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ORIGINAL PAPER

Assessment of the antioxidant and antibacterial properties of red algae (Rhodophyta) from the north coast of Tunisia

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Abstract
Red seaweeds are a rich source of compounds with various bioactive properties, such as antimicrobial, antioxidant, antifouling, antiproliferative, and anticancer activities. In this study, the antioxidant and antibacterial properties of twelve red macroalgae collected from the Tunisian coast were examined. Estimated total phenolic, flavonoid, and tannin contents in methanolic extracts were found to vary among species. *Gracilaria gracilis* presented the highest concentration of total phenolic compounds (19.2 ± 1.88 mg GAE/g dried biomass), *Laurencia obtusa* showed the highest tannin content (18.95 ± 0.84 mg ECat/g DB), and *Sphaerococcus cornopifolius* showed the highest flavonoid content (7.17 ± 0 mg ECat/g DB). Six species showed significant DPPH radical scavenging activities and total antioxidant capacities: *Asparagopsis armata*, *Gracilaria gracilis*, *Hypnea musciformis*, *Laurencia obtusa*, *Pterocladiella capillacea*, and *Sphaerococcus cornopifolius*. Antimicrobial activity was observed for five species. This study therefore highlights the potential use of red seaweed species collected from the Tunisian coast as sources of bioactive compounds.

Keywords Antioxidant · Antibacterial · *Asparagopsis armata* · *Hypnea musciformis* · *Gracilaria gracilis* · *Pterocladiella capillacea*

Introduction

Given the economic and ecological challenges that are currently being encountered worldwide, seaweeds are an important resource to consider in the context of sustainable development. These organisms play an important role in maintaining ecological balance. Moreover, the potential for sustainable seaweed cultivation and bioproduction implies that seaweeds are a highly relevant resource for the "blue growth" strategy (Buschmann et al. 2017). Approximately 12 million tonnes of algae are cultivated per year, around 85% of which is used in food products and for human consumption (FAO 2018).

Several studies have shown that macroalgae can be used in the pharmacological sector, as they produce unusual secondary metabolites with various biological properties, such as cytotoxic, antibiotic, antiviral, anti-inflammatory, and antiparasitic activities (Kolanjinathan 2014; Kumar et al. 2017; Al-Enazi et al. 2018).

The accumulation of large doses of reactive oxygen species (ROS) can disrupt the normal functioning of plant cells (Yalcinkayaa et al. 2019). Thus, plants regulate the physiological production of ROS by producing considerable quantities of several enzymatic and nonenzymatic compounds with antioxidant activities, such as superoxide dismutase enzyme, catalases, peroxidases, vitamins C and E, carotenoids, tannins, phenols, and flavonoids (Xiulan et al. 2019). A large number of potent antioxidant compounds (including phlorotannins, sulfated polysaccharides, carotenoids, and sterols) have already been detected in various macroalgae, making these marine organisms a valuable source of compounds with neuroprotective effects that are useful for treating neurodegenerative diseases such as Alzheimer's or Parkinson's disease (Pangestuti 2011) as well as numerous other health benefits (Fernando et al. 2020).

The Mediterranean Sea is characterized by high seaweed biodiversity. More than 415 seaweed species have been found along the 1300 km of coast in Tunisia, and

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more than 60% of this coastal vegetation belongs to the phylum Rhodophyta (Ben Maiz 1995). Rhodophyta are commonly called red algae because of the predominance of the red pigment R-phycoerythrin in these organisms. They contain large amounts of fiber, protein, and minerals, and are a great source of vitamins. Red algae have been used in phytotherapy due to their medicinal properties; for instance, they exhibit various bioactive properties such as antimicrobial activity against pathogenic strains (Kumar et al. 2017), antioxidant activity (Bouhlal et al. 2013), antiproliferative activity (Neethu et al. 2017), and cytotoxic and anticancer activities (Al-Enazi et al. 2018). These properties of red algae aroused our interest, so various common species of red macroalgae were collected from the north coast of Tunisia to evaluate their potential as a source of valuable bioactive products. The results of this study improve knowledge of the antioxidant and antibacterial properties of seaweeds collected from southern Mediterranean coasts.

Materials and methods

Algae sampling and extraction

Twelve species were collected manually in shallow water (< 2 m depth) from March to April 2014: *Asparagopsis armata* Harvey [Bonnemaisoniales], *Laurencia obtusa* (Hudson) J.V. Lamouroux, *Palisada perforata* (formerly *Laurencia papillosa*) (C. Agardh) Greville, *Ceramium ciliatum* (J. Ellis) Ducluzeau [Ceramiales], *Peyssonnelia squamaria* (S.G. Gmelin) Decaisne [Peyssonneliales], *Sphaerococcus coronopifolius* Stackhouse, *Hypnea musciformis* (Wulfen) J.V. Lamouroux [Gigartinales], *Corallina officinalis* (Linnaeus), *Jania rubens* (Linnaeus) J.V. Lamouroux, *Jania longifurca* Zanardini [Corallinales], *Pterocladiella capillacea* (S.G. Gmelin) Bornet [Gelidiales], and *Gracilaria gracilis* (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham [Gracilariales]. All samples were collected from Cap Zebib (37°15′49.66″N, 10°04′02.85″E) except for the *G. gracilis*, which was collected from Bizerte Lake (37°10′60″N, 9°52′E). In the laboratory, the algae were washed with seawater followed by fresh water in order to remove epiphytes and excess salt. The algae were then dried at ambient temperature in a dry and dark place for 3 days. Next, they were taxonomically identified by morphological characterization according to Fischer et al. (1987) and Cabioch et al. (2006), and a voucher specimen of each species was kept in 2% formaldehyde solution. For each species, a 10 g sample was ground and extracted with MeOH (80%) for 12–16 h at room temperature. All solvents used were of analytical grade.

Determination of the total phenolic content

The total phenolic content of the extracts was assessed using the yellow Folin-Ciocalteu reagent (Dewanto et al. 2002) consisting of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). This reagent generates blue tungsten and molybdenum oxides when it is reduced during phenol oxidation. An aliquot of 125 µl of the algae extract was mixed with 500 µl distilled water and 125 µl Folin-Ciocalteu reagent. Following agitation and 3 min of incubation in the dark at room temperature, 1250 µl Na_2CO_3 (7%) were added, and then the extract was incubated for a further 90 min in the dark. Next, the absorbance of the sample was measured using a Jenway 6405 UV/Vis spectrophotometer at 760 nm. This test was performed in triplicate. In parallel, a standard reference curve was established under the same experimental conditions using gallic acid as a positive control. Gallic acid was used as a reference standard for the calibration curve, and the total phenolic content was expressed in mg gallic acid equivalent per g dried biomass (mg GAE/gDB) via the equation

$$C = (c \times V)/m,$$

where C is the total phenolic content (mg GAE/g DB), c is the concentration of gallic acid determined from the calibration curve (mg/L), V is the volume of the extract (L), and m is the mass of dry material used (g).

Determination of the flavonoid content

The flavonoid content of the methanolic extract was determined by the aluminum chloride colorimetric method (Dewanto et al. 2002). An aliquot of 250 µl of extract was mixed with 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_36H_2O$ were added, and 500 µl of 1 N NaOH were added 5 min later. The final volume was then rounded to 2.5 ml. The last step in this assay was the measurement of absorbance at 510 nm. Catechin was used as a reference standard for the calibration curve, and the flavonoid content was expressed in mg equivalents of catechin per g of dry biomass (mg ECat/g DB). This test was done in triplicate.

Determination of the tannin content

The tannin content of the methanolic extracts was determined by the colorimetric method described by Price et al. (1978). In this method, 50 µl of extract were mixed with 3 ml of 4% vanillin and 1.5 ml of concentrated HCl. After 15 min, the absorbance at 500 nm was measured. This test

was done in triplicate. Catechin was used for the calibration curve. Results are expressed in mg equivalents of catechin per g of dry biomass (mg ECat/g DB).

DPPH radical scavenging

The free-radical scavenging capacity of each extract was analyzed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test according to the method of Farasat et al. (2013). Briefly, 100 µL of each extract at various dilutions (20, 10, 5, 1, 0.5 mg/ml) were mixed with 100 µL of 0.16 mM DPPH solution. The solution was kept at room temperature for 30 min, after which the absorbance was measured in an automated microplate reader at 517 nm. The amount of DPPH radical scavenging performed by the extract was calculated as follows:

$$\%\text{scavenged} = \left(\left(A_0 - A_i\right) \div A_0\right) \times 100,$$

where A_0 is the absorbance of the control and A_i is the absorbance of the sample.

The half-maximal inhibitory concentration (IC_{50}) was calculated by linear regression analysis. Ascorbic acid was used as a positive control. This test was done in triplicate.

Total antioxidant capacity (TAC)

The total antioxidant capacity of each seaweed extract was determined by the phosphomolybdenum method described by Prieto et al. (1999). This assay is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green phosphate/Mo(V) complex. Briefly, in triplicate, an aliquot of 0.2 ml (10 mg/ml) of each methanolic (80%) extract was combined with 2 ml of the reagent solution (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid). Samples were capped and incubated in a thermal block at 95 °C for 90 min. After cooling at room temperature, the absorbance of each solution was measured using a UV–visible spectrophotometer at 695 nm. 0.2 ml of 80% methanol were added as a blank to 2 ml of the reagent solution, which was then incubated under the same conditions as the other samples. Ascorbic acid was used as a reference standard for the calibration curve. The total antioxidant capacity was expressed in mg equivalents of ascorbic acid per g of dry biomass (mg EAA/g DB).

Antimicrobial activity

Seven indicator microorganisms were used for the assay: six bacteria (*Staphylococcus aureus*, *Streptococcus* B, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Enterococcus faecalis*, and *Aeromonas hydrophila*) and one yeast (*Candida albicans*). These are the standard reference bacteria used in the laboratory. Antibacterial tests were performed by the disc diffusion method in agar-plated Petri dishes according to Ismail et al. (2016). Extracts were tested at 1 mg and applied to sterile filter paper discs (6 mm). After evaporating the solvent, the discs were placed on trypto-casein-soy agar (TSA, Bio-Rad) plates inoculated with a strain cultured for 18 h (10^6 bacteria ml^{-1}) in tryptone soy broth (TSB, Bio-Rad). A disc loaded with solvent was simultaneously prepared as a control. Plates were incubated overnight at 30 °C. The test was done in duplicate. Antimicrobial activity was evaluated by measuring the diameter (in mm) of the inhibition zone around the disc after 24 h of incubation. The biocide $CuSO_4$ was used as a positive control at 5 ppm (Hellio et al. 2001).

Statistical analysis

Statistical analysis of the total phenolic content, flavonoid content, tannin content, DPPH radical scavenging capacity (IC_{50}), total antioxidant capacity (TAC), and antimicrobial activity was performed with the XLSTAT 2016.1 software. One-way ANOVAs were performed and significant differences ($p < 0.05$) were determined via Duncan's test. Principal component analysis (PCA) was used to determine correlations between antioxidant activity (DPPH and TAC) and the total phenolic, flavonoid, and tannin contents of the species. The PCA was implemented with SPSS 15.0.

Results

Total phenolic, flavonoid, and tannin contents

The results for the total phenolic contents are given in Fig. 1. They ranged between 0.20 ± 0 and 19.29 ± 1.8 mg GAE/g DB. The extract from *G. gracilis* had a significantly

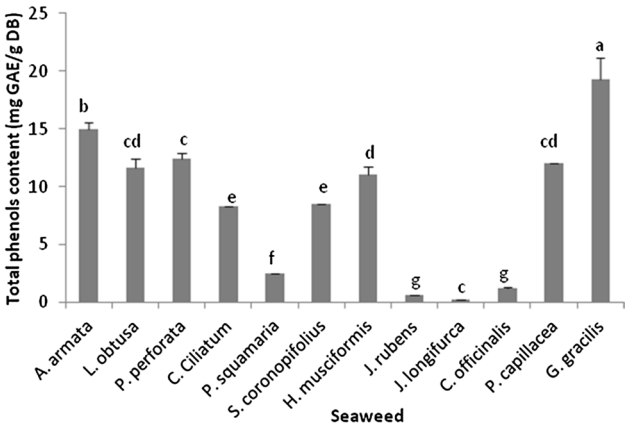

Fig. 1 Total phenolic contents of the seaweed extracts. Bars with different letters are significantly different at $p < 0.05$

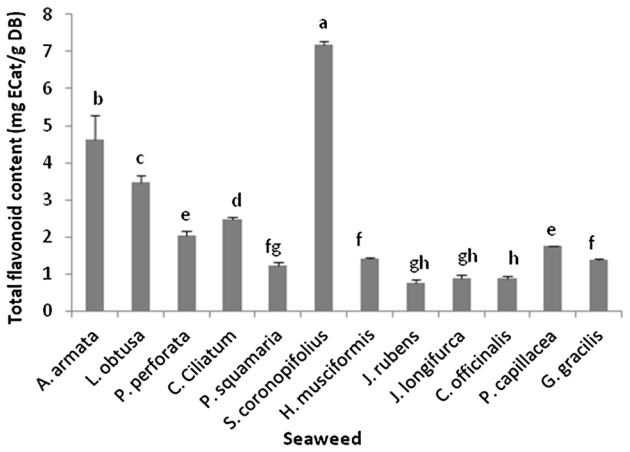

Fig. 2 Flavonoid contents of the seaweed extracts. Bars with different letters are significantly different at $p < 0.05$

higher ($p < 0.05$) total phenolic content (19.29 ± 1.8 mg GAE/g DB) than any other extract. The total phenolic content of the extract from *A. armata* was the second highest (14.95 ± 0.5 mg GAE/g DB), followed by the extract from *P. perforata* (12.45 ± 0.4 mg GAE/g DB). The lowest total phenolic contents were observed for members of the order Corallinales: *C. officinalis*, *J. rubens*, and *J. elongata* (0.59 ± 0.08; 0.20 ± 0.15 and 1.24 ± 0.10 GAE/g DB, respectively).

The flavonoid contents of the seaweed extracts are given in Fig. 2. Flavonoid contents varied from 0.77 ± 0.1 to 7.17 ± 0 mg ECat/g DB, with *S. coronopifolius* and *A. armata* presenting the highest levels (7.17 ± 0 and 4.64 ± 0.63 mg ECat/g DB, respectively). Extracts from members of Corallinales presented significantly lower levels of flavonoids, ranging between 0.77 ± 0.1 and 0.9 ± 0.08 mg ECat/g DB.

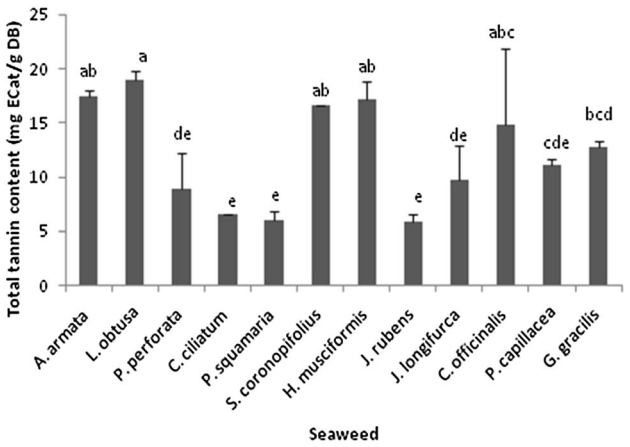

Fig. 3 Tannin contents of the seaweed extracts. Bars with different letters are significantly different at $p < 0.05$

Figure 3 presents the tannin contents of the seaweed extracts. The values obtained ranged between 5.90 ± 3.1 and 18.95 ± 0.8 mg ECat/g DB.

Among the species analyzed, the extract from *L. obtusa* contained a significantly higher ($p < 0.05$) amount of tannin (18.95 ± 0.8 mg ECat/g DB) than the other extracts. The extracts from *A. armata*, *H. musciformis*, and *S. cornopifolius* also presented high tannin contents (17.50 ± 0.5, 17.16 ± 1.7, and 16.62 ± 0 mg ECat/g DB, respectively). However, the extracts from *C. ciliatum*, *P. squamaria*, and *J. rubens* contained significantly lower levels of tannin than the other extracts (6.62 ± 0.01, 5.98 ± 0.8, and 5.90 ± 0.6 mg ECat/g DB, respectively).

DPPH radical scavenging activity and total antioxidant capacity

The radical scavenging activities and total antioxidant capacities of the seaweed extracts are given in Figs. 4 and 5, respectively.

Extracts of *H. musciformis*, *S. coronopifolius*, *A. armata*, *C. ciliatum*, and *P. capillacea* had significantly lower ($p < 0.01$) IC_{50} values (between 13.9 ± 0.3 and 18.6 ± 0.2 mg/ml) than the other extracts, indicating that they had significantly enhanced radical scavenging capacities. The extract from *H. musciformis* displayed the lowest IC_{50} value of all (13.9 ± 0.3 mg/ml); however, a statistical analysis indicated that there was no statistically significant difference between the IC_{50} values obtained for the five most active species.

The highest total antioxidant capacity, 2.02 ± 0.1 mg EAA/g DB, was obtained for the *H. musciformis* extract (Fig. 5). Three other extracts also presented nonnegligible total antioxidant capacities: *L. obtusa*, *A. armata*, and *P. capillacea* (1.22 ± 0.0, 1.05 ± 0.1, and 0.90 ± 0.2 mg EAA/gDB, respectively). The lowest antioxidant capacities were

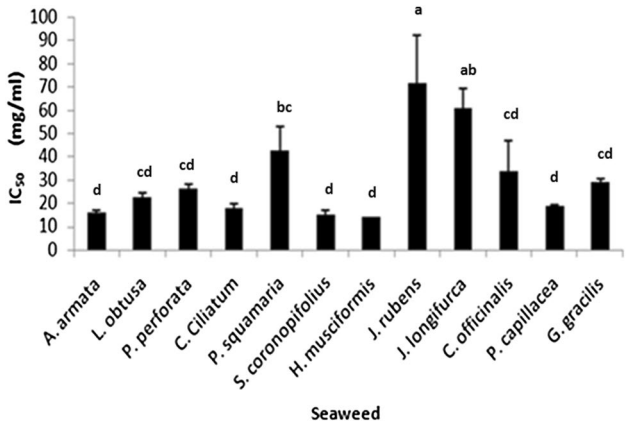

Fig. 4 DPPH radical scavenging activities (IC_{50} values) of the seaweed extracts. Bars with different letters are significantly different at $p < 0.01$

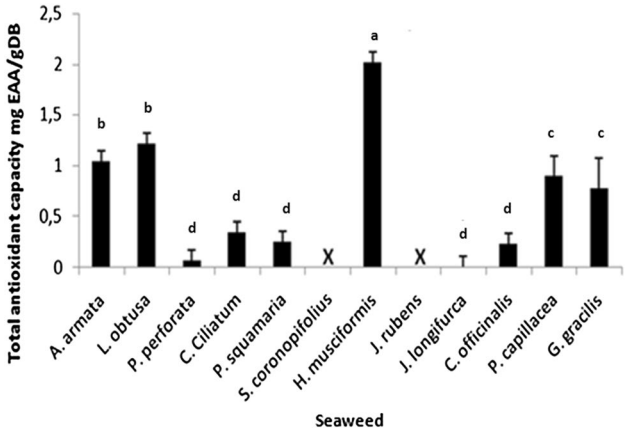

Fig. 5 Total antioxidant capacities of the seaweed extracts. Bars with the same letters are significantly different at $p < 0.01$. Note that the total antioxidant capacities of *S. coronopifolius* and *J. rubens* could not be analyzed due to a lack of extract

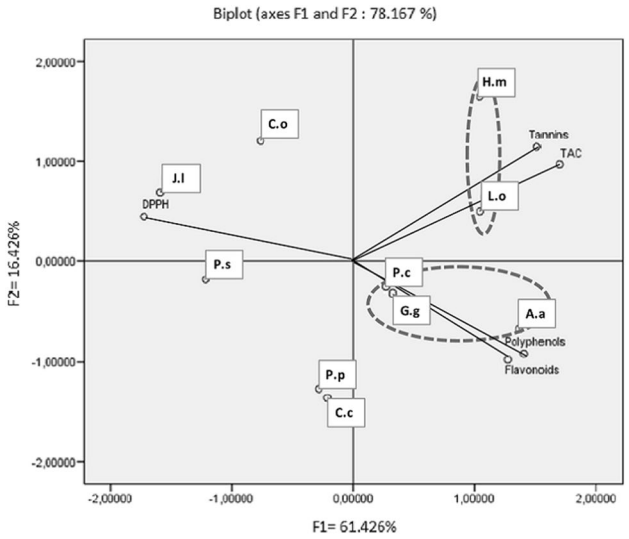

Fig. 6 Principal component analysis (PCA) of the total phenolic, flavonoid, and tannin contents and the antioxidant activities (DPPH, TAC) of seaweed extracts. Each species is distributed according to its variance. *A.a Asparagopsis armata, L.o Laurencia obtusa*, *P.p Palisada perforata*, *C.c Ceramium ciliatum*, *P.s Peyssonnelia squamaria*, *H.m Hypnea musciformis*, *C.o Corallina officinalis* (Linnaeus), *J.l Jania longifurca*, *P.c Pterocladiella capillacea*, *G.g Gracilaria gracilis*. The two species *S. coronopifolius* and *J. rubens* were not taken into account in the PCA analysis due to missing data

observed for the methanol extracts of *J. longifurca* and *P. perforata* (both 0.07 ± 0.1 mg EAA/gDB).

The results from the principal component analysis (PCA) of the total phenolic (TPC), flavonoid (FC), and tannin (TC) contents and the antioxidant activities (DPPH, TAC) of the twelve seaweed extracts are presented in Fig. 6. The first two principal components, F1 and F2, explained 61.4% and 16.4% of the total variance of the data set, respectively. Analysis of the first component (F1) highlighted strong negative correlations of TPC, FC, and TC (on the right) with DPPH (on the left), revealing that the species with the highest DPPH radical scavenging potencies (the lowest IC_{50} values, i.e., *A. armata*, *H. musciformis*, *P. capillacea*, *L. obtusa*, and *G. gracilis*) also showed the highest TPCs, FCs, and TCs. TPC, FC, and TC also showed positive correlations with the total antioxidant capacity (TAC), indicating that the species with the highest gallic acid equivalent contents and catechine equivalent contents were also those with the highest TACs. This analysis confirmed that *A. armata*, *H. musciformis*, *P. capillacea*, *L. obtusa*, and *G. gracilis* are the species with the strongest antioxidant activities.

Antimicrobial activity

Table 1 shows the antimicrobial activities of algae extracts. Data are only shown for algae with antimicrobial activities toward at least one pathogenic strain. The results show that only three pathogenic strains were sensitive to algal extracts (*Pseudomonas cepacia*, *Streptococcus* B, and the yeast *Candida albicans*); *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Enterococcus faecalis*, and *Aeromonas hydrophila* were all resistant to all of the tested extracts. Among the twelve algae species from which extracts were obtained, only five displayed antimicrobial activity: *P. perforata*, *S. coronopifolius*, *J. longifurca*, *H. musciformis*, and *P. capillacea.* Only weak activities were observed, with inhibition zone diameters ranging from 6 to 10 mm. *Streptococcus* B was the most sensitive strain, as it was inhibited by 41% of the extracts. Three extracts inhibited *Candida albicans*—those of *P. perforata*, *H. musciformis*, and *P. capillacea*. Only two extracts—those of *J. longifurca* and *P. capillacea*—displayed activity against *Pseudomonas cepacia*.

Discussion

Total phenolic, flavonoid, and tannin contents

Results showed that the amount of phenolic compounds in the extract depended on the species considered. Natural antioxidants are found in algae as phenolic compounds (flavonoids, xanthones, coumarins, carotenoids, phenolic acid, tannins, anthocyanins, etc.). Polyphenols are a major class of secondary metabolites in macroalgae. Marine polyphenols, or phlorotannins (which are particularly common in brown algae), are a group of a molecules with various structures and degrees of polymerization, and therefore different biological activities (Neethu et al. 2017; Al-Enazi et al. 2018). These metabolites have the capacity to improve food quality and stability, and can also be used as nutraceuticals to terminate free-radical chain reactions in biological systems,

Table 1 Antimicrobial activities of algae extracts (p value = 0.003)

Species	*Pseudomonas cepacia*	*Streptococcus* B	*Candida albicans*
Palisada perforata	–	*8 ± 0.7*	*7*
Sphaerococcus coronopifolius	–	*9 ± 0*	–
Jania longifurca	*8*	*7 ± 0*	–
Hypnea musciformis	–	*7 ± 0*	*7*
Pterocladiella capillacea	*9*	*10 ± 0.7*	*9*
$CuSO_4$	*11*	*9 ± 0*	*10*

−: No activity; +: 6 < inhibition zone diameter ≤ 10 mm, ++: 10 < inhibition zone diameter ≤ 15 mm; +++: inhibition zone diameter > 15 mm; $CuSO_4$: positive control

providing additional health benefits for humans (Fernando et al. 2020). In this study, several seaweed species such as *G. gracilis*, *A. armata*, *H. musciformis*, *S. coronopifolius*, *P. capillacea*, and *L. obtusa* were found to contain significant amounts of phenolic compounds. The highest phenolic content (19.29 ± 1.8 mg GAE/g DB), which occurred in the *G. gracilis* extract, is comparable to the content (21.63 mg GAE/g DB) found by Widowati et al. (2014) for the same species collected from aquaculture ponds in Indonesia. Furthermore, the MeOH extract of *H. musciformis* exhibited a relatively high phenolic content (11 ± 0.7 mg GAE/g DB), which is in agreement with Chakraborty et al. (2015), who observed a content of 9.84 mg GAE/g DB for the same species. Dellai et al. (2013) reported 19.21 mg GAE/g DB of total phenolics for *L. obusta*, which is slightly higher than the content determined in the present study.

We found the amounts of polyphenols in the members of Corallinales to be low. Comparable levels were obtained by Rico et al. (2012), who noted a total phenolic level of 4.64 mg GAE/g DB for *Corallina elongata* collected from the Canary Islands.

Only a few studies have analyzed the flavonoid contents of red macroalgae. Sarojini et al. (2012) investigated the flavonoid contents of fifteen seaweeds, including seven members of Rhodophyta. They found that this taxonomic group gave lower levels of flavonoids (6.03–20.91 mg/g DB) than brown algae (20.72–32.89 mg/g DB) and green algae (8.43–33.39 mg/g DB). The flavonoid content of *C. officinalis* as determined by Ismail (2017) using the aluminum chloride colorimetric technique was 3.48 ± 0.822 mg ECat/g DB, whereas we obtained a value of 0.776 ± 0.1 mg ECat/g DB for this species in the present study. These observed differences between studies may be due to interstudy differences in environmental parameters such as pH, salinity, temperature, geographical location, and biological parameters, which could influence the production of secondary metabolites by these organisms (Heo 2006; Xie et al. 2019).

Tannins are generally defined as high molecular weight polyphenolic compounds (over 1000 kD), and phlorotannins are mostly found in brown algae, where they are used as antioxidative components to overcome oxidative stress (Wei et al. 2003; Gupta and AbuGhannam 2011; Gamze et al. 2014; Creis et al. 2018).

DPPH radical scavenging and total antioxidant capacity

In the present study, the methanol extracts of *H. musciformis*, *A. armata*, *S. coronopifolius*, and *P. capillacea* gave the lowest IC_{50} values. In particular, the MeOH extract of *H. musciformis* showed the highest DPPH antiradical scavenging potency, with an IC_{50} value of 13.9 ± 0.3 mg/ml; this is comparable to the value obtained by Chakraborty et al. (2015) for this species, 15.4 mg/ml. The antioxidant potential of this alga was also highlighted by Chakraborty et al. (2015), who isolated three substituted aryl meroterpenoids with potential antioxidative activities. In addition, the two compounds phloretin and (−)-epicatechin were extracted from the red algae *H. musciformis*; these compounds have high potential applicability in the human food and well-being industries (Rozo et al. 2019). Fellah et al. (2017) showed that seasonal variation had a significant effect on the antioxidant activity of *Sphaerococcus coronopifolius*, with the highest DPPH activity occurring in summer.

Pterocladiella capillacea collected from Tunisian coasts showed significant antiradical activity, and the associated data were in accordance with those reported by Alencar et al. (2018). Those authors demonstrated that fatty acids were responsible for the DPPH radical scavenging capacity.

Antimicrobial activity

The present data for *H. musciformis* and *P. perforata* indicate that extracts of these species inhibit *Streptococcus* B and *Candida albicans* and were in accordance with previous data from Shanab (2007).

Additionally, the present study indicated that *Pseudomonas cepacia* and *Streptococcus* B were inhibited by the methanol extract from *P. capillacea*, which is accordance with the results reported by Mohy El-Din and

El-Ahwany (2016), who found that extracts from the same two species acted against those pathogens. El Kassas and Attia (2014) demonstrated that the observed antibacterial activity against the Gram-positive bacterium *B. subtillus* in *P. capillacea* was due to alkaloid compounds.

According to the present data, the *Sphaerococcus coronopifolius* extract only exhibited activity against *Streptococcus* B. A broader activity spectrum (antimicrobial and antitumor) was described for this species by Rodrigues et al. (2015) and Pinteus et al. (2015). In contrast to our findings, Bouhlal et al. (2012) reported that *S. coronopifolius* presented antibiotic activity against *E. faecalis*. Two tetracyclic diterpenes, ioniols I and II, which possess antibacterial activities against a panel of *Staphylococcus aureus* strains, were extracted from this species collected from the rocky coasts of the island of Corfu in the Ionian Sea (Smyrniotopoulos et al. 2008).

Using PCA, two clusters of bioactive red algae were clearly identified, in agreement with previous conclusions drawn from data analysis. The first cluster consists of *Hypnea musciformis* and *Laurencia obtusa*, which present large amounts of tannins and significant total antioxidant capacities. The second cluster consists of *Pterocladiella capillacea*, *Gracilaria gracilis*, and *Asparagopsis armata*, which present large amounts of total polyphenols and flavonoids and weak DPPH radical scavenging activities (IC_{50} values). Among these species, *A. armata* seems to be particularly rich in flavonoids, tannins, and total polyphenols, and it possesses significant antioxidant activity. Individuals of this species collected from other locations have also been reported to produce these bioactive compounds (Zubia et al. 2009; Pinteus et al. 2015; Neethu et al. 2017). This species is considered to be invasive in the Mediterranean Sea (Pinteus et al. 2016), and should therefore be considered for industrial applications.

The results obtained in this study highlight that *G. gracilis* has a substantial polyphenol content. This species is widely cultivated in Asian countries (FAO 2018), and cultivation data for Tunisia (Ben Said et al. 2018; Chebil Ajjabi et al. 2018) indicate that this species has high potential for development in the southern Mediterranean Sea. In addition, the two species *Hypnea musciformis* and *Pterocladiella capillacea* represent potential candidates for the development of the seaweed-based industry in Tunisia, as these two genera are also cultivated in other parts of the world (FAO 2018).

Conclusions

This work enhances knowledge of the potential uses of seaweeds from southern Mediterranean coasts, which is important because such information is lacking for this region. The results highlight the importance of selecting certain species for biotechnological development applications in Tunisia. The present study found that the levels of certain target molecules such as polyphenols, tannins, and flavonoids varied from one species to another. Thus, five red macroalgae species of interest were identified (*Asparagopsis armata*, *Gracillara gracili*, *Hypnea musciformis*, *Pterocladia capillacea*, and *Laurencia obtusa*). The results obtained indicated that *H. musciformis* and *L. obtusa* present high tannin levels and significant total antioxidant capacities, whereas *P. capillacea*, *G. gracilis*, and *A. armata* possess large amounts of total polyphenols and flavonoids as well as weak DPPH radical scavenging potencies (IC_{50}). It is worth noting that most of the species studied did not exhibit any significant antimicrobial activity.

Seaweeds are a renewable marine resource with multiple possible recovery methods (e.g., CO_2 sequestration, wastewater treatment, and bioremediation in integrated multitrophic aquaculture systems). Their cultivation and exploitation in various industrial and innovative sectors could be a key element of the development of a sustainable "blue economy."

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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