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Full Length Research Paper

Inhibition of the development of pathogenic fungi by extracts of some marine algae from the red sea of Jeddah, Saudi Arabia

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In this study, the predominant marine algae were collected from three different sites in the coastal area of Al-Kumrah at south of the Red sea of Jeddah, during summer and autumn 2009. The different marine algae belonged to Chlorophyta (*Enteromorpha prolifera* and *Ulva reticulata*), Phaeophyta (*Cystoseira myrica*, *Padina pavonica*, *Sargassum portieriatum* and *Turbinaria triquetra*) and Rhodophyta (*Gracilaria multipartita*). Algal extraction was achieved successively by using petroleum ether, diethyl ether, ethyl acetate and methanol. The algae extracts were tested *in vitro* for antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and the yeast *Candida albicans*, by using agar-well diffusion method. The crude extracts of the tested algae revealed differences in their bioactivities. The maximum growth inhibition for fungi was recorded in ethyl acetate extract of *T. triquetra* against *C. albicans* (30 mm), methanol extract of *E. prolifera* (29 mm) and ethyl acetate extract of *Padina pavonica* (28 mm) against *A. fumigatus.* The results clarified that Chlorophyta and Phaeophyta exhibited the highest biological activity against the tested fungi, whereas the lowest was achieved in Rhodophyta. The minimal inhibitory concentrations (MICs) of the crude extracts of the tested algae ranged from 0.5 to 3.0 μg/ml. The results confirmed the potential of seaweed extracts as a natural source of antimicrobial compounds. The antifungal activity of different extracts of marine algae which belongs to Chlorophyta, Phaeophyta and Rhodophyta were examined against *A. flavus, A. fumigatus, A. niger* and the yeast *C. albicans*. The algae belonging to Chlorophyta and Phaeophyta exhibited the highest inhibitory effect against the test pathogenic fungi. The different extracts showed different activities against fungi. The antimicrobial activity depended on both algal species and the efficiency of solvents in the extraction of bioactive substances.

Key words: Green algae, brown algae, red algae, solvent extracts, antifungal activity, minimal inhibitory concentrations (MICs).

INTRODUCTION

Marine macroalgae are potential renewable resources in the marine environment. Marine algae have been identified and grouped into different classes: green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae. More recent reports indicate that in many parts of the world, marine algae are still used in folk medicine for the treatment of a variety of diseases. The screening of algae extracts for biologically active compounds began in the 1950s, with simple antibiotic assays and soon expanded to include testing for products with antiviral (Kashiwagi et al., 1980), antifungal Gonzalez et al., 1982), anti-mitotic (Kosovel et al., 1988), and anti-tumorgenic (Glombitza and Koch, 1989) (activities. The discovery of metabolites with biological activities, from macroalgae, increased significantly in the past three

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decades (Smit, 2004; Bhaskar and Miyashita, 2005).

Marine algae represent a potential source of anti-microbial substances due to their diversity of secondary metabolites activities (Robles and Ballantine, 1999; Gonzalez et al., 2001; Newman et al., 2003; Prasanna and Hema, 2011). Secondary or primary metabolites from these organisms may be potential bioactive compounds of interest in the pharmacological industry (Reichelt and Borowitzka, 1984; Attaway and Zaborsky, 1993; Fitton, 2006). Marine algae are rich in vitamins, minerals, proteins, polysaccharides and fibers (Lahaye, 1991; Darcy-Vrillon, 1993). Many substances obtained from marine algae, such as alginate, carrageenan and agar, as phycocolloids, have been used for decades in medicine and pharmacy (Taskin et al., 2001).

Amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid are among the algal substances with this kind of activity (Mtolera and Semesi, 1996). Marked changes in the chemical constituents were found to occur with change of seasons, environmental conditions, as well as in the various phases of plants growth (Pillai, 1956, 1957a, b). The biological activities of marine algae are thought to be influenced by environmental factors such as water, temperature, nutritive salt, salinity, sea waves current and depth of immersion (Perez et al., 1990; Rioux et al., 2009). The need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms. In the present work, an attempt has been made to evaluate the antifungal characteristics of organic solvent extracts (petroleum ether, diethyl ether, ethyl acetate and methanol) of the dominant marine algae at Al-Kumrah area, Red sea of Jeddah Saudi Arabia. Marine algae were collected during summer and autumn 2009. The MICs of the most effective algal extract was estimated against the tested fungi.

MATERIALS AND METHODS

Study area

The area of study is called Al-Kumrah (Figure 1) and lies about 28 km south of the Red sea of Jeddah. This area is located between Latitude 21° 20' 01.59'' N, Longitude 39° 07' 15.66'' E and Latitude 21° 12' 24.13'' N, Longitude 39° 09' 59.90'' E, and it is characterized by a tropical to subtropical climate. The dominant marine algae were collected from three different sites characterized by different varieties of algae during summer and autumn 2009. The first site of study was dominant with marine algae belonging to Chlorophyta. The second and third sites were one and 8 km far away from the first site, respectively. The second site was dominant with algae of Chlorophyta, whereas algae of Phaeophyta and Rhodophyta were collected from site 3.

Algae samples

Salt, sand and epiphytes were removed from the collected algae using sea water. Algae were transferred to the laboratory, washed several times with fresh water and finally with distilled water. The samples were then spread on string nets and allowed to dry in air. Air dried samples were ground in an electrical mill and stored in stoppard bottles at room temperature. The algae samples were identified according to Aleem (1978, 1981) and Abbott and Hollenberg (1976). The algae were identified as Chlorophyta (*Enteromorpha prolifera* and *Ulva reticulata*), Phaeophyta (*Cystoseira myrica*, *Padina pavonica*, *Sargassum portieriatum* and *Turbinaria triquetra*) and Rhodophyta (*Gracilaria multipartita*).

Preparation of marine algae extract

Successive extractions of the air-dried powdered biomass were prepared in different solvents (petroleum ether, diethyl ether, ethyl acetate and methanol) within a conical flask, plugged with cotton wool and then kept on a shaker at 120 rpm at room temperature (30°C) for 24 h. The extracts were filtered (0.2 μm, Millipore) and concentrated under vacuum on a rotary evaporator at low temperature to get a crude extract of the different solvents. These extracts were stored at -20°C until use.

Microorganisms

The test organisms used in this study (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and the yeast *Candida albicans*) were obtained from King Fahed Hospital in Jeddah. Petri plates were prepared with sterile Sabouraud dextrose agar (SDA) for fungi cultivation. 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 $\times 10^6$ fungus per ml. The flask was shaken well and poured into Petri dishes for solidification.

Antifungal assay

The well-cut diffusion technique was applied according to El Masry et al. (2000). Wells were cut from the plate using a sterile 0.5 cm cork borer. Algal extract (50 μl) was introduced into each well, and the plates were maintained at 4°C for 2 h. The plates were later incubated for 48 days at 27°C. The diameter of the growth inhibition holes caused by the extract of algae was measured in millimeters. Each assay was prepared in triplicate, and the mean values were calculated.

Minimal inhibitory concentrations (MIC)

The minimal inhibitory concentrations were defined as the lowest concentrations of antimicrobials that inhibited the visible growth of microorganisms. This method was performed according to Ter-Laak et al. (1991). The most potent algal extracts were serially diluted and added to sterilize plate containing media that had freshly prepared standard number of cells (1.5×10^8 spores/ml for fungal isolates).

Statistical analysis

All assays were performed in triplicate. The data were expressed as mean values ± SD. The mean values were analyzed by three way analysis of variance (SAS, 1997).

Figure 1. Map of the study area showing the different sites of samples collection at Al-Kumrah, south of the Red sea of Jeddah.

RESULTS AND DISCUSSION

The growth inhibition for testing fungi by the crude extracts of green algae *E. prolifera* (12 to 29 mm) and *U. reticulata* (12 to 20 mm) is recorded in Figure 2. The crude extracts of *E. prolifera* were highly effective against *A. fumigatus* (23 to 29 mm), *A. niger* (16 to 20 mm) and *A. flavus* (13 to 20 mm), followed by that of *U. reticulata* against *C. albicans* (12 to 20 mm). The methanol extracts of *E. prolifera* recorded the largest halo against *A. fumigatus* (29 mm). In agreement to our results, Gonzalez et al. (2001) wrote that methanol extracts of *Enteromorpha* showed antifungal and antibacterial activities. In disagreement with our results, Tüney et al. (2007) reported that diethyl ether extracts of *Enteromorpha, Ulva* and *Gracilaria* appeared to yield better results than those of methanol. The presence of bioactive metabolites in marine algae, which can be soluble in solvent, could be related to the high and low effect of organic extracts against microorganisms (Kolanjinathan and Stella, 2009). The presence of lipophilic and phenolic contents, in particular steroids fatty acids, in *Ulva* organic extract, is related to their antimicrobial activity (Abd El-Baky et al., 2008). Prindle and Wright (1977) and Fung et al. (1997) reported that once phenolic compounds have crossed the cellular membrane of microbe, they could de-nature the enzymes responsible for spore germination in which, interactions with membrane enzymes and proteins would cause an opposite flow of protons, affecting cellular activity or

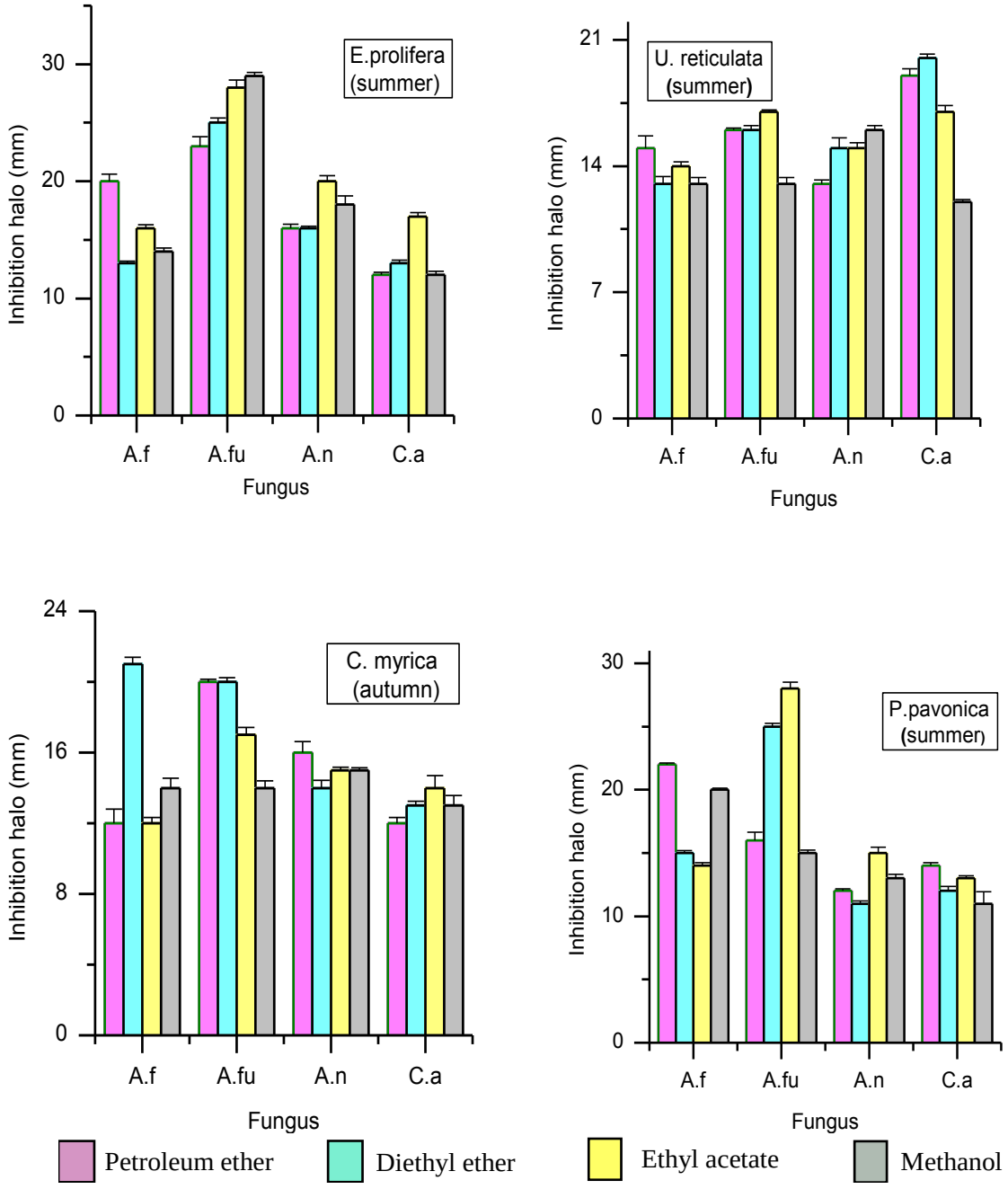

Figure 2. Antifungal activity of extracts of *E. prolifera, U. reticulate, C. myrica* and *P. pavonica* collected from the coastal area of Al-kumrah at south of the Red Sea of Jeddah during summer and autumn (2009). A.f, *Aspergillus flavus*; A.fu, *Aspergillus fumigates*; A.n, *Aspergillus niger*; C.a, *Candida albicans.*

disturb genetic. Figure 2 indicates that the growth of testing fungi was inhibited by different extracts of *C. myrica* (fluctuated between 11 and 21 mm). The highest activity of *C. myrica* extracts was observed in the diethyl ether fraction against *A. flavus* and *A. fumigatus* (21 and 20 mm, respectively). This finding is supported by Abourriche et al. (1999) who reported that the ethyl ether extract of *Cystoseira* showed interesting antimicrobic activities. They mentioned that the genus *Cystoseira* was known to contain diterpenoids, lipids, amino acids, sugars, sterols, enzyme inhibitors, cell division inhibitors, antimicrobic and antitumor constituents.

The results show that the highest antifungal activities in Phaeophyta were observed with extracts of *T. triquetra* (inhibition holes: 11 to 30 mm), *A. fumigatus (*19 to 27 mm) and *A. flavus* (18 to 27 mm) (Figure 3) followed by *P. pavonica* extracts against *A. fumigatus* (15 to 28 mm) and *A. flavus* (14 to 22 mm) (Figure 2). The broadest

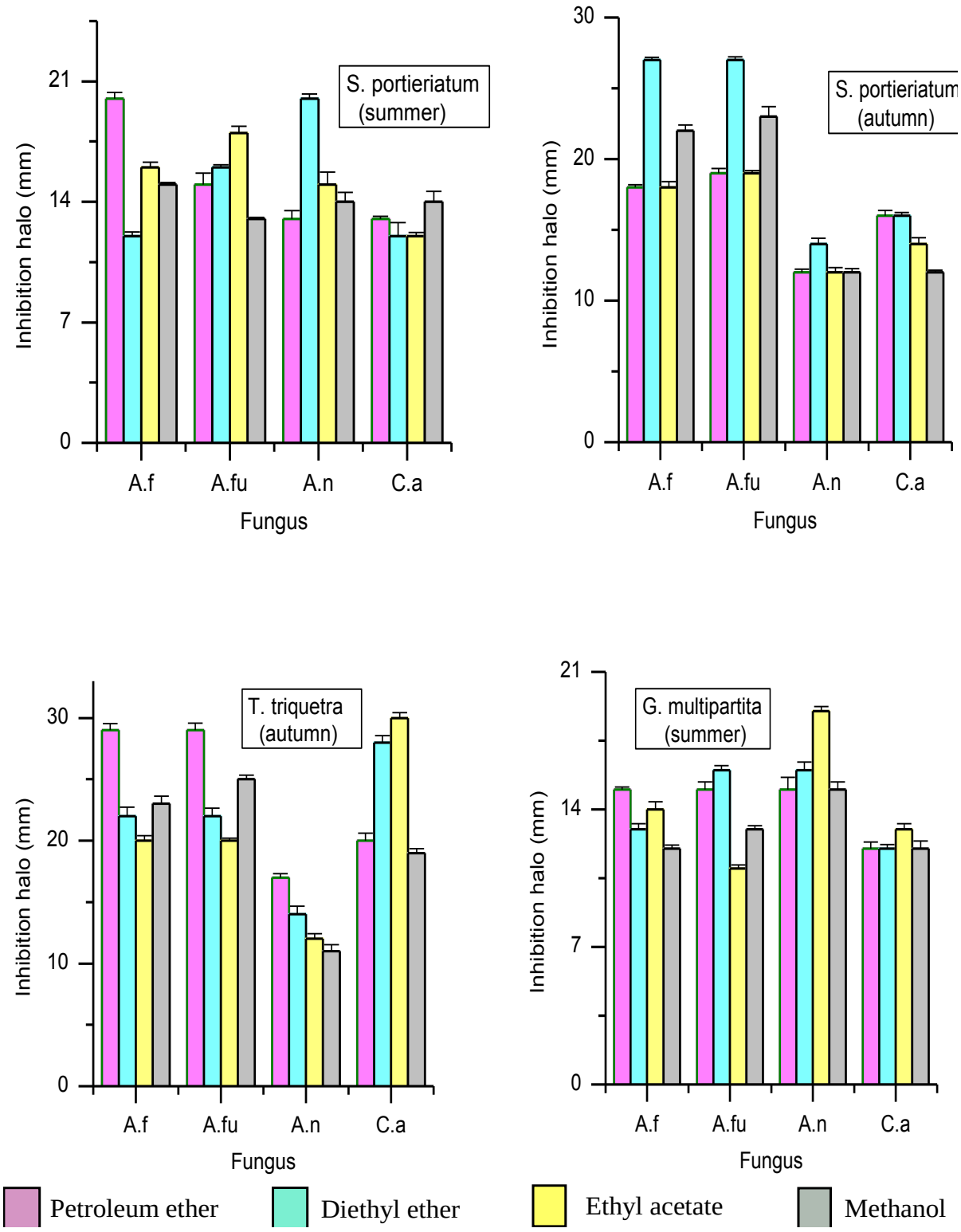

Figure 3. Antifungal activity of extracts of *S. portieriatum*, *T. triquetra* and *G. multipartita* collected from the coastal area of Al-Kumrah at south of the Red sea of Jeddah during summer and autumn (2009). A.f, *Aspergillus flavus*; A.fu, *Aspergillus fumigates*; A.n, *Aspergillus niger*; C.a, *Candida albicans.*

activities were achieved by ethyl acetate extracts for both *T. triquetra* against *C. albicans* (30 mm) and *P. pavonica* against *A. fumigatus* (28 mm). In this connection, Gonzalez et al. (2001) wrote that extracts of *P. pavonica*

Table 1. MIC of the most potent marine algae extracts against the tested fungi.

Algae	*A. flavus*		*A. fumigatus*		*A. niger*		*C. albicans*	
	Solvent	MIC (µg/ml)	Solvent	MIC (µg/ml)	Solvent	MIC (µg/ml)	Solvent	MIC (µg/ml)
E. prolifera (summer)	p	1.75	M	1.50	E	1.50	E	1.50
U. reticulata (summer)	P	1.50	E	2.50	M	2.00	D	1.00
C. myrica (autumn)	D	1.50	P	2.50	P	2.00	E	2.00
P. pavonica (summer)	P	2.00	E	3.00	E	3.00	P	2.50
S. portieriatum (summer)	P	2.00	E	2.50	D	2.50	M	3.00
S. portieriatum (autumn)	D	1.00	D	2.00	D	1.00	D	1.50
T. triquetra (autumn)	P	0.50	P	1.0	P	2.50	E	1.00
G. multipartita (summer)	P	2.00	D	2.0	E	2.00	E	2.25

P, Petroleum ether; D, diethyl ether; E, ethyl acetate; M, methanol.

and *Enteromorpha* showed antifungal and antibacterial activities. Vallinayagam et al. (2009) revealed that antimicrobial activity of the extract of *Padina* may be related to the presence of polyunsaturated alcohol. Tüney et al. (2007) reported that diethyl ether extracts of *Enteromerpha, Ulva* and *Gracilaria* appeared to yield better results than those of methanol. Tuney et al. (2006) recorded that antimicrobial activity depends on both algal species and the efficiency of the extraction method.

The pathogenic fungi *A. flavus* and *A. fumigatus* were more affected by the crude extracts of the brown alga, *S. portieriatum,* collected in autumn (18 to 27 and 19 to 27 mm, respectively) than that found during summer (12 to 20 and 13 to 18 mm, respectively) (Figure 3). Contrary to *A. niger*, different extracts of *S. portieriatum* collected in summer (13 to 20 mm) were more active than that gathered in autumn (12 to 14 mm). Seasonal variations in the chemical constituents have been reported in common marine seaweeds (Kaehler and Kennish, 1996; Kumar, 1993; Mercer et al., 1993). The biochemical composition of algae varies with species, habitats, maturity and environmental conditions (Harrison et al., 1977; Ito and Hori, 1980; Morris et al., 1983; Gatenby et al., 2003). Seaweeds are exposed to seasonal variations of abiotic factors that influence their metabolic responses and levels of proximate constituents (Ordu˜na-Rojaset et al., 2002; Pires-Cavalcante et al., 2011). The activity and inactivity of marine algae against microorganisms could be due to the collection of the algae at different development stages (Ely et al., 2004).

As shown in Figure 3, the lowest antifungal activity was observed with the different extracts of the red alga *G. multipartita* (11 to 19 mm). The ethyl acetate extract of *G. multipartita* showed the most effective results against *A. niger* (19 mm). The efficiency of marine algae belonging to the Rhodophyta agrees with previous results of Mahasneh et al. (1995), Bansemir et al. (2006) and Saeidnia et al. (2009). Vallinayagam et al. (2009) recorded antimicrobial activity of the extract of *Gracilaria* and *Sargassum*. They revealed that this activity may be related to the presence of polyunsaturated esters in *Gracilaria* and *Sargassum.* The present investigation concluded that the marine algae belonging to Chlorophyta and Phaeophyta exhibited the highest inhibitory effect against the test pathogenic fungi, whereas Rhodophyta had the lowest one. In addition, the petroleum ether, diethyl ether, ethyl acetate and methanol extracts showed varied activities against fungi. The most highly active algal species against the tested fungi was represented in the following order: *T. triquetra* (30 mm) > *P. pavonica* (28 mm) > *S. portieriatum* (autumn: 27 mm) > *E. prolifer*a (29 mm) > *U. reticulata* (20 mm) > *C. myrica* (21 mm > *S. portieriatum* (summer, 20 mm) > *G. multipartita* (19 mm).

Evaluation of MICs of the most potent algal extract is reported in Table 1. 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 for testing fungi *A. flavus, A. fumagitus, A. niger* and *C. albicans* were in the range of 0.5 to 3.0 µg/ml. The crude extract of *E. prolifera, U. reticulata, S. portieriatum* (autumn), and *T. triquetra* (autumn) required the lowest MIC values (ranged between 0.5 and 1.5 µg/ml) against tested fungi. Methanol and petroleum ether extracts of *T. triquetra* and *E. prolifera* were more active on *A. fumigatus* (1.0 to 1.5 µg/ml), however the diethyl ether extract from *S. portieriatum* (autumn) and petroleum ether extract of *T. triquetra* required lower MIC value (0.5 to 1.0 µg/ml) against *A. flavus.* Ethyl acetate extract from *E. prolifera* and diethyl ether extract of *S. portieriatum* (autumn) showed high activity on *A. niger* (1.5 and 1.0 µg/ml, respectively). Low MIC values were also observed with petroleum ether extract of *U. reticulata* (1.0 µg/ml) and ethyl acetate extract of *T. triquetra* (1.0 µg/ml) against *C. albicans.* Erika et al. (2011) showed the antifungal activities of the secondary metabolites of five species of *Laurencia* against three strains of pathogenic fungi: *C.*

albicans, *C. parapsilosis* and *Cryptococcus neoformans.* They found that the MIC of algal extracts of *L. bdendroidea* were < 31.25 µg/ml against *C. albicans* with a fungistatic effect; the chloroform extract of *L. catarinensis* showed the same results against *C. parapsilosis* (CP). A fungistatic effect was observed for the methanol extract of *L. aldingenis* against *C. parapsilosis* (MIC < 31.25 µg/ml), *C. neoformans* (CN) (33.9 µg/ml) and CA (65.2 µg/ml). The fungicidal effects of the hexane and chloroform extracts of *L. aldingensis* against CP were indicated by MIC values of 49.0 and 57.8 µg/ml, respectively. Nor Afifah et al. (2011) reported that different extracts with different antimicrobic compounds tend to possess different mechanisms of actions towards the microorganisms, thus inhibiting the growth. A low MIC value suggests that the compound is a strong antimicrobial compound as it can inhibit the microbial growth at low concentration (Hellio et al., 2001). Further assessments of the antifungal properties of these extract against a wide range of fungi and elucidation of the components responsible for the biological activities seems to be imperative for more comprehensive studies.

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