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Evaluation of Antimycotic Activity of Extracts of Marine Algae Collected from Red Sea Coast, Jeddah, Saudi Arabia

Huda Sheikh[1], Amal El-Naggar[1,2*], Danyah Al-Sobahi[3]

[1]Department of Biological Science, Science Faculty for Girls, King Abdulaziz University, Jeddah, KSA
[2]Botany Department, Faculty of Science, Tanta University, Tanta, Egypt
[3]Makkah, KSA
Email: *amalelnagar5@yahoo.com

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Abstract

In the present study, fifteen species of the dominant marine algae were collected during summer 2013 from four selected sites on Red sea coast, Jeddah, Saudi Arabia. The collected species belonged to Chlorophyta, Phaeophyta and Rhodophyta. Crude algal extracts were prepared by successive extractions using different solvents (acetone, ethanol, diethyl ether, ethyl acetate, methanol and petroleum ether). The crude algal extracts were examined for their antifungal efficacy against *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Candida tropicalis* using agar well diffusion method. The algal extracts evoked different patterns of antifungal activities. Results reveal that acetone was the best solvent suited for extraction of bioactive compounds from tested seaweeds with inhibition activity (19.3%) followed by ethyl acetate (17.1%), ethanol (16.4%), petroleum ether (15.9%), diethyl ether (15.85%), and finally methanol (15.4%). Chlorophyta exhibited the highest antimycotic effect followed by Rhodophyta and Phaeophyta. In Chlorophyta, the extracts of *Ulva intestinalis* were the most potent followed by *U. lactuca*, *C. racemosa*, *U. linza* and *U. reticulate*. *Acanthophora spicifera* showed the highest activity in Rhodophyta, followed by *J. rubens*, *D. simplex*, *L. obtusa*, *G. gracilis*, *G. vermicuphylla* and *G. multipartita*. Whereas, *T. triquetra* was the most effective species in Phaeophyta followed by *P. pavonica* and *D. dichotoma*. The minimal inhibitory concentrations (MICs) of the most potent algal extracts were in the range of 0.5 to 4 mg/ml. The results confirmed the antimycotic potentiality of seaweed extracts.

Keywords

Marine Algae, Seaweeds, Antifungal Activity, Solvent Extracts, Minimal Inhibitory Concentrations (MICs)

1. Introduction

The requirements for expansion of alternative antimicrobial agents were explored since the appearance of antibiotic resistant microbes [1]. The screening of extracts or isolated compounds from different natural sources is a common way to discover the bioactive metabolites [2]. Marine environment is a pool of bioactive natural compounds that are not found in terrestrial natural products [3]. The production of secondary metabolites of potential interest from marine algae has been extensively documented [4] [5]. The active metabolites attained from the wide diversity of marine organisms have proved to be the best substitute for conventional pharmaceutical chemicals [1]. Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites with broad spectrum of biological activities. These substances show an appreciable number of distinct biological activities such as antitumoral, antiviral, antifungal, antibacterial, cytotoxic, antidermatophytal, phytotoxic and antiproliferative action [6] [7] [8].

For its importance in human and animal health and the production of agricultural products, the antimycotic activity, has been the subjects of several studies [9]. Green, brown and red algae, along with other biological actions, show antimycotic activity [10], which could be a valuable tool in agricultural applications. *Ulva fasciata* extract is able to effectively reduce the number of colonies in powdery mildew of bean fungi [11]. Significant antifungal effects were detected in brown seaweeds (*Turbinaria conoides*, *Padina gymnospora* and *Sargassum tenerrimum*) [12] and green seaweeds (*Ulva reticulata* and *Ulva lactuca*) [13].

Heptadecane and tetradecane are common major volatile components in several algal species with antifungal activity against *Candida albicans* [14]. *C. albicans* was found to be more susceptible to the methanol extract of *Padina gymnospora* than *Aspergillus niger* [15]. The extracts of *Cystoseira crinita* and *Ulva intestinalis* had antifungal efficacy against *Candida krusei* [16].

The bioactive compounds such as proteins, carbohydrates, fatty acids, steroids, glycosides can be extracted using polar solvents such like methanol, ethyl acetate and chloroform during phytochemical process. Different solvents extracts show different antimicrobial activity depending on their solubility and polarity. Therefore, chemical compounds should be extracted from wide diversity of marine algae in order to optimize their antimicrobial activity by selecting the best solvent system [17].

Candida species are opportunistic pathogens and accounted for a substantial morbidity rate and can result in hospitalization and expensive therapies [18] (Gholampour-Azizi *et al*., 2015). For immune-compromised patients, *Candida albicans* is an important opportunistic fungal pathogen and the major cause of oropharyngeal candidiasis [19] (Steenkamp *et al*., 2007). *Aspergillus* species cause several human diseases, including allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, aspergilloma, and invasive aspergillosis [20] (Henwick and Hetherington, 1992).

Nowadays microbes are increasingly developing resistance against antibiotics and fungicidals in use. Therefore, a large library of novel compounds is required to combat against these drug resistant microbes. Since natural products from seaweeds offer rich source of bioactive molecules, the present work was intended to the evaluate the antifungal efficiency of organic solvents extracts of the most dominant marine algal species from Red Sea coast, Jeddah, Saudi Arabia against some pathogenic fungi. Detection of the best solvents for extraction of antifungal substances and determination of MICs of the most effective extracts were performed.

2. Materials and Methods

2.1. The Study Area

The area of study was Jeddah Corniche which extended for 30 km at the Red Sea Coast. This area is located between Latitude 21˚38'55.71"N, Longitude 39˚6'2.72"E and Latitude 21˚30'27.13"N, Longitude 39˚9'44.39"E, and it is characterized by a tropical to subtropical climate. Four sites were selected; the first site was dominant with marine seaweeds belonging to Chlorophyta. The second, third and fourth sites were 1.7, 5.8 and 17.3 km away from the first site, respectively and were dominant with Chlorophyta, Rhodophyta and Phaeophyta.

2.2. Collection of Marine Algae

In this study, 15 species of dominant seaweeds were collected during summer (2013). Seaweeds were collected by hand from different depths up to one meter. The algae were cleaned from epiphytes, sand and rock debris by seawater. All samples were washed several times with fresh water and finally with distilled water. The cleaned seaweeds were spread on plates, air dried in the shade at room temperature and grounded. The seaweeds were identified according to [21] [22] [23] [24].

2.3. Preparation of Algal Extracts

The dried powdered biomass was successively extracted with different solvents (acetone, ethanol, diethyl ether, ethyl acetate, methanol and petroleum ether) by soaking the material in respective solvents (10 gm:150 ml) and kept on a rotary shaker at 150 rpm at room temperature (30˚C) for 72 hrs. The extracts were filtered using Whatman No. 1 filter paper. The obtained filtrates were taken to dryness by evaporation under reduced pressure in a rotary evaporator. The obtained thick residues (crude extracts) were dissolved in dimethylsulfoxide (DMSO) to final concentration of 100 mg/ml as stalk one and stored at −20˚C.

2.4. Tested Fungi

The tested fungal species used in this study (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and the yeast *Candida albicans*, *Candida tropicalis*) were obtained from King Fahed Hospital in Jeddah. The tested fungal species

were grown in Sabouraud Dextrose Agar medium (SDA) [25]. Antifungal activity of algal extracts was evaluated against the five pathogens. To prepare the inoculums, a portion of each fungus to be tested was inoculated into 10 ml sterile water (saline solution). 1 ml of the suspension was transferred to a flask containing 50 ml warm sterilized medium (45˚C) giving 1×10^6 cell/ml. The flask was shaken well and poured into Petri dishes for solidification.

2.5. Antifungal Assay

2.5.1. The Well-Cut Diffusion Method

Antifungal activity was evaluated using well-cut diffusion technique [26]. Wells were cut from the plate using a sterile 0.5 cm cork borer. Algal extract (50 µl) was introduced into each well. All plates were incubated at 4˚C for 2 hours to slow fungal growth and gives suitable time for the antimicrobial agent to diffuse. The plates were later incubated at 30˚C ± 2˚C for 2 days [27]. After incubation, the diameter of the growth inhibition zone was measured in mm [28]. Each assay was prepared in triplicate, and the mean values were calculated.

2.5.2. Minimal Inhibitory Concentrations (MIC) of Algal Extracts

Minimal inhibitory concentrations were determined according to [29]. Serial dilutions of the most potent algal extracts (mg/ml) were added to sterilized plates containing freshly prepared media with standard number of cells for fungal isolates. The lowest concentration which did not show any visible growth of microorganisms was recorded as minimum inhibitory concentration.

2.5.3. Statistical Analysis

Data are presented as the mean of three replicates ± standard deviation (SD). SAS program (version 6.12) was used for statistical analyses. The obtained results were analyzed statistically to determine the degree of significance using one-way, two-way and/or three-way analysis of variance (ANOVA) at probability level $p \leq 0.05$ levels of significance. Comparison of treatment means was obtained by Tukey's procedure at $p \leq 0.05$.

3. Results and Discussion

Antimicrobial activities of crude extracts of fifteen species of marine seaweeds represented by five Chlorophyta (*Coulerpa racemosa*, *Ulva intestinalis*, *U. lactuca*, *U. linza* and *U. reticulata*), three Phaeophyta (*Turbinaria triquetra*, *Padina pavonica* and *Dictyota dichotoma*) and seven Rhodophyta (*Acanthophora spicifera*, *Digenea simplex*, *Gracilaria gracilis*, *G. multipartita*, *G. vermicuphylla*, *Jania rubens* and *Laurencia obtusa*) were tested against five pathogenic fungi (*Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Candida tropicalis* and *C. albicans*) (**Figures 1-3**). The results concluded that acetone was the most active solvent showed the strongest inhibition against the tested fungi with inhibition activity (19.3%) followed by ethyl acetate (17.1%), ethanol (16.4%), petroleum ether (15.9%), diethyl ether (15.85%), and finally methanol (15.4%) (**Figures 1-3**). In

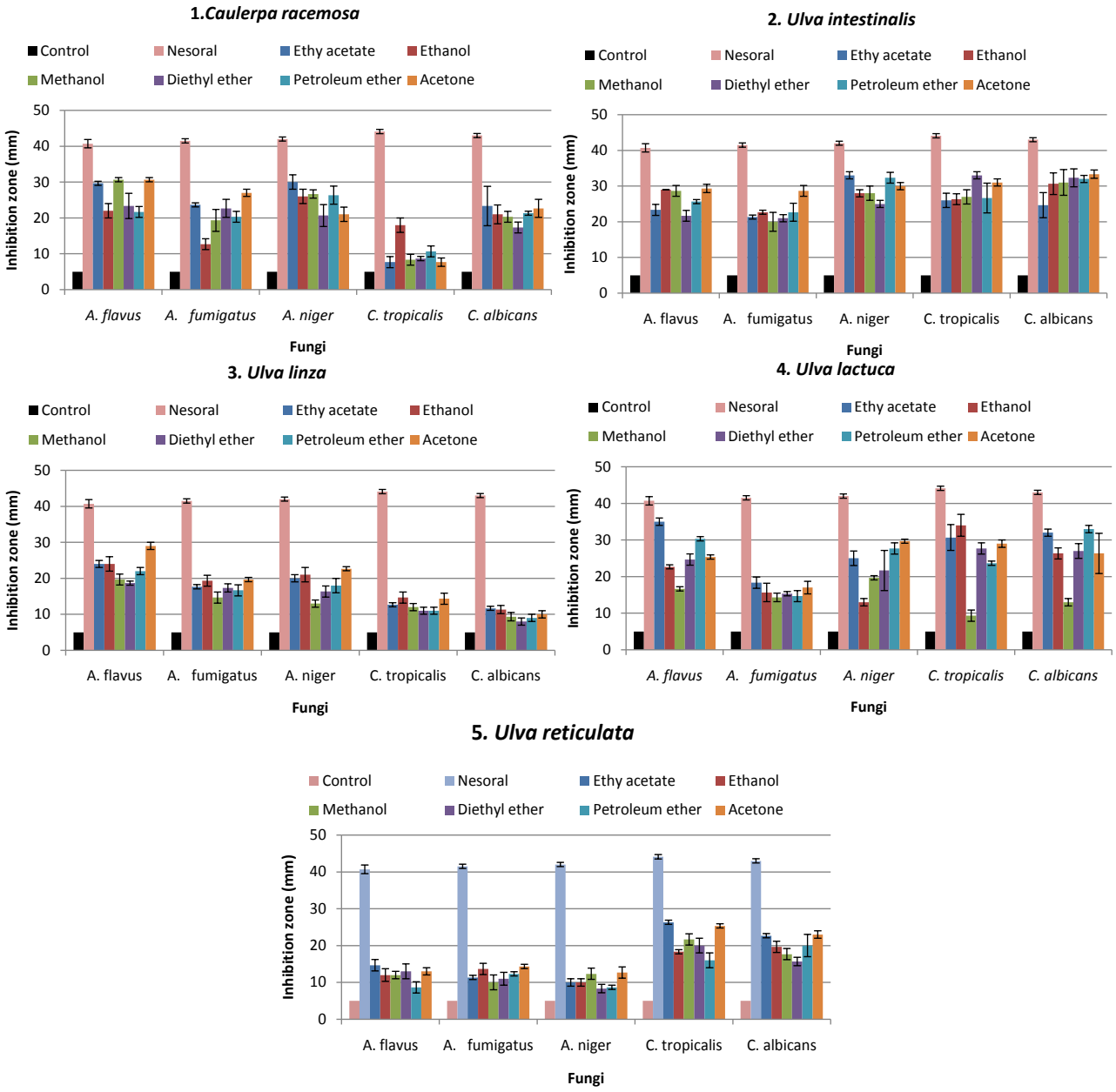

Figure 1. The antifungal activities of different seaweeds from Chlorophyta extracted with different solvents against the tested pathogenic fungi (1. *C. racemosa*, 2. *U. intestinalis*, 3. *U. linza*, 4. *U. lactuca*, 5. *U. reticulata*).

accordance with our results, acetone was the most efficient solvent to yield strong antifungal activity of 10 marine algae [2]. Acetone was the best solvent for extraction of bioactive compounds from nine marine macroalgae from Alexandria, Egypt and recorded the highest antimicrobial activity [30] [31]. This result agreed also with those of Wefky and Ghobrial [32] and Fareed and Khairy [33]. The antimicrobial activity of red and green seaweed extracts significantly increased with ethanol and acetone [34]. In contrast, diethyl ether yields higher antimicrobial activity than methanol, acetone and ethanol for extracting 11 seaweeds species from the coast of Urla [35]. For the preparation of algal

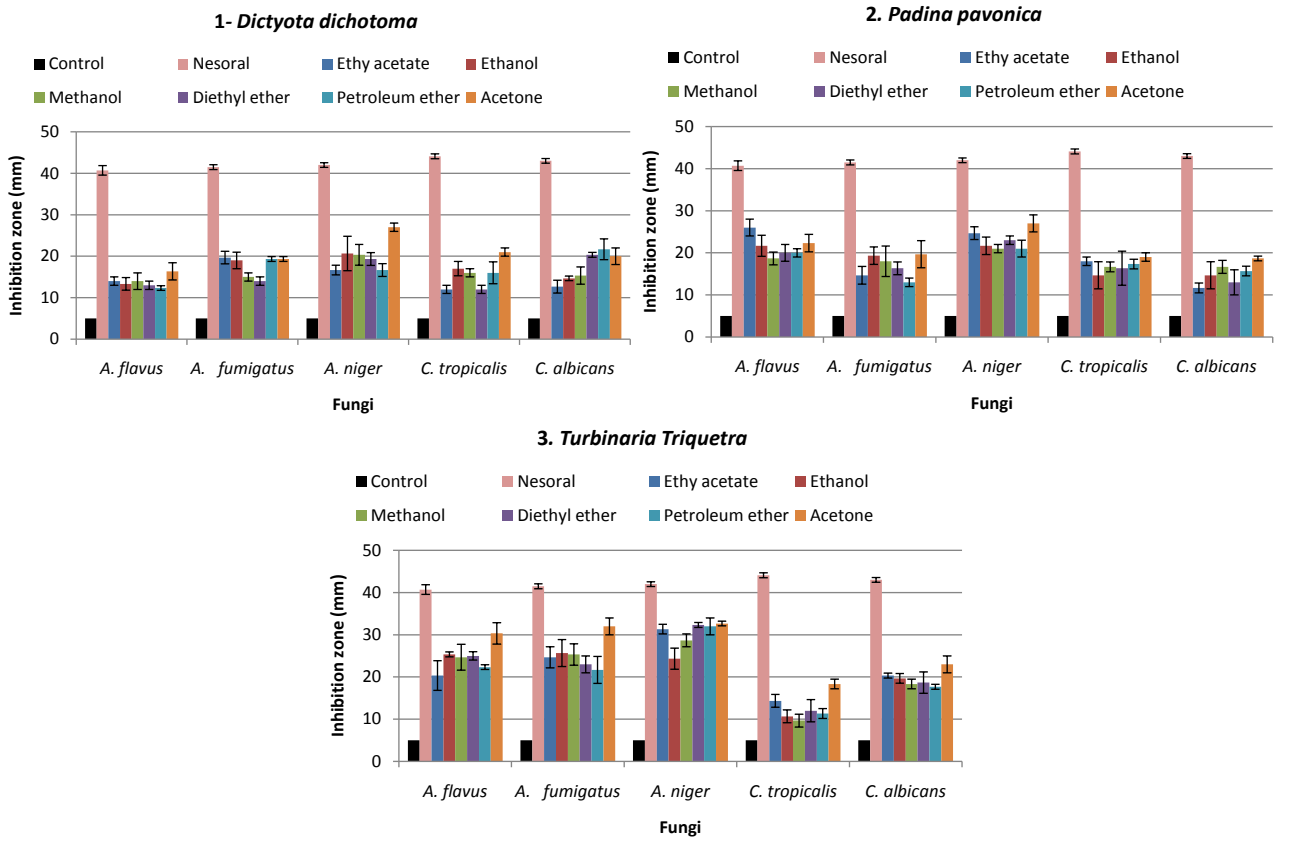

Figure 2. The antifungal activities of different seaweeds from Phaeophyta extracted with different solvents against the tested pathogenic fungi (1. *D. dichotoma*, 2. *P. pavonica*, 3. *Turbinaria triquetra*).

extract with significant antimicrobial effects, chloroform was the most effective followed by ethanol and petroleum ether [36]. These differences referred to the differences in the solubility of bioactive metabolites in the corresponding solvents [30].

Regarding taxonomic groups, the present data show that species of Chlorophyta showed the highest activity against the tested fungi followed by Rhodophyta and Phaeophyta. In agreement with our results, green algae were the most active species than others [31] [37]. *U. lactua* (Chlorophyceae) was more active compared with *J. rubens* (Rhodophyceae) [33]. However, the extracts of red alga *Gracilaria dendroides* were more efficient against the tested bacterial strains followed by green alga *Ulva reticulata*, and brown alga *Dictyota ciliolate* [36]. Moreover, the members of the red algae exhibited the highest antibacterial activity in the screened marine algal species [38] [39]. *Gracilaria fisheri* showed higher antimicrobial activity than *Ulva intestinalis* [40].

In Chlorophyta, the extracts of *Ulva intestinalis* were the most potent followed by *U. lactuca*, *C. racemosa*, *U. linza* and *U. reticulata* (**Figure 1**). Acetone proved to be the best solvent for extraction of antifungal compounds from *U. intestinalis*. *C. albicans* was the most sensitive to the extracts of *U. intestinalis* followed by *A. niger*, *C. tropicalis*, *A. flavus* then *A. fumigatus*. The highest activity (33.3 mm) was detected in acetone extract against *C. albicans*, while the lowest activity

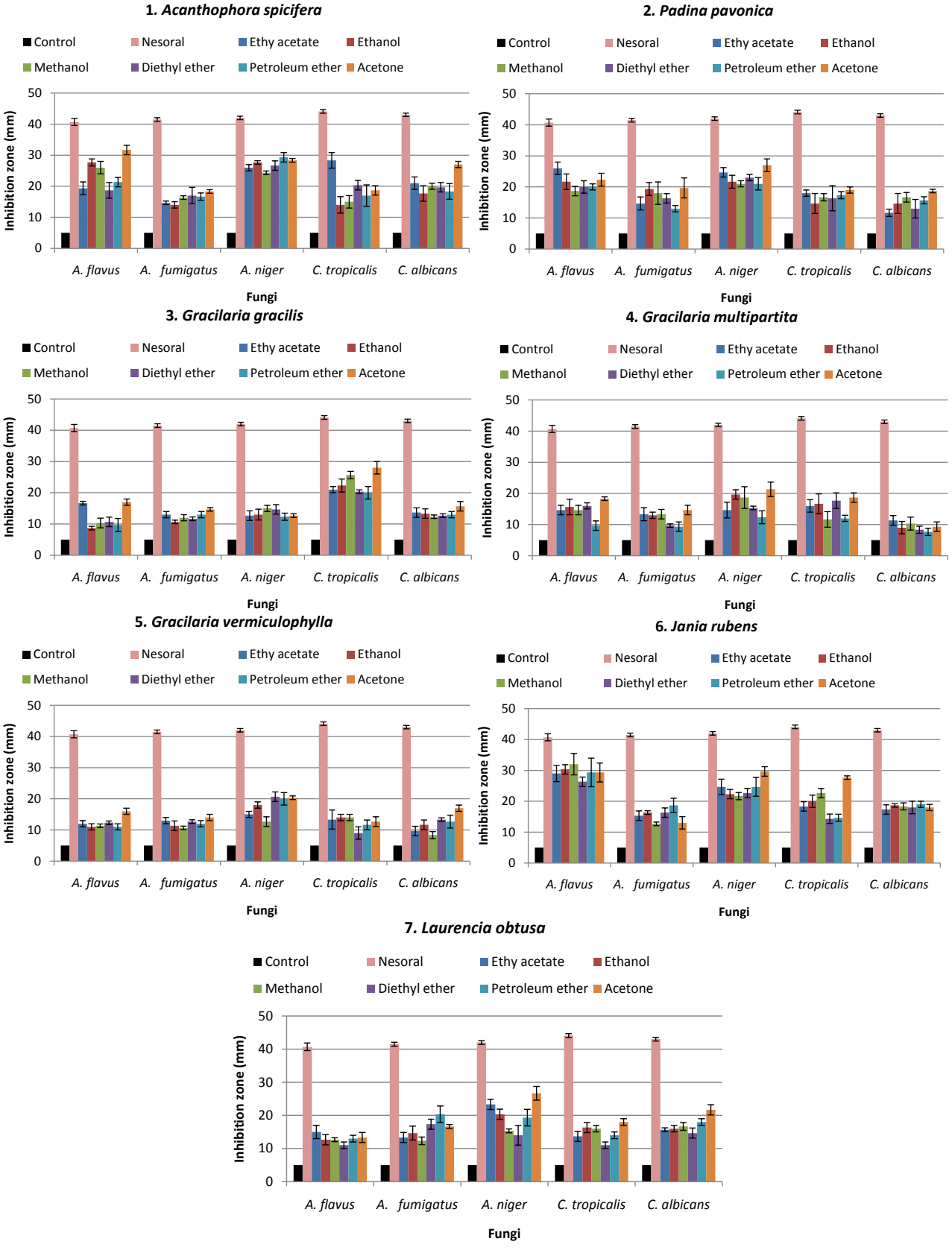

Figure 3. The antifungal activities of different seaweeds from Rhodophyta extracted with different solvents against the tested pathogenic fungi (1. *A. spicifera*, 2. *D. simplex*, 3. *G. gracilis*, 4. *G. multipartita*, 5. *G. vermiculophylla*, 6. *J. rubens*, 7. *L. obtusa*).

(20 mm) was observed in methanol extract against *A. fumigatus* (**Figure 1**). In conformity with our results, *U. fasciata* could be considered promising seaweed for the production of antimicrobial compounds [31]. *Candida albicans* was the most sensitive organism, which was strongly inhibited by acetone extract of *U. lactuca*, *C. racemosa* and *L. farinose* [2]. Ethanolic extract of *Ulva intestinalis* was reported to exhibit significant antibacterial activity and anti-hemolytic activity [41]. Extracts of *Cystoseira crinita* and *Ulva intestinalis* collected from the coastal region of Sinop had antifungal efficacy against *Candida krusei* [19]. However, ethanol extract of *Ulva intestinalis* strongly inhibited *Candida albicans* and *Aspergillus fumigatus* [42]. *U. fasciata* extracts showed antibacterial activity better than antifungal effect [43]. Hexane extracts of *U. intestinalis* showed higher antibacterial activity than methanol, ethanol and dichloromethane [40].

U. lactuca was highly effective (9.3 to 35 mm) against the tested fungi. Ethyl acetate was the potent solvent for extraction the antifungal compounds from *U. lactuca* followed by petroleum ether, acetone, of diethyl ether, ethanol and methanol. Although, ethyl acetate extract yielded the largest hole (35 mm) against *A. flavus*, *C. albicans* was the most sensitive (13 to 32 mm) and *A. fumigatus* was the most tolerant fungus (14.3 to 18.3 mm) to extracts of *U. lactuca* (**Figure 1**). In accordance with our results, *C. albicans* was the most susceptible organism which was strongly inhibited by extracts of *U. lactuca* and *C. racemosa* [2]. *Ulva lactuca* showed the highest mean zone of inhibition against *Candida albicans* and *Aspergillus fumigates* [42]. Whereas, diethyl ether extracts of *Enteromerpha*, *Ulva* and *Gracilaria* appeared to yield better results than those of methanol [35]. The presence of active substances in *U. lactuca* was previously recorded [2] [44]. The antimicrobial activity of *Ulva* organic extracts are related to their lipophilic and phenolic contents, in particular steroids fatty acids [45].

Ethyl acetate was efficient to yield higher inhibitory activities of *C. racemosa* followed by acetone, methanol, ethanol, petroleum ether and diethyl ether. *A. flavus* was the most sensitive (21.7 - 30.7 mm), whereas, *C. tropicalis* (7.7 - 18 mm) was the most resistant pathogen (**Figure 1**). In this connection, ethanolic and lipid soluble extracts of *Caulerpa ashmeadii* and *Caulerpa prolifera* had the broadest spectrum of antimicrobial activity [46]. *Caulepra prolifera* was reported to exhibit significant activity against marine bacterial strains [47]. *C. albicans* was more sensitive than *A. flavus* to acetone extract of *C. racemosa* [2], on the other hand, Chloroform extract of *C. occidentialis* yielded the highest antibacterial activity [36] [48].

Acetone extract of *U. linza* showed the largest halo (29 mm) against *A. flavus* and the lowest one (8 mm) with diethyl ether extract on *C. albicans* which was the most resistant fungus. Acetone was the effective solvent for *U. linza* followed by ethanol and ethyl acetate (**Figure 1**). Although *U. reticulata* showed the lowest hallows among the tested chlorophytes, it showed its highest potency with ethyl acetate and acetone (26.3 and 23 mm) against *C. tropicalis* and *C. albicans*, respectively. The lowest halo was detected in diethyl ether extract on *A. niger* (8.3 mm) which was the most tolerant fungus (**Figure 1**). Species of the genus

Ulva have been demonstrated to metabolize biomolecules with pharmacological potential particularly antifungal and antibacterial activity [49].

The results show that *A. flavus* showed high sensitivity to the extracts of all tested chlorophytes except, *U. reticulata*. On the other hand, it was reported that among the tested 30 marine algal extracts, only acetone extract of *U. lactuca* showed strong inhibitory activity against *A. flavus* which appeared more resistance for other algal extracts [2].

The presence of lipophilic and phenolic compounds, specially steroids fatty acids, in *Ulva* organic extract, is related to their antimicrobial activity [45]. Once phenolic compounds have crossed the cell membrane of microbe, they could de-nature the enzymes responsible for spore germination, interactions with membrane enzymes and proteins lead to an opposite flow of protons, affecting cellular activity and disturb genetic [50].

The antagonistic effects of the algal extracts on the investigated fungi could be attributed to a range of compounds which inhibited the growth of microorganisms and antagonize their infection mechanisms. These involved peptides, alkaloids and phenols [51] and sometimes mono and divalent cations [52]. The presence also of phytohormones, amino acids, total soluble nitrogen and total reducing sugars that might be implicated as allelochemical agents [53]. Algal metabolites may induce specific reactions or modify specific physiological activities either positively or negatively within the microbial pathogen. The presence of bioactive compounds including steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins and triterpenoids extracts in the extract of *U. fasciata* was reported [54].

In Phaeophyta, *T. triquetra* was the most potent species (inhibition holes: 9.7 to 32.7 mm) followed by *P. pavonica* and then *D. dichotoma* (**Figure 2**). Acetone extracts of *T. triquetra* yielded the highest antifungal activities (30.3, 32, 32.7 mm) against *A. flavus*, *A. fumigatus* and *A. niger*, respectively. Whereas, methanol extract introduce the lowest activity (9.7 mm) on *C. tropicalis* as the most resistant pathogen (**Figure 2**). In accordance with our results, it was reported that acetone extract of *T. triquetra* and *P. pavonica* inhibited *C. albicans* [2]. However, acetone extract of *T. conoids* collected from Vedalai coastal waters (Gulf of Mannar Coast) induced mild inhibition against *A. niger* and *A. flavus* and methanol extract had strong antifungal inhibition against *C. albicans* [12]. Methanolic extract of *T. ornata* showed the highest antimicrobial activity, total phenolics and exhibited the highest antioxidative activity than *Sargassum polycystum* [55].

A. niger was the most sensitive fungus to the acetone extract of *P. pavonica* (27 mm), followed by *A. flavus* in ethyl acetate (26 mm), whereas, the lowest hallow was detected in ethyl acetate extract against *C. albicans* (11.7 mm) (**Figure 2**). In agreement with our results, ethyl acetate extract of *P. gymnospora* strongly inhibited *A. niger* whereas, acetone extract was more inhibitory than ethyl acetate against *C. albicans* [12]. The antifungal efficacy of methanol extract

of *P. gymnospora* was confirmed against *A. niger* and *C. albicans* [15]. The antimicrobial activity of *Padina* extract was referred to the presence of polyunsaturated alcohol [56]. The antimicrobial activity depends on both algal species and the efficiency of the extraction method [35]. Based on such fact, it reported that acetone extract of *T. triquetra* and *P. pavonica* showed weak inhibition activity against *C. albicans* [2].

Dictyota dichotoma showed the highest antifungal effect in acetone against *A. niger* (27 mm) and *C. tropicalis* (21 mm), *A. fumigatus* in ethyl acetate (19.7 mm) and *C. albicans* in petroleum ether (21.7 mm) and the lowest effect in diethyl ether against *C. tropicalis* (12 mm) (**Figure 2**). Dictyterpenoids were reported as the biologically active compounds in species of the order dictyotales which control seaweeds herbivores [57]. The highest antifungal effect of *Dictyota sp.* was recorded against *C. albicans* and *C. tropicalis* at MIC 200 mg/ml [58]. It was reported that the strongest antifungal activity of the methanol extract of *Sargassum polycystum*, against *A. niger* and *R. stolonifera* and *R. solani*, whereas, chloroform extract showed the highest activity against *Mucor racemosus*, *Candida albicans* and *Saccharomyces cerevisiae* [17].

The active phytochemical compounds that include steroids, alkaloids, terpenoids, glycosides, phenols, flavonoids, amino acids and oils in brown algae are liable for the antimicrobial efficacy against the human pathogens [59] [60].

In Rhodophyta, *Acanthophora spicifera* showed the highest activity, followed by *J. rubens*, *D. simplex*, *L. obtusa*, *G. gracilis*, *G. vermicuphylla* and *G. multipartita* (**Figure 3**). The highest antifungal effects of *A. specifera* were detected in acetone against *A. flavus* (31.7 mm), in petroleum ether against *A. niger* (29.3 mm) and in ethyl acetate against *C. tropicalis* (28.3 mm). Acetone was the most potent solvent followed by ethanol, methanol, petroleum ether, ethyl acetate and diethyl ether (**Figure 3**). It was concluded that only acetone extract of *A. specifera* inhibited the growth of *C. albicans* [2].

Jania rubens highly affected *A. flavus* (26.3 - 30.3 mm) followed by *A. niger*, *C. tropicalis*, *C. albicans.* The lowest activity was detected in methanol against *A. fumigatus* (12.7 mm). Acetone was the best solvent (**Figure 3**). Acetone extract of *D. simplex* was highly effective against *A. flavus* (33 mm) followed by *C. tropicalis* (32.3 mm), *A. niger* (20.7 mm) and *C. albicans* (15.3 mm).Whereas, methanol and petroleum ether extracts showed the lowest activity against *A. fumigatus* (9 mm) (**Figure 3**). *L. obtusa* in acetone showed the highest inhibition to the growth of *A. niger* (26.7 mm) and *C. albicans* (21.7 mm) and *A. fumigatus* in petroleum ether (20.3 mm), while the lowest inhibition was observed in diethyl ether against *A. flavus* and *C. tropicalis* (11 mm) (**Figure 3**).

Different species of *Gracilaria* showed different patterns of antifungal activities. *G. gracilis* in acetone, methanol, ethanol, highly inhibited the growth of *C. tropicalis* as the most sensitive pathogen (28, 25.7 and 22.3 mm), respectively. The lowest effect was observed in diethyl ether against *A. flavus* (10.7 mm) (**Figure 3**). Except *A. niger* which showed the highest sensitivity (20.7 mm in di-

ethyl ether), all the other tested pathogens showed nearly similar responses to extracts of *G. vermicuphylla.* The lowest activity was observed in methanol (8.3 mm) against *C. albicans* (**Figure 3**). *G. multipartita* showed the highest antifungal effect in acetone (21.3, 18 and 18.3 mm) against *A. niger*, *C. tropicalis* and *A. flavus*, respectively, while the lowest activity was detected in petroleum ether extract (7.7 mm) against *C. albicans* (**Figure 3**). The antimicrobial activity of *Gracilaria* species was reported earlier [61]. The solvent extracts from *G. fisheri* were highly active against *Vibrio harveyi* and increased disease resistance in black tiger shrimp (*Penaeus monodon*) [62]. Dichloromethane and hexane extracts of *G. fisheri* showed the highest antimicrobial activity against *S. aureus* and *B. cereus* [40]. Rhodomelaceae, especially *Gracilaria* species, are known as a potential source of bioactive compounds such as bromophenols and fatty acids [63]. Palmitic acid has been reported to be responsible for the antimicrobial activity of *Gracilaria. G. corticata* exhibited broad spectrum of antimicrobial activity against gram-positive bacteria, gram-negative bacteria and yeast [40] [64] [65].

The statistical analysis using one way Anova confirmed that antimicrobial activities for most treatments were significant. However, two way Anova confirmed that the variation in the antimicrobial activity in relation to seaweeds, solvents and fungal pathogens were significant at $p \leq 0.0001$ (**Table 1**).

The production of antimicrobial substances by the same species varies remarkably; this may be referred to several factors such as the intraspecific variability in the production of secondary metabolites, occasionally related to seasonal variations [66] [67]. Furthermore, the differences in the ability of the extraction

Table 1. Two-way ANOVA to analyze the effect of different solvent extracts of different algal species and their interaction on *A. flavus*, *A. fumigatus*, *A. niger*, *C. tropicalis* and *C. albicans.*

Fungi	Source	df	Sum of squares	Mean squares	F value	p value	R^2
A. flavus	Species	14	10,328.65	737.76	267.74	0.0001	96.39%
	Solvent	5	841.85	168.37	61.10	0.0001	
	Species*Solvent	70	2056.59	29.37	10.66	0.0001	
A. fumigatus	Species	14	4320.91	308.63	119.22	0.0001	92.51%
	Solvent	5	379.67	75.93	29.33	0.0001	
	Species*Solvent	70	1054.37	15.06	5.82	0.0001	
A. niger	Species	14	8084.16	577.4	188.98	0.0001	95.08%
	Solvent	5	656.69	131.33	42.98	0.0001	
	Species*Solvent	70	1906.19	27.23	8.91	0.0001	
C. tropicalis	Species	14	7458.87	532.77	160.19	0.0001	94.91%
	Solvent	5	901.00	180.20	54.18	0.0001	
	Species*Solvent	70	2793.65	39.90	12.00	0.0001	
C. albicans	Species	14	8869.85	633.56	192.20	0.0001	94.83 %
	Solvent	5	464.34	92.86	28.17	0.0001	
	Species*Solvent	70	1553.88	22.19	6.73	0.0001	

ANOVA: analysis of variance; df: degree of freedom; $p \leq 0.05$ indicates significant effect.

protocols to recover the active metabolites and differences in the assay methods that would result in different susceptibilities of the target strains [68]. Finally the collection of the algae at different developmental stages of active growth or sexual maturity [69]. Solvents are always better for extraction of antimicrobial substances from seaweeds when compared with water [17].

MIC test is done to further confirm the antimicrobial activity of new antimicrobic compound, and as an alternative method to test for the susceptibility of organisms towards the extracts. MIC is vital in determining the extract dose needed to inhibit the growth of particular microorganisms. The MIC values of the most potent algal extracts for tested fungi were in the range of 0.5 to 4 mg/ml (**Table 2**). The crude extracts of *U. intestinalis* (0.5 - 1.5 mg/ml), *U. lactuca* (0.5 - 2.5 mg/ml) and *C. racemosa* and *A. spicifera* (1 - 3 mg/ml) recorded the lowest MIC values. Ethyl acetate, diethyl ether and acetone extracts of *U. intestinalis* recorded the lowest MIC value (0.5 mg/ml) against *A. niger*, *C. tropicalis* and *C. albicans*, respectively. Also, *A. flavus* and *C. tropicalis* and *C. albicans* inhibited by 0.5 mg/ml of ethyl acetate, ethanol and ethyl acetate extracts of *U. lactuca*, respectively. *A. flavus* inhibited by 1 mg/ml of acetone extracts of *C. racemosa*, *U. intestinalis* and *A. spicifera*, whereas, *A. niger* inhibited by 1 mg/ml of ethyl acetate extract of *C. racemosa*, acetone extract of *U. linza* and petroleum ether extract of *A. spicifera*. Acetone extracts of *T. triquetra* was more active on *A. flavus*, *A. fumigatus* and *A. niger* (1.5 mg/ml). Also, ethyl acetate extract of *P.*

Table 2. MIC (mg/ml) of the most potent marine algae extracts against the tested pathogenic fungi.

Algae	*A. flavus*		*A. fumigatus*		*A. niger*		*C. tropicalis*		*C. albicans*	
	Solvent	MIC (mg/ml)	Solvent	MIC (mg/ml)	Solvent	MIC (mg/ml)	Solvent	MIC (mg/ml)	Solvent	MIC (mg/ml)
Nizoral		0.5		0.5		0.25		0.25		0.25
C. racemosa	A	1	A	2	E.a	1	E	3	E.a	2.5
U. intestinalis	A	1	A	1.5	E.a	0.5	D.e	0.5	A	0.5
U. lactuca	E.a	0.5	E.a	2.5	A	1	E	0.5	E.a	0.5
Ulva linza	A	2	A	3	A	2.5	E	3	E.a	3.5
U. reticulata	E.a	3	A	3.5	A	4	E.a.	2	A	2.5
D. dichotoma	A	3	E.a	2.5	A	2	A	2.5	D.e	2.5
P. pavonica	E.a	1.5	A	2.5	A	2	A	2.5	A	3
T. triquetra	A	1.5	A	1.5	A	1.5	A	3	A	2.5
A. spicifera	A	1	A	3	P.e	1	E.a	1.5	A	1.5
D. simplex	A	1.5	E	4	A	2.5	A	1.5	A	4
G. gracilis	A	3	A	3	M	3	A	2	A	3
G. multipartita	A	3	A	3	A	2.5	A	3	E.a.	3.5
G. vermiculophylla	A	3	A	3.5	D.e.	2.5	E, M	3.5	A	3
J. rubens	E	1.5	P.e	3	A	1.5	A	2	P.e	3
L. obtusa	E.a.	3	P.e.	2.5	A	2	A	3	A	2.5

*A, Acetone; E, Ethanol; E.a, Ethyl acetate; D.e, Diethyl ether; M, Methanol; P.e, Petroleum ether.

pavonica, ethanol extract of *J. rubens*, acetone extract of *D. simplex* inhibited *A. flavus* by 1.5 mg/ml. *C. tropicalis* showed high sensitivity (1.5 mg/ml) to acetone extract of *D. simplex* and ethyl acetate extract of *A. spicifera* whereas, *C. albicans* was inhibited by the same concentration of acetone extract of *A. spicifera.*

In agreement with our results, it was reported that *U. lactuca* recorded lower MIC values (4 - 32 mg/ml) than *Ulva reticulata* (4 - 64 mg/ml) against some pathogenic fungi and the lowest MIC (4 mg) recorded against *C. albicans* and *C. glabrata* [13]. The MIC of *Laurencia bdendroidea* extracts were <31.25 μg/ml against *C. albicans* with a fungistatic effect [70]. A low MIC value suggests that the compound is a strong antimicrobial compound as it can inhibit the microbial growth at low concentration [71]. *Ulva intestinalis* recorded the lowest MIC (3.9 mg/ml) against *Candida albicans* followed *by Aspergillus fumigatus* (7.81 mg/ml) [42], while *Ulva lactuca* recorded the lowest MIC (0.98 mg/ml) against *Candida albicans* and (3.9 mg/ml) against *Aspergillus fumigatus*. The highest antifungal effect of *Dictyota sp.* was recorded against *C. albicans* and *C. tropicalis* at MIC 200 mg/ml [58].

4. Conclusion

The results proved the promising antimycotic potency of solvent extracts of marine algae from Red Sea coast, Saudi Arabia and suggest that the active antifungal compounds in seaweeds are found to be interesting. Thus exploration of such biological agents might be a probable resource of an array of biologically active compounds and the present results will ensure a starting point for exploiting natural bioactive substances presents in the extracts of marine algae. Such compounds may serve as leads in the development of new pharmaceuticals. Consequently, our future research direction is toward isolation, purification and identification of the bioactive ingredients to understand their bio prospects.

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