

Research Article



Radical Scavenging Activity and Phenolic Contents of Brown Seaweeds Harvested from the Coast of Sidi Bouzid (El Jadida, Morocco)

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ABSTRACT

The aim of this work is to quantify the total phenolic compounds, flavonoids and condensed tannins in different extracts of seven brown seaweeds collected from the coast of Sidi Bouzid El Jadida and to evaluate their radical scavenging activity. The antioxidant activity of the thirty five organic extracts obtained from seven seaweeds species was evaluated by the DPPH radical scavenging method. Butylated hydroxytoluene (BHT) was used as a standard antioxidant. The quantification of total phenolic compounds was carried out, according to the Folin-Ciocalteu method. The quantitative estimation of flavonoids was assessed by the method of aluminium trichloride and condensed tannins by the method of iron trichloride. Higher total phenolic content was observed in ethyl acetate extract of *Bifurcaria bifurcata*, among all seaweeds extracts, dichloromethanolic extract of *Bifurcaria bifurcata* showed higher condensed tannins and ethyl acetate extract of *Cystoseira tamariscifolia* showed higher flavonoids content. The highest DPPH scavenging activity was found in methanolic extract of *Bifurcaria bifurcata* and in methanol water extract of *Fucus spiralis*. The results obtained indicate that *Bifurcaria bifurcata*, *Fucus spiralis* and *Cystoseira tamariscifolia* could be considered as a potential source of bioactive compounds.

Keywords: Brown algae, total phenolic compounds, flavonoids, condensed tannins, DPPH radical scavenging activity.

INTRODUCTION

Numerous reports show that macroalgae collected in coast of Morocco present a broad range of biological activities such as antibacterial¹⁻⁴, anti-inflammatory⁵⁻⁷ Cytotoxic⁸ and antioxidant⁹. However, reports on the antioxidant properties of seaweed extracts from Morocco are very limited.

Interest in natural antioxidants, in relation to their therapeutic properties, has increased considerably. Scientific research in various specialties has been developed for the extraction, quantification and identification of these compounds from several natural substances, such as medicinal plants and agri-food products¹⁰. Numerous studies have revealed the antioxidant activity in different seaweeds over the world¹¹⁻¹⁴.

Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signalling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species¹⁵.

Reactive oxygen species (ROS) refers to an array of metabolites derived from molecular oxygen. These cellular renegades damage DNA, proteins, lipids and corrode cell membrane. Such molecular mayhem plays a major role in the development of various diseases such as cancer, atherosclerosis and respiratory ailments¹⁶.

Antioxidant activity has the subject of intensive investigations due to the ever-increasing demand by the food and pharmaceutical industries to develop natural bioactive anti-aging and anti-carcinogenic compounds

that demonstrate measurable health benefits. The aim of this study is to evaluate radical scavenging potential and phenolic content of seven moroccan brown algae harvested from coastlines of Sidi Bouzid El Jadida, Morocco.

MATERIALS AND METHODS

Plant materiel

Brown algae were collected manually, during the low water spring tides, along the coast of El Jadida (33°-33°16'09"N, 8°30'-8°45'W), in April 2015. The samples were washed three times in tap water to remove any attached salt, epiphytes and sand, then rinsed carefully with fresh distilled water, air dried, ground into powder material and stored before extraction.

The algae investigated were identified from fresh species as: *Sargassum muticum*, *Fucus spiralis*, *Cystoseira tamariscifolia*, *Sargassum vulgare*, *Cystoseira humilis*, *Bifurcaria bifurcata* and *Laminaria digitata*.

Preparation of extracts

For each species, the prepared algae powder is extracted in a five solvent: methanol (M), dichlorometane (DC), ethyl acetate (EA), methanol/water (MW) (60/40) and the dichloromethane/methanol (DC/M) (50/50) at a rate of 1 g of alga powder/5 ml of solvent during 72 hours at ambient temperature according to the extraction protocol described by Caccamese and Azzolina.¹⁷ The extracts are then filtered on Whatman paper N°1 and then evaporated in a rotary evaporator. The dry extracts obtained are stored at 4 °C. For methanol water extract



the result evaporated was lyophilised until used for the biological tests.

Phytochemical screening of algal extract

Analysis was used to determine the content: total phenolic compounds, flavonoids and condensed tannins.

Total concentration of phenolic in the crude extract was determined by a modification of the method of Bray and al.¹⁸ 100 µL of test solutions (samples or standards) were added to 2mL of Na₂CO₃ (0,2%), after 2 min, 100 µL of Folin-Ciocalteu diluted by half of the methanol reagent were added and allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm on spectrophotometer. The blank consisted of all reagents and solvents without test compounds or standard. The standard was gallic acid prepared in concentrations of 0.01 mg/ml to 1.0 mg/ml. The phenolic concentrations were determined by comparison with the standard calibration curve.

The amounts of condensed tannins are estimated using the Vanillin method¹⁹. 50 µL of the algal extract is added to 1500 µL of the Vanillin/methanol solution (4%) and the contents are mixed. Then, 750 µL of the hydrochloric acid (HCl) 1N is added and allowed to react at room temperature for 20 minutes. Absorbance was measured at 550 nm on spectrophotometer. The concentration of the tannins is estimated in milligrams (mg) equivalents of catechin per gram (g) of extract.

The determination of the flavonoids content was carried out according to the method described by Dehpour and al.²⁰: 500 µL of each extract are added to 1500 µL of methanol (95%), 100 µL of AlCl₃ (10 %), 100 µL of sodium acetate 1 M and 2.8 mL of distilled water. The mixture is stirred and then incubated in the dark and at room temperature for 30 minutes. The blank is made by replacing the extract with methanol (95%). The absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in mg quercetin equivalent/g extract by reference to the calibration curve of quercetin.

1,1diphenyl,2,picrylhydrazyl (DPPH) scavenging assay

The scavenger effect of the free radical DPPH was measured according to the protocol described by Bounatirou and al.²¹ Different concentration of the samples studied was used at the same time as the synthetic antioxidant BHT (Butylated hydroxytoluen). The DPPH solution was prepared by solubilising 2.4 mg of DPPH in 100 mL of methanol. 100 µL of each extract as well as the positive control are added to 2 mL of the DPPH solution. The mixture is left in the dark for 30 min at room temperature and then absorbance is measured at 517 nm. The radical scavenging activity was estimated according to the equation:

Percentage inhibition

$$(\%) = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / (\text{Absorbance of control})] \times 100}{}$$

All in vitro experimental results are presented as mean ± standard deviation of the mean from three parallel replicates.

RESULTS AND DISCUSSION

Total phenolic content

The present study indicated that the total phenolic content was different between algal species and in terms of solvents used for extraction. The amount of total phenolic varied from 3.18±0.821 to 29.33±0.258 mg GAE/g of extract (Fig.1). According to the recent study, the differences in the total phenolic content of different solutions may be due to the relative solubility of such compounds in these extracts. The solubility of polyphenols depends on the type of solvent uses, their polymerisation degree, their interaction with other constituents and the formation of insoluble complexes²².

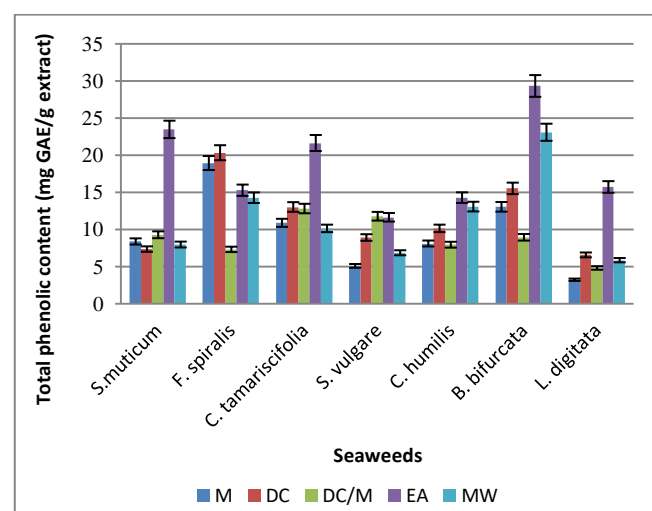


Figure 1: Total phenolic content of different extracts of algae studied.

M: methanol; DC: dichloromethane; DC/M: dichloromethane/methanol (50/50); EA: ethyl acetate; MW: methanol/water (60/40). Each point represents the mean of three replicates.

Bifurcaria bifurcata, *Cystoseira tamariscifolia* and *Sargassum muticum* extracted by ethyl acetate contained higher amount of polyphenols than other brown algae, respectively 29.33±0.258; 23.47±0.417 and 21.63±0.270 mg EAG/g extract (Fig. 1).

In the case of *Fucus spiralis*, the high values of phenolic content were found in dichloromethane (20.32±0.030 mg EAG/g extract). As reported by the earlier studies, all the *Fucus* species had high total phenolic content.²³⁻²⁴ These results are consistent with those reported by Chernane and al.²⁵ who found that dichloromethane fraction of *Fucus spiralis* and ethyl acetate fraction of *Bifurcaria bifurcata* showed higher phenolic content (29.79 and 24.44mg GAE/g extract) when compared to the other solvent fractions.

Previous studies on brown seaweeds have reported the ability of these organisms to produce antioxidant compounds. In this regard, Chernane and al.²⁵ brown



seaweeds (*Bifurcaria bifurcata* and *Fucus spiralis*) and green seaweeds (*Enteromorpha intestinalis* and *Ulva rigida*) indicated the highest amount of polyphenols in brown algae than in the green ones. Brown algae have also revealed more antioxidant power than red algae.²⁶

The highest values of the content of polyphenols were recorded for most of the algae who were extracted by the acetate of ethyl (fig.1). Similarly, Duan and al.²⁷ observed higher phenolic content (73.7 GAE/g extract) in ethyl acetate soluble fraction of red algae (*Polysiphonia urceolata*). These results showed that the best solvent used for the extraction of polyphenols from brown algae was the ethyl acetate.

Flavonoid content

Amount of the flavonoids was very high in ethyl acetate extract for all species of algae (Fig. 2), with a high value observed in extract of *Cystoseira tamariscifolia* (13.22±0.423 mg EQ/g extract).

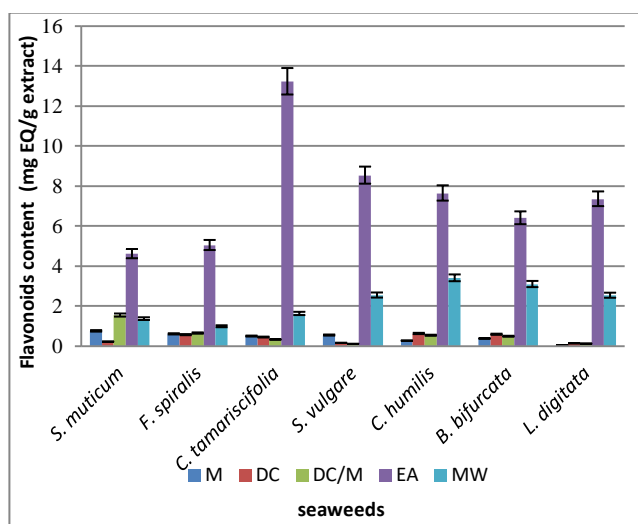


Figure 2: Flavonoids content of different extracts of algae studied.

M: methanol; DC: dichloromethane; DC/M: dichloromethane/methanol (50/50); EA: ethyl acetate; MW: methanol/water (60/40). Each point represents the mean of three replicates.

On other hand, methanol/water extract has been found to be rich in flavonoids with 2.53±0.12; 2.54±0.097; 3.09±0.054 and 3.40±0.043 mg EQ/g of extract respectively in *Laminaria digitata*, *Sargassum vulgare*, *Bifurcaria bifurcata* and *Cystoseira humilis*. However, very low value of flavonoid compounds was observed in the methanolic extract of *Laminaria digitata* (0.03±0.006 mg EQ /g extract). Many studies have confirmed that the flavonoid yield is influenced by the polarity of the organic solvent and its capacity to have a good solubility of flavonoid hydroxyl groups²⁸.

Condensed tannins

The levels of condensed tannins in the seaweed extracts were ranged from 0.73±0.021 mg EC/g extract in *Laminaria digitata* to 9.42±0.011 mg EC/g extract in *Bifurcaria bifurcata*. The maximum content was recorded

in dichloromethanolic extract and the minimum in methanolic water extract (Fig.3).

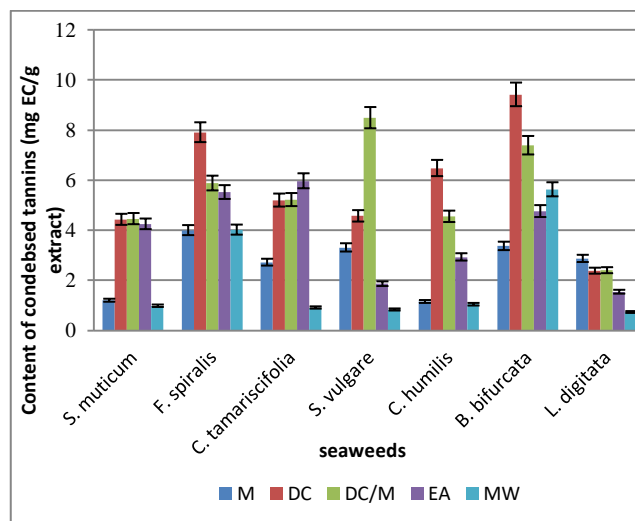


Figure 3: Condensed tannins content of different extracts of algae studied. M: methanol;

DC: dichloromethane; DC/M: dichloromethane/methanol (50/50); EA: ethyl acetate; MW: methanol/water (60/40). Each point represents the mean of three replicates.

Ethyl acetate extract gave the highest yield of total polyphenols, while dichloromethane gave the highest yield of tannins. The highest yields of flavonoids were obtained with ethyl acetate extract.

Radical scavenging activity

The radical scavenging activity using DPPH has been reported in percentage inhibition (%) (Fig.4). DPPH is a stable free radical widely used for evaluating natural antioxidants, algae, algal products (29-30-31) or other natural compounds²⁷.

DPPH radical scavenging activity differed between the algae species tested. *Bifurcaria bifurcata* and *Fucus spiralis* were the ones with the highest values (Fig.4). In this test, *Bifurcaria bifurcata* exhibited the highest percentage of inhibition (81.36±0.067%) for the methanol extract, which is also the most rich in phenolic compounds. Similar results have been reported by Chiboub and al.³² in the ethyl acetate extract obtained from the brown algae *Cystoseira sedoides* who is the richest in phenolic compound.

As presented in Fig.4, methanol extract from *Bifurcaria bifurcata* showed a very important scavenging activity (81.36±0.067%) followed by *Cystoseira tamariscifolia* (50±1.033%) and *Fucus spiralis* (37.74±0.789%). Dichloromethanolic extract of *Cystoseira tamariscifolia* showed an important scavenging activity (56.8±1.064%), followed by *Bifurcaria bifurcata* (52.56±1.112%) and *Fucus spiralis* (28±0.615%).

Dichloromethanolic and methanolic mixture extract from *Cystoseira tamariscifolia* showed an important scavenging activity (63.32±0.818%), followed by *Bifurcaria bifurcata* (50.19±0.921%) and *Sargassum vulgare* (46.18±0.358%).

Ethyl acetate extract from *Bifurcaria bifurcata* showed an important scavenging activity (51.35±0.420%) followed by *Fucus spiralis* (50.32±0.211%) and *Cystoseira tamariscifolia* (49.52±0.550%).

The maximum scavenging activity of *Fucus spiralis* (78.28±0.455%) was observed with aqueous methanolic extract, followed by *Sargassum vulgare* (53.11±1.601%) and *Cystoseira tamariscifolia* (48.16±0.791%). All solvent extract of *Laminaria digitata* showed relatively less scavenging potentials (less than 36%).

Depending upon their solubility and polarity, different solvents shows different DPPH radical scavenging activity.

Earlier studies have shown that a change in extractant polarity influences its efficacy to extract a specific group of antioxidant compounds³³⁻³⁶. Concentration dependency of radical scavenging activity was confirmed for different species of algae studied. The antioxidant activity increased with increasing concentration in all the extract²⁵.

Table 1 shows that the best values (EC₅₀) were found in methanolic extract of *Bifurcaria bifurcata* (234.1±0.20 µg.mL⁻¹) and in methanol water extract of *Fucus spiralis* (334.85±0.12 µg.mL⁻¹).

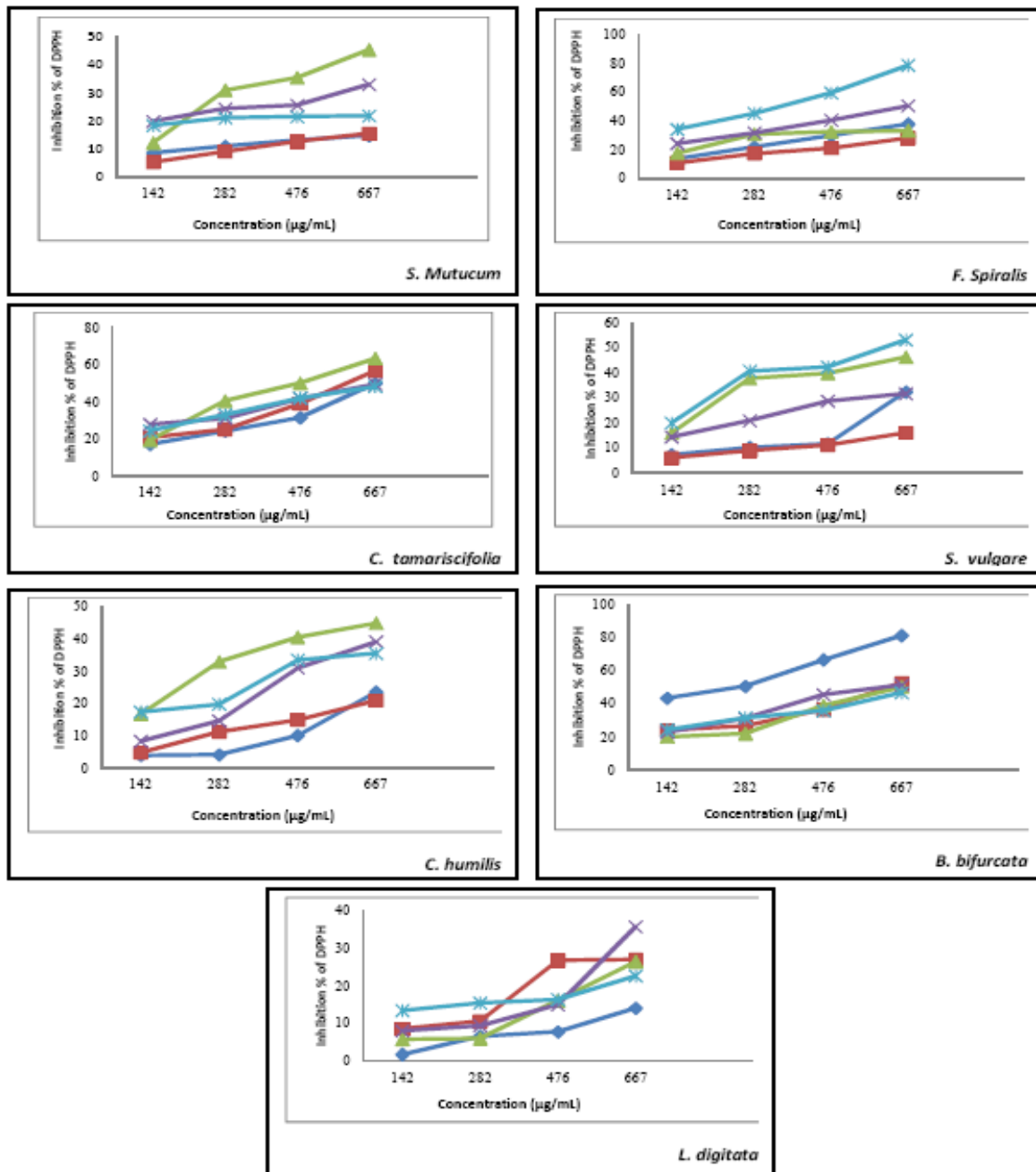


Figure 4: DPPH radical scavenging activity (% of inhibition) of different algae species tested.

M: methanol; DC: dichloromethane; DC/M: dichloromethane/methanol (50/50); EA: ethyl acetate; MW: methanol/water (60/40). Each point represents the mean of three replicates.

Table 1: Evaluation of the antioxidant activity in the DPPH assay (EC₅₀) of seaweeds extracts. Each value represents the mean of three replicates.

Seaweed Solvent	EC ₅₀ (µg/mL)				
	M	DC	DC+M	EA	MW
<i>S. muticum</i>	>1000	>1000	732.7±0.23	>1000	>1000
<i>F. spiralis</i>	950±0.28	>1000	>1000	667.34±0.20	334.85±0.12
<i>C. tamariscifolia</i>	712.86±0.21	605.55±0.32	482.05±0.22	681.16±0.14	683.11±0.16
<i>S. vulgare</i>	>1000	>1000	700±0.15	>1000	597.81±0.13
<i>C. humilis</i>	>1000	>1000	722±0.11	821.69±0.18	>1000
<i>B. bifurcata</i>	234.10±0.20	678.14±0.10	679.29±0.15	618.03±0.19	781.75±0.12
<i>L. digitata</i>	>1000	>1000	>1000	>1000	>1000

All the EC₅₀ values obtained were however relatively lower than that of standard compound (BHT: EC₅₀=11.5±0.19 µg/mL). Our results were in agreement with those found by Bouhlal and al.⁹ (BHT: EC₅₀=11.43±0.17µg/mL). According to the radical scavenging capacities related by EC₅₀ values, in most studies the standard antioxidants BHA, BHT, ascorbic acid and tocopherol were superior than the algal extracts³¹.

A moderate activity was found for *Cystoseira tamariscifolia* and *Sargassum vulgare* extract, which neutralized 50% of free radicals at the concentration 482.05±0.22 and 597.81±0.13 µg/mL, respectively. A low activity was observed in *Sargassum muticum* (EC₅₀: 732±0.23 µg/mL) and *Cystoseira humilis* (EC₅₀: 722±0.11 µg/mL), in this study the EC₅₀ values were ranged from 234.10±0.20 to 950±0.28 µg/mL for DPPH radical scavenging assay for the brown seaweeds.

According to the earlier reports, brown algae in general have a better DPPH radical scavenging effect than red seaweeds, and *Fucus* species are the best DPPH scavengers²³⁻²⁴. Furthermore, fucoxanthin, which is found in brown seaweed have been reported to be the responsible of the radical scavenging activity³⁷.

The scavenging effect of the tested extracts at concentration of 142, 285, 476 and 667 µg/mL decreased in the order of: *Bifurcaria bifurcata* methanol extract (81.36±0.067%) > *Fucus spiralis* aqueous methanolic extract (78.28±0.455%) > *Cystoseira tamariscifolia* dichloromethane/methanolic extract (63,32±0,818%) > *Sargassum vulgare* methanol water extract (53.11±1.601%) (Fig. 4).

Methanol extract of *Sargassum muticum* and *Laminaria digitata* and dichloromethanolic extract of *Sargassum vulgare* showed a low activity (less than 17%) at the concentration 667µg/mL. Chandini and al. (14) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range of 17.79 - 23.16% at an extract concentration of 1000 µg/mL.

It was observed that the extracts containing high levels of total phenolic content were potent DPPH radical scavengers, confirming that algal polyphenols have a strong antioxidant activity³⁸⁻⁴⁰.

Therefore, other materials present in the extracts of seaweed, such as small molecular weight polysaccharides, pigments, proteins, or peptides may contribute to the free radical scavenging activity.

CONCLUSION

It can conclude that most extracts from marine algae evaluated exhibit an important radical scavenging activity. The extracts of *Bifurcaria bifurcata* contain high levels of total phenolic compounds and a very important radical scavenging activity. It is well-known that phenolic compounds present in the algae are mainly responsible for the antioxidant activity of seaweeds. According to this study, an important relationship was found between the radical scavenging activity and phenolic content and indicated that phenolic compounds could be major contributors to antioxidant activity.

To explore the suitability of different extracting solvents with different polarity, we have compared the total phenolic content and radical scavenging properties of methanol, dichloromethane, dichloromethane/methanol, ethyl acetate and methanol/water extracts. Methanolic extract of *Bifurcaria bifurcata* showed the highest scavenging activity against DPPH. Ethyl acetate extract showed the highest phenolic compounds. The data on extraction procedures and radical scavenging activity assessment obtained in this experiment single out methanol as the most promising sources for the isolation of natural radical scavenging compounds of *Bifurcaria bifurcata*.

These results obtained remain important as the first step in screening antioxidant activity by DPPH radical of algae studied. Further scientific work in our laboratory is in progress to determine the antioxidant activity by other tests.

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