Saudi Journal of Biological Sciences (2017) **24**, 162–169

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Antimicrobial, antioxidant properties and chemical composition of seaweeds collected from Saudi Arabia (Red Sea and Arabian Gulf)

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Received 24 March 2016; revised 26 May 2016; accepted 31 May 2016
Available online 16 June 2016

KEYWORDS

Sargassum latifolium B;
Sargassum platycarpum A;
Cladophora socialis;
Free radical scavenging activity;
Phenol

Abstract The present study demonstrates the antibacterial activity of selected brown and green marine algae collected from Saudi Arabia Red Sea and Arabian Gulf. The methanolic and acetone extracts were tested against gram positive, gram negative bacteria and *Candida albicans* in an attempt to be used as an alternative to commonly used antibiotics. Both brown seaweed species *Sargassum latifolium B* and *Sargassum platycarpum A* methanolic extracts were found to be active against gram positive than gram negative; however, *S. latifolium* acetone extract gave the highest inhibitory activity against *Salmonella* sp. On the other hand, *Cladophorasocialis* organic extract demonstrated higher antibacterial activity than the fresh extract but both *C. socialis* extracts revealed decreased activity compared to *Sargassum* extracts. *Cladophora* methanolic extract showed an obvious effect on methicillin resistant *Staphylococcus aureus* (MRSA). The present work shows a comparable therapeutic potency of the tested seaweed members *Sargassum* and *Cladophora* extracts in treating human microbial pathogens to synthetic chemical antibiotics. A remarkable higher antioxidant DPPH free radical scavenging effect was recorded with *Sargassum* sp. compared to *Cladophora* sp.

FTIR Infrared Spectrometer analysis together with the high performance liquid chromatography provided a detailed description of the possible functional constituents and the major chemical components present in marine macroalgae particularly in brown seaweeds to be mainly of phenolic nature to which the potent antimicrobial activity is being attributed.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

As a consequence of an increasing demand for biodiversity in seeking therapeutic drugs from natural products, there is now a greater interest in marine organisms, especially algae. Seaweeds or marine algae are primitive non-flowering plants

http://dx.doi.org/10.1016/j.sjbs.2016.05.018
1319-562X

without true root stem and leaves. They are divided into three major groups Rhodophytae (red algae), Chlorophytae (green algae) and Pheaophytae (brown algae) depending on their nutrient and chemical composition.

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites (Gonzalez et al., 2001; Smit, 2004) characterized by a broad spectrum of biological activities; antiviral, antibacterial (Chakraborthy et al., 2010a), antifungal and anti tumoric activities that have been detected in green, brown and red algae giving an alternative approach to the use of the synthetic antimicrobial agents. However, the potent antimicrobial effect of seaweeds resides in the efficiency of the extraction method (Tuney et al., 2006), the algal species (Valchos et al., 1997) and the solvents being used (Cox et al., 2010).

Moreover, higher medicinal effect was obtained from dry seaweeds samples than from fresh samples as indicated by many studies which reported that extracts prepared from fresh seaweeds showed negligible antimicrobial activity compared to that obtained from dried seaweeds (Padmini Sreenivasa Rao et al., 1986; Campos-Takaki et al., 1988; Manivannan et al., 2011). In addition, Salvador et al. (2007) revealed that algal sample preparation method greatly affect the bioactivity of tested algae (e.g. lyophilization generally allows greater compound extraction and hence gives highest antimicrobial activity).

Many investigations have demonstrated that a high dietary intake of natural phenols with the presence of several types of antioxidants such as flavonoids (Moraes-de-Souza et al., 2008) commonly found in plants and seaweeds is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancer (Hodgson and Croft, 2006; Halliwell, 2007; Yan and Asmah, 2010). Since seaweeds are known to contain a wide variety of bioactive compounds as such offering a rich source of new drugs with potentially lower toxicity.

The present study aimed to test the *in vitro* antimicrobial effects of the three selected marine brown and green algae identified as *Sargassum latifolium B*, *Sargassum platycarpum A* (collected from the red sea) and *Cladophora socialis* (collected from the Arabian Gulf), Saudi Arabia. The dried acetone and methanol algae extracts were tested against some known human pathogenic gram positive and gram negative bacteria, some of which are antibiotic resistant such as methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Salmonella* sp., as for the antifungal activity, samples were tested against one yeast isolate *Candida albicans*. Additionally, the DPPH antioxidant effects of the tested algae were also evaluated.

2. Materials and methods

2.1. Seaweed collection and processing

Green algae were collected from the Red Sea, Jeddah, KSA whereas the Brown marine seaweeds were taken from the Arabian Gulf, Khobar, KSA; both were collected on February, 2014.

Algal samples were handpicked, washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. They were transported in an icebox to the microbiology laboratory, KSU, Riyadh, identified with the help of the Botany department as *C. socialis* (green algae), *S. latifolium B* and *S. platycarpum A* (brown algae) respectively (Fig. 1). Samples were washed thoroughly using tap water and then with sterile distilled water to be air dried at room temperature for 1 week. Dried samples were cut into small pieces and ground with a blender into powder to be analyzed and screened for their therapeutic effects. *Cladophora* fresh green algal samples were processed immediately for their antimicrobial activity as a comparative assay.

2.2. Extract preparation

10–20 g of each of the dried algal samples was extracted with 50–100 ml of acetone and methanol separately depending on the sample availability. Samples then were kept in a rotator

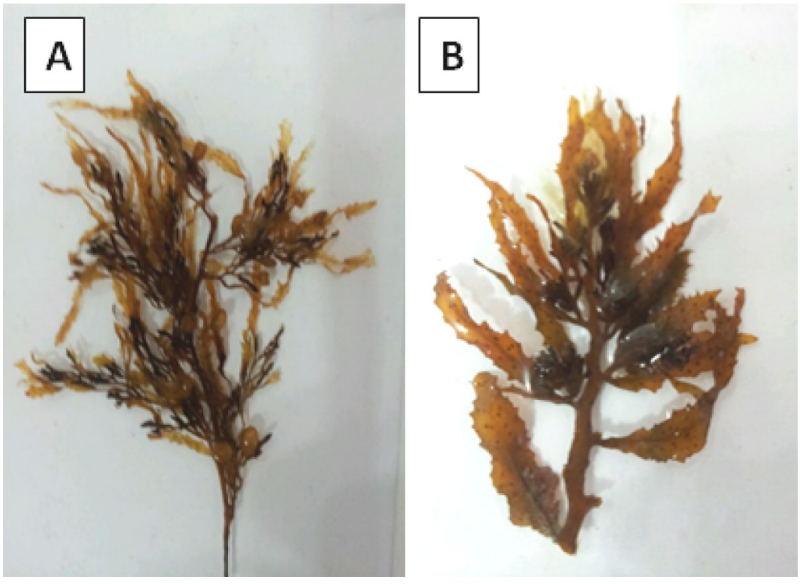


Figure 1 Samples A: *Sargassum platycarpum A*; B: *Sargassum latifolium B*.

shaker for 7 days at 150 rpm and 25 °C. Similarly fresh *C. socialis* samples were extracted and incubated as mentioned above. Prior to incubation, both samples dried and fresh were filtered using a Millipore filter unit of 0.45 μm pore size (Millipore, USA) and kept at −4 °C in sterile microcentrifuge tubes for further use.

2.3. Microbial isolates preparation

Tested organisms were common human pathogens, some of which are antibiotic resistant, comprised of 5 standard gram positive bacteria such as *S. aureus* ATCC 25923, *Staphylococcus xylosus* obtained from the Microbiological Resources Center (Cairo, Egypt), methicillin resistant *S. aureus* MRSA ATCC 12498, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633; 4 gram negative bacteria as follows *Escherichia coli* ATCC 25966, *P. aeruginosa* ATCC 27853, *Salmonella* sp. (clinical isolate) and *Klebsiella pneumoniae* ATCC 700603, and one yeast isolate *C. albicans* ATCC 60193. All cultures were obtained from King Khalid University Hospital, Microbiology Laboratory, Riyadh, KSA. Isolates were kept fresh on nutrient agar plates (Oxoid) and on nutrient broth (Oxoid) with 15% glycerol and stored at −4 °C for future use.

Small inoculum of each of the pure, fresh cultures was added to 5 ml sterile distilled water making a 0.5 MacFarland bacterial suspension, to be inoculated on Muller Hinton agar plate surfaces. Yeast suspension was prepared similarly and inoculated onto Muller Hinton agar plates.

2.4. Antimicrobial assay

Antimicrobial activity was carried out *in vitro* using the agar well diffusion technique (Berghe and Vlietinek, 1991). Small inoculums of each of the prepared bacterial and yeast suspensions were spread with a sterile cotton swab evenly onto Muller Hinton agar plate surfaces (Oxoid). Prior to inoculation plates were kept to rest at room temperature allowing the absorption of the microbial inoculums. Four equidistant wells on each MH plates were made, using a 5–7 mm diameter sterile cork borer, corresponding to the dry and fresh *C. socialis*, *S. latifolium B* and *S. platycarpum A* algal methanol and acetone extracts. Wells were loaded with 100 μl of the appropriate seaweed extracts. Prior to inoculation, plates were kept to rest at room temperature for 30 min for a better absorption; plates were then incubated at 37 °C for 18–24 h. All tests were performed in duplicate. The antimicrobial activity was indicated by measuring the inhibition zone around each well. Mean diameter values were calculated from duplicate runs of each assay. The efficiency of the algal extracts being tested was compared with standard antibiotic disks, methanol and acetone used as positive and negative controls, respectively.

2.5. The Fourier Transform Infrared Spectrometer (FTIR) analysis

The Fourier Transform Infrared (FTIR) Spectrometer was obtained from analytical instrumentation facility. A qualitative and preliminary analysis of the main functional groups on the *C. socialis*, *S. latifolium B* and *S. platycarpum A* surface samples were prepared to be then analyzed and recorded using a Nicolet 6700 FT-IR instrument (Thermo scientific). FTIR spectrum of each of the two samples was obtained separately by KBr pellet method. About 0.1 g of dried algal biomass was mixed with KBr (0.1 g) and compacted in pellet form. FTIR spectra were then recorded in the wavelength between 4000 and 500 cm^{-1}. Data were plotted on standard software provided with the instrument.

2.6. Estimating antioxidant activity

2.6.1. DPPH free radical scavenging assay

The antioxidant potency of the different algal extracts was determined using 2,2-diphenyl-1-picrlyhydrazyl (DPPH) free radicals scavenging technique. 150 μl DPPH solution (4.3 mg dissolved in 3.3 ml methanol) was added to 3 ml methanol and the absorbance was taken immediately at 517 nm for control reading. Different volumes of test sample (20, 50,100, and 150 μl) were taken and diluted with methanol up to 3 ml to be screened with 150 μl DPPH solution added to each test tube. The mixture was vortexed and kept at room temperature for 0 and 5 min in the dark. Absorbance was taken at 517 nm spectrophotometrically using methanol as a blank. The percentage of DPPH free radicals scavenging activity was calculated with the following formula:

$$I = [1 - (A2 - A1)/A0] \times 100\%.$$

where A0 control absorbance (methanol), A1 sample absorbance (methanol + sample + DPPH) and A2 test absorbance (methanol + sample without DPPH). All reactions were carried out in duplicates and the degree of purple color formation and decolorization indicates the free radial scavenging activity of the algal extracts (Viturro et al., 1999). The antioxidant effects of the tested extracts will then be compared to that produced by ascorbic acid as standard antioxidant.

3. Results

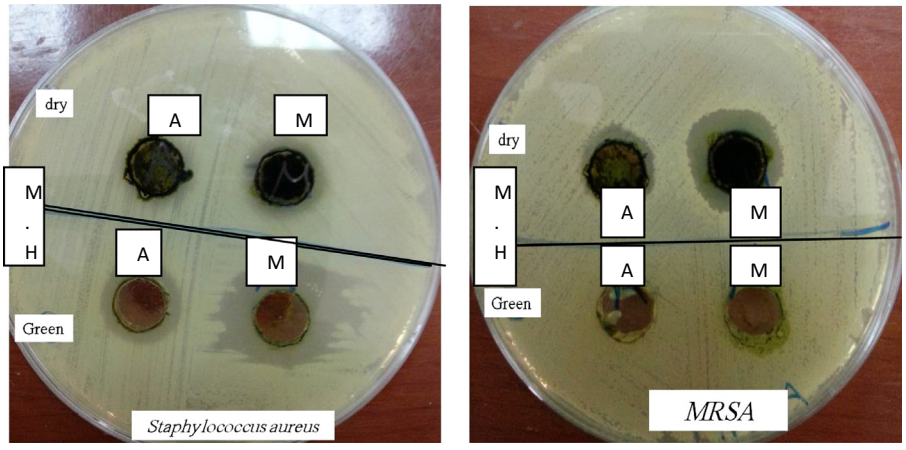
Table 1 demonstrates the *in vitro* antimicrobial activity of the dried seaweeds; *S. latifolium B* and *S. platycarpum A* and *C. socialis*, methanol and acetone extracts. It can be easily noticed that these extracts exhibited higher antimicrobial activity compared to that obtained by the green fresh *Cladophora* algal samples indicated as + or − signs where + + + is relative to an inhibition zone >10 mm; + + indicating a measurement >7 mm and + equals 7 mm. Among the dried extracts, *S. latifolium B* methanolic extract was the most effective showing the largest inhibition zone particularly with *Salmonella* sp. followed by *S. latifolium A* and then *Cladophora* (Figs. 2 and 3). Moreover, both methanol and acetone algal extracts showed variable antibacterial effect against some isolates, but higher when compared with the standard antibiotic disks being used (Table 1).

3.1. FTIR analysis

The exact mechanism and the compounds responsible for this antimicrobial activity are still unclear. Many studies suggested that the higher phenol content of marine macroalgae may affect bacterial growth and metabolism; they could have an activating or inhibiting effect on the microbial growth according to their constitution and concentration (Reguant et al.,

Table 1 Data for the potent antimicrobial activity of *Sargassum* sp. and *Cladophora socialis* respectively. Larger inhibition zones were referred to by (+ + +), moderate (+ +), low (+) and no activity (–) A: acetone; M: methanol.

No.	Pathogenic	Algal species and organic extracting								
		Sargassum latifolium		*Sargassum platycarpum*		*Cladophora socialis* $_{green}$		*Cladophora socialis*$_{dry}$		Antibiotic disk (µg)
	Bacterial isolates	*A*	*M*	*A*	*M*	*A*	*M*	*A*	*M*	
1	*K. pneumoniae*	–	–	–	–	–	–	–	–	MEM 10 (10 mm)
2	*E. coli (25966)*	+ +	+ +	+ +	+ +	–	–	–	+	MEM 10 (8 mm)
3	*P. aeruginosa*	+ +	+	+ +	+ + +	–	–	–	–	MEM 10 (8.5 mm)
4	*Salmonella* sp.	+ + +	+ +	+ +	+ +	–	–	–	–	AMC 30 (7.5 mm)
5	*E. feacalis*	+ +	+	+ + +	+ +	–	–	–	–	AMC 30 (7 mm)
6	*MRSA*	+ +	+ + +	+	+ +	–	–	+ +	+ + +	MEM 10 (8 mm) MXF 5 (7 mm) AMC 30 (10 mm)
7	*B. subtilis*	+ +	+ + +	+ +	+ + +	–	+ +	+ +	+ + +	MEM 10 (8 mm)
8	*Staph. aureus*	+ +	+ + +	+	+ +	+ +	+ + +		+ +	MEM 10 (9 mm)
9	*Staph. xylosus*	+ + +	+	+	+ + +	–	–	–	+ +	MEM 10 (9 mm)
	Yeast									
1	*C. albicans*	–	+	+ +	–	–	–	–	–	



(A) (B)

Figure 2 *Cladophora socialis* dry and fresh, acetone and methanol extracts activity against *Staphylococcus aureus* and MRSA. Inhibition zones around the wells were noticed indicating the effect of both green algal species extracts. MRSA: methicillin resistant *Staphylococcus aureus*, A: acetone; M: methanol.

2000; Alberto et al., 2001). This was emphasized by analyzing the major chemical constituents of both algal cells being processed using the FTIR which indicated highest O–H absorption ranging between 3400 cm^{-1} and 3300 cm^{-1} related to the main chemical groups phenols namely hydroxyl amide I, amide II and primary amine groups present on the cell walls, mainly of *Sargassum* sp., to which this larger inhibitory effect is observed. Higher absorption was observed for both *Sargassum* species ranging between 3421 cm^{-1} (Fig. 4) and 3409 cm^{-1} (Fig. 5) respectively whereas in *Cladophora* highest hydroxyl peak was read at 3348 cm^{-1} (Fig. 6).

3.2. DPPH radical scavenging activity

Screening the antioxidant activity by free radical scavenging assay showed that crude extracts of the seaweeds, revealed a comparable antioxidant activity. Increased activity was observed with increased algal extract concentration and increased time (5 min interval), where maximal values were obtained with *S. latifolium B* at a 150 µg/ml compared to the *C. socialis* samples. Results indicated 66% scavenging activity of the *S. latifolium B* crude extracts followed by *C. socialis (65%) S. platycarpum A* (60%) compared with the reference control ascorbic acid having a 92% free radical scavenging activity (Fig. 7). It was noted as well that higher antioxidant activity was observed with dried samples than with fresh ones (59%); supporting as such the finding of dried samples having a significant antimicrobial activity correlated to the high phenolic constituent presence and to an increased scavenging free radical activity.

4. Discussion

Marine organisms are a rich source of novel and biologically active metabolites, producing potential bioactive compounds of interest in the pharmaceutical industry. Both, the solvent

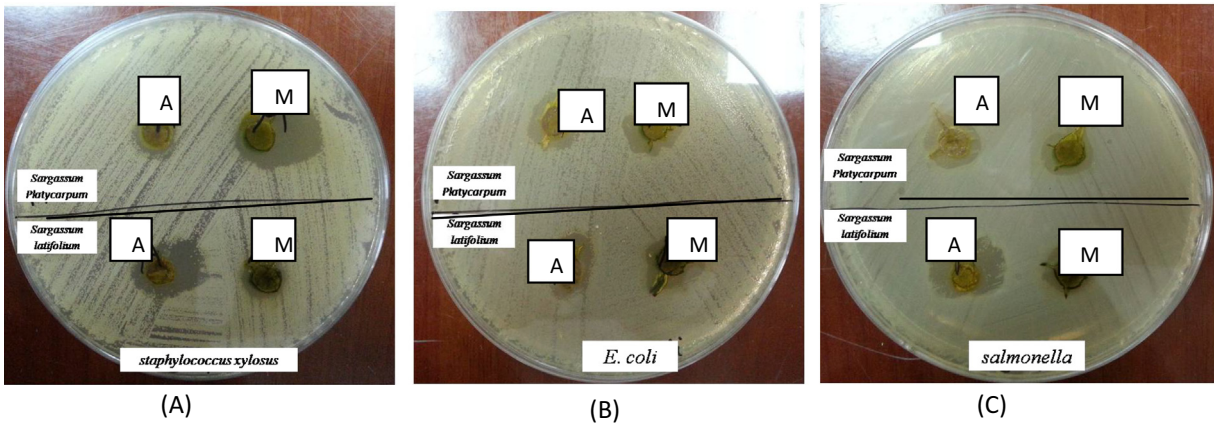


Figure 3 The potent activity of *Sargassum latifolium B* and *Sargassum platycarpum A* against gram positive and gram negative bacteria *S. xylosus*, *E. coli* and *Salmonella* sp. respectively. Both extracts acetone and methanol were active where larger inhibition zones were measured around the wells. A: acetone; M: methanol.

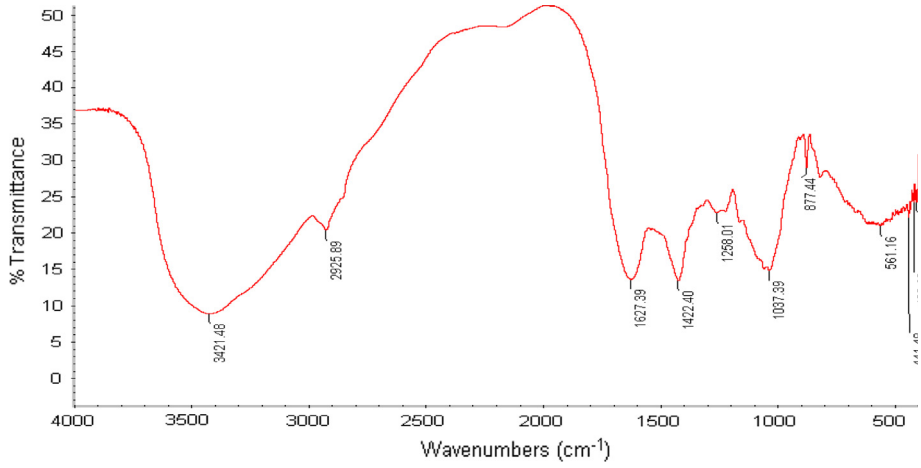


Figure 4 *Sargassum latifolium B* FTIR spectrum showing the highest peaks of phenolic nature responsible for the potent antibacterial activity.

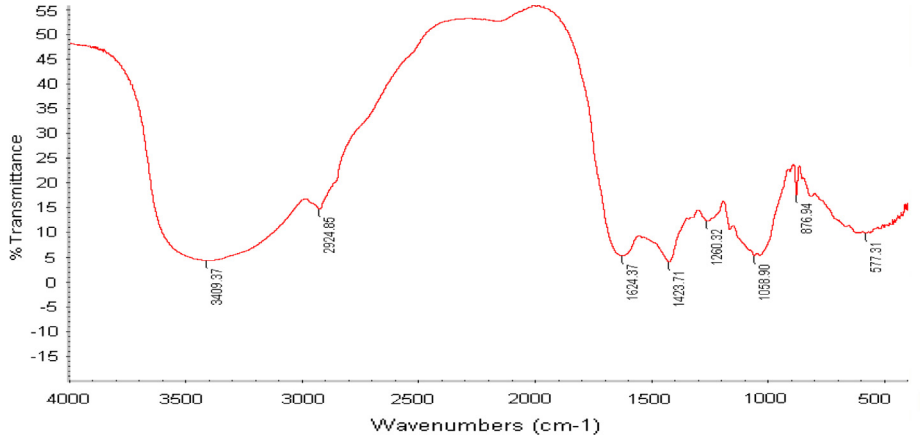


Figure 5 *Sargassum platycarpum a* FTIR analysis indicating phenol to be the major chemical constituent of this algal extract revealed by the highest peak.

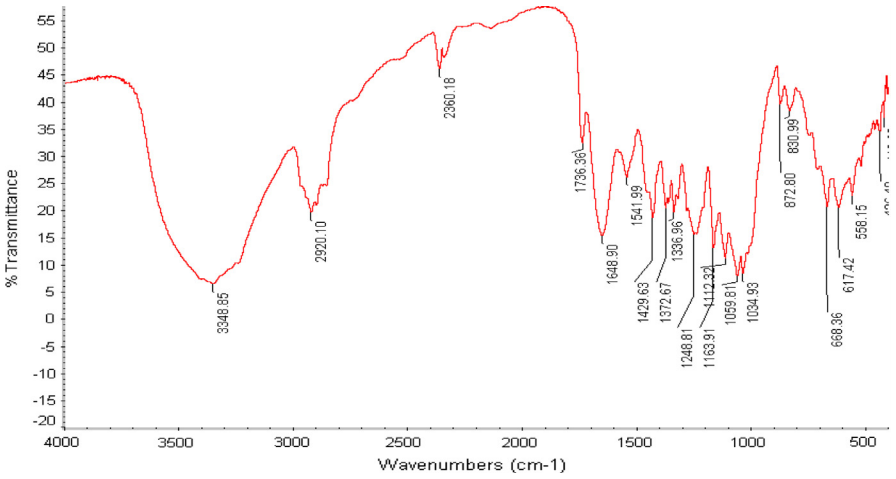


Figure 6 *Cladophora socialis* infra red result revealing the highest peaks of phenolic nature to which the microbial inhibitory effect is being correlated.

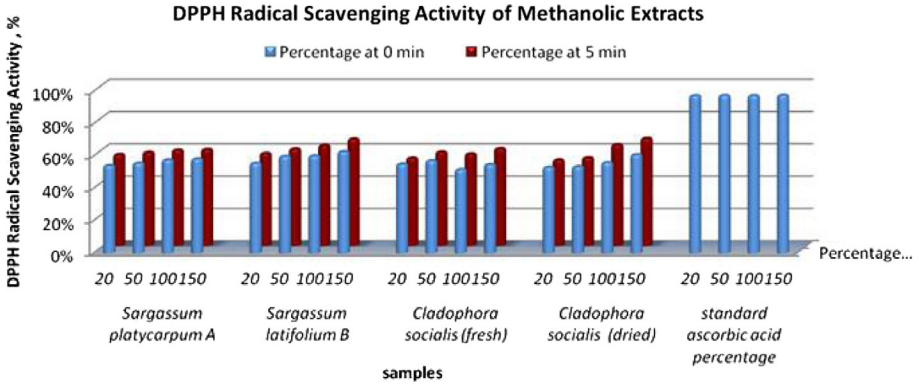


Figure 7 Total antioxidant activity of crude extracts at different concentrations of 20, 50, 100, and 150 (μg/ml).

extracts and the constituents of various algae have been shown to have *in vitro* antibacterial activity against gram positive and gram negative bacteria (Ely et al., 2004; Manivannan et al., 2011).

Phenol compounds suggested to be the major chemical components of algal cells could have an activating or inhibiting effect on microbial growth depending on their constitution and concentration (Alberto et al., 2001; Reguant et al., 2000). The potency of this activity could also depend on the solvents being used as well as on the type of seaweeds, since some bioactive metabolites present in the algal materials have the ability to be soluble in one solvent but not in another (Manilal et al., 2009; Rangaiah et al., 2010; Salem et al., 2011). Cox et al. (2010) revealed that the extraction of antimicrobials from different species was solvent dependent; methanol was a good solvent for extraction of antimicrobials from brown seaweeds; whereas acetone was better for green species. Manilal et al. (2009) and Rangaiah et al. (2010) found that higher antimicrobial activity was yielded with methanolic seaweeds extracts; Viachosi et al. (2001) reported that extracts of the Phaeophyta exhibited the highest level of antibacterial activity followed by the Rhodophyta and then the Chlorophyta. Kandhasamy and Arunachalam (2008) also reported that brown and green algae were more active compared to other groups of marine macroalgae, all these pervious findings supported our results; where higher antimicrobial activity were obtained with methanol brown seaweed *Sargassum* species followed by the green *C. socialis* extracts compared to the acetone extracts of the two seaweeds respectively. Our results indicated as well, that a potent inhibitory effect was precisely observed on gram positive than on gram negative bacteria, except with *Salmonella* sp. which was highly affected by *S. latifolium b* acetone extract.

The difference in susceptibility among tested bacterial isolates could be due to the difference in the cell wall structure and their composition (Taskin et al., 2007). In gram negative, bacteria the outer membrane acts as a barrier to many environmental substances including antibiotics (Tortora et al., 2001). This high inhibition gives a promising indication of developing a potent drug from marine natural sources used in treating human pathogens. Furthermore, the method of extraction, time and place of collecting algal samples, all together, play a critical role in determining the effectiveness of the seaweed extracts in study (Padmini Sreenivasa Rao et al., 1986). Higher antimicrobial activity was revealed with dried seaweeds compared to fresh extracts; lyophilization of algal samples, gives a greater compound extraction; whereas in fresh seaweeds where higher water content is present, produces diluted extracts with lower or even negligible inhibitory activity (Campos-Takaki et al., 1988; Padmini Sreenivasa Rao et al., 1986).

FTIR Chemical analysis revealed that the major constituents particularly in *Sargassum* species were of phenolic nature (Reguant et al., 2000; Alberto et al., 2001) to which both potent antimicrobial and antioxidant activities are associated. This is indicated by highest absorption of hydroxyl amide groups, relative to phenol compounds, ranging between 3421 cm^{-1} (Fig. 4) and 3409 cm^{-1} (Fig. 5) for *S. latifolium B* and *S. platycarpum A* respectively whereas *C. socialis* showed a lower absorption at 3348 cm^{-1}; explaining and correlating the highest antioxidant and antimicrobial activities of *S. latifolium B* followed by *S. platycarpum A* compared to the lowest *C. socialis* activity. However, the mechanism of action still is not clear, further studies should be conducted.

During the study, all three seaweed extracts showed antioxidant activity to various degrees. The scavenging effect of the tested extracts at concentration of 20, 50, 100, and 150 µl on the DPPH radical decreased in the order of: *S. latifolium B* > *C. socialis* (dried) > *S. platycarpum* A > *C. socialis* (fresh) and were 66.3363 ± 0.031373, 65.97175 ± 0.018087, 60.08202 ± 0.03065 and 59.54659 ± 0.007432, respectively. Several studies have demonstrated a highly significant correlation between the phenolic content and the antioxidant activity in seaweeds extracts (Zhang et al., 2007). Higher radical scavenging activity was found in *Sargassum wightii* (brown algae) having the greater phenolic constituent (Duan et al., 2006; Nahas et al., 2007) in agreement with our findings where higher phenolic content and increased antioxidant activity were observed in brown algae *Sargassum* species.

5. Conclusion

Various algal species, variable solvents and different methods of extraction together with the presence of phenols as the major chemical constituent correlated to high antioxidant activity, all of these parameters make seaweeds a potent antimicrobial agents replacing the synthetic chemical products and giving new promising horizons to the pharmaceutical industry. In the present investigation, *S. latifolium B and S. platycarpum A*, collected from the Red Sea and *Cladophora* collected from the Arabian Gulf, were both found to be potent antimicrobial agents mainly against gram positive bacteria; *S. latifolium B* exhibited a unique higher growth inhibition against *Salmonella* sp. All of these tested seaweeds offer opportunities for producing new types of bioactive compounds; however, the mechanism of inhibition and stability of the extracts still are not clear and more studies should be involved.

Acknowledgement

This research project was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges Deanship of Scientific Research, King Saud University.

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