Research Article

Phenolic Compounds and Radical Scavenging Activity of Red Seaweeds Harvested from the Atlantic Coast of Sidi Bouzid Morocco

Laila Hsaine*, Nazha Samri, Soukaina ElKafhi, Samira Etahiri, Samira Khlifi
Marine Biotechnology and the Environment Laboratory (BIOMARE) Faculty of Science, El Jadida-Morocco.
***Corresponding author's E-mail:** laila.hsaine@gmail.com

Received: 25-03-2019; **Revised:** 22-04-2019; **Accepted:** 01-05-2019.

ABSTRACT

Differents extracts from eight macroalgae species were tested for total phenolic compounds (TPC), flavonoids, condensed tannins contents and for their radical scavenging activity, in vitro, using DPPH free radical. Dichloromethane and methanol mixture extract of *Boergeseniella thuyoides* and ethyl acetate extract of *Gracilaria multipartita* showed higher phenolic content (22.93mg GAE/g extract). Ethyl acetate extract of *Boergeseniella thuyoides* and *Bornetia secundiflora* showed higher flavonoids content (2.45 and 1.21mg QE/g extract). Dichloromethane extract of *Boergeseniella thuyoides* and *Bornetia secundiflora* showed higher condensed tannins content (1.15 and 1.05mg CE/g extract). Among all algae tested, methanol extract of *Boergeseniella thuyoides* exhibited the higher scavenging ability on DPPH (EC50= 85µg.ml-1). It can be concluded that *Boergeseniella thuyoides* is a promising seaweed for more detailed investigations of its antioxidant properties and application possibilities.

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

INTRODUCTION

The Moroccan coast extends over 3500 km, 2900 km of Atlantic coastline and 500 km of Mediterranean coastline. It contains important variety and quantity of algal species among which 500 species were identified by many works[1-4].

Marine resources have attracted worldwide attentions in the search for bioactive substances to develop new drugs and healthy foods, thanks to their low toxicity and high bioactivities[5]. Therefore, new interest has been developed to search natural and safe antioxidative agents from marine sources[6]. Antioxidant activity is focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anticarcinogenic natural bioactive compounds, which possess health benefits[7, 8].

The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.). Those compounds are widely distributed in plants or seaweeds and are known to exhibit higher antioxidative activities[7].

Antioxidants are substances wich possess the ability to protect the body, cells and tissues from continuous threat and damage caused by free radical and reactive oxygen species, which are produced during normal oxygen metabolism or induced by exogenous factors[9]. These cellular renegades damage DNA, proteins, and lipids, and corroding cell membrane[10].

On another side, and in order to lower the risk of oxidative deterioration, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been added to many food products[11]. These compounds are suspected to possess some undesirable effects for human health[12].

In this study, eight macroalgae species harvested from the atlanticcoast of Morocco were examined for their phenolic compounds and their radical scavenging activity.

MATERIALS AND METHODS

Plant materials

Eight red algae (*Corallina elongata, Boergeseniella thuyoides, Laurencia pinnatifida, Caulcanthus ustulatus, Bornetia secundiflora, Gracilaria multipartita, Gelidium pulchellum and Corallina officinalis)* were collected manually, during the low water spring tides, along thecoast of Sidi Bouzid El Jadida (33° 33° 16' 09'N, 8° 30' 8° 45'W), during 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 and stored before extraction.

Preparation of seaweed extracts

For each species, powder is extracted in a different solvent: methanol (M), dichlorometane (DC), ethyl acetate (EA), methanol/water (MW) (60/40) and the dichloromethane/methanol mixture (DC/M) (50/50) at a rate of 1g of alga powder /5 ml of solvent according to the extraction protocol described by Caccamese et al.[13]. The extracts are then filtered on whatman paper and then evaporated in a rotary evaporator. The dry extracts obtained are stored at 4°C until used for the biological tests. For methanol water extract, the result evaporated was lyophilised and stored.

Phytochemical screening of algal extract

The method used in this experiment was the addition of certain reagents giving a positive reaction. This analysis determined the concentration of total phenolic compounds, flavonoids and condensed tannins.

Total phenolic content

The concentration of phenolic content in the crude extract was determined by slight modified method of Bray and Thorpe[14]. Dried samples and standard were prepared with various solvents. A volume of 100µl of test solutions (samples or standards) were added to 2.0ml of Na_2CO_3 (0.2%). After 2 minutes, 100µl of Folin-Ciocalteau reagent (50%) were added and allowed to stand at room temperature for 30 minutes. Absorbance was measured at 750nm. The blank consisted of all reagents and solvents without test compounds or standard. The standard was gallic acid prepared in concentrations of 0.01mg/ml to 1.0mg/mL. The phenolic concentrations were determined by comparison with the standard calibration curve.

Condensed tannins

The condensed tannins contents were measured using the vanillin assay described by Julkunen-Titto[15]. A volume of 50 µl of the test solutions (sample or standard) was added and mixed to 1500 µl of vanillin/methanol solution (4%, m/v). Then, 750µl of concentrated hydrochloric acid was added. After incubation for 20 minutes at room temperature, the absorbance was measured at 550nm against a blank. The amount of total condensed tannins (three replicates per sample) was expressed as milligrams of catechin equivalent per gram of extract (mg CE/g extract) from the calibration curve.

Flavonoids

The concentration of flavonoids was determined according to the method described by Dehpeur et al.[16], 500µl of each extract are added to 1500ml methanol (95%), 100µl of $AlCl_3$ (10%, m/v), 100µl of sodium acetate 1M and 2.8ml of distilled water. The mixture is incubated in the dark at ambient temperature for 30 minutes. The white is done by replacement of the extract by methanol (95%) and the absorbance is measured at 415nm using a UV spectrophotometer. The results areexpressed in milligram quercetin equivalent per gram of algae extract (mg QE/g extract) in referring to the calibration curve of quercetin.

Evaluation of radical-scavenging activity

The scavenger effect of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) has been measured according to the protocol described by Bounatirou et al.[17]. Differents concentrations of the samples and BHT (butylated hydroxytoluen) as standard were tested.

The solution of DPPH was prepared by solubilisation of 2.4mg of DPPH in 100ml of methanol, 100µl of each sample extract as well as the positive control are added to 2ml of the solution of DPPH, the mixture is left in the dark for 30 minutes at room temperature, the absorbance is measuredat 517nm. The radical scavenging activity has been estimated according to the equation:

Percentage inhibition
(%) = [(Absorbance of control − Absorbance of sample)/(Absorbance of control)] × 100

The extract concentration providing 50% of the activity of free radical scavenging (EC_{50}) was calculated from the graph of DPPH percentage reductions depending on the concentration.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

Differences in total phenolic contents were noticed between the different seaweed species and between different solvents used for extraction for the same species. So, the recovery of phenolic contents in different samples is influenced by the polarity of extracting solvents and the solubility of this compound in the solvent used for the extraction process[18-19].

Total phenolic content was affected by the extracting solvent, among all the algae extracts, dichloromethane/methanol mixture and Ethyl acetate extracts were found to be the most efficient solvent compared with the other solvent systems (Fig.1).

The highest phenolic content was found in *Boergeseniella thuyoides* extracted by dichloromethane and methanol mixture with value of 22.93mg GAE/g of dry extract. Similar results have been reported by Dellai et al.[20], in the methanolic extract obtained from the red algae *Laurencia obusta*, and the lowest TPC values was observed in *Laurencia pinnatifida* extracted with aqueous methanolic (3.82mg GAE/g extract). In fact, species belonging to the family of Rhodomelaceae are known for their richness in phenolic compounds, especially bromophenols[21-22].

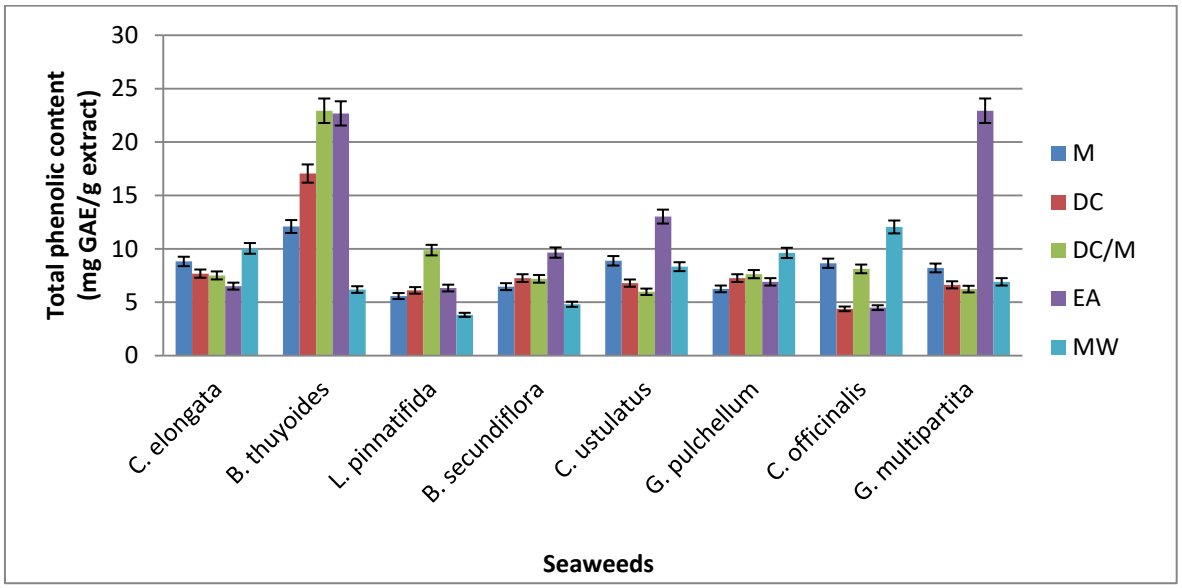


Figure 1: Total phenolic content of different extract of algae studied
M: methanol, DC: dichoromethane, DC/M: dichloromethane methanol mixture,
EA: ethyl acetate, MW: methanol water. Each point represents the mean of tree replicates.

The amount of total phenolics varied from 3.82 to 22.93mg equivalent GAE/g of extract. Chew and al.[23] reported that phenol content can vary considerably depending on the variety of algae; it was found that *Kappaphycus alvarezzi* contained 1.15mg GAE/g while *Padina antillarum* contained levels of 24.30mg GAE/g. Our values were lower than those reported by Wang et al.[24] who found TPC of 138, 169 and 176mg GAE/g of water extract for *Ascophyllum nodosum*, *Fucus serratus* and *Fucus vesiculosus*, respectively. However, values in the present work were higher than those reported by Sabeena Farvin and Jacobsen[25] who reported TPC ranging from 0.11 to 6.1mg GAE/g of water extract in several seaweed species.

Boergeseniella thuyoides showed higher phenolic content for almost of the solvents used for the extraction with the exception of the water methanol solvent. The results obtained are in accordance with those demonstrated that the phenolics compounds are generally more soluble in polar organic solvent than in water[24]. Other compounds such as water soluble polysaccharides, proteins and organic acid were simultaneously extracted when using water as the only extractant[26].

Gracilaria multipartita contained a high value in phenolic compounds (22.93mg GAE/g extract) when it extracted by the ethyl acetate, compared to other solvents. Similar result was founding by Chiboub et al.[27] who observed higher phenolic content (21.2mg GAE/g extract) in ethyl acetate dry extract of red algae, *Halopteris scoparia*.

Flavonoids content

The flavonoids quantification showed a large variability depending on the species and on the organic solvent used for the extraction. The result of flavonoides content of the five solvent extracts of all seaweeds studied is given in figure 2.

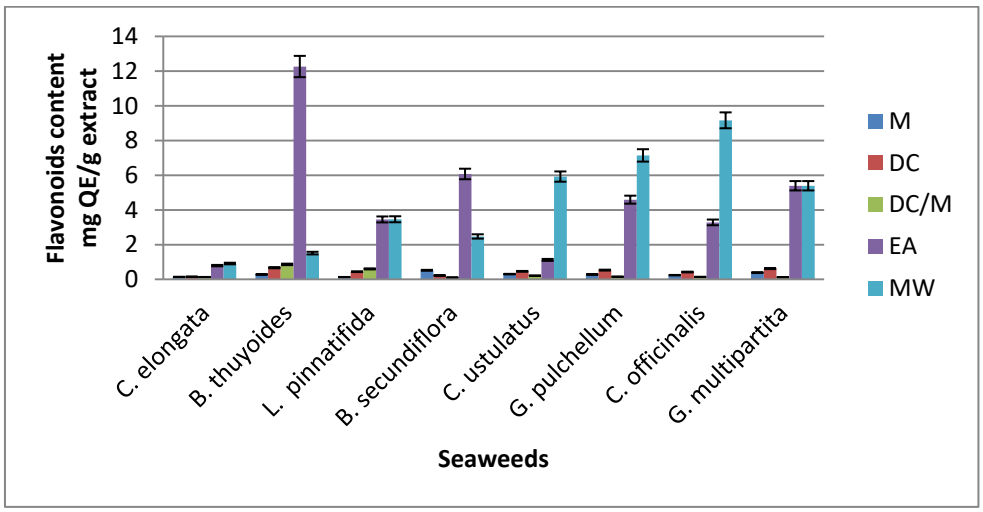


Figure 2: Flavonoids contents of different extract of red algae studied
M: methanol, DC: dichoromethane, DC/M: dichloromethane methanol mixture,
EA: ethyl acetate, MW: methanol water. Each point represents the mean of tree replicates

The highest amount of flavomoids was found in *Boergeseniella thuyoides* with 12.26mg QE/g of the ethyl acetate extract, Similar results was found by Sameeh et al.[28], who reported that *Padina pavonica* conteined a high value of flavonoids content (12.09mg QE/g). While the methanol dichromethane mixture extract of *Bornetia secundiflora* presented the lowest quantity with an amount of 0.1mg QE/g of extract.

Condensed tannins content

As shown in figure 3, condensed tannins contents varied from 0.27 to 5.77mg CE/g extract. Dichloromethane and dicloromethane methanol mixture extract has been found to be rich in condensed tannins. By against, methanol water extract showed the low values of condensed tannins in almost algae studied.

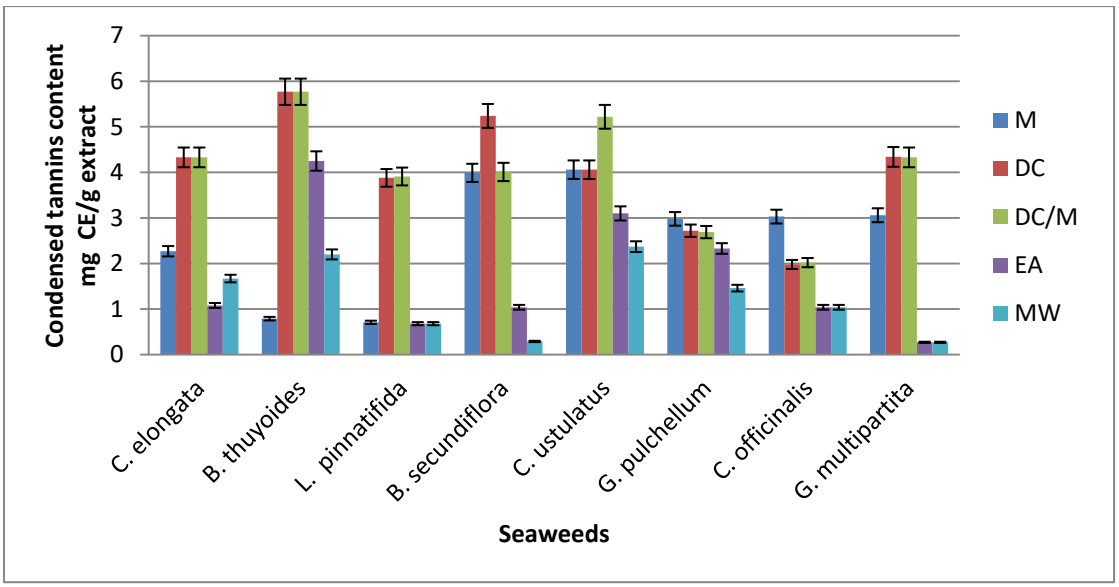
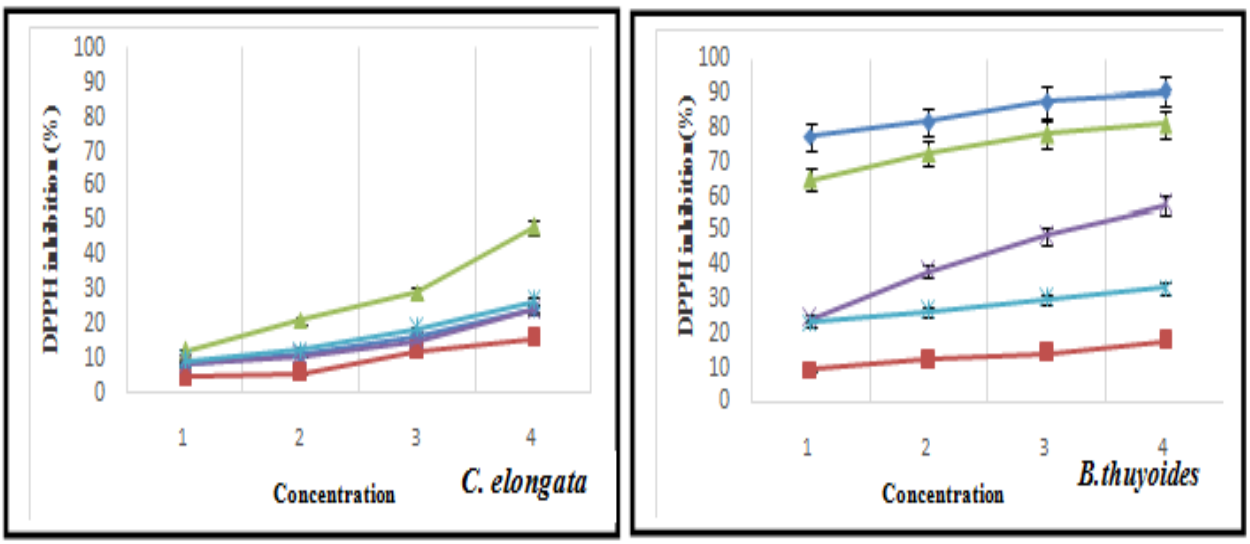


Figure 3: Condensed tannins content of differents seaweed extracts tested
M: methanol, DC: dichoromethane, DC/M: dichloromethane methanol mixture,
EA: ethyl acetate, MW: methanol water. Each point represents the mean of tree replicates.

To summarise, methanol and dichloromethane mixture extracts show the highest yield of total phenolic compouds, while dichloromethane gave the highest yield of condensed tannins and the highest yields of flavonoids were obtained with ethyle acetate extract. In the present results *Boergeseniella thuyoides* showed total phenolic, flavonoids and condensed tannins contents higher than other seaweeds studied, this difference perhaps could be attributed to genetic factors[28].

DPPH radical scavenging

The DPPH radical scavenging activity has been reported in percentage of DPPH inhibition (%). DPPH is a synthetic stable free radical widely used for evaluating natural antioxidants, algae or algal products[29-30-31]



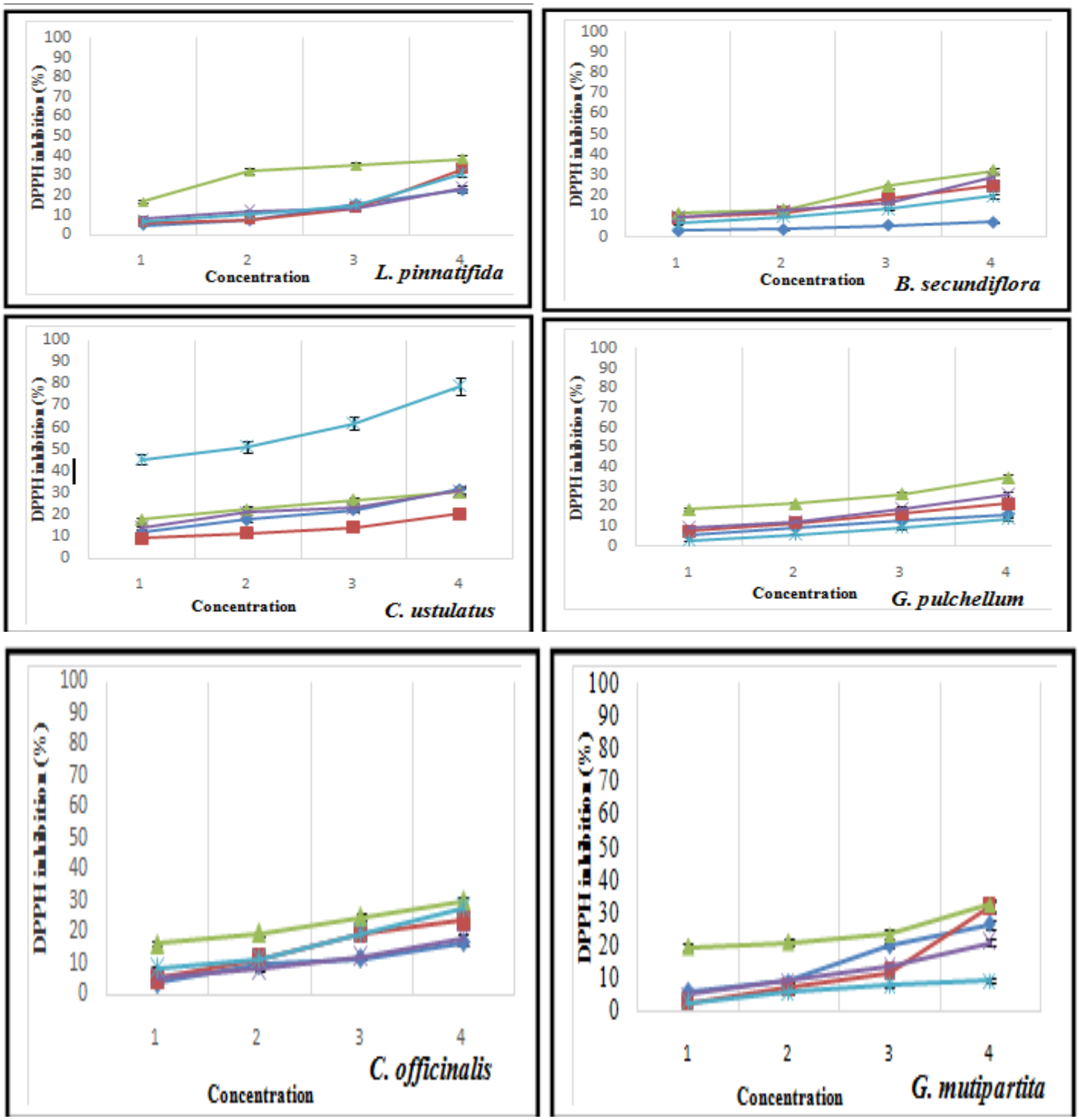


Figure4: DPPH radical scavenging activity differed between the algae species tested

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

1: 142µg.mL^{-1}, 2: 285µg.mL^{-1}, 3: 476µg.mL^{-1}, 4: 667µg.mL^{-1}

Radical scavenging activity was evaluated in differents extracts from eight species of red seaweeds (Fig. 4). The ability of a compound to scavenge DPPH radicals is dependent on their ability to pair with the unpaired electron of a radical[32].

Differences in the activity among different seaweed species and among different solvent extracts were observed. As shown in Figure 4; methanol extract from *Boergeseniella thuyoides* showed a higher scavenging activity (90.91%). Dichloromethane and methanol mixture extract from *Boergeseniella thuyoides* showed a higher scavenging activity (81.06%), followed by *Corallina elongata* which showed a moderate scanvenging activity (48.13%). Ethyl acetate extract from *Boergeseniella thuyoides* showed a moderate scavenging activity (57.78%). While the methanol water extract from *Caulacanthus ustulatus* showed a higher scavenging activity (78.88%). Furthermore, methanol, dichloromethane, ethyl Acetate and methanol water extracts of *Corallina elongata, Laurencia pinnatifida,*

Bornetia secundiflora, Gelidium pulchellum and *Gracilaria multipartita* as well as dichloromethane extract *of Boergeseniella thuyoides* and *Caulcanthus ustulatus* showed relatively less scavenging potentials (less than 30%).

The EC_{50} is obtained by interpolation from a linear regression analysis, a lower EC_{50} indicates a higher antioxidant activity of a compound. The radical scavenging activity of all the seaweed extracts (table.1) was lower than that of the reference compound BHT (EC_{50}=11.5µg.ml^{-1}) similar result was found by Rhimou et al.[33]. BHT is a single compound whereas seaweed extracts contained a mixture of compounds of different natures that interact with each other[34].

According to the obtained results (Table 1), the methanol and dichloromethane methanol mixture extracts of *Boergeseniella thuyoides* have a high scavenging activity with EC_{50} of 85 and 95µg.ml^{-1}, respectively. However, methanol water extract and dichloromethane extract of *Boergeseniella thuyoides* showed no activity (EC_{50}>1000µg.ml^{-1}). A moderate activity was found for the same algae in ethyl acetate extract with EC_{50}=524.91µg.ml^{-1}. Rhimou et al.[33] evaluated the antioxydant activity of methanolic extracts from selected morrocan red seaweeds and reported that the methanol extracts of *Pterosiphonia complanata* and *Boergeseniella thuyoides* had a high scavenging activity with EC_{50} of 96µg.ml^{-1} and 132µg.ml^{-1}, respectively.

Table 1: Evaluation of the antioxidant activity in the DPPH assay (EC_{50}) of seaweeds extracts. M: methanol, DC: dichoromethane, DC/M: dichloromethane methanol mixture,

EA: ethyl acetate, MW: methanol water. Each value represents the mean of three replicates

	EC_{50} (µg/ml)				
Solvent / Seaweed	M	DC	DC+M	EA	MW
Corallina Elongata	>1000	>1000	740.49±0.21	>1000	>1000
Boergeseniella thuyoides	85±0.23	>1000	95.0±0.26	524.91±0.12	>1000
Laurencia pinnatifida	>1000	>1000	>1000	>1000	>1000
Bornetia secundiflora	>1000	>1000	>1000	>1000	>1000
Caulacanthus ustulatus	>1000	>1000	>1000	>1000	250±0.18
Gracilaria multipartita	>1000	>1000	>1000	>1000	>1000
Gelidium pullchelum	>1000	>1000	>1000	>1000	>1000
Corallina officinalis	>1000	>1000	>1000	>1000	>1000

Methanol water extract of *caulacanthus ustulatus* showed a moderate scavenging activity with EC_{50} of 250µg.ml^{-1}; however the same algae extracted by other solvents showed no efficient antioxidant activity (EC_{50}> 1000µg.ml^{-1}). Dichloromethane methanol mixture extract of *Corallina elongata* showed a moderate scavenging activity with EC_{50} of 740.49µg.ml^{-1}; however the same algae extracted by other solvents showed no efficient antioxidant activity.

Among all extracts of algae tested those of *Corallina elongata, Boergeseniella thuyoides, and Caulacanthus ustulatus* participated in the transformation of DPPH radical in reduced form with an EC_{50} between 85 and 740.49µg.ml^{-1}. While no activity was showed by the methanolic, ethyl acetate and dichloromethane extracts of *Corallina elongata*, *Corallina officinalis*, *Bornetia secundiflora*, *Gracilaria multipartita*, *Gelidium pulchellum*, *Laurencia pinnatifida*.

The percentage of inhibition increases with the concentration of the extract in all samples so the radical scavenging activity is dose-dependent[35]. Different extraction solvents, according to their polarity, may have extracted various compounds including pigments (chlorophyll a, b, carotenoids), alkaloids, and phenolic compounds which can participate in the great antioxidant activity[36]. This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of algal extract[37-38-39-40].

In this study, we noticed that most of seaweeds contained phenolic constituents in various proportion, and showed various radical scavenging activity. There is some extracts containing a high levels of polyphenols and were also potent DPPH radical scavengers. Dichloromethane and methanol mixture and ethyl acetate extracts of *Boergeseniella thuyoides* conteined high levels of total phenolic and exhibited a high radical scavenging activity. This result is agreement with the previous study which has reported that there is a direct relationship between the radical scavenging activity and the total phenolic content in some vegetable, herbs[41]. However, some extracts conteined a higher value in total phenolic but they exhibit a low radical scavenging activity (Dichloromethane extract of *Boergeseniella thuyoides*

and Ethyl acetate extracts of *Gracilaria multipartita and caulacanthus ustulatus)*. few studies as Heo and Cha[42]. reported that the antioxidant activity (determined by different methods), using large number of algal species (10 green and 25 brown seaweed species) may not necessarily be correlated with the total phenolic content in each algal extract[43].

Methanol extract and methanol water of *Boergeseniella thuyoides* and *Caulacanthus ustulatus* exhibited a high value of DPPH radical scavengers but conteined a less value of TPC compared to other solvent extracts. These results may indicate the possible participation of other active substances which exhibit antioxidant activity such as pigments (chlorophyll, carotenoids) and low molecular weight polysaccharides.

The highest free radical scavenging activity was observed in different algae species belonging to different phyla[44] but this potential is still considered weak in comparison with terrestrial aromatic and medicinal plants as shown on chamomile for example[45]. The antiradical activity characterizes the ability of compounds to react with free radical while antioxidant activity represents the ability to inhibit the process of oxidation[46]. So, only one assay cannot determine total antioxidant potential, the results obtained for the radical scavenging activity must be completed by other methods to determine antioxidant activity of the extracts studied.

CONCLUSION

The results obtained allowed us to conclude that extraction with various solvents of varying polarities affects the content of total phenolic compounds, flavonoids, condensed tannins and radical scavenging activity. The highest content of polyphenols compounds, flavonoids and condensed tannins were obtained in *Boergeseniella thuyoides* extracts, the same algae was seen to be the most effective extract in terms of radical scavenging activity.

Based upon the results obtained in the present study, it is concluded that methanolic extract of *Boergeseniella thuyoides* and the ethyl acetate extract *of Caulacanthus ustulatus* contains a less TPC as the same exhibited a high value of DPPH radical scavenging activity, these results suggest that 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. Hence, Methanol extract was seen the best solvent used for determination of radical scavenging activity of algae studied.

Pending further analysis and experimentation will be conducted to evaluate antioxidant activity of the seaweeds studied; theses naturals seaweed extracts may have potential applications for use in the food, pharmaceutical and cosmetic industries.

REFERENCES

1. Benhissoune S, Boudouresque C, Verlaque M. A check list of marine seaweeds of the Mediterranean and Atlantic Coast of Morocco. I. Chlorophyceae willes L. Botanica Marina 44, 2001, 171-182
2. Benhissoune S, Boudouresque C-F, Verlaque M. A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. II. Phaeophyceae. Botanica Marina 45, 2002, 217-230.
3. Benhissoune S, Boudouresque C-F, Perret-Boudouresque M, Verlaque M. A checklist of the seaweeds of the Mediterranean and Atlantic coasts of Morocco. III. Rhodophyceae (Excluding Ceramiales). Botanica Marina 45, 2002, 391-412.
4. Bengueddour Y, El Hani S, El Ibaoui H, El Ayadi R, Brhadd N. Etude de l'activité antioxydante de Gelidium Sesquipedale par chromatographie liquide haute performance. Revue «Nature &Technologie». B-Sciences Agronomiques et Biologiques 10, 2014; 29-33.
5. Wang BJ, Wu XH, Zhang M, Xu, Chang HM, Lu XY, Ren XY. In vitro antioxidant and antitumor activities of different sulphated polysaccharides isolated from three algae. Carbohydrate Polymers 89, 2012, 31-35.
6. Khairy HM, El-Sheikh M. Antioxidant activity and mineral composition of three Mediterranean common seaweeds from Abu-Qir Bay, Egypt. Saudi Journal of Biological Sciences 22, 2015, 623–630.
7. Heo SJ, Park EJ, Lee KW, Jeon YJ. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresource Technology 96, 2005; 1613–1623.
8. Murugan K, Iyer VV. Antioxidant and Antiproliferative Activities of Marine Algae, Gracilaria edulis and Enteromorpha lingulata, from Chennai Coast. International. Journal of Cancer Research 8, 2012, 15-26.
9. Martinez VI, Periago MJ, Ros G. Nutritional importance of phenolic compounds in the diet. Archivos Latino americanos Nutricion 50, 2000, 5-18.
10. Park PJ, Shahidi F, Jeon YJ. Antioxidant activities of enzymatic extracts from an edible seaweed Sargassum horneri using ESR spectrometry. Journal of Food Lipids 11, 2004, 15-27.
11. O'Sullivan AM, O'Callaghan YC, O'Grady MN, Queguineur B, Hanniffy D, Troy DJ. et al. In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. Food Chemistry 126, 2011, 1064–1070.
12. Benhammou N, Bekkara FA, Panovska TK. Antioxidant activity of methanolic extracts and some bioactive compounds of Atriplexhalimus.Comptes Rendus Chimie 12, 2009, 1259–1266.

13. Caccamese S, Azzolina R. Screening for antimicrobial activities in marine algae from eastern Sicily. Planta Medica 37, 1979, 333-339

14. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. In: Method of Biochemical Analysis (Ed.) Glick, D. Interscience Publishers, Inc, 1, 1954, 27-52.

15. Julkunen-Titto R. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry 33, 1985, 213–217.

16. Dehpour AA, Ibrahim zadeh MA, seyed Fazel N, Seyed Mohammad N. Antioxydant activity of the methanol extract of Ferula assafoetida and its essential oil composition. Grasas Y Aceites 2009. p, 405-412.

17. Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. Et Link. Food Chemistry 105, 2007, 146-155.

18. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115, 2009, 785–788

19. Sulaiman SF, Sajak AAB, Supriatno KLO, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis 24, 2011, 506–515.

20. Dellai A, Laajili S, Le morvan V, Robert J, Bouraoui A. Antiproliferative activity and phenolics of the Mediterranean seaweed Laurencia obusta. Industrial Crops and Products 47, 2013, 252-255.

21. De Carvalho LR, Roque NF.Halogenated and/or sulfated phenols from marine macroalgae. Quimica Nova 23, 2000, 757-764.

22. Zhao J, Fan X, Wang S, Li S, Shang S, Yang Y. et al. Bromophenol derivatives from the red alga Rhodomelaconfervoides. Journal of Naural Products 67, 2004, 1032-1035.

23. Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT Food Science and Technology 41, 2008, 1067–1072.

24. Wang T, Jo´ nsdo´ ttir R, O´ lafsdo´ ttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chemistry 116, 2009, 240–248.

25. Sabeena Farvin KH, Jacobsen C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chemistry 138, 2013, 1670–1681.

26. Chrinos R, Rogez H, Campos D, Pedreschi R, Larondelle RY. Optimisation of extraction conditions of antioxydant phenolic compounds from mashua(tropacolum tuberosum ruiz et pavon) tubers. Separation and purification technology 55 (2), 2007, 217-225.

27. Chiboub O, Ktari L, Sifaoui I, López-Arencibia A, Reyes-Batlle M, Mejri M. et al. In vitro amoebicidal and antioxidant activities of some Tunisian seaweed, Experimental Parasitology 183, 2017, 76-80.

28. Sameeh MY, Mohamed AA, Elazzazy AM. Polyphenolic contents and antimicrobial activity of different extracts of Padina boryanaThivy and Enteromorpha sp marine algae. Journal of Applied Pharmaceutical Science 6(9), 2016, 087-092.

29. Kang K, Park Y, Hye JH, Seong HK, Jeong GL, Shin HC. Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agents against vascular risk factors. Archives of Pharmacal Research 26, 2003, 286–293.

30. Kuda T, Kunii T, Goto H, Suzuki T, Yano T. Varieties of antioxidant and antibacterial properties of Ecklonia stolonifera and Eckloniakurome products harvested and processed in the Noto Peninsula, Japan. Food Chemistry 103, 2007, 900–905.

31. Balboa EM, Conde M, Moure M, Falque E, Dominguez H. In vitro antioxidant properties of crude extracts and compounds from brown algae. Food Chemistry 138, 2013, 1764–1785.

32. Park PJ, Shahidi F, Jeon YJ. Antioxidant activities of enzymatic extracts from an edible seaweed Sargassum horneri using ESR spectrometry. Journal of Food Lipids 11, 2004, 15-27.

33. Rhimou B, Hassane R, Nathalie B. Antioxidant activity of Rhodophyceae extracts from Atlantic and Mediterranean Coasts of Morocco.African Journal of Plant Science 7(3), 2013, 110-117.

34. Rubén A, Paulo EM, Ruben D, Javier C, Daniel F, José ML. Proximate composition, phenolic content and in vitro antioxidant activity of aqueous extracts of the seaweeds *Ascophyllum nodosum, Bifurcaria bifurcata* and *Fucus vesiculosus*. Effect of addition of the extracts on the oxidative stability of canola oil under accelerated storage conditions. Food Research International 99, 2017, 986–994.

35. Khaled N, Mawlawi H, Chbani A. Antioxidant and antifungal activities of Padina pavonica and Sargassum vulgare from the Lebanese Mediterranean coast. Advances in Environmental Biology 6, 2012, 42-48

36. Shanab, S.M.M., Shalaby, E.A., El-Fayoumy, E.A. *Enteromorpha compressa* exhibits potent antioxidant activity. Journal of Biomedecine and Biotechnology 2011, 2011, 1-11.

37. Marinova EM, Yanishlieva NV. Antioxidant activities of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chemistry 58, 1997, 245–248.

38. Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. Lebennsmittel-Wissenschaft und-Technologie 37, 2004, 717–721.

39. Yuan YV, Bone DE, Carrington MF. Antioxidant activity of dulse (palmariapalmata) extract evaluated in vitro. Food chemistry 91, 2005, 485-494

40. Yang J, Chen C, Zhao S, Ge F, Liu D. Effect of solvents on the antioxidant activity of walnut (Juglans regia L.) shell extracts. Journal of Food and Nutrition Resrarch 2, 2014, 621-626.

41. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activityand total phenolics in selected fruits, vegetables, and grainproducts. Journal of Agricultural and Food Chemistry 46, 1998, 4113-4117

42. Heo, S.J., Cha, S.H. Antioxidant activities of chlorophyta and phaeophyta from Jeju Island. Algae 20, 2005, 251–260.

43. Kahkonen MP, Hopia AI, Vuorela HJ. Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 57, 1999, 792-797.

44. Bianco EM, Lenita K, Jessica L, Priscila K, Aline ,J Camila MB. et al. Antimicrobial (including antimollicutes), antioxidant and anticholinesterase activities of Brazilian and Spanish marine organisms e evaluation of extracts and pure compounds. Revista. Brasileira de Farmacognosia 25, 2015, 668-676.

45. Sebai H, Jabri MA, Souli A, Hosni K, Rtibi K, Tebourbi O. et al. Chemical composition, antioxidant properties and hepatoprotective effects of chamomile (Matricariarecutita L.) decoction extract against alcohol induced oxidative stress in rat. General, Physiology and Biophysics 34, 2015, 263-275.

46. Moharram HA, Youssef MM. Methods for Determining the Antioxidant Activity. Alexandria Journal of Food Sciences & Technology, 11, 2014, 31-42.

Source of Support: Nil, **Conflict of Interest:** None.