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Full Length Research Paper

Antibacterial activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia

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In the present study, marine algae were collected from the southern coast of Jeddah, Saudi Arabia during summer and autumn 2009. The antibacterial activities of petroleum ether, diethyl ether, ethyl acetate and methanol extracts of marine algae belonging to the Chlorophyta, Phaeophyta and Rhodophyta were studied. Their crude extracts were tested against different types of Gram-positive bacteria (*Bacillus subtilis*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureu)* and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). All marine algae extracts tested exhibited a broad spectrum of antibacterial activity. The maximum inhibition activities were shown for extracts of *Padina pavonica* and *Turbinaria triquetra*. The growth inhibitions of bacteria by S*argassum portieriatum* extracts were higher in samples collected during autumn than that investigated in summer. The maximum inhibitory effect of *Gracilaria multipartita* was observed in the petroleum ether extract against *B. subtilis* and *E. coli.* The ethyl acetate and petroleum ether extract of *Enteromorpha prolifera* and *Ulva reticulata* showed strong activity against the tested bacteria. The tested microorganisms that were susceptible to the most effective extracts were further tested for the minimum inhibitory concentration (MIC). The MIC of the tested microorganisms was between 0.5 and 1.25 µg/ml. The results of the present study confirmed the potential use of marine algae as a good source of antibacterial agent.

Key words: Chlorophyta, Phaeophyta, Rhodphyta, gram-positive bacteria, gram-negative bacteria, solvent extract, minimum inhibitory concentration (MIC).

INTRODUCTION

Bacterial infection causes a high rate of mortality in human population and aquaculture organisms (Kand hasamy and Arunachalam, 2008). Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics (Lavanya and Veerappan, 2011). It becomes a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki et al., 1999). Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives (Smith et al., 1994). Moreover, the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut (Idose et al., 1968). Marine algae represent an inexhaustible reservoir of raw materials used in pharmaceutical, medicine, food industries and cosmetics (Badea et al., 2009). Marine algae serve as an important source of bioactive natural substances (Vijayabaskar and Shiyamala, 2011; Villarreal-Gómez et al., 2010). Special attention has been reported on antibacterial activities

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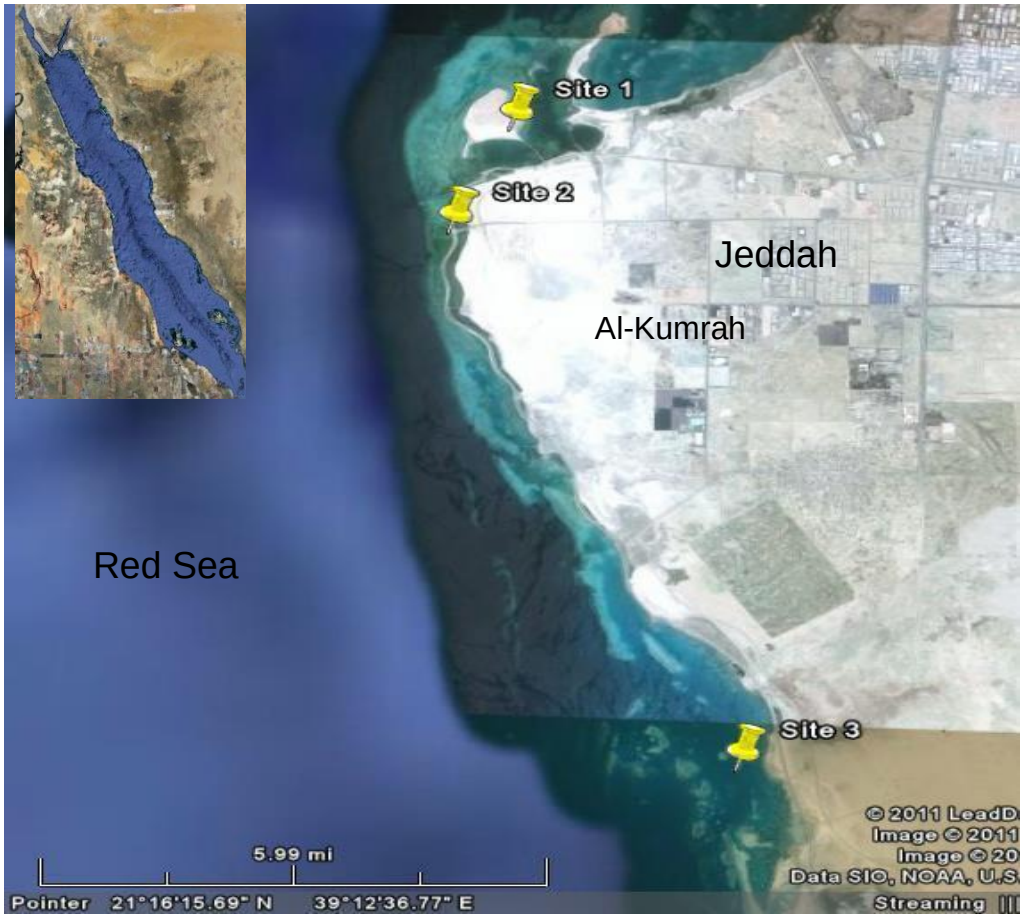

Figure 1. Map of the study area showing the Red Sea in the south of Jeddah.

related to marine algae against several pathogens (Siddhanta et al., 1997).

The extracts and active constituents of various marine algae have been shown to have antibacterial activity against Gram positive and Gram negative bacteria (Lima-Filho et al., 2002; Paul et al., 2006). The antimicrobial compounds derived from the marine algae consist of a diverse group of chemical compounds (Nor Afifah et al., 2010). Season and habitat of collection age of marine algae have an influence on their metabolic responses, the nature and levels of proximate constituents (Ordu˜na-Rojas et al., 2002). The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases (Chanda et al., 2010). Approximately, 2500 new metabolites were reported from a variety of marine organisms during the years from 1977 to 1987 (Arul Senthil et al., 2008). Many substances obtained from marine algae such as alginate, carra-geenan and agar as phycocolliods have been used for decades in medicine and pharmacy (Taskin et al., 2001). Chemical structure types include sterols (Ahmed et al., 1993), isoprenoide, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, fatty acids and acrylic acid can be counted (Mtolera and Semesi, 1996).

In this study, we aim to screen petroleum ether, diethyl ether, ethyl acetate and methanol extracts of some marine algae that were abundant in the Red Sea of Jeddah during summer and autumn 2009 to evaluate their antibacterial activities. Further studies are being carried out now on the same species or other species of marine algae which appeared at the same localities during winter and spring 2010, with the aim of identifying novel species with antimicrobial activities.

MATERIALS AND METHODS

Study area

Study area (Figure 1) lies about 28 km south of Jeddah. It extends for about 8 km and 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. The study area is located in the central part of the Red Sea which is characterized by a tropical to subtropical climate. The dominant marine algae were collected at three different sites. The first site of study is located at the middle of the reef. The

second and third sites are 1 and 8 Km far away from the first site, respectively. Algal samples were collected during summer and autumn 2009.

Algal materials

The algal species were dominant at the three different sites during summer and autumn 2009. The algae were identified according to Aleem (1978, 1981) and Abbott and Hollenberg (1976). The algae belong to the Chlorophyta (*Enteromorpha prolifera* J. Agardh and *Ulva reticulata* Forsskål, summer), Phaeophyta (*Cystosiera myrica* C. Agardh*, a*utumn; *Padina pavonica* (Linnaeus), summer)*, Turbinaria triquetra* (J.Agardh)*, Sarrgassum portieriatum* C. Agardh*,* summer and autumn) and Rhodophyta (*Gracillaria multipartite* J. Agardh*,* summer). Algal materials were cleaned up from epiphytes and non-living matrixes in running tap water and rinsed many times in distilled water. The samples were 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.

Algal extract

Successive extraction was carried out by soaking the algae in different solvents (petroleum ether, diethyl ether, ethyl acetate and methanol) within a conical flask (50 g dry algae/750 ml solvent) and then kept on a shaker at 120 rpm at room temperature (30°C) for 24 h. The mixture was filtered by muslin cloth and by Whatman No.1 filter paper. The obtained filtrates were taken to dryness under reduced pressure at 40°C. The obtained thick residues were dissolved in dimethylsulfoxide (DMSO). The algal extracts were filtered using 0.2 µm Millipore filter and stored at -20°C.

Test microorganisms

The antimicrobial activity of petroleum ether, diethyl ether, ethyl acetate and methanol of marine algae extracts were examined against Gram-positive bacteria (*Bacillus subtilis*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus)* and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The organisms used in this study were obtained from King Fahed Hospital in Jeddah.

Antibacterial assay

The different pathogenic bacteria were grown on nutrient agar and blood agar media. The nutrient medium was sterilized, and 25 ml was poured into sterile cabbed test tubes. Test tubes were cooled to room temperature and 0.5 ml of the uniform mixture of inoculum was introduced to each tube giving 1×10^8 bacteria per ml. The tubes were mixed using a vortex mixer for 15 to 30 s. The test tube content was poured into a sterile Petri-dish for solidification (Mtolera and Semesi, 1996).

Testing procedures

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. 50 µl algal extract (2 µg/ml) was introduced into each well and the plates were maintained at 4°C for 2 h. The plates were later incubated for 24 h at 37°C. The diameter of the growth inhibition halos caused by the extract of algae was measured in millimeters. Each assay was triplicated and the main values were recorded.

The minimal inhibitory concentration (MIC)

Each antimicrobial agent was serially diluted and the experiment was conducted as described by Ter Laak et al. (1991). The minimal inhibitory concentration (MIC) was recorded as the lowest concentrations inhibiting visible growth.

Statistical analysis

All assays were done in triplicate. All data were expressed as mean values ± SD, the mean values being analyzed by three ways analysis of variance using the Statistical Analysis System (SAS) (1997) computer program in Biostatistics.

RESULTS AND DISCUSSION

In the present study, the crude extract of all tested marine algae evoked strong antibacterial activities and inhibited all tested bacteria (Figures 2 and 3). The maximum biological activities of the green algae were observed in ethyl acetate extract of *E. prolifera* against MRSA (inhibition halo: 25 mm) and petroleum ether extracts of *U. reticulata* against *E. coli, K. pneumoniae* and MRSA (25, 23 and 20 mm, respectively) (Figure 2). At the same time, *B. subtilis* was highly inhibited (17 to 21 mm) by different extracts of *the U. reticulata.* A similar observation was recorded by Thillairajasekar et al. (2009) who found good antimicrobial activity in ethyl acetate extract of algae. Also, they reported that hexane and ethyl acetate extracts of *Ulva* and *Gracilaria* showed the presence of myristic and palmitic acid, linoleic acid, oleic acid, lauric, stearic and myristic acid, which are known to have potent antibacterial and antifungal agents. Kim et al. (2007) reported that *Ulva lactuca* exhibited a broad-spectrum of antibacterial activity, especially against MRSA. A large number of *Ulva* extracts products have been found to have antibacterial activity, many of these structures identified as fatty acids, hydroxyl unsaturated fatty acids, ghycolipids, steroids, phenolics and terpenoids (Awad, 2000). The antimicrobial activity of *Ulva* organic extract is apparently related to their lipophelic and phenolic contents (Abd El-Baky et al., 2008). González et al. (2001) found that the algal extracts such as *Enteromorpha ramulosa* and *Dictyopteris membranacea* were active against Gram-positive and Gram-negative bacteria. The results clarified that the minimum inhibition halos (11 to 15, 9 to 15 and 11 to 14 mm) were found in extracts of *E. prolifera* against *B. subtilis*, *S. aureus* and *K. pneumoniae*, respectively. However, *U. reticulata* extracts recorded the minimum activities (12 to 15 and 13 to 16 mm) against *S. aureus* and *P. aeruginosa*, respectively. Seaweed extracts in different solvents exhibited different antimicrobial activities (Rangaiaha et al., 2010).

As obtained from Figure 2, the different extracts of the brown algae, *C. myrica* and *P. pavonica* exhibited good biological activities against the tested bacteria (inhibition halos ranged between 11 to 23 and 11 to 35 mm,

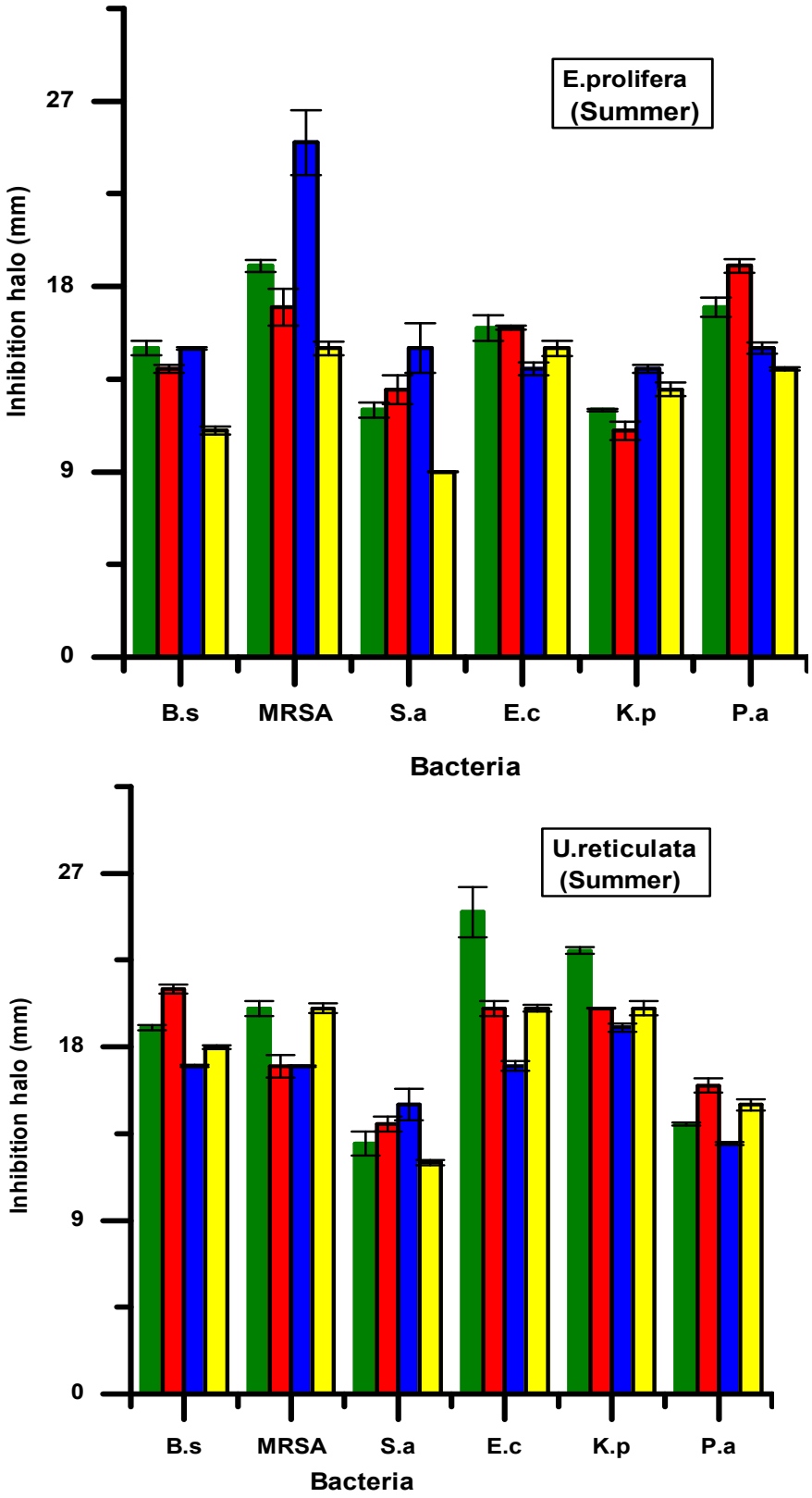

Figure 2. Antibacterial activity of extracts of some marine algae collected from Red Sea of Jeddah during summer and autumn (2009).
B.s, *B. subtilis*; **MRSA,** Methicillin-Resistant *Staphylococcus aureus*; **S.a,** *Staphylococcus aureus*; **E.c,** *Escherichia coli*; **K.p,** *Klebsiella pneumonia*; **P.a,** *Pseudomonas aeruginosa.*

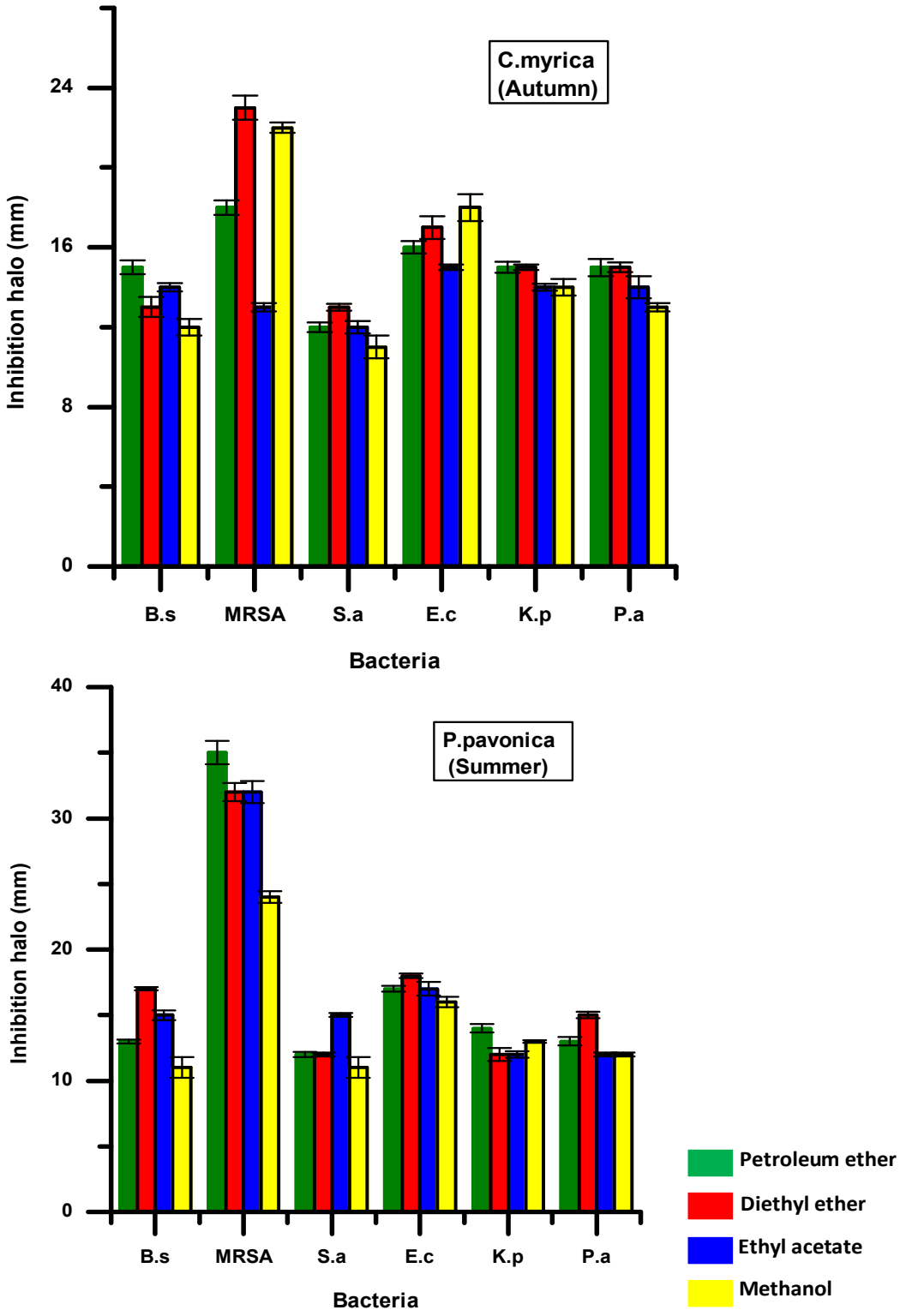

Figure 2. Contd.

respectively). The maximum inhibition activities were shown for diethyl ether and methanol extracts of *C. myrica* (23 and 22 mm, respectively) and for all tested extracts of *P. pavonica* (24 to 35 mm) against MRSA. Moreau *et al.* (1984) found that *Cystoseira mediterranea* and *Cystoseira usneoides* were strongly active versus *S.*

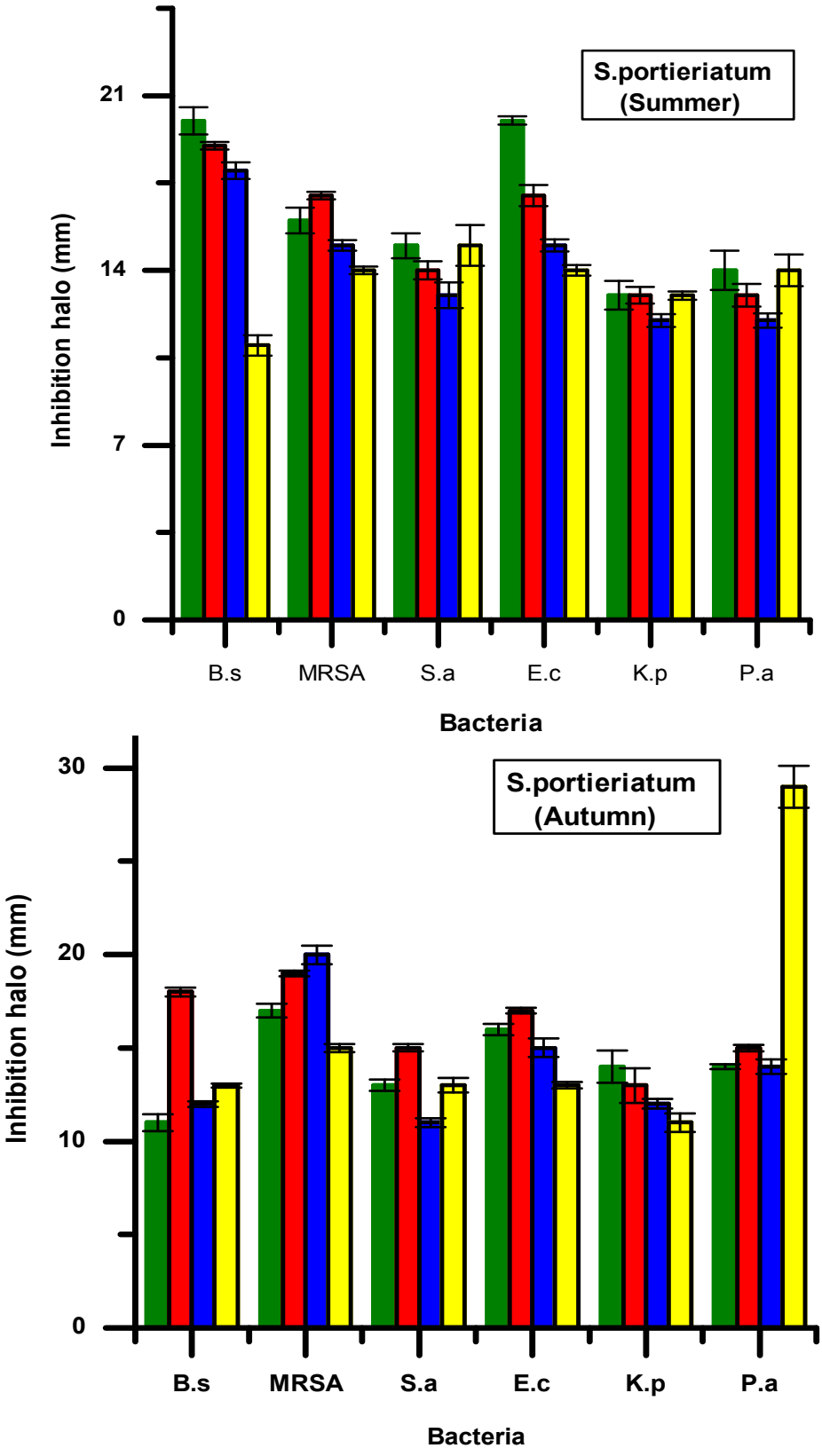

Figure 3. Antibacterial activity of extracts of some marine algae collected from Red Sea of Jeddah during summer and autumn (2009).
B.s, *B. subtilis*; **MRSA,** Methicillin-Resistant *Staphylococcus aureus*; **S.a,** *Staphylococcus aureus*; **E.c,** *Escherichia coli*; **K.p,** *Klebsiella pneumonia*; **P.a,** *Pseudomonas aeruginosa*.

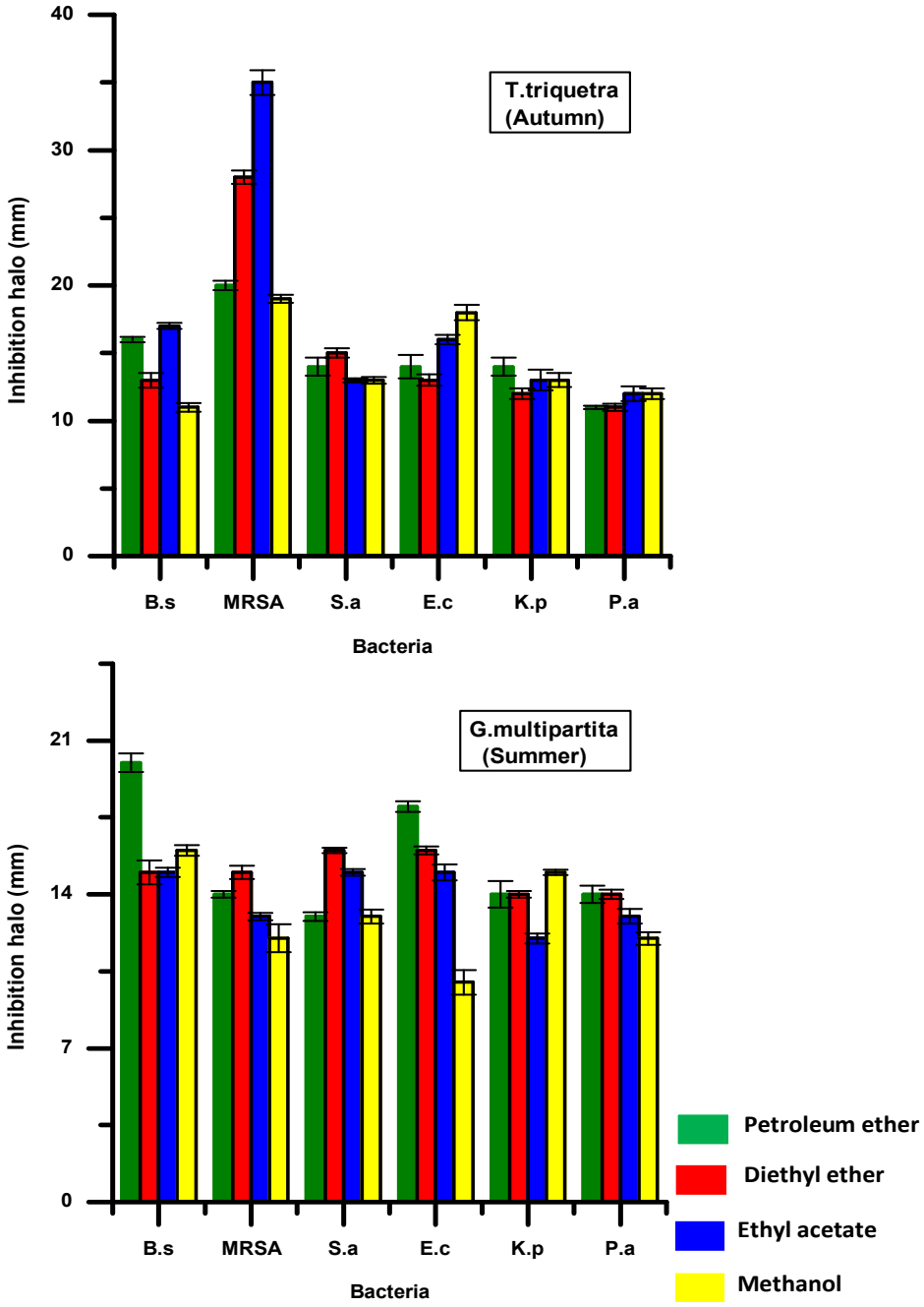

Figure 3. Contd.

aureus.

In the present study, there was a clear distinction between antibacterial effects of *S. portieriatum* which was collected in summer and autumn (Figure 3). Approximately, the growth inhibition of bacteria by the extract of *S. portieriatum* that was collected in autumn was higher (ranged between 11 to 29 mm) than that found in sample of summer (11 to 20 mm). This result may be explained by Kantachumpoo and Anong (2010) who found that the crude extract from most of the brown species did not show antimicrobial activity, possibly due to the samples been collected during the summer. The maximum antibacterial activities of *S. portieriatum* were represented in methanol extract (autumn) against *P. aeruginosa* (29 mm) and in the petroleum ether extracts (summer) against *E. coli* and *B. subtilis* (20 mm). A similar observation was recorded by Das et al. (2005). They examined methanol extracts of some algae and showed moderate to high activity against bacteria. In contrast to our results, Ibtissam et al. (2009) reported non efficiency of the

methanol extracts of *Sargassum vulgare*, which did not show antibacterial activity against *E. coli and S. aureus* growth. Salvador et al. (2007) reported that brown and red seaweeds collected in the spring, and autumn inhibited microorganisms. The biological activities of marine algae are thought to be influenced by environmental factors (Lobban and Harrison, 1997). The activity and inactivity of marine algae against microorganisms could due to the reproductive state and seasonality (Moreau et al., 1984; Ely et al., 2004). The extraction protocol and the harvest period are other important factors (Edwards et al., 2006; Rajasulochana et al., 2009).

As shown in Figure 3 the different extracts of *T. triquetra* recorded the highest inhibition against MRSA (19 to 35 mm), especially ethyl acetate extract. In *T. triquetra* extracts, the moderate inhibition halos were observed against *E. coli* and *B. subtilis* (13 to 18 and 11 to 17, respectively). On the other hand, *S. aureus*, *K. pneumonia* and *P. aeruginosa* were less sensitive to inhibitory action induced by *T. triquetra* extracts (13 to 15, 12 to14 and 11 to12 mm, respectively). The high and low effect of organic extract against microorganisms could be related to the presence of bioactive metabolites, which can be soluble in solvent (Sastry and Rao, 1994; Kolanjinathan and Stella, 2009; Manivannan et al., 2011). Vijayabaskar and Shiyamala (2011) showed that the marine algae extract of *Turbinaria ornata* and *Sargassum wightii* possessed noticeable activity against positive and negative bacteria and could be utilized as a good source of antimicrobial agent in pharmaceutical industry.

Among all tested algae, the red alga *G. multipartita* extracts showed the lowest inhibitory effect (10 to 20 mm) against all tested bacteria (Figure 3). The maximum inhibitory effect of *G. multipartita* was observed in the petroleum ether extract against *B. subtilis* and *E. coli* (20 and 18 mm, respectively). In accordance with the present results, Wong and Cheung (2001) found that the antimicrobial activity of red seaweeds was lower than that of the green seaweeds, as the result of high phenolic contents in the green seaweeds. The present results are in contrast with Lavanya and Veerappan (2011) who found that the maximum inhibition zone was obtained from methanol extracts against *E. faecalis* and *Proteus mirabilis* (6 mm). In contrast to our results, Reichelt and Borowitzka (1984) and Mahasneh et al. (1995) showed higher degrees of antibacterial activity rather than extracts were for red algae.

Conclusion

The extracts of testing marine algae showed antibacterial activities in the following order: Phaeophyta > Chlorophyta > Rhodophyta. The most highly active algal species against tested bacteria was represented in the following order: *T. triquetra* (35 mm) > *P. pavonica*(35 mm,) > *S. portieriatum* (autumn: 29 mm) > *E. prolifer*a (25 mm) > *U. reticulata* (25 mm) > *C. myrica* (23 mm) > *S. portieriatum* (summer, 20 mm) > *G. multipartita* (20 mm). However, the responses of pathogenic bacteria strains to the different algal extracts were MRSA > *E. coli* > *B. sabtilis* > *P. aeruginosa* > *K. pneumoniae* > *S. aureus.* In addition, petroleum ether, diethyl and ethyl acetate were the best solvents, for extracting the bioactive compounds, followed by methanol extract against the selected pathogens. Caccamese (1985) has reported that the brown algal extracts showed higher activity than the extracts of red algae. But Vallinayagam et al. (2009) recorded that the red alga showed higher activity than the brown algae and green alga. The remarkable differences between our results and the results obtained in previous studies may be due to several environmental factors. The large diameter of zone inhibition represent the high sensitivity of the microorganisms to the seaweed extracts and vice versa (Rebecca et al., 2012).

The MIC values of the most effective algal extracts against tested bacteria are shown in Table 1. The MIC values of testing algal extract for inhibition of Gram-positive and Gram-negative bacteria ranged from 0.5 to 2.5 ug/ml. The petroleum ether extracts from *S. portieriatum* (summer) and *G. multipartita* (summer) were the most effective against *B. subitilis* (MIC: 1.25 ug/ml), whereas MRSA was highly inhibited by ethyl acetate extract of *E. prolifera* (summer) and petroleum ether extract of *P. pavonica* (summer) (MIC: 1-1.25 ug/ml). The gram- positive bacteria *S. aurues* was more inhibited with diethyl ether extract of *T. triquetra* (autumn) and *G. multipartita* (summer) and the MIC were 0.5 and 2.5 ug/ml, respectively. The petroleum ether extracts from *U. reticulata*, *S. portieriatum* (summer) and *C. myrica* were more active against Gram-negative bacteria *E. coli* and *K. pneumoniae* (MICs were ranged between 0.5 to 1.5 ug/ml). The diethyl ether extract of *E. prolifera* and methanol extract of *S. portieriatum* (autumn) exhibited the maximum growth inhibition against *P. aeruginosa,* and the MIC were 1.25 and 0.75 ug/ml, respectively. Chiao-Wei et al. (2011) recorded that Gram-positive bacteria especially *B. cereus* was more susceptible to the seaweed extracts (MIC=0.130 to 0.065 mg/ml). Generally, they found that *S. polycystum* extracts exhibited higher bacteriostatic activity (lower MICs) against all the tested bacterial strains when compared with *P. australis*. Salem et al. (2011) found that MIC of seaweed extracts was ranging from 5 mg/ml to 50 mg/ml. The antimicrobial activity of various extracts of *Halimeda discoidea* was previously evaluated by Nor Afifah et al. (2010). They showed that the hexane extract was active against eight bacteria with MIC values ranged between 0.25 to1.00 µg/ml.

It can be concluded that, the crude extract of all tested marine algae evoked strong antibacterial activities and can be used for development of anti-pathogenic drugs in the pharmaceutical industries. The brown marine species

Table 1. MIC of the highest antibacterial activity of some marine algae extracts.

Bacteria	Algae	Solvent	MIC (ug/ml)
Gram (+) ve			
B. subtilis	*S. portieriatum* (summer)	P	1.25
	G. multipartita (summer)	P	1.25
MRSA	*E. prolifera* (summer)	E	1.0
	P. pavonica (summer)	P	1.25
S. aurues	*T. triquetra* (autumn)	D	0.5
	G. multipartita (summer)	D	2.5
Gram (-) ve			
E. coli	*U. reticulata* (summer)	P	1.25
	S. portieriatum (summer)	P	1.25
K. pneumonia	*U. reticulata* (summer)	P	1.5
	C. myrica (autumn)	P	0.5
P. aeruginosa	*E. prolifera* (summer)	D	1.25
	S. portieriatum (autumn)	M	0.75

P, Petroleum ether; **D,** Diethyl ether; **E,** Ethyl acetate; **M,** Methanol.

were the highly active against the tested bacteria. Study in this field is required for isolation and characterization of the antibacterial compounds from marine algae.

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