimicrobial properties (Pinteus *et al*., 2015). The antifungal activity of *P. gymnospora* extract in dichloromethane and ethanolic extract was effectiveagainst *C. albicans*, with the largest inhibition zone measuring 15.00 mm (Guedes *et al*., 2012).These outcomes are consistent with the findings from the agar well diffusion tests in the current investigation. *A. fumigatus, A. flavus, A.terreus,* and *A.niger* are the most common species (Sugui *et al*., 2014).*C. Scalpeliformis* did not show antifungal activity against *C. albicans, C.neoformans* and *A.niger* according to Sasikala and Geetha Ramani,2017. In contrast to *C. albicans, C. crassa* and *C. serrulata* both displayed 8 mm. In the present study, crude methanol extract of *C.scalpelliformis* inhibited 11.5 to 9.5mm against *C.albicans*. A wide range of clinical diseases, from minor and superficial infections to serious and invasive illnesses with a death rate of over 80%, can be caused by *Aspergillus* species. The most common invasive aspergillosis manifestation is thought to be pulmonary aspergillosis.The antimicrobial properties of *C.scalpelliformis* against *C. albicans, A. flavus, A. terreus,* and *A.niger* were determined by Karthick *et al*.,2014 using chloroform, benzene, acetone, diethyl ether, and methanol extracts. Regarding the components causing the antifungal activity, extracts from *C. crinita* and *C. sedoides* in both ethyl acetate and chloroform showed notable activity, strongly indicating that several compounds of different nature were actives as antifungal agents.*C. scalpelliformis* demonstrated strong antifungal activity against *A. flavus* in the current investigation, with 16.5 and 15.35 mm zone of inhibition. In comparison to other fungal strains, it exhibited extremely strong activity against *A. flavus*. *Caulerpa* sp. seaweed extract demonstrated a very strong activity in inhibiting the fungus *A. flavus*, according to research by Julyasih and Purnawati, 2019.According to Lavanya. and Veerappan, (2012), *Caulerpa scalpelliformis's* acetone extract showed the lowest (2 mm) activities against *C. krusei*, while methanol extract showed the lowest (2 mm) activities against *R. solani* and *C. albicans* and diethyl ether extract against *A. flavus*. Wefky and Ghobrial, (2008)used methanol, ethanol, and acetone solvents to report the antifungal activity of five seaweeds against strains of *A. flavus* and *A. niger* fungal. Acetonic *L. pinnatifida* and *S. hystrix* extracts had the strongest antifungal effect, with Zone of inhibition 16 and 26 mm, respectively, according to the results of the earlier study. On the other hand, *Eisenia bicyclis* edible brown seaweed extract was shown to have antifungal activity against eight strains of *Candida* by Kim *et al*., 2014. According to the earlier study, Zone of inhibition varied between 20 and 24 mm when using methanol extracts.The extracts of *S. vulgare* ethanol and cyclohexane were found to have a 7 and 12 mm zone of inhibition against *A. niger*. In contrast, no action was seen when the same strain was treated with extracts of acetone, chloroform, and ethyl acetate (Khallil *et al*., 2015). In the present study, *C.scalpelliformis* exhibited high anti-fungal activity against *A.niger* with the zone of inhibition 13.4mm.The following components of crude seaweed extracts are believed to have antifungal properties: alkaloids, flavonoids, steroids/triterpenoids, and tannins (Singh and Ali, 2011).According to Manivannan *et al*., 2011, *Padina gymnospora's* methanol extract showed the highest activity (20.00mm) against *C. neoformans* and the lowest activity (12.00mm and 13.66mm) against *A.niger* and*C. albicans*. Maximum activity (15.00mm) against *C. albicans* and minimum activity (5.66mm) against *A.niger*

were demonstrated by the methanol extract of *Sargassum tenerrimum*. In terms of *A. flavus*, the chloroform extract was most active (14.66mm). According to Manigandan and Kolanjinathan, (2014), the acetone extract of green seaweed *Ulva lactuca* had the highest activity against *A. niger* (12.0 mm zone of inhibition), followed by *A. flavus* (11.0 mm), and *C. albicans* (10.0 mm) had the lowest zone of inhibition. In the present study, *C.scalpelliformis* methanol extract showed highest anti-fungal activity against *A.niger* (13.4mm) and *A.flavus* (16.5mm) than *C.albicans* (11.5mm) (Table 2). Amphotericin B was the positive control medication used in this investigation. Similar to the positive control drug Amphotericin B in the current study, *C. scalpelliformis* demonstrated strong fungal activity against *A. fumigatus*, *A. niger*, and *A. flavus* compared to *C.albicans* and *C.neoformans*. Common clinical situations are the primary uses for Amphotericin B (Amp B). Moreover, Amp B is regarded as the preferred medication for the treatment of fungus infection.Patients requiring an increased dosage of Amp B may experience severe side effects, including renal damage, as a result of the drug's poor permeability across the membrane. Reducing the dose of medications used to treat mycoses may result in fewer side effects when using different types of latex in conjunction with antifungal medications like amphotericin-B (Hartsel and Weiland, 2003). Since natural products have been demonstrated to be a fantastic source of novel chemical entities, the *C. scalpelliformis* methanol extract screening in this study has opened the way to the discovery of novelanti-candidal, anti-aspergillus and anti-cryptococcal compounds.

5. CONCLUSION

Anti-fungal medications are desperately needed since invasive fungal infections (IFI) pose a major threat to human health. The chosen marine macro algae have exceptional fungicidal activity against human fungal pathogens, and this is the first report from this study site, According to the current study the seaweed (*C.scalpelliformis*) found along the Periyathalai coast is rich in compounds that are biologically active against fungi that cause infections.It can be concluded from the results of the present study that *C.scalpelliformis* has a high potential to be used in the pharmaceutical industry to improve health owing to its different compounds with antifungal activity.

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