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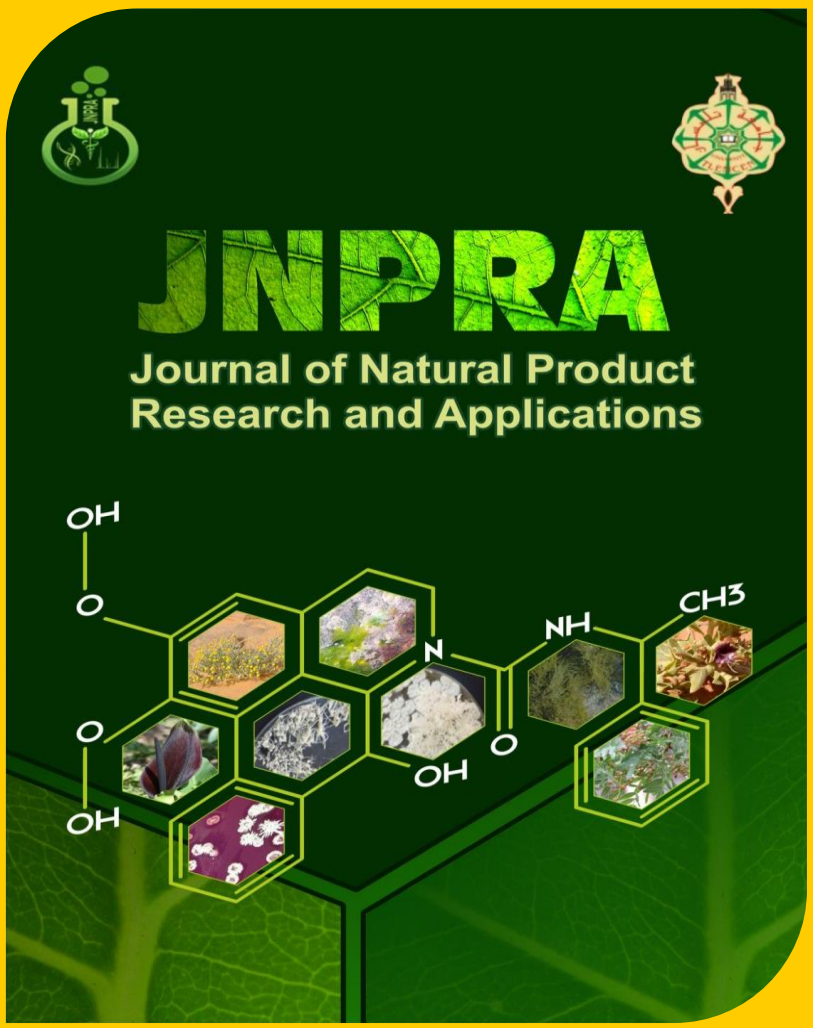

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Research Article

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Antioxidant Activity of Brown Seaweed (*Padina pavonica* (L.) Extracts From the Algerian Mediterranean Coast

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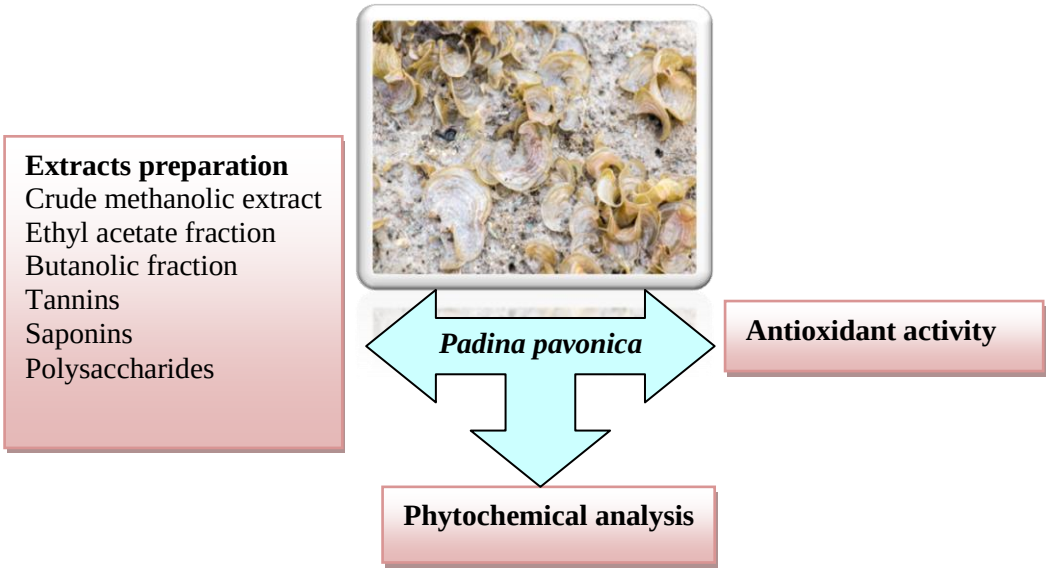
Highlights

- The crude methanolic extract from *Padina pavonica* was prepared and their total phenolic, flavonoid and proanthocyanidin contents were determined.
- Ethyl acetate and butanolic fractions, tannins, saponins and polysaccharides were also extracted.
- The antioxidant activity of all extract, using three assays were determined.

Graphical Abstract



Abstract
The crude methanolic extract, ethyl acetate and butanolic fractions, tannins, saponins and polysaccharides of the marine brown alga, *Padina pavonica*, growing on the Algerian Mediterranean Coast were measured for antioxidant activity, using total antioxidant capacity (TAC), reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. Total phenolic, flavonoid and proanthocyanidin contents were determined. The total phenolic, proanthocyanidin and flavonoid contents of methanolic extract were about of 2.007 ± 0.104 mg GAE/g DM, 4.611 ± 0.346 mg CE/g DM and 1.132 ± 0.091 mg CE/g DM, respectively.
The total antioxidant capacity was higher (4.365 ± 0.452 mg AAE/g DM) in crude methanolic extract. For the other two tests, the tannins and ethyl acetate fraction presented high activities compared to the other extracts with EC_{50} about 1.430 ± 0.000 and 1.615 ± 0.009 mg/mL), respectively for the reducing power and 5.718 ± 1.111 and 5.848 ± 0.186 mg/mL for the DPPH radical scavenging. The seaweed extracts displayed moderate antioxidant activity compared to ascorbic acid.

Keywords: *Padina pavonica*; antioxidant activity; total antioxidant capacity; reducing power; DPPH radical scavenging.

1. Introduction

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds such as carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals with various biological activities (Kumar Chandini et al., 2008). Marine algae present a source of novel bioactive substances. Several research revealed that marine algal originated compounds exhibit various biological activities involved as antimicrobial, antivirus, antioxidant, antitumor and anti-inflammatory (Wijesekara et al., 2011; Demirel et al., 2012). Recently, there is a considerable interest of marine algae in the food industry as well as pharmaceutical industry for the development of antioxidants from natural sources.
About 6000 species of seaweeds have been identified and are grouped into different classes viz., green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae (Kumar Chandini et al., 2008). Brown algae are economically valuable seaweeds as a source of raw material for the extraction of polysaccharides (e.g. laminarin, alginate and fucoidan), mannitol and phlorotannins. Bioactive metabolites of brown algae are phenolic compounds, such as phlorotannins (oligomers of phloroglucinol), which play ecological roles, such as antifouling substances, antioxidant, anti-plasmin inhibitor, chemical defenses against pathogens, epiphytes and grazers, and are photoprotection against solar radiation (UV radiation) (Demirel et al., 2012).
Padina pavonica, commonly known as Peacocks tail, is a brown alga belonging to Phaeophyceae class, growing abundantly in the Mediterranean Sea. This alga is mainly abundant from June to September. The mature species are frond as thin, flattish and often concave, or almost funnel shaped (Sari and Tuzen, 2009; Ismail-Ben Ali et al., 2010). Previous studies reported the biological potentialities of *P. pavonica* (Ktari et al., 2001). The test of antibacterial activity showed that the petroleum ether extract of *P. pavonica* presented an important activity against *klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Chbani et al., 2011). The ethyl acetate fraction showed the highest antioxidant activity (42.5%) and a significant antifungal activity against *Candida glabrata* (Naja et al., 2012). *P. pavonica* was rich in stimulating natural substances for test crops growth without causing damage to the soil. This Alga was incorporated as an organic fertilizer of plant growth. Furthermore, the aqueous extracts of this alga proved endowed with a strong antifungal capacity against the fungi tested (Omezzine et al., 2009; Chbani et al., 2013). Other authors evaluated the natural

biomass available (Ksouri et al., 2008) and abiotic and biotic factors (weight, height, diameter, biomass, fertility) of *P. pavonica* (Ben Said et al., 2002). Kamenarska et al. (2002) recorded that this species was rich in sterol and lipid compounds which were significant components of cellular membranes and responsible for a great number of cellular functions. The principal sterol of *P. pavonica* in Mediterranean area was fucosterol (24-hydroperoxy-24-vinyl-cholesterol) and as being responsible for the cytotoxic activity (Ktari and Guyot, 1999).

In this paper, we report the antioxidative potential of brown alga *P. pavonica* of the Algerian Mediterranean Coast by measuring the total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power assay. The total phenolic, flavonoid and proanthocyanidin contents in various extracts were also determined.

2. Materials and methods

2.1. Plant material

The marine alga, *P. pavonica* was collected from Aïn Témouchent region situated in the Northwest Coast of Algeria (Latitude 35°18′ N, Longitude 1°28′ O), during Mai 2012. The samples were washed thoroughly with seawater and then freshwater to remove epiphytes and other dirt particles, followed by shade–drying one week. Dried alga was ground in an electric mixer and stored at 4 °C for further experiments. The algal sample was identified by Dr. BENGUEDDA Wacila from Tlemcen University according to Fischer et al. (1987), and voucher specimens are deposited in Natural Products Laboratory Herbarium of Tlemcen University (Algeria).

2.2. Preparation of extracts

2.2.1. *Crude methanolic extract*

Dried seaweed material (1g) was extracted in 20 mL of methanol for 48 h at room temperature. Then, the extraction was filtered through a glass funnel and Whatman No. 0.45 µm filter paper. The filtrate was concentrated under reduced pressure at 40°C using HAHNVAPOR Rotavapor HS-2005V.

2.2.2. Ethyl acetate and butanolic fractions

The sample of *P. pavonica* was directly extracted with methanol at room temperature (20 mL/48 h). Then, the suspension was filtered and the solvent eliminated under vacuum. The residue was dissolved in 10 mL of boiling water, and then successively partitioned with 10 mL of ethyl acetate and 10 mL of n-butanol, respectively. After separation and evaporation, the organic phases were weighed and stored before use (Bekkara et al., 1998).

2.2.3. Tannins

Tannins extraction from alga was obtained according to the method of Zhang et al. (2008). The powder (5 g) was extracted with 100 mL of acetone-water (70/30, v/v), and the mixture was stirred continuously for 72 h, at room temperature. Then, the mixture was filtered and evaporated under vacuum at 40 °C to remove acetone. The remaining solution was washed with 30 mL of dichloromethane to remove lipid soluble substances. After the elimination of dichloromethane under reduced pressure, the aqueous phase was extracted with 30 mL of ethyl acetate. This process was repeated twice. Then, the organic phases recovered (ethyl acetate) containing tannins were mixed and evaporated to dryness. The residue obtained was weighed and preserved until uses.

2.2.4. Saponins

Saponins were extracted according to the method of Applebaum et al. (1969). Powdered alga was delipidated during 2 h by 75 mL of n-hexane. After elimination of the organic phase, the precipitate obtained was macerated in 25 mL of absolute ethanol under magnetic agitation at ambient temperature during 24 h. The ethanolic phase was evaporated at 40 °C by the rotavapor. The dry residue was extracted two times with 50 mL of a distilled water/petroleum ether mixture (v/v) heated at 50 °C in a water-bath during 30 min. The aqueous phases were mixed, and then, treated by 5 mL of butanol during 30 min. The organic phase, evaporated at 40 °C, was weighed and dissolved in 3 mL of methanol.

2.2.5. Polysaccharides

The extraction of polysaccharides was determined by the method prescribed by Zhenfei et al. (2012). The powdered alga (5 g) was defatted with 50 mL anhydrous ethanol at 60 °C for 3 h under stirring. After filtration, 100 mL of distilled water are added to the dry powder of the alga at 100 °C under stirring for 2 times and 3 h for each time. After centrifugation, the supernatant was concentrated to about a quarter of the original volume by evaporation and precipitated with 20% ethanol containing 0.2 % $CaCl_2$ at 4 °C overnight. After evaporation, the precipitate was weighed and dissolved in 4 mL of distilled water.

2.3. Determination of total phenolic content

The total phenolic content in methanolic extract was determined by spectrometry using ‘‘Folin-Ciocalteu’’ reagent assay (Singleton and Rossi, 1965). A volume of 200 µL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times in water, and 0.8 mL of 7.5 % sodium carbonate solution in a test tube. After 30 min of stirring, the absorbance was measured at 765 nm using a Specord 200 plus UV/VIS spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). All tests were carried out in triplicate.

2.4. Determination of total flavonoids

The total flavonoid content in methanolic extract was determined by a colorimetric assay using a method described by Zhishen et al. (1999). Briefly, 500 µL of methanolic extract was mixed with 1500 µL of distilled water in a test tube, followed by addition of 150 µL of a 5% (w/v) $NaNO_2$ solution, at time zero. After 5 min, 150 µL of $AlCl_3$ at 10% (m/v) was added. After 6 minutes of incubation, at room temperature, 500 µL of NaOH (1 M) were added. The mixture was homogenized immediately after the end of the addition. The absorbance of the solution was measured at 510 nm against the blank. Total flavonoid contents were calculated as catechin equivalents from a calibration curve of catechin and as expressed as mg CE/ g DM). All tests were carried out in triplicate.

2.5. Determination of total proanthocyanidin content

The total proanthocyanidin content in methanolic extract were measured using the vanillin assay described by Julkunen-Titto (1985). To 50 µL of methanolic extract, 1500 µL of vanillin/methanol solution (4%, w/v) was added, and the solution was homogenized. Then, 750 µL of concentrated HCl were added and allowed to react at room temperature for 20 min. The absorbance at 550 nm was measured against the blank. The amount of total proanthocyanidin content was expressed as milligrams of catechin equivalents per gram of dry matter (mg CE/ g DM) from the calibration curve. All tests were carried out in triplicate.

2.6. Total antioxidant capacity

The total antioxidant capacity (TAC) of brown alga extracts was evaluated by the phosphomolybdenum method of Prieto et al. (1999). A 0.3 mL aliquot of extract was mixed with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. Then, the samples were cooled down to room temperature and the mixture's overall absorbance was measured at 695 nm against a blank. The antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalents per gram of dry matter (mg AAE/ g DM).

2.7. Reducing power

The reducing power of *P. pavonica* extracts was determined according to the method of Oyaizu (1986). Various concentrations of the extracts (mg/mL) in distilled water were mixed with a phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1 % of potassium ferricyanide water solution (2.5 mL, $K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. Aliquots of trichloracetic acid (2.5 mL, 10%) were added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared $FeCl_3$ solution (0.5 mL, 0.1 %). The absorbance was measured at 700 nm. An increased absorbance of the reaction mixture was taken to mean an increased reducing power. Ascorbic acid was used as a positive control. EC_{50} value (mg/mL) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

2.8. DPPH radical scavenging assay

The free radical scavenging activity was measured by a modified $DPPH^{\bullet}$ assay (Sanchez-Moreno et al., 1998). Fifty microliters of various concentrations of the extracts in methanol were added to 1.950 mL of a 0.025 g/L methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was measured against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100$$

Where $A_{control}$ is the absorbance value of DPPH, A_{sample} is the absorbance value of the sample and DPPH.

The extract concentration providing 50% inhibition (EC_{50}) was calculated from the plotted graph of inhibition percentage against extract concentrations. The ascorbic acid methanolic solution was used as positive control.

2.9. Statistical analysis

All analyses of antioxidant activity were performed in twice. Data were expressed as means ± standard derivation (SD) using Excel programme and Microcal Origin 6.

3. Results and Discussion

3.1. Extract yields

Yields of crude methanolic extract and fractions of *P. pavonica* are shown in Table 1. The highest yield was observed in crude methanolic extract (4.18 %) followed by the polysaccharides extract (3.48 %). This result was lower than others studies reported by Kokabi et al. (2013) with methanolic extract (6.56 %) and Naja et al. (2012) with ethyl acetate fraction of *P. pavonica* (28.76 %).

Table 1. Yields of bioactive compound.

Bioactive compound	Yields (%)
Methanolic extract	4.18
Ethyl acetate fraction	1.86
Butanolic fraction	0.48
Tannins	0.40
Saponins	0.25
Polysaccharides	3.48

3.2. Total phenolic, flavonoid and proanthocyanidin contents

The total phenolic, flavonoid and proanthocyanidin contents were presented in Table 2. The methanolic extract contain higher amount of proanthocyanidin content (4.611 ± 0.346 mg CE/ g) than total phenolic (2.0077 ± 0.104 mg GAE/ g) and flavonoids (1.132 ± 0.0919 mg CE/g). These results found in our study were correlated with the phenolic content of *P. pavonica* (2.181 ± 0.50 mg GAE/g), reported by Demirel et al. (2012), but tenfold higher (10.55 ± 0.23 mg GAE/g) in the study found by Naja et al. (2012).

Table 2. Total phenolic, flavonoid and proanthocyanidin contents.

	Total phenolics (mg GAE/g DM)	Total proanthocyanidins (mg CE/g DM)	Total flavonoids (mg CE/g DM)
Methanolic extract	2.007 ± 0.104	4.611 ± 0.346	1.132 ± 0.091

Values are means ± SD of three replicate determinations.

3.3. Total antioxidant capacity

The TAC test is based on the assessment of the ability of the sample to donate electrons, thus neutralizing compounds such as free radicals, like reactive oxygen species (Teodosio Melo et al., 2013). Total antioxidant capacity of different extracts from *P. pavonica* is presented in Table 3. The methanolic extract exhibited higher value of total antioxidant capacity (4.365 ± 0.452 mg AAE/g DM), followed by ethyl acetate fraction (2.511 ± 0.094 mg AAE/g) and butanolic fraction (1.761 ± 0.149 mg AAE/g). For the others extracts, this capacity is a very weak.

Table 3. Total antioxidant capacity (TAC) and EC_{50} (mg/mL) concentrations of reducing power and DPPH radical scavenging of *P. pavonica* extracts.

Bioactive compound	**TAC (mg AAE/g DM)**	**EC_{50} (mg/mL)**	
		Reducing power	**DPPH test**
Methanolic extract	4.365 ± 0.452	2.498 ± 0.004	21.329 ± 1.935
Ethyl acetate fraction	2.511 ± 0.094	1.615 ± 0.009	5.848 ± 0.186
Butanolic fraction	1.761 ± 0.149	3.841 ± 0.048	10.309 ± 0.298
Tannins	0.562 ± 0.045	1.430 ± 0.000	5.718 ± 1.111
Saponins	0.065 ± 0.005	6.189 ± 0.744	8.911 ± 1.296
Polysaccharides	0.883 ± 0.023	15.950 ± 0.237	9.663 ± 0.428
Ascorbic acid		0.063 ± 0.002	0.090 ± 0.002

3.4. Reducing power

In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. The presence of reductants in the solution causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Aidi Wannes et al., 2010). Table 3 showed the Fe^{3+} reducing power ability of alga extracts. The tannins (EC_{50} = 1.430 ± 0.000 mg/mL) and ethyl acetate fraction (EC_{50} = 1.615 ± 0.009 mg/mL) had higher reducing power when compared to the others extracts but still lower than that of the ascorbic acid (EC_{50} = 0.063 ± 0.002 mg/mL).

3.5. DPPH radical scavenging assay

The electron donation ability of the obtained extracts was measured by bleaching of the purple-colored solution of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). Lower EC_{50} value indicated higher antioxidant activity (Table 3). Among the tested extracts, the tannins exhibited higher DPPH radical scavenging activity of 5.718 ± 1.111 mg/mL followed by ethyl acetate fraction (5.848 ± 0.186 mg/mL). However, the antioxidant activities to scavenge DPPH free radical of all extracts were lower than that of ascorbic acid (EC_{50} = 0.090 ± 0.002 mg/mL). This result is in agreement with those reported by Naja et al. (2012) and Kokabi et al. (2013). These authors were demonstrated that the ethyl acetate extract of *P. pavonica* shown to have a better scavenging activity to DPPH free radical when compared to the others extracts. Several studies have demonstrated the correlation between polyphenol content and radical scavenging activity. The presence of hydrophilic polyphenols in seaweeds such as phlorotannins, which are bipolar in nature, and mostly found in brown seaweeds, could function as antioxidative components and thus assist the algae to overcome oxidative stress (Indu and Seenivasan, 2013)

4. Conclusion

From the present finding, it was concluded that *P. pavonica* can be utilized as a source of natural antioxidant compounds in food and medical industries. The results show that ethyl acetate fraction and tannins exhibit higher antioxidant activities when compared to others extracts. This activity is probably related to the presence of phenolic compounds in these extracts. Further study is necessary for isolation and characterization of the specific chemical, which is responsible for the antioxidant activity and can be used to treat various oxidative stress-related diseases.

Author Contribution Statement

K. Kerzabi-Kanoun: Carried out the experiment and wrote the manuscript; N. Belyagoubi-Benhammou and L. Belyagoubi: analysed the data and corrected the manuscript; M. Benmahdjoub and G. Aissaoui: Contributed in experiment and discussion of results; W. Benghedda: identified the algal species; F. Atik- Bekkara: Designed and supervised this research.

Conflict of interest

The authors declare that they have no conflict of interest.

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