



Original article

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Antioxidant and antimicrobial activities of *Padina pavonica* and *Enteromorpha* sp. from the Tunisian Mediterranean coast

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ABSTRACT

Objective: To examine the antioxidant and the antimicrobial activities of the marine seaweeds *Padina pavonica* (*P. pavonica*) and *Enteromorpha* sp. from the Tunisian Mediterranean coast.

Methods: The acetone and water were used for algae extraction to envisage the antimicrobial activity versus Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and against four *Candida*. The microdilution method was used to evaluate this activity. *In vitro*, total phenolic content and the antioxidant activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and 2,2'-azino-bis-(3-ethylthiazoline)-6-sulfonic acid (ABTS) were also studied.

Results: The highest amount of phenolic compound was found in the *P. pavonica* acetic extract [(90.61 ± 0.11) mg catechin equivalent/g extract]. This brown algae sample demonstrated greater DPPH and ABTS radical scavenging ability potential in comparison to other green seaweed, *Enteromorpha* sp. The maximum antimicrobial activity was shown by the *P. pavonica* acetic extract against all the pathogenic strains tested (minimum inhibitory concentrations = minimum inhibitory bactericidal = minimum inhibitory fungicidal concentrations = 0.04 mg/mL). Those activities might be due to phenolic substances present in this fraction.

Conclusions: The present results highlight the possible use of *P. pavonica* as source of antioxidant and antimicrobial compounds.

1. Introduction

Oceans account for 71% of the earth's surface and are the largest remaining reservoirs of bioactive compounds[1]. Seaweeds are a great and varied group of freshwater and marine organisms with more than 10 000 various species described to date[2]. Marine macroalgae are capable to adapt to the extreme and changing marine environmental conditions such as temperature, salinity, radiation, nutrients and combination of oxygen and light concentration by producing secondary metabolites[3]. Macroalgae were listed containing wide spectrum of biological activities with wealthy

pharmacological potential, which can benefit human health[4,5]. They are at the moment in different phases of clinical assays due to their high content of polysaccharides, antioxidants, alkaloids, carotenoids, protein, dietary fibre, vitamins, terpenes, essential fatty acids, minerals as well as phenolic compounds making them interesting *candidates* to be utilized as food supplement, food additives, and a source of vitamins[6,7].

The algal genus *Padina* is well defined widely distributed all over the tropics and easily recognized[8]. A few species of *Padina* spp have been traditionally used as food source in some coastal civilizations, such as salt replacement for high blood pressure patients or seasoning in dry flake forms[9], and for treatment of goiter[10]. The *Enteromorpha* species have tubular thalli with sunken spaces that contain nutrient reserve compounds and dissolved organics. Several of these species are tolerant to heavy metals, thus, they often utilized as pollution indicators[11]. *Enteromorpha* sp. is recommended for human consumption because it has several beneficial components, such as mineral, protein, essential amino

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acids, essential fatty acid, and fiber[12]. *Enteromorpha* species are commonly processed to obtain dried green lavers. These lavers are lightly roasted, crushed and utilized as coating or topping in soups or other foods. They are equally available in powder shapes for like uses[13].

Synthetic antioxidant and antimicrobial components are generally used in food industry for its quality and for preserving food but these substances have been suspected of exerting carcinogenic effect and their toxicity. Hence, the aim of the present work was to study the antioxidant activity of the green and the brown macroalgal species [*Enteromorpha* sp. and *Padina pavonica* (*P. pavonica*), respectively] from Tunisian coast. To evaluate the efficiency of these two seaweeds extracts for producing antibacterial and antifungal activities against pathogenic bacteria and fungi was our second objective. The correlations between the total phenol content of samples prepared and its antimicrobial and antioxidant activities were investigated.

2. Materials and methods

2.1. Chemicals

Sodium phosphate buffer (pH 7), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), potassium peroxodisulfate, Folin-Ciocalteu reagent, Trolox, acetone, dimethylsulfoxide, Mueller-Hinton agar, potato dextrose agar, the 96 well microtiter plates with round bottom, and gentamicin were purchased from Sigma-Aldrich (Germany).

2.2. Algae collection and preparation of algal extracts

The two marine algae samples, brown algae, *P. pavonica* and green algae, *Enteromorpha* sp. were collected by hand picking in shallow water from Monastir coast, Tunisia. The algal samples were washed thoroughly with seawater to remove all the impurities, epiphytes and sand particles and then washed thoroughly using tap water. The specimens were deposited in the Herbarium of Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Team: Medicinal Chemistry and Natural Products, Faculty of Sciences of Monastir, University of Monastir, Tunisia. The fresh seaweeds (100 g) were cut into small pieces and extracted successively with acetone and water at 100 °C for 2 h using a reflux condenser under reduced pressure. The resulting four extracts were filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness using a rotary evaporator, to yield the acetone and aqueous extracts. The extract of each specimen was poured into vials and weighed. The vials were labeled and kept in refrigerator at 4 °C for further experiments. The extractive values were calculated on fresh weight basis from the following formulae:

Extractive value (yield %) = Weight of fresh algae/Weight taken after extraction × 100

2.3. Quantification of total phenolic content (TPC)

The TPC of seaweed extracts was determined using the protocol of Velioglu et al.[14] which uses Folin-Ciocalteu reagent. A volume of 100 µL of sample was mixed with 750 µL of Folin-Ciocalteu reagent

and allowed to stand for 5 min at room temperature. After incubation, 0.75 mL of Na₂CO₃ (saturated sodium carbonate solution) was added and the reaction was mixed thoroughly and allowed to stand for 90 min at 25 °C in the dark. Absorbance of all the extracts solutions was measured at 725 nm using a spectrophotometer. The TPC of macroalgae samples was expressed as mg catechin equivalents (CE) per g of extract. Calibration curve was prepared using catechin stock solution.

2.4. DPPH radical scavenging activity

The scavenging effects of the macroalgae extracts for the DPPH radical were monitored according to the method of Hatano et al.[15] with minor modifications. DPPH working solution was prepared with dilution in ethanol until final concentration at 60 µmol/L. An aliquot of 500 µL of each marine algae extract was mixed with 500 µL DPPH. The mixture was incubated in dark for 30 min and then the absorbance was recorded at 517 nm in spectrophotometer. Trolox was referred as the antioxidant standard. The IC₅₀ was calculated by the linear regression analysis and expressed as mean of determination. The percentage of inhibition of DPPH radical scavenging activity was determined using the formula:

$$\% \text{ Inhibition of DPPH radical scavenging activity} = (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100$$

where OD_{control} is the absorbance of the control (without sample) and OD_{sample} is the absorbance in the presence of the sample/standard. All the experiments were repeated thrice. The higher the IC₅₀ value is, the lower the scavenging activity is.

2.5. ABTS radical cation scavenging activity

ABTS radical cation scavenging activity of the sample was determined by Re et al.[16] with minor modification. ABTS was prepared by mixing 7 mmol/L ABTS solution with 2.45 mmol/L potassium peroxodisulfate 16 h prior to use in darkness at room temperature. A 10 µL aliquot of the samples were mixed with ABTS reagent and absorbance of these solutions was measured at 734 nm after 20 min. Trolox was used as standard.

$$\% \text{ Inhibition of ABTS radical} = (\text{A}_{\text{control}} - \text{A}_{\text{sample}})/\text{A}_{\text{control}} \times 100$$

where A_{control} is the absorption of the control reaction (holding all reagent unless the test sample), and A_{sample} is the absorption of the extract tested.

2.6. Antimicrobial activity

2.6.1. Microbial strains

In vitro antimicrobial susceptibility tests were performed using a panel of strains including two Gram-negative strains *Escherichia coli* (*E. coli*) ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 279503, two Gram-positive bacteria *Enterococcus faecalis* (*E. faecalis*) ATCC 29212 and *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and four *Candida* ATCC reference strains, *Candida albicans* (*C. albicans*) ATCC 90028, *Candida glabrata* (*C. glabrata*) ATCC 90030, *Candida krusei* (*C. krusei*) ATCC 6258, and *Candida parapsilosis* (*C. parapsilosis*) ATCC 22019. These microorganisms were obtained from the Microbiology Laboratory of Monastir,

University Hospital. The bacterial pathogens were maintained on Mueller-Hinton agar medium while *Candida* strains were maintained on potato dextrose agar at 4 °C. A loopful of sample from each pure culture was inoculated in sterile nutrient broth and kept overnight at 37 °C for growth.

2.6.2. Determination of minimum inhibitory concentration (MIC)

The standard micro-dilution method in 96-well plates was carried out to evaluate the MIC. The MIC was recorded as the lowest concentration of antimicrobials extract with no visible growth of bacteria or *Candida* after incubation at 37 °C for 18 to 24 h[17]. The method described by Kitzberger *et al.*[18] was followed.

The seaweeds extracts were dissolved in water containing 5% dimethylsulfoxide and the initial extract concentration was 10 mg/mL. 125 µL of each extract was serially diluted two-fold with Mueller-Hinton broth, ranging the concentrations between 10 to 0.02 mg/mL. Each well was inoculated with 62 µL of tested strains which were adjusted to McFarland 0.5 turbidity (about 10⁶ cells/mL) and then diluted to 1:100 for broth dilution method. Wells without inoculums added were used as controls, and positive control (gentamicin) was added to the inoculated growth medium without the substances.

2.6.3. Determination of minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC)

The MBC and the MFC were determined by subculture of all concentrations that had no detectable growth (100 µL) on the surface of freshly prepared blood agar at 37 °C for 18 to 24 h. The lowest concentration that completely inhibited bacterial and candidal growth after incubation was considered as the MBC for bacteria and MFC for fungi. Three independent experiments were performed.

2.7. Statistical analysis

All the data were presented as mean ± SD. The mean values were calculated based on the data taken from at least three independent experiments conducted on separate days using freshly prepared reagents.

3. Results

3.1. Yield of extracts

The acetonic extract of *Enteromorpha* sp. had the highest yield of 12% followed by the *P. pavonica* acetonic extract (9%), and its aqueous extract (7%). The aqueous extract of *Enteromorpha* sp. yielded the lowest (6%).

3.2. TPC

In this paper, the phenolic content was determined using Folin-Ciocalteu reagent and was expressed as CE/g extract. Thereby, the TPC of the four extracts of *P. pavonica* and *Enteromorpha* sp. were evaluated and the results were shown in Figure 1. The TPC varied between (1.00 ± 0.13) mg CE/g (*Enteromorpha* sp. aqueous extract) and (90.61 ± 0.11) mg CE/g (*P. pavonica* acetonic extract). The *P.*

pavonica extracts presented the highest values of TPC.

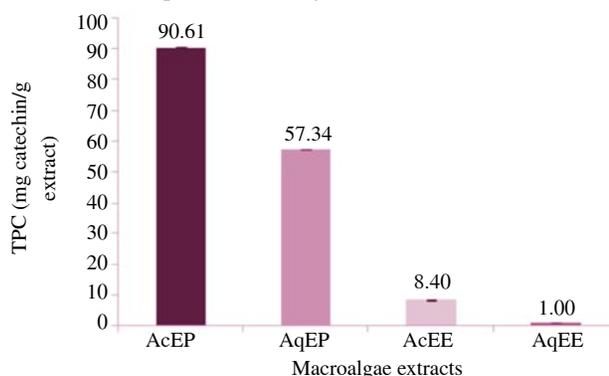


Figure 1. TPC of extracts of *P. pavonica* and *Enteromorpha* sp.

AcEP: Acetonic extract of *P. pavonica*; AqEP: Aqueous extract of *P. pavonica*; AcEE: Acetonic extract of *Enteromorpha* sp.; AqEE: Aqueous extract of *Enteromorpha* sp.

In the present study, *P. pavonica* showed that the TPC was higher than *Enteromorpha* sp. and this could be attributed to genetic factors.

3.3. DPPH radical scavenging activity

Free radical scavenging ability of acetonic and aqueous extracts of the two seaweeds studied were evaluated through the change of absorbance caused by the reduction of DPPH radical and the results were expressed in Figure 2 and Table 1. The acetonic extract of *P. pavonica* presented the highest scavenging activity with an inhibition percentage of 72.92% ± 0.10% at 1 mg/mL [IC₅₀ = (0.35 ± 0.01) mg/mL] followed by the aqueous extract (47.20% ± 0.02%).

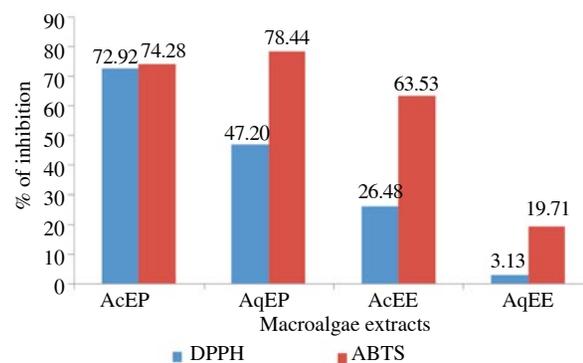


Figure 2. Antioxidant activity of *P. pavonica* and *Enteromorpha* sp. extracts.

AcEP: Acetonic extract of *P. pavonica*; AqEP: Aqueous extract of *P. pavonica*; AcEE: Acetonic extract of *Enteromorpha* sp.; AqEE: Aqueous extract of *Enteromorpha* sp.

Concerning the extracts of the green algae, *Enteromorpha* sp., it presented a moderate scavenging activity with an inhibition percentage of 26.48% ± 0.01% (acetonic extract) and 3.13% ± 0.03% (aqueous extract).

3.4. ABTS assay

All seaweeds extracts possessed free radical scavenging activity but at different levels (Figure 1). At 1 mg/mL, the highest activity was obtained from the *P. pavonica* aqueous extract, with the inhibition percentage value of 78.44% ± 0.01% and IC₅₀ = (0.01 ± 0.01) mg/mL, followed by the acetone extract of this species

Table 1Antioxidant activity of *P. pavonica* and *Enteromorpha* sp. extracts.

| Extracts | DPPH radical | | ABTS radical | |
|---|--------------------------|------------------------------------|--------------------------|------------------------------------|
| | IC ₅₀ (mg/mL) | Scavenging activity (%) at 1 mg/mL | IC ₅₀ (mg/mL) | Scavenging activity (%) at 1 mg/mL |
| Acetonic extract of <i>P. pavonica</i> | 0.35 ± 0.01 | 72.92 ± 0.10 | 0.01 ± 0.00 | 74.28 ± 0.02 |
| Aqueous extract of <i>P. pavonica</i> | - | 47.20 ± 0.02 | 0.01 ± 0.01 | 78.44 ± 0.01 |
| Acetonic extract of <i>Enteromorpha</i> sp. | - | 26.48 ± 0.01 | 0.03 ± 0.01 | 63.53 ± 0.10 |
| Aqueous extract of <i>Enteromorpha</i> sp. | - | 3.13 ± 0.03 | - | 19.71 ± 0.03 |
| Trolox | 0.02 ± 0.01 | 95.60 ± 0.20 | 0.02 ± 0.01 | 94.01 ± 0.10 |

[74.28% ± 0.02%; IC₅₀ = (0.01 ± 0.00) mg/mL] (Table 1). ABTS activity was lowest in *Enteromorpha* sp. extracts; thus, its acetonic extract showed 63.53% ± 0.10% with IC₅₀ value of (0.03 ± 0.01) mg/mL, while the aqueous extract showed a weak antioxidant activity (19.71% ± 0.03%). The lowest activity of *Enteromorpha* sp. extracts may be due to low level of phenols.

In the two tests studied in this work (DPPH and ABTS), the antioxidant activity of the brown algae *P. pavonica* extracts was higher than the green algae *Enteromorpha* sp. extracts.

3.5. Correlation analysis

In order to examine possible association between antioxidant activity and polyphenolic content in algae extracts, correlation coefficient (R^2) was evaluated. The correlation coefficients were exposed in Figure 3. A positive correlation between the values for the antioxidant activity (ABTS and DPPH) and total polyphenolic content was noted ($R^2 = 0.533$ and $R^2 = 0.933$, respectively). Thus the interesting antioxidant activity of these macroalgae was due to the presence of polyphenolic compounds in its extracts. Similarly, a good correlation was observed between the two tests used in this work, DPPH and ABTS ($R^2 = 0.714$) (Figure 4).

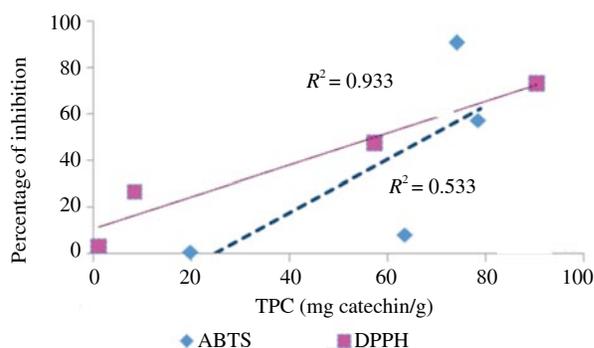


Figure 3. Correlation between TPC and antioxidant activity of macroalgae extracts.

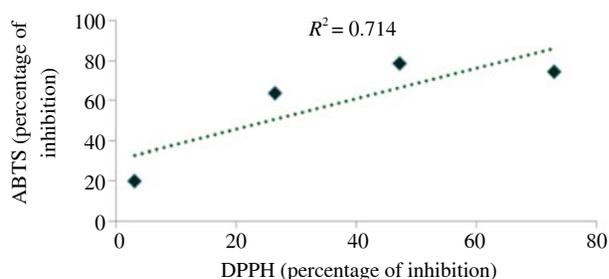


Figure 4. Correlation between antioxidant activity in DPPH and ABTS tests.

3.6. MIC, MBC, and MFC

The extracts of *Enteromorpha* sp. and *P. pavonica* were tested for

their antimicrobial activity against eight strains of microorganisms, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 279503, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 by microdilution method. The results of antimicrobial activity against the tested pathogens were shown in Figure 5. The algal aqueous and acetonic extracts displayed different degrees of antimicrobial activity against different bacteria and fungi and the values of MICs varied from 0.04 to 5 mg/mL. The *P. pavonica* acetonic extract exhibited strong inhibition against all bacteria and fungi strains tested with MIC = MBC = 0.04 mg/mL, which was higher than all other extracts and more active than the standard gentamicin used against *P. aeruginosa* (MBC = 0.5 mg/mL) (Figure 5B). The minimum concentration essential to kill a microbe should be equal to or greater than the MIC for the microorganism. In this study, antimicrobial activity of acetonic extracts was higher than that of aqueous extracts. In present study, aqueous extract from *Enteromorpha* sp., exhibited antimicrobial activity against *E. coli* and *S. aureus* (MICs = 5 and 2.5 mg/mL, respectively).

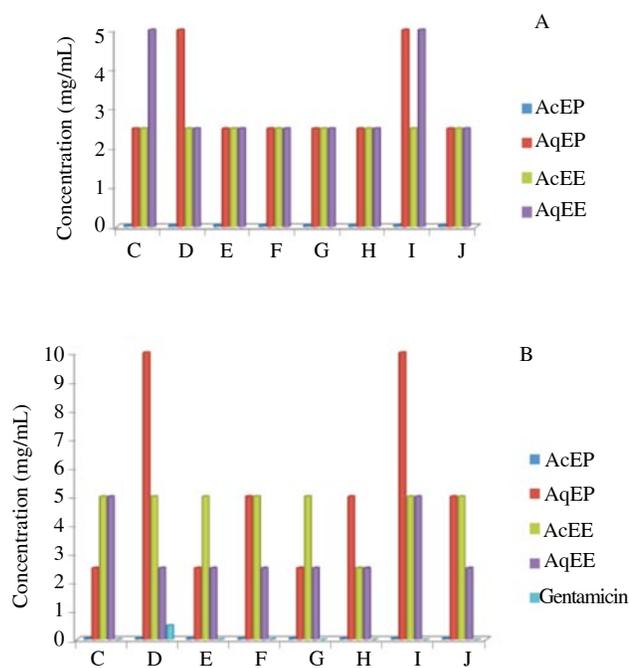


Figure 5. The *in vitro* MIC (A) and MBC (B) values of extracts of tested algae.

AcEP: Acetonic extract of *P. pavonica*; AqEP: Aqueous extract of *P. pavonica*; AcEE: Acetonic extract of *Enteromorpha* sp.; AqEE: Aqueous extract of *Enteromorpha* sp.; C: *E. coli* ATCC 25922; D: *P. aeruginosa* ATCC 279503; E: *E. faecalis* ATCC 29212; F: *S. aureus* ATCC 25923; G: *C. albicans* ATCC 90028; J: *C. glabrata* ATCC 90030; H: *C. krusei* ATCC 6258; I: *C. parapsilosis* ATCC 22019.

3.7. Correlation between the values of antimicrobial activities and total polyphenolic content

To evaluate a possible association between polyphenolic content and antimicrobial activity in macroalgae extracts, correlation coefficient (R^2) was evaluated (Figure 6). There was a strong correlation between the level of polyphenols with antibacterial activity against *E. coli* and *E. faecalis* ($R^2 = 0.983$ and 0.625 , respectively), whereas no correlation was observed against *S. aureus* and *P. aeruginosa* ($R^2 = 0.275$ and $R^2 = 0.016$, respectively). In addition, a positive correlation was found between the phenolic content in extracts with antifungal activity versus *C. albicans* ($R^2 = 0.625$) and *C. glabrata* ($R^2 = 0.651$). To the best of our knowledge, there were no reports concerning the correlation analysis between the TPC and antimicrobial activity of algae extracts. This was the first work which examined this study.

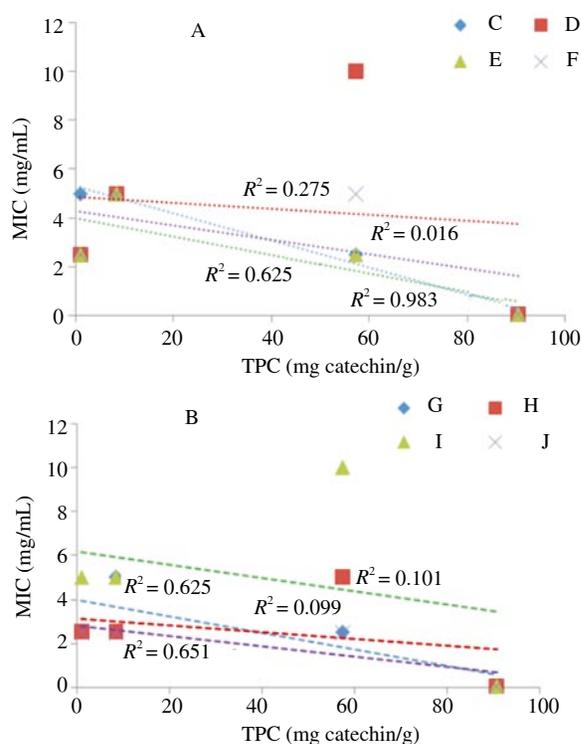


Figure 6. Correlation between the values of antibacterial (A) and anticandidal (B) activities of algae extracts and polyphenols content. C: *E. coli* ATCC 25922; D: *P. aeruginosa* ATCC 279503; E: *E. faecalis* ATCC 29212; F: *S. aureus* ATCC 25923; G: *C. albicans* ATCC 90028; J: *C. glabrata* ATCC 90030; H: *C. krusei* ATCC 6258; I: *C. parapsilosis* ATCC 22019.

4. Discussion

Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds[19]. Phenolic compounds are considered as one of the most important classes of natural products. Phenolics act as antibacterial, anti-allergic, anti-diabetes and antioxidants by inhibiting enzymes involved in radical generation and anti-HIV activities[20,21]. Folin-Ciocalteu reagent determines total phenols, producing blue color by reducing yellow heteropolyphosphomolybdate-tungstate anions[22]. Sameeh *et al.*[23]

found also that the TPC of Saudian seaweeds, *Padina boryana* Thivy acetone extract [(15.22 ± 0.13) mg/g gallic acid equivalent] was higher than the acetone extract of *Enteromorpha* sp. [(5.17 ± 0.11) mg/g gallic acid equivalent]. Hamza *et al.*[24] presented an interesting TPC for the methanolic extract of Saudian *P. pavonica* (55 mg gallic acid equivalent/g). Another team from Lebanon who examined the TPC of methanolic extract of *P. pavonica*, found a value of (10.76 ± 0.87) mg gallic acid equivalent/g extract. Pinteus *et al.*[25] presented a value of (44.61 ± 0.01) mg gallic acid equivalent/g extract for the methanolic extract of *P. pavonica* from Portugal. The TPC of methanolic extract of *Enteromorpha* sp. from India was (5.72 ± 0.13) mg gallic acid equivalent/g. This result is near to ours. The methanolic extract of *Enteromorpha spirulina* from Ireland showed an important total flavonoid content (49.75 mg gallic acid equivalent/g)[26]. The existence of phenolic compounds in green and brown macroalgae is understood to be associated with their protective mechanisms during some adverse conditions. Superior quantity of phenolic content is generated during the hot climate and during the early stage of the growth in order to prevent the photooxidative damage and sea grazers, respectively. The existence of different results in the TPC between the same species of algae from different countries could be due to the growth period, geographic location, genetic diversity and the difference in the solvent used[27].

DPPH has been used widely as a free radical to assess reducing substances and is a useful reagent for studying the free radical scavenging activities of compounds[28]. Pinteus *et al.*[25] have shown that the antioxidant activity of *P. pavonica* methanolic extract has almost the same value found in the *P. pavonica* acetone extract studied in this paper ($IC_{50} = 0.338$ mg/mL). Kokabi *et al.*[29] showed also that the methanolic extract from *P. pavonica* from Iran showed a value close to that found by our team ($IC_{50} = 410 \pm 4.1$ µg/mL). Another study about the antioxidant activity of *P. pavonica* methanolic extract from Lebanon showed an inhibition percentage lower than our results (10.80% ± 0.08%) at 1 mg/mL[30]. In another study, a moderate DPPH scavenging activity of the methanolic extract of the brown algae *P. pavonica* was found (36%) [31]. When comparing the antioxidant activity of the green seaweed *Enteromorpha* sp. with literature, many works demonstrated similar results to ours, such as Sivaramakrishnan *et al.*[31] who showed that the percent of inhibition of the DPPH scavenging activity of Indian *Enteromorpha* sp. methanolic extract was 28% at 20 mg/mL, while Yanti *et al.*[32], proved a moderate antioxidant potential of Indonesian *Enteromorpha* sp. ethanolic extract (percent of inhibition = 27% ± 3% at 0.25 mg/mL). Trolox used in this study, is a synthetic antioxidant and can be used as a good indicator for comparing scavenging activity between the samples. None of the studied extracts showed higher results than Trolox standard at 1 mg/mL [percent of inhibition = 95.60% ± 0.01% and $IC_{50} = (0.02 \pm 0.01)$ mg/mL]. Nevertheless, Trolox was pure and can have higher antioxidant activity by comparing to the crude samples. The difference in the DPPH radical scavenging activity of each sample in various extracts included that the extracting solvent utilized will affect the radical scavenging potentiality. This could be due to the

different polarities of every antioxidant compound group existing in the macroalgae[33].

A few works in the literature examined the antioxidant activity of *Enteromorpha* sp. and *P. pavonica* using the ABTS test, like Sivaramakrishnan *et al.*[31], who showed that the *Enteromorpha* sp. methanolic extract has an inhibition percentage of the order of 30% at 2 mg/mL.

An extract is bactericidal when the ratio MBC/MIC \leq 4 and bacteriostatic when this ratio is $>$ 4[34]. Thus, it seems to be that antibacterial effects obtained with all seaweeds extracts tested against the pathogenic strains proved to have bactericidal activity.

Alghazeer *et al.*[35] found that the aqueous extract of *Enteromorpha* sp. algae had a value of MICs = 200 mg/mL against *E. coli* and *S. aureus* strains. Sahgal *et al.*[36] demonstrated that the differences of the MIC value between samples may be due to the morphological structure of the bacterial cells and their composition in the cells. The reduction in growth possibly occurred due to interference by active substances in the sample[37]. The Gram-positive *S. aureus* and *E. faecalis* strains were more susceptible to extracts tested. This could be due to the more complex composition and structure of Gram-negative bacterial cell wall[38]. Salem *et al.*[39] indicated that the outer membrane and the thick murein layer of Gram-negative act as a barrier, avoiding the entrance of environmental substances as inhibitors and antibiotics. Our results are confirmed by the work of Ismail and Tan[40] who treated the antibacterial activity of dichloromethane/methanol extract of Tunisian *P. pavonica* and demonstrated that the Gram-positive bacteria such as *S. aureus* and *E. faecalis* were strongly inhibited by this extract. On the other hand, the Gram-negative bacteria, *E. coli* and *E. faecalis* were resistant toward this extract. Antibacterial and antifungal activities depend on algal species and on the method of extraction of their active substances as well as seasons of the year, location and temperature of the water. The difference between our results and those found in the literature can be attributed to seasonal or location variations.

El Shoubaky and Salem[41] tested the antibacterial activity of the acetone extracts of brown algae like *P. pavonica* and *Hormophysa triquetra* and showed its strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. Kayalvizhi *et al.*[42] showed that the acetone extract of brown seaweed was mentioned as highly efficient solvent versus fungi and bacteria when compared to else solvents. Wefky and Ghobrial[43] have shown also, that the use of acetone solvent in extracting the bioactive antimicrobial substances gave a high antimicrobial activity versus various pathogens. Same thing occurred for Delaraisassi *et al.*[44] who reported that extracts prepared with acetone showed the best activity.

Marine algae have numerous bioactive compounds like antimicrobial and antioxidant components. The results obtained in the present paper clearly proved that the acetonic extract derived from *P. pavonica* is fairly active fraction for *in vitro* DPPH and ABTS free radicals scavenging activity. Moreover, the results suggest that polyphenolic content could be major contributors to the interesting antioxidant activity of this selected brown seaweed from the coast

of Tunisia. This same sample showed important antibacterial and anticandidal activities. The present work will assure a starting point for exploiting natural bioactive components present in the macroalgae extracts, thus our findings appear useful for further studies aiming to isolate and identify the active substances which are responsible for higher antimicrobial and antioxidant activity, as well as to evaluate the effects of each individual compound on pathogenic strains.

Conflict of interest statement

We declare that we have no conflict of interest.

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