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In-vitro Antioxidant Activity of Five Selected Species of Libyan Algae

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A B S T R A C T

The main objective of this study was to investigate the i*n vitro* antioxidant activities of three selected green (*Codium tomentosum*, *Enteromorpha linza*), red *(Gelidium sesquipedale*) and brown (*Cystoseira spicata*, *Padina pavonica*) seaweeds from western of Tripoli coast, Libya. After methanol extraction, polyphenol, flavonoids, and condensed tannins contents were assessed. Methanol extracts of the seaweeds were investigated for their total antioxidant activity, reducing power, DPPH radical scavenging activity, NO radical scavenging activity, deoxyribose scavenging activity, and nitric oxide radical inhibition assay. Brown seaweed especially *C. spicata* exhibited good antioxidant activity when compared to red and green seaweeds. The maximum antioxidant activity was shown by the methanol extract of *C. spicata* for DPPH and nitric oxide radicals scavenging assay (66%, and 53% respectively). Also *C. spicata* extract showed a noticeable protection levels against lipid peroxidation. However, extract of *E. linza* exhibited the lowest antioxidant activity. Macroalgae can be considered as a good source of natural antioxidants.

Keywords: Seaweeds, brown algae, red algae, green algae, Free radicals, antioxidant activity.

A R T I C L E I N F O

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1. Introduction

Macro algae are rich sources of natural bioactive products as they are able to produce a great variety of secondary metabolites characterized by a wide range of biological activities. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents. But the lack of oxidative damage in the structural components of seaweeds and their stability to oxidation during storage suggest that their cells have antioxidative defense systems [1]. Therefore, secondary metabolites, which produced by algae under environment stress, are capable of scavenging or inactivating free radicals in the human body and thereby may prevent many diseases [2].

An antioxidant can be any substance that is able to inhibit oxidation when present at low concentrations, that is, more reactive than an oxidizable substrate [3]. Seaweeds are considered to be a rich source of antioxidants [4,5,6]. The antioxidant properties of its components has been related to the protection against cancer, coronary heart disease (CHD), inflammatory troubles, neurological deterioration, and aging [7, 8]. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit the progression of carcinoma formation [9]. Further from these findings, a recent study has suggested that algae extracts can improve the glycaemic control in a non-insulin dependent diabetic mouse model [10].

Several studies have investigated the antioxidant activity of natural products in marine and freshwater algae [11,12]. The antioxidant activity of red seaweed extracts is correlated with their polyphenol content [5,6]. The brown algae possess the capability of scavenging free radicals and stabilizing lipid peroxidation due to their hydroxyl groups present in polyphenols like fucoxanthin [13]. Antioxidant activities have been attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity, radical scavenging [14,15].

In addition to health issue, antioxidants from natural sources increase the shelf-life of foods. In food sector, the free radical peroxidation of lipids is the predominant cause of food decay, destruction of vitamins and rancidity during storage and transformation [16]. Therefore, many products with antioxidant properties especially from natural sources are widely used to increase the shelf life of foodstuffs [17]. Furthermore, beside their use in the food and pharmaceutical industries, algal antioxidants are used in the cosmetic industry in anti-aging products [18].

In Libya, seaweeds have not been receiving appropriate attention in the past and the availability of seaweed pharmaceutical data is still rare in comparison with that of plants [2]. Seaweeds are a promising renewable natural resource in Libya especially many of seaweeds species are found in abundance around the Libyan coastline. Several reports have revealed seaweeds to be rich sources of natural antioxidant compounds [6,19,20]. However, reports on the antioxidant activity of seaweed extracts from Libya are very limited [4,21]. Hence, the current study aimed to investigate the antioxidant capacity, total phenolics, flavonoids, alkaloids and condensed tannins of five common species of seaweeds from the west coast of Libya for possible applications in medicine, dietary supplements, cosmetics, or food industries.

2. Materials and Methods

Chemicals and reagents: DPPH (1,1–diphenyl – 1,2 – picryl hydrazyl), Gallic acid, Rutin , potassium ferricyanide, trichloroacetic acid (TCA), aluminum chloride, Ferric chloride, sodium nitroprusside, sulphanilamide, napthylethylenediamine dihydrochloride, ammonium molybdate, ammonium persulphate, ascorbic acid, sodium nitrite, thiobarbituric acid (TBA), Folin & Ciocalteu's phenol reagent and all solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Algae Material and extraction

Five seaweed samples (*C. tomentosum*, *C. spicata*, *E. linza*, *P. pavonica*, *G. sesquipedale*) were collected in September 2014 from Western Libyan Coast, and authenticated from the Department of Botany, Faculty of Science, University of Tripoli, Libya. All samples were brought to the laboratory in plastic bags containing sea water to prevent evaporation. Few collected seaweeds were preserved for identification. Algae samples were cleaned such that epiphytes and necrotic parts were removed. Samples were rinsed with sterile water and shade dried for 7-14 days and ground thoroughly to powder in a kitchen-type blender. The extraction of the sample was carried out using methanol on a rotary shaker at 150 rpm at room temperature (25-30˚C) for 72 hours. The extracts were pooled and filtered using filter paper (Whatmann No.1); the obtained filtrate was evaporated, and the residues were stored at -20˚C.

Phytochemical screening

The freshly prepared methanolic extracts of the selected algae were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff's and Mayer's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions, and saponins with the ability to produce suds. These tests were identified by characteristic color changes using standard procedures [22].

Determination of Polyphenols, flavonoids and condensed tannin content

The total phenolic compounds in plants were determined using the Folin- Ciocalteu reagent according to the method of Marinova (2005) [23]. 200 µl of the sample (0.1 g/ml) in triplicate was incubated with 1 ml of diluted Folin-Ciocalteu reagent (1:2 with water) for 5 min. 1 ml of 7% Na_2CO_3 was added to the reaction mixture which was incubated again for 90 min. thereafter, the absorbance was read at 750 nm using Jenway UV-VIS 6305 spectrophotometer. The total phenolic content is expressed

as gallic acid equivalent (GAE) in milligrams per gram of dry sample. Total flavonoid content was determined using assay described by Zhishen J. et al., 1999 [24] with slight modifications. Briefly, 1 ml of algal extract (0.1g/ml) was diluted with 4 mL of water and 0.3 mL of $NaNO_2$ (5% w/v) was added. After 5 min, 0.3 mL of $AlCl_3$ (10% w/v) was added followed by the addition of 2 mL of NaOH (1 M) six min later. The volume was increased to 10 mL by adding 2.4 mL distilled water and the sample incubated at RT for 15 min. The absorbance was taken at 510 nm. The assay was performed in triplicate, and the flavonoids content was determined by interpolating the absorbance of the samples against a calibration curve constructed with rutin standard (1.0–5.0 mg/mL) and expressed as milligrams of rutin equivalent per gram of extract (mg Rutin/g).

Total condensed tannin content was determined according to the method of Julkunen-Titto (1985) [25]. Briefly, a 50 µl aliquot of each extract was mixed with 1.5 ml of 4% vanillin (prepared with methanol) and then 750 µl of concentrated HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. The absorbance against blank was read at 500 nm. Tannic acid was used to prepare the standard curve and results were expressed as mg tannic acid equivalents (TE)/g extract.

***In-vitro* Antioxidant evaluation**

Reducing Power

The reducing capacity of plant extracts were investigated according to the method of Oyaizu (1986) [26]. various concentrations of algal extracts (6.25-50 mg /ml was mixed with 2.5 ml of phosphate buffer (2 M, *p*H 6.6) and 2.5 ml of potassium ferric cyanide (1%), and the mixture was incubated at 50°C for 20 min. After which, then the mixture was incubated at 50 ^{0}C for 20 min. 1.5 ml of 10% TCA was added to the reaction mixture which was centrifuged at 1000×g for 10 min. The supernatant (0.5 ml) was mixed with distilled water (1 ml), $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measure at 665 nm. The higher the absorbance of the reaction mixture the greater is the reducing power.

Evaluation of total antioxidant capacity (TAC) by Phosphomolybdenum method: The total antioxidant capacity was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. 1999 [27]. A 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After that, the absorbance of the green phosphate/Mo complex was measured at 695 nm. The higher absorbance value indicated higher antioxidant activity.

DPPH free radical-scavenging activity

The free radical scavenging activity of plant extracts was measured by DPPH according to Wong, et al., 2006 method [28]. Briefly, 40µl of methanolic extract of algae (25 mg/m) was added to 3 ml of DPPH (0.1 mM) in methanol solution, shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance was measured at 517 nm using a UV- visible spectrophotometer (UV-VIS 6305 model, Jenway, Germany).

The percent of DPPH scavenging effect was calculated as follows

$$\% \text{ DPPH} = ([A_C - A_S]/A_C) \times 100$$

Where A_C was the absorbance of the control reaction and As was the absorbance in the presence of the sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically according to Garrat, 1964 [29] method. 1 ml of Sodium nitroprusside (10 mM) in phosphate buffer was added to 0.5 ml of sample (1.25 mg/ml) and incubated at 25 ºC for 150 minutes. Thereafter, 0.5 ml of the reaction mixture containing nitrite ions was removed and added 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid), shaken and allowed to stand for 5 min. then 1 ml of naphthylethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. The absorbance of the mixture was measured at 540 nm against the corresponding blank solutions at 546 nm. The percentage of scavenging activity was measured with reference to ascorbic acid as standard.

Deoxyribose radical scavenging activity

An assay mixture containing EDTA (1 mM), $FeCl_3$ (10 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) was added to the seaweed extracts (12.5mg/mL) dissolved in distilled water with ascorbic acid (1 mM) in 50 mM phosphate buffer. The mixture was incubated at 37˚C for 1 hour and 1.0 mL of the incubated mixture was mixed with 1 mL of 10% TCA and 1 mL of 0.4% TBA (in glacial acetic acid, pH adjusted by NaOH) to develop the pink chromagen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is expressed as percentage inhibition of deoxyribose degradation and was calculated as previously reported [30].

Thiobarbituric Acid Reactive Assay (TBARS)

Thiobarbituric Acid Reactive Assay (TBARS) The assay was performed as described by Halliwell Gutteridge (1999) [31]. 4mg of the algal extracts were taken in a test tubes and were evaporated to dryness at 80°C .1 ml of 0.15M potassium chloride was added to the tubes and followed by 0.5ml of linoleic acid (1mg) with 0.15 M KCl. Peroxidation was initiated by the addition of 100 µl of 2mM ferric chloride. After incubating the tubes for 30min at 37°C, the peroxidation reaction was stopped by adding 2ml of ice-cold HCL (0.25N) containing 15% TCA & 0.38% TBA. The samples were kept at 80°C for 1 hr, cooled and centrifuged at 7500rpm. The absorbance of the supernatant, containing TBA-MDA complex was read at 532nm. The anti-lipid peroxidation activity (ALP%) was calculated using the formula :

$$\text{ALP\%} = ([A_C - A_S]/A_C) \times 100$$

Where A_C was the absorbance of the control reaction and As was the absorbance in the presence of the sample.

Statistical analysis

Data were expressed as means ± standard deviations (SD) of triplicate determinations. All statistical analyses were carried out using SPSS V.16 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL). Statistical differences between extract activities were determined

using ANOVA followed by Least Significant Difference (LSD) testing. Differences were considered statistically significant when $P<0.05$. The Pearsons correlation analysis was performed between antioxidant activity, total phenolic and flavonoids contents.

3. Results and Discussion

Phytochemical screening

The phytochemical analysis of the selected seaweeds was performed and the results were tabulated according to the presence or absence of the phytochemicals in the algae. '+' indicates the presence of the phytochemicals and '–' indicates the absence of the phytochemicals. Preliminary phytochemical screening of the seaweeds showed the presence of flavonoids, tannins, saponins and alkaloids with respectable amount in *C. spicat*a and *G. sesquipedale*. Alkalaoids, flavonoids Tannins and saponins were found in all tested algae with various amount (Table 1). Subsequent quantification showed the presence of 125.93±28.15, 21.93±21.57, 264±38, 1283.33±21.93, 365.33±28.30 mg GAE/g DW of phenolics, and 1632.91± 87.40, 827.96±125.31, 906.20±52.05, 740±95.26 and 887.5 ±40.46 mg Rutin /g DW of flavonoids and 608.88±20.36, 857.77±120.98, 286.66±9.42, 440±26.66, and 471.11±3 3.55 mg tannic acid/g DW of condensed tannins in methanol extract of *C. spicata, P. pavonica, E. linza, G. sesqulpedale and C. tomentosum* respectively (Table 5).

A Significant high amount of phenolics, flavonoids and condensed tannins were found in *C.spicata* extract as compared with the other extracts. Previous results are in agreement with previous studies which found that brown algae has higher amount of phenolics and flavonoids than green algae [32]. Also the red and green species contained lower total condensed tannins than the brown seaweeds [33]. Phenolic, flavonoids compounds are found in seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties [20, 34]. Algae phenolics including flavonoids are known to possess strong antioxidant properties. Flavonoids are oxidized by the radicals, resulting in more stable, less-reactive radicals. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical [35]. Beside phenols and flavonoids, alkaloids have been reported as good antioxidant reagent [36,37]. Therefore, the quantity of alkaloids in tested algae was estimated according to Shamsa et al., 2008 [38].

The total alkaloid content in algal extracts is presented in the table 3 and was expressed in milligram atropine equivalent (mg CE). A yellow-colored complex with a maximum absorption was obtained by *G. sesqulpedale* (155±19.7) indicating high content of alkaloids while *P. pavonica* showed the lowest amount of alkaloids compared to others. The results are not reflecting the actual amount of alkaloids as the reagent (BCG) can react with a specific class of alkaloids (alkaloids that have nitrogen inside their structure) and amine or amid alkaloids do not react with this reagent [39].

Table 3: Total alkaloids in tested plant materials (100 g) by BCG-complex formation.

Algae	Amount (mg)
C. spicata	52.75^{c}±15.06
P. pavonica	44^{d}±9.8
E. linza	48.36^{c}±11.2
G. sesqulpedale	155^{a}±19.7
C. tomentosum	122^{b}±23.3

Each value is presented as mean ± SD (n = 3). Means Within each column with different letters (a-d) differ significantly (P <0.05). nt: not tested

Antioxidant activity

Antioxidant activity should not be concluded on a single antioxidant test model and in practice several in vitro test procedures are carried out for evaluating antioxidant activities with the samples of interest. Antioxidant analysis methods like scavenging activity of nitric oxide, hydrogen peroxide, hydroxyl radicals, free radical scavenging (DPPH), phosphomolybdenum ability and reducing power were carried out.

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the seaweeds extract and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphor-molybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [28]. The maximum antioxidant activity was exhibited significantly by the methanol extract of *C. spicata* 199.38±12.73 (199.38 mg of Ascorbic acid/g of seaweed dry weight) and the lowest activity was recorded in the green algae *E linza* (144.05 mg of Ascorbic acid/g of seaweed dry weight). Similarly, findings also showed that the maximum total antioxidant activity was shown by the brown algae compared to the green algae and red algae [40].

The Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in milligram per gram of extract (Figure 1). Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [41]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [42].

In the current study, the reducing power was found to increase in dose dependent manner. Maximum antioxidant activity was observed in the extracts of highest concentration (50 mg/ml) for all the samples. The reducing ability was found to be higher for the *C. spicata* extract as compared with other extracts followed by *P. pavonica* extract (Figure 2). These results are in concord with Matsukawa et al. (1997) [43], who found that the antioxidant activity of brown algae was greater than to that of red or green groups. *C. spicata* can be considered as a potent source of natural antioxidants as it acts as a good indicator of its potential antioxidant property (Figure 2).

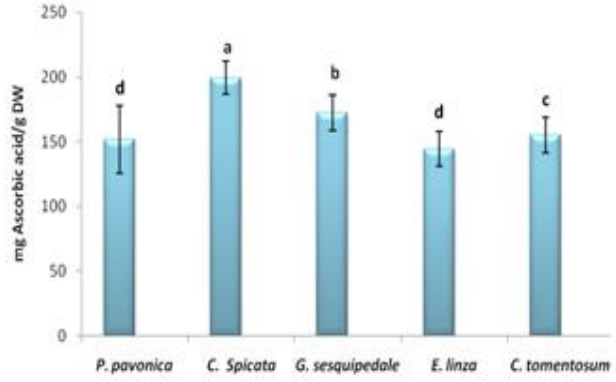

Figure 1: Total antioxidant capacity (TAC) by the methanolic extracts of selected Algae. Each value is represented as mean ±SD (n=3). Means with different letters (for each concentration level) are significantly different at $P < 0.05$.

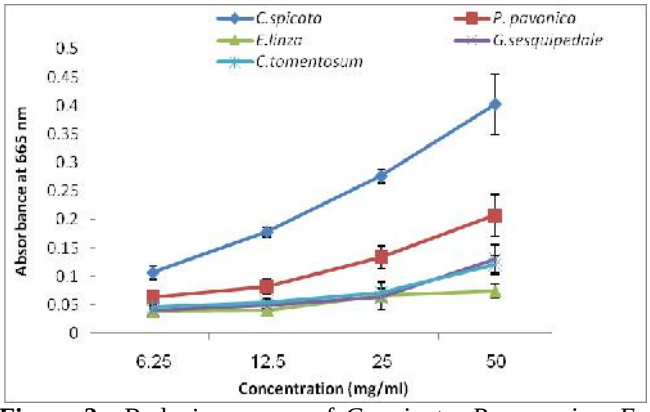

Figure 2: Reducing power of *C. spicata, P. pavonica, E. linza, G. sesqulpedale, C. tomentosum* extracts. Each value is represented as mean ±SD (n=3).

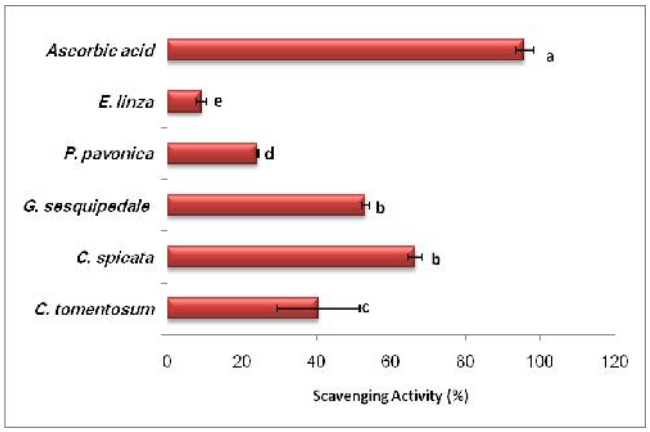

Figure 3: DPPH radical scavenging activity (% ± SD) of methanol extracts of algae and ascorbic acid at concentration 25 mg/ml. Each value is expressed as the mean ±SE (n=3). Means with different letters (for each concentration level) are significantly different at P <0.05.

The free radical scavenging activity of seaweed extracts was assessed by the DPPH assay and ascorbic acid was used as a standard (Figure 3). A significant decrease in the concentration of DPPH radical was observed due to the scavenging ability of the seaweeds especially in presence of *C. spicata* extract ($P<0.05$). However The free radical scavenging activity of ascorbic acid was obtained a significant higher affect as compared to tested algae ($P<0.001$). The radical scavenging ability of methanolic extract of *C. tomentosum , P. pavonica, C. spicata, E. linza, G. sesqulpedale* and ascorbic acid at 25 mg/mL was observed to be 40%, 24%, 66%, 9%, 53% and 95% respectively (Figure 3). The results of the present study are in line with Wang *et al.* (2009) and Yan *et al.* (1999) [34,44], who also found that brown algae contained higher amounts of polyphenols and DPPH radical scavenging activity than red and green algae.

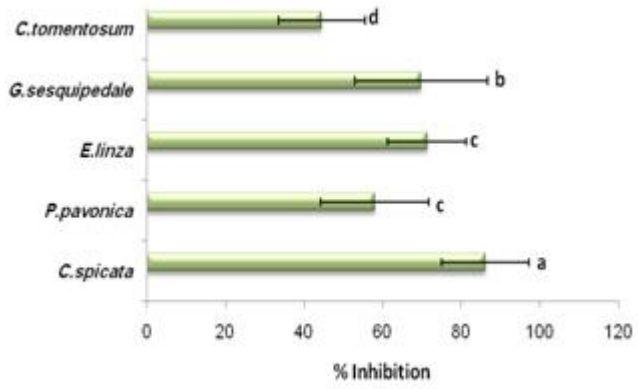

Figure 4: Effect of algal extracts, at 4mg/mL on anti-lipid peroxidation. Each value is the mean ± SD (n=3).

Much available evidence in the literature suggests the protective effects of seaweeds against oxidative stress in target tissues and lipid oxidation in foods [9]. Some of these active anti-lipid peroxidation compounds from marine algae were identified such as phlorotannins in *Sargassum kjellamanianum* [45] and fucoxanthin in *Hijikia fusiformis* [46]. The maximum value of suppression of lipid peroxidation was recorded in *C. spicata* extract (86%) which was significantly greater as compared with other extracts ($P<0.05$). whereas the less value was observed in *C.tomentosum* (44%) (Figure 4).

NO is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citrulline with the formation of NO via a five electron oxidative reaction [47]. These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of with extracts lead to suppression of NO• release and that may be attributed to a direct NO• scavenging effect as all the seaweed extracts decreased the amount of nitrite generated from the degradation of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. Nitric oxide scavenging ability of *seaweeds extracts* was increased with increasing the extract concentrations (0.062, 0.25, 0.5 and 1 mg mL^{-1}), the maximum value was obtained at 1 mg/ml by *C. spicata* (56%) followed by *G. sesqulpedale* (53.26%), C. *tomentosum* (44.64%), E. *linza* (35.73%), and *P. pavonica* (27.49%) (Figure 5). It is to be noted that *C. spicata* shows

a greater inhibition comparative to other algae extracts but less than ascorbic acid which has shown 68.5% inhibition of NO. In previous studies suggested that the nitric oxide scavenging activity of algal extracts is related to their content of flavonoids and phenolic compounds [47,48].

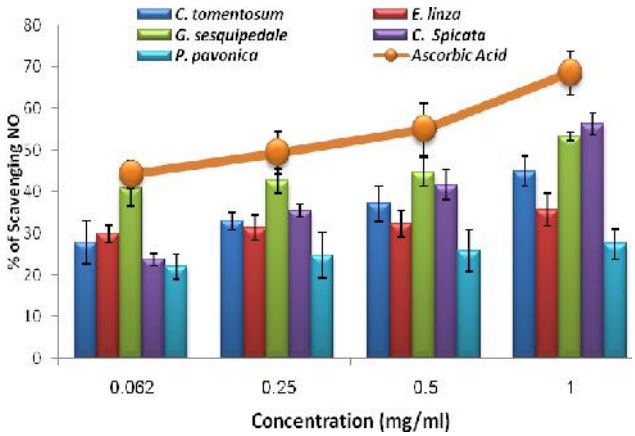

Figure (5): Effect of algal extracts and ascorbic acid at 0.062, 0.25, 0.5 and 1 m/mL on free radical scavenging toward NO radicals. Values are means of triplicate determinations ± standard deviation.

In the test of deoxyribose, there is liberation of the hydroxyl radical which the most active of reactive oxygen derivatives [44, 49]. The effect of methanol extracts of *C. spicata*, C. *tomentosum*, *E. linza*, *G. sesqulpedale* and *P. pavonica* involving in the inhibition of the formation of OH radical in order to prevent oxidative degradation of the deoxyribose was evaluated (Figure 6). Extract of *C. spicata* showed highly significant of inhibition of producing OH radical (56%) as compared with other extracts (P<0.001), while the extract of *E. linza* gave a weak degradation oxidative deoxyribose with percentage of inhibition of 8%.

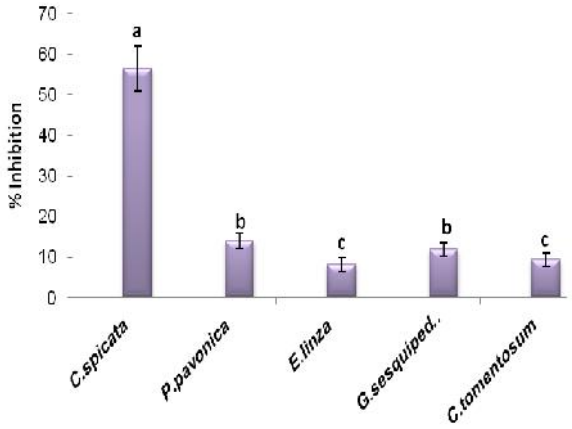

Figure 6: Deoxyribose radical scavenging activity of algal extracts at 12.5 mg/ml. Values are means of triplicate determinations ± standard deviation. Mean values followed by different superscript in the same column are significantly (P < 0.05) different.

The correlation between bioactive compounds and antioxidant activity

The antioxidant property of various seaweeds such as edible brown, green and red seaweeds has been correlated to their phenolic content [50]. This activity is believed to be mainly due to their redox properties [51,52] which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides. In our study, Pearson's correlation coefficient was considered positively high if $0.60 \leq r \leq 0.97$.

Table 1: Preliminary phytochemical screening of crude extract of some algae from Libyan coast

Algae	*C. tomentosum*	*C. spicata*	*E. linza*	*P. pavonica*	*G. sesquipedale*
Flavonoids	-	++	-	-	+
Tannins	-	+++	-	-	+++
Saponins	+	++	+	++	+
Alkaloids	+	+++	++	+++	++

(+++) high; (++) medium; (+) poor; (-) no found

Table 2: Total condensed tannins and total flavonoids content of methanol extracts of tested algae.

Algae	TP (mg Galic acid/g DW)	TF (mg Rutin/gDW)	CT (mg tannic acid/gDW)
C. spicata	125.93±28.15^{a}	163.91±87.40^{a}	85.77±12.98^{a}
P. pavonica	21.33±2.57^{c}	82.96±125.31^{c}	60.88±10.36^{b}
E. linza	26.4±3.8^{c}	90.20±52.05^{b}	28.66±5.42^{d}
G. sesqulpedale	128.33±21.93^{a}	74.0±95.26^{d}	44.0±12.66^{c}
C. tomentosum	36.33±28.30^{b}	88.5±40.46^{b}	47.11±13.55^{c}

Each value is presented as mean ± SD (n = 3). Means within each column with different letters (a-d) differ significantly (P <0.05).

Table 4: Pearson's correlation coefficients between the variables.

Scavenging activity	TP (mg Galic acid/g DW)	TF (mg Rutin/gDW)	CT (mg tannic acid/gDW)	Alkaloids (mg)
DPHH	0.781*	0.325	0.273	0.271
NO radical	0.621 *	0.148	0.117	0.411
Phosphomolybdenum	0.766*	0.633*	0.643*	0.052
Deoxyribose radical	0.372	0.933**	0.796*	0.367

Anti-lipid peroxidation	0.538	0.516	0.197	0.112

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

All samples showed no significant correlation with their alkaloid contents. However, the highly positive correlation between total phenolic content and DPPH scavenging activity (r = 0.781, $P<0.05$), total antioxidant activity (r= 0.740, $P<0.05$) as well as with NO scavenging activity (r= 0.621, $P<0.05$). Based on these data it could be concluded that antioxidant capacities in algae extracts by DPPH methods might be estimated indirectly by determining their total phenolic content. The positive and high correlation between flavonoids content and deoxyribose scavenging activities were observed (r= 0.933, $P<0.01$) (Table 4). Also a positive correlation between flavonoids content and total antioxidant activity (r = 0.633, $P<0.05$) was obtained. These results are in accordance with other reports in the literature, which have demonstrated that phenolic compounds were one of the most effective antioxidant compounds in marine algae [53,54] . Algae generally have higher antioxidant activity due to higher contents of nonenzymatic antioxidant components such as ascorbic acid, reduced glutathione, phenols and flavonoids [55].

4. Conclusion

The results of the present study indicated that Libyan seaweeds; *C. spicata*, C. *tomentosum*, *E. linza*, *G. sesqulpedale* and *P. pavonica*, successfully displayed antioxidant activity and positively correlated with its phenolics, condensed tannins and flavonoids. However, it was found that the different algal extracts were remarkably different in antioxidant capacity. The maximum antioxidant activity was seen in the brown algae *C. spicata* as well as an interesting high content of most active compounds (Phenolics, flavonoids and condensed tannins). This is a promising finding, as there may be a potential to utilize such extracts in food products to act as antioxidants which could enhance food quality, and also as antimicrobial agents, which could potentially increase the shelf life and safety of a wide range of food products as well as for health supplements to alleviate oxidative stress.

5. Acknowledgement

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Conflict of Interest: None declared

6. References

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