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Antioxidant and Antifungal activities of *Padina Pavonica* and *Sargassum Vulgare* from the Lebanese Mediterranean Coast

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ABSTRACT

The aim of our investigation was to evaluate the antioxidant, the antifungal activities and the phenolic content of extracts from two brown algae *Padina Pavonica* and *Sargassum Vulgare* from the Lebanese coast. Two different extraction methods with methanol, followed by fractionation (petroleum ether, ethyl acetate, butanol and aqueous) were carried out. Antioxidant activities were evaluated using the DPPH method. The method of disk diffusion was used to evaluate the antifungal activity of seaweed extracts against Candida. The total phenolic content was determined with the Folin–Ciocalteu method. The ethyl acetate fraction of the algae *Padina Pavonica* showed a significant antifungal activity against *Candida glabrata* (diameter of inhibition = 16 mm) and *Candida krusei* (diameter of inhibition = 14 mm). The ethyl acetate fraction of the algae *Padina Pavonica* showed the highest antioxidant activity (42.5%); those activities may be due to phenolic compounds present in significant amounts in this fraction (8.98 GAE/g). Also, on the other hand, the petroleum ether fraction of the algae *Sargassum Vulgare* had the highest antioxidant activity (40.6%), which seems to be due to lipids because the phenolic content in this fraction is lower (6.10 GAE/g). No antifungal activity was detected with all extract of *Sargassum Vulgare*.

Key words: Lebanese Coast, Brown algae, *Padina Pavonica, Sargassum Vulgare,* Antioxidant activity, Antifungal activity.

Introduction

Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and grouped into different classes: green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae [9]. It has been reported that seaweeds serve as an important source of bioactive natural substances. In fact, the discovery of metabolites with biological activities, from macroalgae, has increased significantly in the past three decades [23,1].

On the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids, mycosporine-like amino acids (mycosporine-glycine) and catechins (*e.g*., catechin, epigallocatechin, epigallocatechin), gallate, phlorotannins (*e.g*., phloroglucinol) and tocopherols.

Furthermore, antioxidants from natural sources increase the shelf-life of foods [22]. Therefore, consumption of antioxidant and/or addition of antioxidant in food materials protect the body as well as foods against. Many researchers have reported various types of antioxidants in different kinds of higher plants [4;18]. More recent reports revealed seaweeds to be a rich source of antioxidant compounds [8,13,17,20]. Brown-algal polyphenols phlorotannins worked as antioxidants, antimicrobial compounds [5,8,10,13,16,28]

Bianchi and Morri estimate that more than 8500 macroscopic marine species should live in the Mediterranean Sea, which constitutes 4–18 % of the world's marine species (depending on different estimates of global diversity).

Further, reports on the antioxidant properties of seaweed extracts from Lebanon and other biological activities are very limited.

Recently, an antibacterial activity of *Padina Pavonica* from Lebanon against clinical strains of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaricus* and *Enterococcus faecalis*) was carried out [6].

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Hence, the present study was aimed to investigate and evaluated the antioxidant and antifungal activities of crude extracts and their different fractions of *Padina Pavonica* and *Sargassum Vulgare* using two different extraction methods compared to two synthetic antioxidants, α tocopherols and butylated hydroxyanisole (BHA). Furthermore the relationship between antioxidant activity and total phenolic content were also considered in this study.

Materials And Methods

2.1. Chemicals:

2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocatteau's phenol reagent, Gallic acid were purchased from Sigma-Aldrich.

All other chemicals and solvents, such as methanol, petroleum ether, ethyl acetate and n-butanol were of the highest analytical grade and were purchased from common sources.

2.2 Marine algal material:

The marine algae *Padina Pavonica* and *Sargassum Vulgare* used for this study was freshly collected from the third island of Tripoli-Lebanon (N34.46864 E35.80238) at 1.5 Km off the coast, in the spring of 2010. Samples collected were gently rinsed with filtered seawater and immediately transported to the laboratory, then rinsed with sterile distilled water and air dried for 6 days at room temperature in the darkness and then ground into a fine powder in a mixer grinder (Kandhasamy et al. 2008). Samples were packed and stored in a refrigerator until the experiments were carried out.

2.3. Microorganisms:

The four fungal species strains of Candida used were *Candida albicans* LHN 099, *Candida glabrata* LHN 093, *Candida krusei* LHN 063 and *Candida tropicalis* LHN 098. The microorganisms were obtained from the collection of Department of Microbiology-Lebanese University - Azm Center for Biotechnology Research in Tripoli.

2.4. Preparation of marine algal extract and fractions:

Two methods were used in the extraction phase. In the first one, 10 g marine algal were macerated for three days in 100 ml methanol at room temperature in an orbital shaker; the second extraction was also in methanol but using a Soxhlet extractor for six hours.

The total extracts were filtered, and the obtained filtrates were concentrated under reduced pressure to dryness, yielding the crude extract which was then suspended in 90% aqueous methanol. The solution was partitioned with 3× 100 ml of petroleum ether and the solution of aqueous methanol was evaporated under reduced pressure to a semisolid, dissolved in 200 ml distilled water, then successively partitioned with 3× 100 ml of ethyl acetate and 3× 100 ml of n-butanol, respectively. The resulting four extracts were evaporated to dryness in vacuum, to yield the petroleum ether, ethyl acetate, n-butanol soluble fractions and aqueous residue [5].

2.5. Antifungal activity test:

Antifungal activity was carried out against *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida tropicalis*. The microorganisms were grown on Sabouraud dextrose agar (Merck, Germany) plates at 24°C for 48 hours prior to seeding onto the Sabouraud dextrose agar (Merck, Germany). One or several colonies of similar morphology of each Candida were transferred into sterile distilled water and adjusted to the 0.5 McFarland turbidity standards. The inocula of the respective fungi were seeded on Sabouraud dextrose agar. The sterile disks were impregnated with different extracts and then dried (25µl/disc). The sterile filter disks 6.4 mm in diameter were placed on inoculated agar medium and incubated at 24°C for 48 hours. Nystatin (100 U/disc) was used as positive antifungal control. Methanol solvent (100%) without algal extract was also used as negative control.

The diameter (mm) of the growth inhibition halos caused by the different extracts was measured. All the assays were carried out in triplicate.

2.6. Total phenolic content:

The total phenolic of the extract and fractions were determined with Folin–Ciocalteu reagent using the method of Singleton et al. [24].

20 µl of sample were mixed with 300 µl of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteau's phenol reagent were added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark.

Absorbance of all the sample solutions was measured at 765 nm using a spectrophotometer. Phenolic contents are expressed as Gallic acid equivalents per gram (GAE/g) of extract. Calibration curve is prepared using Gallic acid stock solution.

2.7. DPPH radical scavenging activity:

The scavenging effects of samples for DPPH radical were monitored according to the method of Yen and Chen [25].

2.0 ml aliquot of the test sample was added to 2.0 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark, and its

absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using the follow equation given by [8]

$$\% \text{ of inhibition} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Where the $A_{control}$ is the absorbance of the control (DPPH solution without sample), the A_{sample} is the absorbance of the test sample (DPPH solution plus test sample).

Synthetic antioxidants, BHA, α-Tocopherol were used as positive controls.

Results And Discussion

3.1. Extract and fractions yield:

Yields of total methanolic extract and fractions of two brown seaweeds after room temperature (RT) and Soxhlet extraction are shown in table 1.

In *Padina Pavonica*, the ethyl acetate fraction showed the highest yield (28.76%) and (28.63%), while in *Sargassum Vulgare*, the petroleum ether fraction showed the highest yield (27.31%) and (27.12%).

The yield of the total fraction by extraction with Soxhlet or room temperature in two algae showed no significant difference, which shows that the extraction can be done at room temperature or at high temperature using Soxhlet but it is better performed at a room temperature extraction despite the time it needs because the temperature may degrade certain sensitive products.

3.2. Antifungal activity test:

The antifungal activity of total methanol extracts of the two algal species and fractions is summarized in Table 2. The antifungal activity was classified from less active (+:10 mm ≤ Diameter of inhibition < 16 mm), to moderately active (++: 16 mm ≤ diameter of inhibition <20 mm), to highly active (+++: diameter of inhibition ≥ 20 mm) and non active (- : diameter of inhibition < 10 mm.

No antifungal activity was detected from total extract and fractions of *Sargassum Vulgare*, while ethyl acetate fraction from *Padina pavonica* showed a moderate activity against *Candida glabrata* (diameter of inhibition=16 mm) and a lesser activity against *Candida krusei* (diameter of inhibition=14 mm)

3.3. Total phenolic content:

The phenolic content was determined using Folin – Ciocalteu reagent and was expressed as gallic acid equivalents (GAE) as shown in Table 3.

The results showed that the phenolic content of the crude extract of *Sargassum Vulgare* was higher than that of the crude extract of *Padina Pavonica*, which proposes that *Sargassum Vulgare* contains more of these compounds as *Padina Pavonica* although the fraction Ethyl acetate of the latter showed a greater level which is consistent with the literature. [8]. The content of phenolic compounds showed no significant difference between the extraction by Soxhlet and the room temperature with the same solvent, showing that this level does not depend on the extraction method and depends on the polarity of solvents used.

Ethyl acetate extract of *Padina Pavonica* showed the highest concentration of phenolic compounds (8.90 mg GAE /g and 8.98 mg GAE/ g).

In Sargassum *Vulgare*, the butanol extract showed the highest content (7.15 and 7.00 mg GAE/g), demonstrating that several phenolic compounds were extracted.

These results are confirmed by TLC (thin layer chromatography) and a simple spectroscopic study recordings of absorption spectra for the ethyl acetate fraction of *Padina Pavonica* and *Sargassum Vulgare* fraction of butanol between 200 and 800 nm show peaks between 265 nm and 270 nm, which confirms the presence of phenolic compounds (maximal absorbance of phenol is between 270 and 275 nm).

DPPH radical scavenging activity:

DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds [19,21,26].

DPPH radical scavenging activities of *Padina Pavonica* and *Sargassum Vulgare* are summarized in Table 4.

Table 4a showed that the highest percentage of DPPH radical scavenging of *Sargassum Vulgare* was observed in petroleum ether fraction (40.6%) at concentration 1mg/L and at room temperature. While table 4b showed that the highest percentage of DPPH radical scavenging of *Padina Pavonica* was observed in the ethyl acetate fraction (42.9%) at 1 mg/L and also at room temperature. These results showed that compounds with medium polarity have the strongest radical scavenging activity in *Padina Pavonica*

Almost all fractions (except aqueous fraction) showed greater activity than crude extract itself which ensures the need for purification fractionation of crude extract. Indeed this is probably due to interactions between the compounds present in the extract that can exert an antagonistic effect between them.

The correlation between the concentration of the extract and the percentage of inhibition was studied: The percentage of inhibition increases with the concentration of the extract in all samples so the antioxidant activity is dose-dependent.

TLC of bioactive compounds:

The petroleum ether fraction of *Sargassum Vulgare* and the ethyl acetate fraction of *Padina Pavonica* are subjected to thin-layer chromatography on silica gel plate with a solvent system of cyclohexane/methanol/chloroform (50:30:20 v/v). The spots were visualized by spraying the plates with $FeCl_3$ solution. Phenols with hydroxyl function gave a blue spot, those with two hydroxyl groups turn green, and the other phenolic appeared red or brown.

Table 1: Yield of total extract (as % w/w of algae on dry weight basis) and fractions (as % of total methanolic extract)

Algae	*Padina Pavonica*		*Sargassum Vulgare*	
Extract	*RT extract*	*Soxhlet extract*	RT extract	Soxhlet extract
Total	11.70±0.10	11.9±0.30	9.6±0.43	10.1±0.17
Petroleum ether	19.73±0.1	27.31±0.08	19.16±0.02	27.12±0.44
Ethyl acetate	28.76±0.29	28.63± 0.60	20.77±1.23	20.50±3.3
butanol	25.44± 0.9	25.58±1.0	26.53±0.45	26.88±2.1
Aqueous	26.10 ±0.1	26.62±0.77	25.38 ± 1.23	25.51±0.65

Each value is presented as mean ± standard deviation (n = 3)

Table 2: Antifungal activity of algal species

Candida	*Albicans*	*Glabrata*	*Krusei*	*Tropicalis*
Algae extract				
Padina Pavonica				
Total	-	-	-	-
Petroleum ether	-	-	-	-
Ethyl acetate	-	++	+	-
Butanol aqueous	-	-	-	-
Sargassum Vulgare				
Total	-	-	-	-
Petroleum ether	-	-	-	-
Ethyl acetate	-	-	-	-
Butanol aqueous	-	-	-	-

Table 3: Total phenolic content (mg Gallic acid equivalents (GAE /g extract) of total extract and fractions.

Algae	*Padina Pavonica*		*Sargassum Vulgare*	
Extract	*RT extract*	*Soxhlet extract*	RT extract	Soxhlet extract
Total	10.55±0.23	10.76±0.87	12.71±0.54	12.66±0.65
Petroleum ether	4.77±0.43	4.71±0.2	5.94±0.65	6.10±0.76
Ethyl acetate	8.90±0.54	8.98±0.43	5.77±0.33	5.79±0.12
butanol	6.23±1.0	6.38±1.2	7.15±0.97	7.00±0.95
Aqueous	5.90±0.23	6.04±0.21	6.11±0.14	5.97±0.17

Each value is presented as mean ± standard deviation (n = 3)

Table 4a: DPPH radical scavenging activity (%) of total extract and fractions obtained from Padina Pavonica

Concentration (µg/ml)	200	250	500	1000
RT extract				
Total	2.95±0.42	3.55±0.23	4.8±0.12	12.9±0.26
Petroleum ether	6.4±0.66	6.85±0.32	11.25±1.21	20.3±1.98
Ethyl acetate	7.45±1.2	8.65±2.1	14.75±2.12	42.9±0.87
Butanol	3.65±0.08	3.95±1.4	10.0±0.07	17.7±0.76
Aqueous	0.25±0.02	0.9±0.05	3.5±0.07	5.15±0.12
Soxhlet extract				
Total	2.4±0.02	3.05±0.09	4.15±1.2	10.8±0.08
Petroleum ether	6.0±0.06	6.3±0.45	10.0±0.11	20.2±0.90
Ethyl acetate	7.1±0.55	8.45±0.43	14.1±1.32	42.0±1.65
Butanol	3.2±0.87	3.35±0.07	9.15±0.04	15.0±1.0
Aqueous	0.2±0.02	0.55±0.9	2.9±0.95	4.85±0.04
BHA	92.44±0.2	93.02±0.5	95.72±0.75	97.34±0.03
α-Tocopherol	89.73±0.03	90.00±0.12	93.45±0.08	95.69±0.23

Rf values of spots from the TLC chromatograms of the fractions after spraying are shown in Table 5. Therefore, on the basis of TLC analysis, at least four types of antioxidant compounds were identified in the ethyl acetate fraction of *Padina Pavonica* and five types of antioxidant compounds were identified in the petroleum ether fraction of *Sargassum Vulgare*

Table 4b: DPPH radical scavenging activity (%) of total extract and fractions obtained from Sargassum Vulgare

Concentration (µg/ml)	200	250	500	1000
RT extract				
Total	2.95±0.42	3.55±0.23	4.8±0.12	12.9±0.26
Petroleum ether	6.4±0.66	6.85±0.32	11.25±1.21	20.3±1.98
Ethyl acetate	7.45±1.2	8.65±2.1	14.75±2.12	42.9±0.87
Butanol	3.65±0.08	3.95±1.4	10.0±0.07	17.7±0.76
Aqueous	0.25±0.02	0.9±0.05	3.5±0.07	5.15±0.12
Soxhlet extract				
Total	2.4±0.02	3.05±0.09	4.15±1.2	10.8±0.08
Petroleum ether	6.0±0.06	6.3±0.45	10.0±0.11	20.2±0.90
Ethyl acetate	7.1±0.55	8.45±0.43	14.1±1.32	42.0±1.65
Butanol	3.2±0.87	3.35±0.07	9.15±0.04	15.0±1.0
Aqueous	0.2±0.02	0.55±0.9	2.9±0.95	4.85±0.04
BHA	92.44±0.2	93.02±0.5	95.72±0.75	97.34±0.03
α-Tocopherol	89.73±0.03	90.00±0.12	93.45±0.08	95.69±0.23

Each value is presented as mean ± standard deviation (n = 3)

Table 5: *Rf Values of Spots Separated on TLC Plate from different fractions*

Fraction	Rf	Color	Fraction	Rf	Color
Padina Pavonica			*Sargassum Vulgare*		
Petroleum ether	0.73 0.71 0.65	Green Green Yellowish green	Petroleum ether	0.92 0.90 0.84 0.72 0.67	Yellowish green Green Yellowish green Gray Green
Ethyl acetate	0.90 0.85 0.80 0.71	Brown Orange Orange Gray	Ethyl acetate	0.62 0.56	Blue green
Butanol	0.59 0.55	Brown Brown	Butanol	0.61 0.50	Yellow Brown

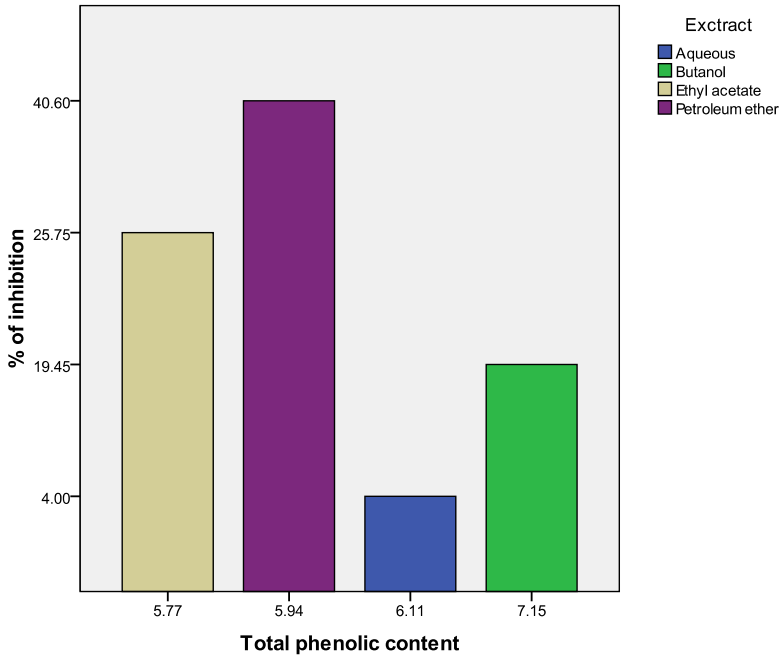

Fig. 1: Total phenolic content (mg Gallic acid equivalents (GAE /g extract) VS DPPH radical scavenging activity (%) in Sargassum Vulgare

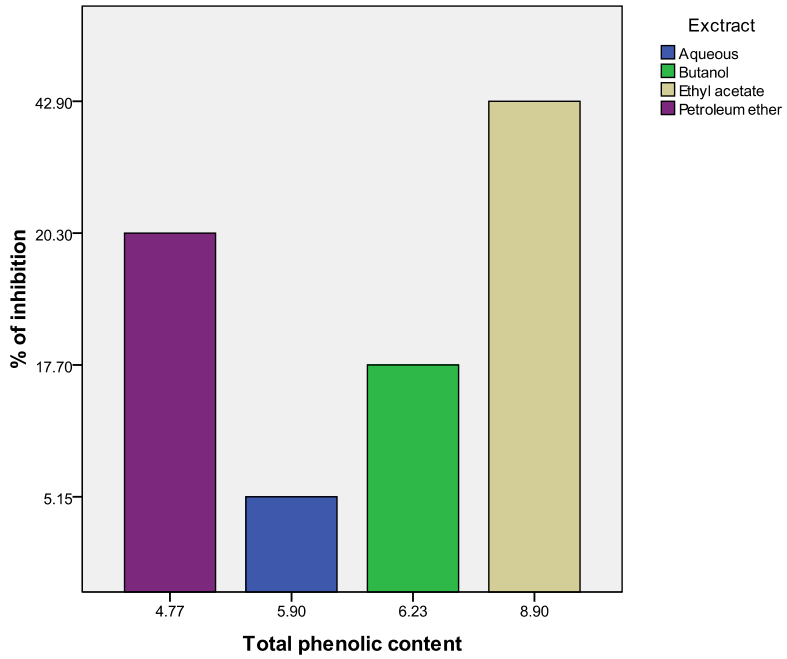

Fig. 2: Total phenolic content (mg Gallic acid equivalents (GAE /g extract) VS DPPH radical scavenging activity (%) in Padina Pavonica

Total Phenolic compounds and antioxidant activity :

Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids [11]. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants.

[4].

Quantitative comparison of total radical scavenging capacities and the total phenolic content is carried out; the results are shown in figure 1and 2. Results suggest that the antioxidant activity in *Padina Pavonica* may be due to phenolic compounds; in contrast, the antioxidant activity in *Sargassum Vulgare* may be due to phenolic compounds and/or other molecules not of phenolic nature.

Conclusion:

The results obtained in the present study clearly demonstrate that the ethyl acetate extract derived from *Padina Pavonica,* and petroleum ether extract derived from *Sargassum Vulgare* are fairly active fractions for *in vitro* DPPH free radical scavenging activity. In addition, the results suggest that phenolic compounds might be major contributors to the antioxidant activities of the two selected seaweeds from the coast of Lebanon.

Ethyl acetate fraction from *Padina Pavonica* showed a moderate activity against *Candida glabrata.*

The findings of the current work appear useful for further research aiming to isolate and identify the specific compounds which is responsible for higher antioxidant activity.

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