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"Gheorghe Asachi" Technical University of Iasi, Romania

POLYPHENOLS CONTENT, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF SEAWEEDS FROM THE PERSIAN GULF

Shima Ebrahimi[1], Sedigheh Babaei[1]*, Mahmood Naseri[1], Mohammad-Taghi Golmakani[2]

[1]Department of Natural Resources and Environmental Engineering, School of Agriculture, Shiraz University, Shiraz, Iran
[2]Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Iran

Abstract

The Persian Gulf is a unique environment in comparison with other marine bodies because of its high temperatures and salinity levels. It hosts enormous amounts of green, red and brown macroalgae. In this study, phenolic compounds, antioxidant and antibacterial activities were evaluated in *Caulerpa sertularioides*, *Padina distromatica* and *Sargassum boveanum* extracts. Different solvents were used for the measurements. Statistical analyses showed the highest extraction yield was found in *C. sertularioides* aqueous extract. The highest phenolic content was found in *S. boveanum* aqueous extract, consistent with the DPPH IC_{50} results. According to the IC_{50} value of the *S. boveanum* aqueous extract, water is more efficient in extracting phenolic compounds in comparison with n-hexane and ethyl acetate. The HPLC analysis showed different polyphenols such as syringic acid, myricetin and gallic acid. The gallic acid increased in yield when higher levels of solvent polarity were present. Moreover, n-hexane extracts generated the largest inhibition zone against bacterial growth. In conclusion, the *S. boveanum* aqueous extract can be recommended as a safe, natural additive of antioxidant potential for formulating functional foods. Also, n-hexane extracts can have antibacterial effects when incorporated in foodstuffs.

Key words: Caulerpa, macroalgae, natural antioxidant, Padina, polyphenol

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1. Introduction

The global production of seaweed (macroalgae) through aquaculture reached about 34.6 million tons in 2019 based on Food and Agriculture Organization (FAO, 2021). The high amounts of macroalgae aquaculture in the past decade indicates special attention to these aquatics. Seaweeds are rich in minerals, vitamins, amino acids, sulfated polysaccharides, lipids (PUFA), and polyphenols (Lee et al., 2013). These nutritional contents play big roles in the production of antioxidants and antibacterial substances, bioactive compounds in medicine, biological stimulants and probiotics.

The Persian Gulf spans from 24° to 30° 30' N latitude and from 48° to 56° 25' E longitude in Iran. The high temperature and high salinity of its waters make it a strategic and unique habitat in comparison with other water bodies in the world. The Gulf is home to numerous genera of green, red and brown macroalgae such as *Sargassum*, *Gracilaria*, *Acanthophora*, *Laurencia*, *Padina*, *Caulerpa*, *Hypnea* and *Enteromorpha*. Seaweeds are greatly exposed to light and high concentrations of oxygen throughout their life cycle. Light and oxygen stimulate the production of free radicals (Balboa et al., 2013). The antioxidant properties of seaweed are attributed to the presence of phenolic compounds, e.g. flavonoids such as anthocyanins, flavonoids and flavones and non-flavonoids such as phenolic acids, lignin's and acetylenes (Singh et al., 2009). The antioxidant activity of polyphenols is highly dependent on phenol rings which act as electron carriers for proxy, superoxide anions and hydroxyl radicals (Wang et al.,

* Author to whom all correspondence should be addressed: e-mail: Babaei.Sedigheh@gmail.com; S-babaei@shirazu.ac.ir; Phone: +987136138172; Fax: +987144113131

2009). Some of these polyphenol compounds such as gallic acid, coumaric acid, myricetin, catechins, etc., exist in seaweed extracts (López et al., 2011).

Using different solvents for extraction shows that the chemical properties of the solvent and their different structures make each solvent/material system exhibit a different unpredictable extraction behavior (Al-Farsi and Lee, 2008). For example, organic solvents can extract low polarity compounds, while water can extract compounds with higher polarity (Sonibare and Abegunde, 2012).

Studies on *Turbinaria conoides* and *Padina tetrastomatic* (Sachindra et al., 2010), *Atypocaulon scoparium* (López et al., 2011), *Sargassum angustifolium* (Babakhani Lashkan et al., 2012) and *Sargassum siliquastrum* (Cho et al., 2011) indicated that the types of extraction method and solvent have significant effects on antioxidant activity and total phenolic content (TPC) of seaweed extracts. In this regard, similar results have been reported regarding the antibacterial properties of various seaweed extracts (Rosaline et al., 2012; Sujatha et al., 2019). Research showed that active metabolites from seaweed extracts can be used instead of antibiotics and, thus, prevent the growth of bacteria, fungi and viruses.

The present research focused on proximate analysis, polyphenol content, antioxidant and antibacterial properties of two brown macroalgae (*S. boveanum* and *P. distromatica*) and a green macroalga (*C. sertularioides*) from the Persian Gulf. The extractions were carried out by water, n-hexane and ethyl acetate as solvents. It was hypothesized that each solvent can result in a different yield of extract and constituents.

2. Material and methods

2.1. Material

The chemicals being used in this research were, namely, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazine (DPPH), tryptic soy broth culture medium (TSB), plate count agar, n-hexane, ethyl acetate, methanol, ethanol, chloroform, Folin-Ciocalteu, calcium carbonate, gallic acid, sodium carbonate, physiologic serum, sodium hydroxide, sulfuric acid, boric acid, methyl red, hydrochloric acid, tetracycline and cephalexin and 0.45 µ filter. All chemicals, reagents and standards with premium quality were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Two Gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), along with one Gram-positive bacterium *Staphylococcus aureus* (ATCC 25923) were included in this study. They were supplied from the Pathobiology Department of the School of Veterinary Sciences, Shiraz University.

2.2. Equipment

Several types of equipment were used in this research, including an incubator (503, Arian Azmateb, Iran), vortex (Nargha Tajhiz, Iran), microbial hood (JTLVC2X, Jal Tajhiz, Iran), spectrophotometer (T7, PG Instruments Limited, Spain), oven (KM23S, Fara Azma, Iran), autoclave (RT-2 Reyhanteb, Iran), scale (ENTRIS224-1S, Sartorius, Germany), Kjeldahl apparatus (V50, Bakhshi, Iran), centrifuge (K241R, Benchtop Centrifuges, United Kingdom), ELISA (Cytation 3 imaging, Viragene, Iran), freeze dryer (FD-5003-B, LTD, Dena Vacuum Industry Co., Iran), vacuum-evaporator (Fara Azma, Iran), caliper (Tricle Brand, China), HPLC (KNAUER/AZURA system, Germany), heater (RH B2, IKA, Germany), refrigerator (JTCL 300, Jaltajhiz, Iran), milli-Q water purification system (Direct- QUV3, France).

2.3. Preparation of macroalgae

P. distromatica, *S. boveanum* and *C. sertularioides* were purchased from the Fars Science and Technology Park, Abdf company-algae bank, Shiraz, Iran (along the coast of the Persian Gulf). Macroalgae were first cleaned and then washed several times using fresh water. They were dried in an oven at 37-38 °C before being powdered and stored in plastic bags at -20 °C.

2.4. Proximate analysis of macroalgae

Proximate analysis of each alga included evaluations of moisture (934.01), protein (955.04), ash (920.153) and fat (991.36). The evaluations were performed according to the AOAC method (AOAC, 2005).

Total carbohydrate content was calculated according to sugar cubes starch method (Van Beneden et al., 1999).

2.5. Preparation of macroalgae extract

First, 50 g of algae powder was mixed with 500 mL n-hexane or 500 mL ethyl acetate (Babakhani et al., 2012). The containers were shaken using a magnetic stirrer in the dark for 24 hours at room temperature. The extracts were filtered by Whatman No.1 filter paper for the removal of algal particles. They were condensed in a vacuum-evaporator at 37°C. The dried extracts were kept in the freezer at -20°C. To prepare the water extract, 30 g of algae powder was mixed with 600 mL sterile distilled water (1:20 w: v) and was autoclaved at 121 °C for 20 minutes. After cooling down, the mixture passed through a filter paper and then dried using a freeze-dryer. The dried extract was stored at -20 °C for further analysis (Yang et al., 2017). The extraction yield was calculated according to Eq. (1).

$$Extraction\ yield(\%) = [Weight\ of\ dry\ extract(g) / / weight\ of\ dry\ a\lg ae(g)] \times 100 \quad (1)$$

2.6. Total phenolic content

First, 100 µL of different concentrations of the extract were mixed with 750 µL of Folin-Ciocalteu reagent. After being vortexed for 10 minutes, they were mixed with 750 µL sodium carbonate (2%). The reaction solution was stored for 45 minutes in the dark at room temperature.

The absorbance was read at 765 nm by a spectrophotometer (Farvin and Jacobsen, 2013). A calibration curve of gallic acid (ranging from 0.005 to 0.2 mg/ mL) was prepared. The results were calculated by the regression equation of the calibration curve (y = 4.8416x + 0.0749; correlation coefficient R^2 = 0.9926) and expressed as mg GAE/ g DW extract.

2.7. Phenolic compounds by HPLC system

Approximately, 100 mg/mL of each macroalgal solution as condensed extract was centrifuged at 2000 g for 20 min at 4 °C. The supernatant was collected accordingly. Before injection, the supernatants were filtered through a 45 µm nylon syringe filter (López et al., 2011).

The identification and measurement of polyphenolic compounds were performed using high-performance liquid chromatography (HPLC). The samples were injected into the HPLC KNAUER/AZURA system (Germany) equipped with a diode array detector (DAD) and connected to the Claritychrom software. The separation was made by a reverse-phase Agilent Zorbax C_{18} (100 mm-10 µm) analytical column. The flow rate was 1.0 mL/ min at 27 °C. The monitoring was set at (I) 270 nm for gallic acid, protocatechuic acid, catechin, vanillic acid, epicatechin and syringic acid, (II) 324 nm for chlorogenic acid, gentisic acid, caffeic acid, coumaric acid and ferulic acid, and (III) 373 nm for rutin, myricetin and quercetin quantification (López et al., 2011).

2.8. DPPH free radical scavenging activity

DPPH is a violet-colored compound that consists of free radicals due to the presence of phenyl groups in its structure. Its color usually changes to yellow by taking an electron from the antioxidant compound. The DPPH free radical scavenging activities of macroalgal extracts were measured according to a method used by Wang et al. (2009). First, 1 mL of different concentrations (ranging from 0.00001 to 5.5 mg/ mL) of the condensed extracts were prepared in the corresponding solvent. Then, 1 mL of the DPPH solution was added to each sample and the control. The resultant solution was stored in the dark for 30 minutes. Finally, its absorbance was read at 517 nm wavelength by spectrophotometry (T7, PG Instruments Limited, Spain). The percentage of inhibition (%) was obtained according to the (Eq. 2). BHT was used as a synthetic antioxidant.

$$Inhibitory(\%) = [Absorbance\ of\ control - Absorbance\ of\ sample / / Abosrbance\ of\ control)] \times 100 \quad (2)$$

2.9. Antibacterial activity of seaweed extract (Disc diffusion method)

E. coli, *P. aeruginosa* and *S. aureus* were incubated in the TSB medium for 24 hours at 35 °C before being centrifuged. A concentration of 0.5 McFarland (10^7 units / mL) bacteria were prepared by means of a physiologic serum and then placed on an agar plate medium. Then, blank disks were stained with different concentrations of seaweed extracts (2, 5, 10, 20, 50, 100, and 150 mg/ mL) and added to the agar plate. The plates were incubated at 37 °C for 24 hours. The diameter of the inhibition zone (DIZ) was measured using a digital caliper (Usharani et al., 2015). Cephalexin and Tetracycline antibiotics were used as positive controls.

2.10. Statistical analysis

Data analysis of algae extracts characteristic (extraction yield, antioxidant activity, total phenolic content, and antibacterial activity) were carried out using one-way ANOVA with Duncan's multiple range test at a 0.95 level of probability, after investigating the normal distribution (Kolmogorov–Smirnov test) and homogeneity of variance using IBM SPSS Statistics version 22, Armonk, NY, software and *SigmaXL*, Version 8.0. (2019). Kitchener, ON, Canada: SigmaXL. All parameters were tested in triplicates.

3. Results and discussion

3.1. Chemical composition of macroalgae

According to Table 1, the lowest moisture content was detected in *S. boveanum* (80 ± 1%). The carbohydrate (20.6 ± 2.5%) and ash contents (46.2 ± 1.1%) in *P. distromatica* were higher than the contents in *S. boveanum* and *C. sertularioides*. The amounts of lipids were very low (2-5%) in all macroalgae. The proximate analysis of different species of Sargassum, Caulerpa and Padina results in agreement with the present outcome (Azad and Xiang, 2012; Manas et al., 2017). The protein content is known to differ among macroalgae, measuring 9.21% in *Caulerpa racemosa* (Manas et al., 2017), 10.04% in *Padina minor* (Azad and Xiang, 2012) and 6.3% in *Sargassum* sp. (Casas-Valdez et al., 2006). This indicated a low protein content in these macroalgae. The chemical composition of seaweeds is completely dependent on the species, physiological conditions, geographical differences, availability of light and mineral, as well as stress caused by waves and tides (Hafezieh et al., 2014).

Table 1. Proximate composition of different macroalgae (as wet weight (%))

Macroalgae	*Composition (%)*					
	Moisture	***Dry matter***	***Protein***	***Carbohydrate***	***Fat***	***Ash***
Sargassum	80 ± 1^{b}	20 ± 1^{a}	5.1 ± 0.3^{a}	12.3 ± 3^{b}	2.2 ± 1.3^{b}	22.1 ± 0.3^{c}
Padina	84.6 ± 0.2^{b}	15.4 ± 0.2^{a}	6 ± 0.5^{a}	20.6 ± 2.5^{a}	3 ± 1.1^{ab}	46.2 ± 1.1^{a}
Caulerpa	90.5 ± 4.5^{a}	9.5 ± 4.5^{b}	7.5 ± 2.5^{a}	11.8 ± 0.1^{b}	5.7 ± 2.2^{a}	31.3 ± 1.1^{b}

All experiments were performed in triplicate. Values are expressed as mean ± SD. Small letters represent a significant difference between different macroalgae species

3.2. Extraction yield

According to Fig. 1a, the extraction by hot water resulted in a higher yield, as compared to extraction by n-hexane and ethyl acetate, especially in *C. sertularioides* (18.5 ± 1.3%) ($P< 0.05$). Meanwhile, as a matter of algae species, *C. sertularioides* generated a higher extraction yield than *S. boveanum* and *P. distromatica* ($P < 0.05$). In comparison with other extraction methods, aqueous extraction has reportedly resulted in higher yields when extracting from *Sargassum muticum*, *Ulva lactuca*, *Gracilaria vermiculophylla* (Farvin and Jacobsen, 2013), *Sargassum siliquastrum* (Cho et al., 2007), *Padina boergesenii* (Kumar and Sudha, 2012) and *Himanthalia elongate* (Plaza et al., 2010).The ethyl acetate and n-hexane extraction yields of three macroalgae were lower than water extraction yields (Fig. 1a).

A lower extraction yield by organic solvents was also reported in *Sargassum siliquastrum* (0.21%) (Cho et al., 2007) and *Himanthalia elongate* (3.4%) (Plaza et al., 2010) when n-hexane was used. Similar results were obtained in extractions from *Padina boergesenii* (7.9%) (Kumar and Sudha, 2012) and *Enteromorpha prolifera* (5.5%) (Cho et al., 2011) when using ethyl acetate as solvent. A higher extraction efficiency depends on methods and solvent penetrance into the tissues (Chen et al., 2011; Kumar and Sudha, 2012). It was observed that algae species, solvent type and their interaction had significant effects on extraction yields ($P < 0.05$), as suggested previously in the case of *Tunisian Phormidium* (Belhaj et al., 2017).

3.3. Total phenolic content

According to Fig 1, the TPC was higher when using the water extraction method, compared to ethyl acetate and n-hexane extractions. Meanwhile, the effect of algae species showed that TPC is generally higher in *S. boveanum* than in *P. distromatica* and *C. sertularioides*. Based on the results (Fig. 1b), TPC was significantly higher in the extracts when using the water extraction method on *S. boveanum* (28 ± 2.7 mg GAE/ g DW extract), *P. distromatica* (19 ± 3.9 mg GAE/ g DW extract) and *C. sertularioides* (9 ± 0.8 mg GAE/ g DW extract), as compared to n-hexane and ethyl acetate extracts ($P < 0.05$). A high TPC has reportedly occurred in the water extract of *Stypocaulon scoparium* (López et al., 2011), *Fucus vesiculosus* (Wang et al., 2009) and *Sargassum angustifolium* (Babakhani et al., 2012). In the present study, TPC of the ethyl acetate extracts were higher than those of the n-hexane extracts. This is in agreement with previous studies on *Bangia atropurpurea* and *Chlorella vulgaris* (Punampalam et al., 2018). A study on *Gracilaria edulis* and *Padina tetrastomatica* also generated similar results (Sachindra et al., 2010). Cho et al. (2011) reported that solvent polarity enables a higher extraction yield of phenolic compounds in comparison with nonpolar solvents such as n-hexane. A high TPC is the main reason for the high antioxidant activity of some extracts, especially when using polar solvents (O'Sullivan et al., 2011). Algae species and solvent type have reportedly had significant effects on the TPC of *Enteromorpha intestinalis* macroalgae (Hashem Dabbaghian et al., 2016).

3.4. Polyphenol compounds

The amount and type of polyphenols in each of the three macroalgae differed, especially when extracted by three different solvents (Table 2). The results showed that gallic acid, coumaric acid and myricetin could be successfully isolated in most of the extracts. The amounts of gallic acid (1086.5 ± 0.2 mg/ 100 g DW algae) and myricetin (1375.3 ± 0.1 mg/ 100 g DW algae) in the water extracts of *S. boveanum* and *C. sertularioides* were higher than in other treatments. The highest amounts of coumaric acid were observed in *S. boveanum* and *P. distromatica* water extracts, respectively. Vanillic acid, epicatechin, chlorogenic, gentisic acid and caffeic acid were observed in lower amounts, as compared to other phenolic acids in all treatment groups. According to Table 2, ethyl acetate was able to extract a wider array of polyphenol compounds from all three macroalgae.

Few reports are available on the identification of polyphenols in algae compounds by HPLC techniques. However, polyphenolic compounds such as flavonols and catechins are well-known in different algae (Farvin and Jacobsen, 2013; López et al., 2011). As observed in Table 2, the polarity of solvent can change its capacity to dissolve special polyphenol compounds.

In this study, aqueous extract as a polar solvent gave an increase in the content of polyphenols especially gallic acid (Table 2). This is in good agreement with previous findings about *Stypocaulon scoparium* water extract (López et al., 2011).

Different factors, such as geographic origin, genetic, harvest time, plant organ and extraction methods could change the phenolic composition (Bettaieb Rebey et al., 2012).

3.5. DPPH free radical scavenging

Seaweeds are a rich source of phenols, which differ in terms of structure and number of hydroxyl groups. Depending on the polarity and molecular characteristics of phenolic compounds, they have different antioxidant activities (China et al., 2012; Wang et al., 2009). Hydroxyl groups in phenolic compounds reduce intermediates by giving electrons or binding to the active site of tyrosinase (Nirmal and Benjakul, 2009). According to the present results, there is a positive relationship between the free radical scavenging activity and TPC in different macroalgae extracts ($y = -71304x^2 + 4290.6x + 22.45$; correlation coefficient $R^2 = 0.5245$), whereas a variation in phenolic compounds cannot increase antioxidant activity (Table 2). Previous studies have shown a significant relationship between antioxidant capacity and phenolic content in algae extracts *of Stypocaulon scoparium* (López et al., 2011), *Enteromorpha prolifera* (Cho et al., 2011), *Bangia atropurpurea* (Punampalam et al., 2018) and *E. intestinalis* (Hashem Dabbaghian et al., 2016).

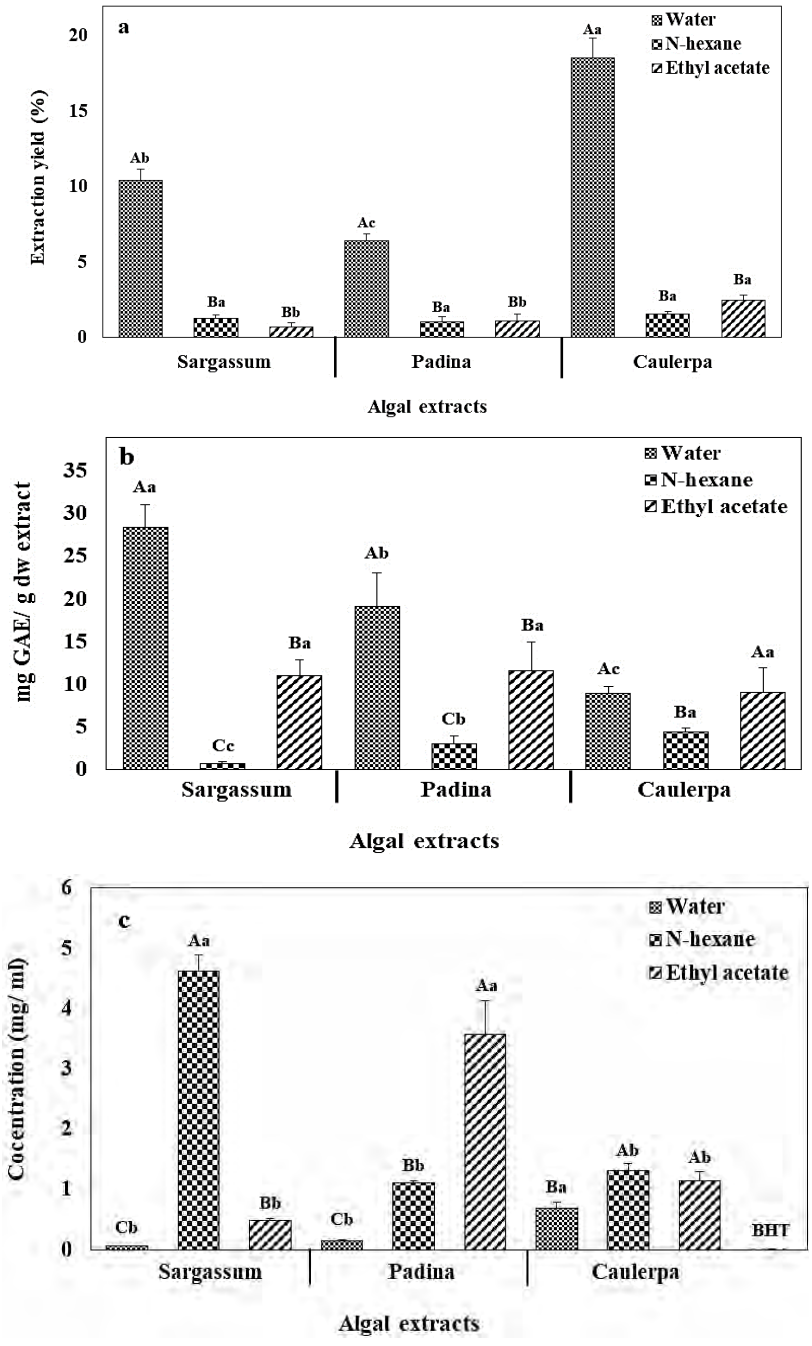

Fig. 1. a) Extraction yields, b) total phenolic content (TPC), c) IC50 of DPPH radical scavenging activity of different macroalgae extracts after being obtained by various solvents. The capital letters represent a significant difference between different solvents as per macroalgae species. Small letters represent a significant difference between different macroalgae species as per solvent

Table 2. Polyphenol contents of Sargassum, Padina, and Caulerpa extracts, as determined by HPLC (mg/ 100 g DW algae)

Polyphenols	***Sargassum***			***Padina***			***Caulerpa***		
	Water	***N-hexane***	***Ethyl acetate***	***Water***	***N-hexane***	***Ethyl acetate***	***Water***	***N-hexane***	***Ethyl acetate***
Gallic acid	1086.5±0.2	-	24.8±0.1	-	76.6±0.0	40.7±0.1	766.6±0.2	84.4±0.1	92.9±0.01
Protocatechuic	342.8±0.1	-	-	-	-	-	-	-	-
Catechin	340.1±0.0	-	-	-	-	36.2±0.1	-	-	-
Vanillic acid	-	-	-	-	-	-	-	-	-
Epicatechin	-	-	-	-	-	-	-	-	-
Syringic acid	358.7±0.2	55.2±0.0	29.6±0.0	-	-	36.3±0.1	-	-	204.0±0.1
Chlorogenic acid	-	-	27.1±0.0	-	-	-	-	-	-
Gentisic acid	-	-	26.4±0.0	-	-	36.2±0.0	-	-	-
Caffeic acid	-	-	-	-	-	-	-	-	-
Coumaric acid	670.1±0.0	46.8±0.0	-	220.2±0.1	38.6±0.1	36.1±0.0	-	-	81.8±0.0
Ferulic acid	-	-	27.5±0.1	-	-	-	-	-	-
Rutin	-	-	29.4±0.0	-	-	-	-	50.9±0.1	81.1±0.0
Myricetin	795.5±0.0	-	27.0±0.0	-	33.6±0.0	81.3±0.0	1375.3±0.1	50.9±0.0	81.2±0.0
Quercetin	-	137.4±0.1	77.4±0.1	440.9±0.2	65.6±0.0	-	-	104.8±0.1	-

Mean ± SD of three measurements

Antioxidant properties may be more dependent on TPC, the total amounts of each phenolic compound and non-phenolic compound in an extract. Moreover, previous research suggests that high antioxidant activity in seaweeds indicate the presence of other non-phenolic compounds and polysaccharides, alginates, fucans and laminarins (Cox et al., 2010) which are abundant in brown seaweeds (Ahn et al., 2004).

The IC_{50} value of the water extract was lower than that of the n-hexane and ethyl acetate extracts (Fig. 1c). According to Fig. 1c, the IC_{50} value of brown macroalgae (*S. boveanum* and *P. distromatica* water extracts) were 0.06 and 0.15 mg/ mL, respectively ($P \geq 0.05$), being lower than the value observed in the *C. sertularioides* water extract (0.7 mg/ mL) ($P < 0.05$). In previous studies, the IC_{50} values of water extracts of macroalgae were reportedly 1 mg/ mL in *Polysiphonia fucoides*, 1 mg/ mL in *Fucus vesiculosus* (Farvin and Jacobsen, 2013), 103.73 mg/ mL in *Ulva lactaca* and 15.05 mg/ mL in *Caulerpa racemosa* (Yangthong et al., 2009).

Polar solvents such as methanol and ethanol are highly able to extract carotenoids, flavonoids, and vitamin C (Farvin and Jacobsen, 2013). This may lead to a higher antioxidant potential. Moreover, Rupérez et al. (2002) reported an algal species (*Fucus vesiculosus*) from which sulfated polysaccharides were extracted by water and showed the highest potential of antioxidant activity by a ferric reducing antioxidant power assay (FRAP). Therefore, water can extract high molecular weight polysaccharides, proteins and peptides which have antioxidant properties.

3.6. Antibacterial activity

The antibacterial activities of algal extracts are shown in Table 3. The bacterial growth decreased in response to higher algal extract concentrations, while some extracts had no significant effect on bacterial growth. It was shown that the inhibition zone of n-hexane-assisted extraction was higher than that of water- and ethyl acetate-assisted extractions (Table 3). Moreover, as a matter of algae species, *S. boveanum* had the highest antibacterial activity ($P < 0.05$). The broadest inhibition zone was caused by n-hexane-assisted extracts ($P < 0.05$), especially by the *S. boveanum* n-hexane extract against *E. coli* growth (15.1± 0.1 mm). In contrast, *S. boveanum* and *P. distromatica* water extracts had no antibacterial activity against *E. coli* and *S. aureus* – consistent with the effect of the water extract of *Sargassum glaucescens* (Peymani et al., 2013) on *E. coli* growth. Therefore, it is likely that the antibacterial compounds in the macroalgae are non-polar compounds (Kandhasamy and Arunachalam, 2008) which can dissolve in n-hexane.

The n-hexane (DIZ = 19 ± 2.9 mm) and ethyl acetate (DIZ = 9.2 ±1 mm) extracts of *P. distromatica* showed an antibacterial effect on *P. aeruginosa* in 150 mg/ mL concentration. Rosaline et al. (2012) showed that n-hexane and ethyl-acetate extracts of *Padina pavonica* have no antibacterial activity against *P. aeruginosa* growth. This discrepancy between the results of similar research can be due to the secondary metabolite activities of seaweed species (Golchin Manshadi et al., 2015) and the composition of saturated and unsaturated fatty acids (Al-Saif et al., 2014). In general, Al-Saif et al. (2014) found that organic solvents have a much higher efficiency than water in extracting antibacterial compounds from seaweeds.

4. Conclusions

The extraction yield, TPC, HPLC and IC50 values of DPPH free radical scavenging show hot water extracts are more efficient in isolating polar bioactive molecules and polyphenols, especially gallic acid. Moreover, *S. boveanum* performed better than other algal species with regard to these parameters.

Table 3. Mean diameter (mm) inhibition zone of macroalgal extracts against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* growth

Bacteria	*Concentration (mg/mL)*	*Sargassum*			*Padina*			*Caulerpa*		
		Water	*N-hexane*	*Ethyl acetate*	*Water*	*N-hexane*	*Ethyl acetate*	*Water*	*N-hexane*	*Ethyl acetate*
Escherichia coli	2	NIZ	9.1 ± 0.8	NIZ	NIZ	NIZ	8.2 ± 0.3	8.2 ± 0.2	9.1 ± 1	8.3 ± 1.2
	5	NIZ	8.8 ± 0.1	NIZ	NIZ	NIZ	9.1 ± 0.3	8.7 ± 0.6	9.8 ± 0.6	8.6 ± 0.1
	10	NIZ	9.2 ± 0.4	NIZ	NIZ	NIZ	9.3 ± 0.6	9.5 ± 2.3	9.4 ± 0.6	8.1 ± 0.8
	20	NIZ	10.7 ± 0.5	9.5 ± 0.99	NIZ	NIZ	8.6 ± 0.3	8.9 ± 1.4	11.3 ± 0.7	7.8 ± 0.7
	50	NIZ	11.9 ± 2.6	8.8 ± 0.2	NIZ	NIZ	9.3 ± 0.6	8.8 ± 1.1	11.4 ± 0.3	8.9 ± 0.04
	100	NIZ	13.9 ± 2.2	9.4 ± 0.01	NIZ	NIZ	9.4 ± 0.6	9.2 ± 0.9	12 ± 0.7	8.6 ± 0.2
	150	NIZ	15.1 ± 0.1^{Aa}	10.2 ± 0.4^{Ba}	NIZ	NIZ	10 ± 1^{Aa}	9.5 ± 2.3^{Ba}	13 ± 1.8^{Ab}	9.2 ± 0.5^{Ba}
Staphylococcus aureus	2	NIZ	8.7 ± 0.5	NIZ	NIZ	15.8 ±1.3	7.9 ± 0.4	8.5 ± 0.3	9.7 ± 0.8	8.4 ± 1.2
	5	NIZ	9.1 ± 0.1	NIZ	NIZ	15.9 ± 1.7	8.6 ± 0.4	9.7 ± 1.9	9.9 ± 2.1	8.6 ± 0.3
	10	NIZ	9.7 ± 0.3	8.7 ± 0.4	NIZ	14.8 ± 1.8	8 ± 1.2	9 ± 1	11.4 ± 1.9	8.3 ± 1
	20	NIZ	9.8 ± 0.9	9.1 ± 0.3	NIZ	18.4 ± 0.9	8.3 ± 0.3	9.3 ± 1.9	11.1 ± 1.2	8.8 ± 1.1
	50	NIZ	9.6 ± 0.6	8.4 ± 0.4	NIZ	16.2 ± 2.8	8.7 ± 0.0	8.1 ± 0.6	11.4 ± 1.3	9.3 ± 0.3
	100	NIZ	10.3 ± 0.4	8.6 ± 0.6	NIZ	14.5 ± 3.6	8.8 ± 0.1	9.2 ± 2.3	12.3 ± 1.3	9.3 ± 0.3
	150	NIZ	14.6 ± 1.1^{Ab}	10 ± 0.3^{Ba}	NIZ	18.4 ± 1.4^{Aa}	9 ± 0.8^{Ba}	9.1 ± 0.9^{Ba}	13 ± 1.3^{Ab}	9.7 ± 0.04^{Ba}
Pseudomonas aeruginosa	2	8.7 ± 0.1	9.1 ± 0.8	8.9 ± 0.3	8.6 ± 0.3	NIZ	7.9 ± 0.8	8.3 ± 1.5	NIZ	8.8 ± 0.2
	5	9.9 ± 1.2	10.3 ± 1.5	8.4 ± 0.4	9.7 ± 1	NIZ	8.4 ± 0.3	8.9 ± 1.7	NIZ	8.5 ± 1.3
	10	10.4±0.4	9.8 ± 0.9	8.6 ± 0.3	9.6 ± 0.2	NIZ	8.1 ± 0.1	8.3 ± 1.3	NIZ	8 ± 0.9
	20	10.8±0.8	12.4 ± 0.4	8.8 ± 0.2	10.5 ± 1.3	NIZ	8.4 ± 0.4	8.2 ± 0.4	9.3 ± 1.2	8 ± 0.06
	50	12.2±0.4	11.4 ± 0.5	8.5 ± 0.2	9.9 ± 0.6	11.7 ± 0.9	8 ± 0.4	8.6 ± 0.4	9.5 ± 1.3	8 ± 0.06
	100	13.2±0.8	11 ± 1.1	9 ± 0.6	11 ± 0.1	15.9 ± 1.4	8.8 ± 1.3	9 ± 0.9	9.3 ± 1.6	8.4 ± 0.0
	150	14 ± 1.1^{Aa}	12.5 ± 0.1^{Bb}	10.1 ± 0.3^{Ca}	10.7 ± 1^{Bb}	19 ± 2.9^{Aa}	9.2 ± 1^{Ba}	10 ± 1^{ABb}	11 ± 1.2^{Ab}	9.1 ± 0.3^{Ba}

All experiments were performed in triplicate. Values are expressed as mean ± SD. NIZ: No Inhibition Zone. Capital letters represent significant differences between different solvents as per macroalgae species and small letters represent significant differences between different macroalgae species as per solvent

N-hexane extracts showed the strongest inhibitory effects on bacterial growth, probably due to the antibacterial property of semi or non-polar compounds.

The *S. boveanum* water extract can be considered as a source of bioactive compounds for the production of functional foods, although its interaction with food components should be investigated in future research. Moreover, n-hexane extracts can be used as antibacterial agents in foods intended for storage, although their direct effects on such foods need to be examined.

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