Research Article

Phenolic Compounds and Antioxidant Activity of Nine Seaweeds on the Coast of El Jadida-Morocco

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Received: 05-03-2020; **Revised:** 18-06-2020; **Accepted:** 25-06-2020.

ABSTRACT

This study lies within the scope of the valorization of seaweed extracts from Moroccan coast El Jadida (sidi Bouzid), as antioxidants. The methods applied to measure the antioxidant activity were trapping of the free radicals byusing the 1, 1'-diphenyl-2-picrylhydrazyl (DPPH°), total antioxidant capacity (TAC) and the estimation of total phenolic content. The results obtained demonstrate that quantitative estimation of flavonoids, condensed tannins and total phenolic compounds showed variability in the richness of the algae studied in these compounds. The free radical scavenging evaluation and antioxidant capacity method indicated that the red alga *Halopitys incurvus* had a good antioxidant activity (IC50 = 63.51±0.44 µg/mL), than the BHT (IC50 =11,072±0,22 µg/mL).

Keywords: Seaweeds, Total phenolic compounds, flavonoids, condensed tannins, radical scavenging activity (DPPH), total antioxidant capacity (TAC).

INTRODUCTION

The marine organisms constitute an infinite source of active molecules with an original chemical structure[1]. Seaweed is an important source of bioactive natural substances, which are synthesized by metabolic pathways different from those observed in terrestrial environments[2]. Among marine organisms, algae, which are most often attached to a substrate, develop chemical defenses to prevent colonization by other species, including microorganisms[3]. Algae are much less known than terrestrial plants and much more difficult to apprehend. They occupy a large part of the aquatic environment, in particular marine and submarine, and constitute a set of extremely diverse organisms[4].

Algae have been used since the dawn of time, during the XXe century; the researchers were interested in the biological properties of algae[4]. Numerous reports show that macro algae collected in coast of Morocco present a broad range of biological activities such as antibacterial[5-6]and antioxidants[2-7], so they can prevent and fight against pathologies related to the formation of highly toxic derivatives such as singlet oxygen and free radicals[8].

Several bioactive molecules have been isolated and identified from marine algae[9-10-11], most of which contain phenolic compounds such as catechins and flavonoids[12-13], Phlorotannins[14], tocopherols and ascorbic acid known for their antioxidant activity[15]. This activity have been reported through various methods of reactive oxygen species scavenging and the inhibition of lipid peroxidation[16-18] among others.

The objective of the present study is to investigate the antioxidant properties of nine marine algae using phytochemical analysis, DPPH scavenging test and total antioxidant capacity essay.

MATERIALS AND METHODS

Table 1: Algae samples used in this work

Orders	Scientific name	types	Collection date
Gelidiales	*Gelidium sesquipedale*	Red algae	30/03/2017
Gigartinales	*Hypnea musciformis*		30/03/2017
	Plocamium cartilagineum		30/03/2017
	Gigartina teedii		30/03/2017
Bonnema isoniales	*Asparagopsis armata*		30/03/2017
Ceramiales	*Halopitys incurvus*		28/02/2017
Gracilariales	*Gracilaria cervicornis*		30/03/2017
Ulvales bryopsidales	*Ulva lactuca Codium elongatum*	Green algae	28/02/2017 30/03/2017

Chemicals used

Butylated hydroxytoluene (BHT), 1,1'-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, sodium carbonate, Folin reagent, sodium acetate, aluminum chloride, hydrochloric acid (HCL), vanillin, sulfuric acid, sodium phosphate, ammonium molybdate, ascorbic acid were purchased from Sigma-Aldrich**.**

Algal materials

Seaweeds were collected by hand-picking during February to March 2017 from the coast of El Jadidasidi Bouzid (33 ° 33 °16'09''N, 8 °30' 8 °45'W). The algae were cleaned, washed in distilled water, air dried at room temperature, powdered and stored.

Preparation of extracts

Each powder from dried algae was extracted in different solvents, namely Methanol (M), Methanol/ Dichloromethane (50/50) (M/DC), Dichloromethane (DC) and Methanol/Water (50/50) (M/W), as described by Caccamese and Azolina[19]. The resulting extracts were concentrated by drying in a rotary evaporator under reduced pressure at 45°C. A crude extract obtained was stored at 4°C until utilization.

Evaluation of total phenolic contents

Total phenolic contents of different solvent extracts were measured using Folin Ciocalteu's method as described by Taga and al[20]. 100 µl aliquot of sample were mixed with 2 ml of 0.2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin Ciocalteu's phenol reagent were added, mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. The absorbance was measured at 750 nm and total phenolic content was calculated using gallic acid curve and expressed as gallic acid equivalent per gram of extract (mg GAE/g).

Determination of flavonoid contents

Flavonoids were estimated using the method of Dehpeur and al[21]. Briefly, 0.5 ml of seaweed extract in different solvent were added to 0.1 ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (Perkin Elmer). The flavonoid contents were calculated as quercetin equivalent per gram of extract (mg QE/g) from the calibration curve.

Determination of condensed tannin contents

The amounts of tannins were estimated using the method described by Price and al[22]. Condensed tannins were determined by vanillin under acidic conditions[7].This method is based on the ability of reacting with the vanillin units of condensed tannins in the presence of acid to produce a colored complex measured at 500 nm. The results were expressed as catechin equivalent per gram of extract (mg CE/g).

Antioxidant assays

Evaluation of free radical scavenging activity by DPPH method

Antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine (DPPH-H) with discoloration (yellow). The free radical-scavenging activity of Algae was measured as described by Bounatirou and al[23]. 0.1 ml of seaweed extract, diluted in pure methanol, at different concentrations, were added to 2 ml of DPPH (2.4 mg in 100 ml of methanol), the mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm; the synthetic antioxidant BHT was used as standard. For each sample, the radical scavenging activity was calculated as a DPPH percentage of inhibition:

$$(\%) = 100\ (A0 - A1)\ /A0.$$

A0: Absorbance of control, A1: Absorbance of sample.

Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity of the seaweed extracts was evaluated by the phosphomolybdenum method according to the procedure described by Prieto and al[24]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm; the methanol was used as the blank. The antioxidant activity is expressed as ascorbic acid equivalent per gram of extract (mg AAE/g).

Statistical analyses

The experiments were carried out in triplicate. The results are given as mean ± standard deviation (SD). The statistical analysis was performed using the Software Package for Social Sciences (SPSS, version 20.0, IBM Inc, USA). All tests were considered to be statistically significant at P<0, 05.

RESULTS AND DISCUSSION

Total phenolic content

The results showed that the polyphénols contents differ significantly depending of the solvent polarity (p <0,05). Total phenolic content of 36 algal extracts studied varied from 3,645±0,044 to 45,63 ±0,023 mg EAG/g of extract (Fig. 1).

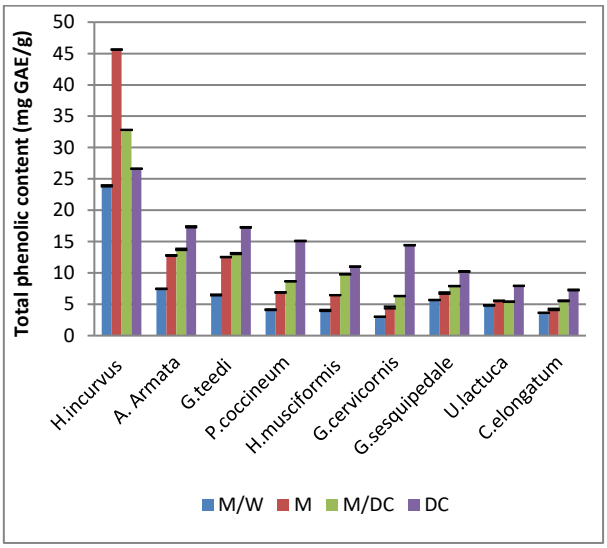

Figure 1: Total phenolic content of different extracts of algae studied.

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

The methanolic extract of *Halopitys incurvus* revealed the highest level of phenolic compounds (45,63±0,023mg EAG/g of extract), wearing other algae ($p<0,05$); when the methanol/dichloromethanolic, dichloromethanolic and hydromethanolic extracts contains the respective values of 32,82±0,034, 26,63±0,051,and 23,894±0,119mg EAG/g of total polyphénols.

For the dichloro methanolic extracts of *Asparagopsis armata, Gigartina teedii* and *Plocamium cartilagineum* also have an important phenolic content with the respective values of 17.36±0.112, 17.26±0.072 and 15.1±0.058mg EAG/g of extract.

Regarding green algae, the high value of phenolic content was found in dichloromethanolic extract of the *Ulva lactuca* (7.94±0.045 mg EAG/g), comparable with the results of Chernane andal[25] who found that the dichloro methanolic fraction of *Ulva rigida* had a higher values of (6.29 mg GAE/g of extract).

Determination of flavonoids

The results of the quantitative study of the flavonoid contents of the 36 different algae extracts are presented in the figure 2.

Statistically the difference between the levels in flavonoids depending on the algae is highly significant ($p<0.01$). the flavonoid content of the algae by the various solvents (Fig. 2) show that the extracts of red alga *Halopitys incurvus* had the highest content of flavonoids, namely an average of 18.64±0.057mg QE/g (methanolic extract); 18.16±0.011mg QE/g (methanol / dichloromethanolic extract); 17.12±0.03mg QE/g (dichloromethanolic extract) and 16.1±0.04mg QE/g (hydromethanolic extract). Thus, methanol / dichloromethanolic and dichloromethanolic extracts of *Plocamium cartilagineum* has an important flavonoid content noticed with the respective values of 8, 6±0, 077and 8, 68±0, 092 mg QE/g of extract.

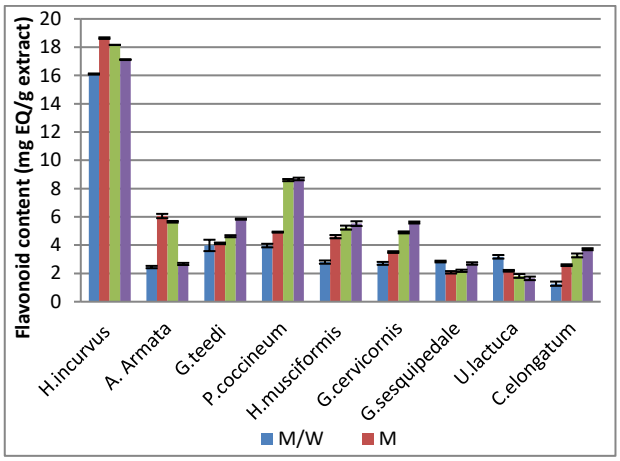

Figure 2: Flavonoid content of different algae studied extracts.

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

Condensed tannins

We note that the condensed tannins content varies from one alga to another and from one extraction solvent to another. This variation, highly significant ($P<0.01$), can be explained in the differences that exist in the chemical composition between the algal tissues and dissolution in the extraction medium.

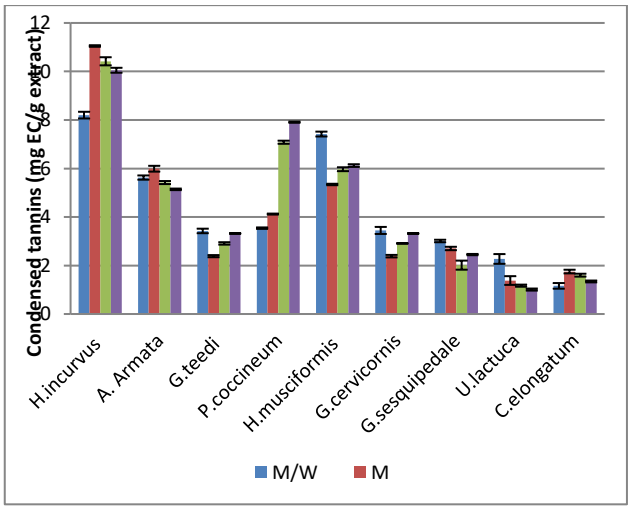

Figure 3: Condensed tannin content of different algae studied extracts.

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

The *Halopitys incurvus* seaweed has been marked by its highest levels of condensed tannins with respective

values of 11.05±0,032 mg CE/g (methanolic extract), 10.42±0.167mg CE/g (methanol/ dichloromethanolic extract), 10.05±0.102mg CE/g (dichloromethanolic extract) and 8.2±0.138mg CE/g (hydromethanolic extract). These results, obtained with *Halopitys incurvus,* confirm our precedent researches on the red alga which have a large amount of condensed tannin such as *Boergeseniella thuyoides*[26].

The analysis of condensed tannins shows that the dicloromethanolic extracts obtained from the *Plocamium cartilagineum* and *Asparagopsis armata* have substantial condensed tannins content by respective values of 7.91±0.025mg CE/gand 5.14±0.032mg CE/gextract.

Regarding the methanol/dichloromethane extract it is noted the *Plocamium cartilagineum* has a significant content of condensed tannins with the value of 7.082±0.0643 mg CE/g.

Antioxidant assay for DPPH radical-scavenging activity

This method is widely used as a fast, reliable and reproducible parameter for investigating in vitro antioxidant activity of pure compounds as well as alga extracts. It is based on the reduction of the alcoholic solution of DPPH in the presence of a hydrogen donor antioxidant due to the formation of the non-radical form DPPH-H by the reaction[27-28-29].

DPPH radical scavenging activity show that dicloromethanolic extracts of *Halopitys incurvus* exhibited the highest percentage of inhibition with the value of 99.46±0.031% (Fig4)**.** The hydromethanolic, methanolic and methanol/dicloromethanolic extracts of *Halopitys incurvus* showed a very important scavenging activity with the respective values of 93.28±0.13%, 85.45±0.084% and 82.18±0.051%. Concerning *Gigartina teedii,* the methanol/dicloromethanolic and methanolic extracts showed an important scavenging activity with respective values of 77.16±0.071% and 65.09±0.081%.

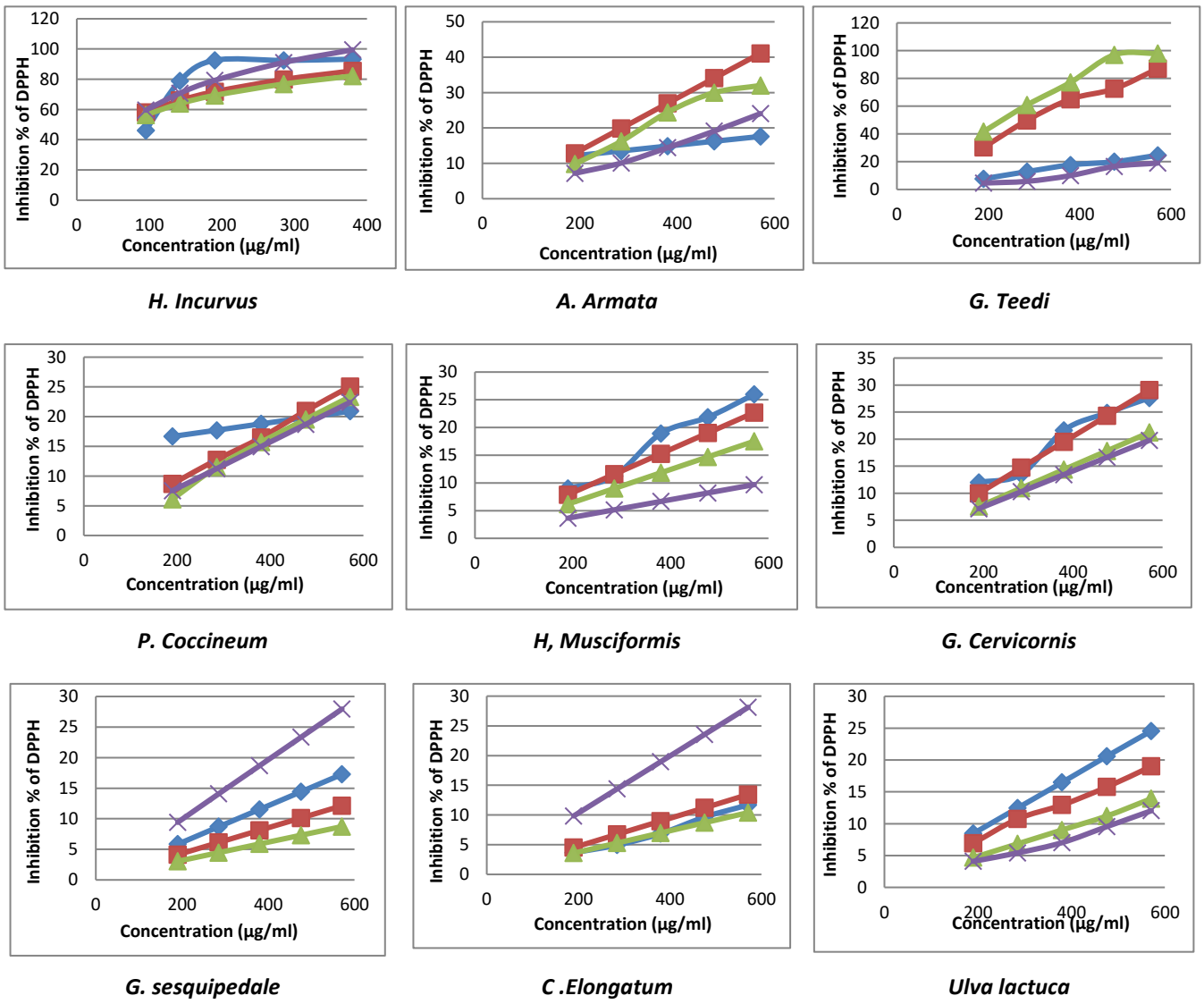

Figure 4: DPPH radical scavenging activity (% of inhibition) of different algae species tested.

According to the results obtained by the DPPH test, it was possible to band together the algae extracts in four groups with different IC_{50} values (Table 2):

The first group whose IC_{50} values <200µg/ml show a powerful strong antioxidant, which includes the methanolic, methanol / dicloromethanolic, dicloromethanolic and Hydro-methanolic extracts of *Halopitys incurvus*, with the following respective values of 63.51±0.44; 68.697±0,87; 70.028±0.74 and 96.14±0.12 µg/ml. In comparison with the research found in the literature, our study shows that the methanolic extract of *Halopitys incurvus* that was harvested on 2017 is more active than the methanolic extract of *Halopitys incurvus* that was harvested on 2007 which shows a value of IC_{50}> 1000µg/ml[9].

Table 2: Evaluation of the antioxidant activity in the DPPH assay (EC_{50}) of seaweeds extracts.

		EC_{50} (µg/mL)			
	samples	**M/W**	**M**	**M/DC**	**DC**
	BHT	**11,072±0,22**			
Red algae	*Halopitys incurvus*	96,14±0,12	63,51±0,44	68,697±0,87	70,028±0,74
	Gigartina teedi	>1000	303,5±1,01	240,67±2,07	>1000
	Asparagopsis Armata	>1000	690±1,22	860,25±233	>1000
	Plocamium coccineum	>1000	>1000	>1000	>1000
	Hypnea musciformis	>1000	>1000	>1000	>1000
	Gracilaria cervicornis	>1000	>1000	>1000	>1000
	Gelidium sesquipedale	>1000	>1000	>1000	>1000
Green algae	*Codium elongatum*	>1000	>1000	>1000	>1000
	Ulva lactuca	>1000	>1000	>1000	>1000

Each value represents the mean of three replicates with standard deviation (± SD)

M: Methanol; **DC**: Dichloromethane; **M/DC**: methanol/dichloromethane; **M/W**: Methanol/Water

The second groups are characterized by the IC50 values between 200 and 600µg/ml and indicate an average antiradical power involved: the methanolic and methanol/dicloromethanolic extract of *Gigartina teedi* with the respective values of 303.5±1.01 and 240.67±2.07µg/ml.

The third group presents the IC_{50} values between 600 and 1000 µg/ml and therefore low antioxidant activity, for example methanolic and methanol/dicloromethanolic extract of *Asparagopsis Armata* with respective values of 690±1.22 and 860.25±0.233µg/ml.

Finally, the last group has showed highest IC_{50} values >1000µg/ml, which indicates the absence of antiradical power, included the extracts of *Ulva lactuca, Gelidium sesquipedale, Codium elongatum, Gracilaria cervicornis, Hypnea musciformis,*and *Plocamium coccineum.*Regarding the methanolic extract of *Asparagopsis Armata* that was harvested on 2017, our study shows an IC_{50}value of 690±1.22µg/ml also more active than the methanolic extract of *Asparagopsis Armata* that was harvested on 2007 which shows an IC_{50} value of about 862.45±0.11µg/ml[30].

Evaluation of total antioxidant capacity (TAC)

As we can see in the figure 5, all extracts show a different antioxidant activity. The dicloromethanolic, methanol/dicloromethanolic, methanolic and Hydro-methanolic extracts of red algae, *Halopitys incurvus* hold the best record of antioxidant capacity by the order of 62.43±1.01; 56.82±1.11; 48.78±1.219 and 40.39±0.02mg AAE/g extract.

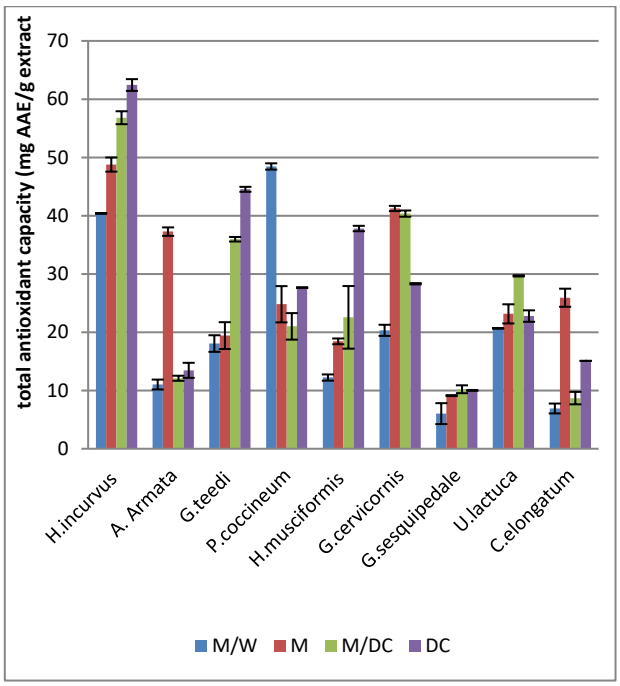

Figure 5: total antioxidant capacity of different extracts of algae studied.

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

The Hydro-methanolic extracts of *Plocamium coccineum, Gracilaria cervicornis* and *Gigartina teedii*have a good total antioxidant activity with the respective valuesof 48.45±0.54;20.32±0.96 and 18.055±1.425mg AAE/g extract. Also the dicloromethanolic extracts of *Gigartina teedii*, *Hypnea musciformis, Gracilaria cervicornis* and *Plocamium coccineum who* exhibited the following respective values of 44,53±0,425;37,8±0,465; 28,32±0,11 and 27,66±0,07mg EAA/g extract.

CONCLUSION

The results obtained in the present study confirm the existence of a certain correlation between the content of phenolic compounds and the antiradical activity, in fact the methanolic extract of the*Halopitys incurvus*alga showed high levels of total polyphénols and was also recorded as extract that have the greatest reductive activity of DPPH, the same results were highlighted in many of the similar works[31-7-26].

REFERENCES

1. Mark L, Wells P, Potin J, Craigie S, John A, Raven S, Sabeeha S, Katherine E, Helliwell AG, Mary EC, Susan HB. Algae as nutritional and functional food sources: revisiting our understanding, Journal of Applied Phycology 29, 2017, 949-982.
2. Matanjun P, Mohamed S, Mustapha NM and Muhammad K. Nutrient Content of Tropical Edible Seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum, Journal of Applied Phycology 21, 2008, 75-80.
3. Paul VJ, Fenical W. Natural products chemistry and chemical defence in tropical marine algae of the phylum chlorophyta, Bioorganism Marina Chemistry 1, 1987, 1-37.
4. Person J, Lando D, Mathieu D. Algues, filières du futur : Livre Turquoise, Édition : Adebioihech-Romainville, 163, 2011, 2-160.
5. Etahiri S, butel-ponce V, el kouri AK, assobhei O, zaoui D, guyot M. Antibacterial activity of marinealgae from the atlantic coast of morocco, Marine life 13, 2003, 3-9.
6. Lakhdar F, Boujaber N, Oumaskour K, Assbhei O, Etahiri S. Inhibitive activity of 17 marinealgae from the coast of El Jadida-Morocco against erwinia chrysanthemi, International Journal of Pharmacy and Pharmaceutical Sciences 10, 2015, 376-380.
7. Samri N, Hsaine L, El Kafhi, S, Khlifi S, Etahiri S. Radical Scavenging Activity and Phenolic Contents of Brown Seaweeds Harvested from the Coast of Sidi Bouzid (El Jadida, Morocco), International Journal of Pharmaceutical Sciences 54, 2019, 116-122.
8. Dodet B. La chasse aux radicaux libres oxygénés, Biofutur 101, 1991, 23-34.
9. Nagappan T, Vairappan CS. Nutritional and Bioactive Properties of Three Edible Species of Green Algae,*Genus Caulerpa* (Caulerpaceae), Journal of Applied Phycology 26, 2014, 1019-1027
10. Athukorala Y, Lee KW, Shahidi F, Heu MS, Kim HT, Lee JS, Jeon YJ.Antioxidant efficacy of extracts of an edible red alga (Grateloupia filicina) in linoleic acid and fish oil, J. Food. Lipids 10, 2003, 313-327.
11. Aneiros A, Garateix A. Bioactive peptides from marine sources: pharmacological properties and isolation procedures, Journal of Chromatography 803, 2004, 41-53.
12. Yoshie-Stark Y, Hsieh YP and Suzuki T. Distribution of flavonoids and related compounds from seaweeds in Japan,Journal of the Tokyo College of Fisheries 89, 2003,1-6.
13. Santoso J, Yoshie Y and Suzuki T. The distribution and profile of nutrients and catechins of some Indonesian seaweeds, Fisheries Science68, 2002, 1647-1648.
14. Koivikko R, Lopone J, Pihlaja K, Jormalainem V. Highperformance liquid chromatographic analysis of phlorotannins from the brown alga Fucus vesiculosus, Phytochemical Analysis 4, 2007, 326-332.
15. Ragubeer N, Beukes DR and Limson JL. Critical assessment of voltammetry for rapid screening of antioxidants in marine algae, Food Chemistry 121, 2010, 227-232.
16. Yan X, Nagata T, Fan X .Antioxidative activities in some common sea weeds, Plant Food. Hum. Nutr, 52, 1998, 253-262.
17. Heo SJ, Lee KW, Song CB, Jeon YJ. Antioxidant activity of enzymatic extracts from brown seaweeds, Bioresour. Technol 18, 2003, 71-81.
18. Siriwardhana N, Lee KW, Kim SH, Ha JH, Park GT, Jeon YJ. Lipid peroxidation inhibition of *Hizikia fusiformis* methanolic extract on fish oil and Limoleic, Food science and Technology international 10, 2004, 65-72.
19. Guiry MD and Guiry GM. AlgaeBase. World-wide electronic publication. National University of Ireland, Galway 35,2014, 105-115.
20. Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants, Journal of American Oil Chemistry Society 61, 1984, 928-931.
21. Dehpour A, Ibrahimzadeh MA, Seyed FN et Seyed M. Antioxydant activity of the methanol extract of ferula assafoetida and its essential oil composition, Grasas y aceites 60, 2009, 405-412.
22. Price ML, Van Scoyoc S and Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain,*J. Agric. Food Chem* 26, 1978, 1214–1218.
23. Bounatirou S, Smiti S, Miguel MG, Rejeb MN, Neffati M, Costa MM, Faleiro L, Figueiredo AC, Barroso JG, and Pedro

LG. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff, Et Link, Food Chem 105, 2007, 146-155.

24. Prieto P, Pineda M, Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex : Specific application to the determination of Vitamin E, Anal. Biochem, 269, 1999, 337-41.

25. Chernane H, mansori M, latique S, el kaoua M. Evaluation of antioxidant capacity of methanol extract and its solvent fractions obtained from four moroccan macro algae species. European Scientific Journal, 10, 1014, 1857-7881**.**

26. Hsaine L, Samri N, El Kafhi S, Etahiri S, Khlifi S. Phenolic Compounds and Radical Scavenging Activity of Red Seaweeds Harvested from the Atlantic Coast of Sidi Bouzid Morocco, International Journal of Pharmaceutical Sciences, 56, 2019, 73-81.

27. Shon MY, Kim TH and Sung NJ. Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts, Food Chem 82, 2003, 593-597.

28. Duan XJ, zhang WW, li XM, wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *polysiphonia urceolata*, food chemistry, 95, 2006, 37-43.

29. Burda S and oleszek W. antioxidant and antiradical activities of flavonoids, J Agric food chem, 49*,* 2001, 2774-2779.

30. Bouhlal R, riadi H and bourgougnon N, antioxidant activity of rhodophyceae extracts from atlantic and Mediterranean coasts of morocco, African Journal of Plant Science, 7, 2012, 110-117.

31. Bozan B, Tosun G, Ozcan d. Study of polyphenol content in the seeds of red grape (Vitis vinifera L.) varieties cultivated in Turkey and their antiradical activity, 109, 2008, 426-30.

Source of Support: None declared.

Conflict of Interest: None declared.