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Unveiling the Antioxidant Potential of Halophyte Plants and Seaweeds for Health Applications

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Abstract: Halophyte plants and seaweed are described in the literature as rich sources of antioxidant compounds that can be used in the pharmaceutical and food industries. In this work, we studied the antioxidant composition of five species of halophytic plants (*Suaeda vera* Forssk, *Portulaca oleracea* L., *Inula crithmoides* L., *Salicornia ramosissima* (Hook.f.) J. Woods and *Sarcocornia perennis* (Mill.) A.J.Scott) and three seaweeds (*Gracilaria gracilis* (Stackhouse) Steentoft, L.Irvine and Farnham, *Fucus spiralis* L. and *Ulva rigida* C. Agardh) collected in Sado Estuary, Portugal. In the case of the plants, different parts of the plant were also assessed. Various extraction procedures were also performed to understand which methods were most suitable for extracting the various antioxidant compounds. Therefore, the aim of this study was to characterize the antioxidant compounds in halophytes and seaweed using various methods (ABTS, DPPH and FRAP), as well as the phenolic (TPC) and flavonoid (TFC) contents in the different extracts obtained. The amount of soluble protein in each extract was also determined. The results show that methanolic extracts generally have a higher antioxidant capacity, while the highest soluble protein content was observed in aqueous extracts. The seaweed *Fucus Spiralis* showed the highest antioxidant content, while in halophytic plants the highest antioxidant content was detected in the leaves. In general, this work confirms the potential of halophytes and seaweed as sources of antioxidant compounds for use in the food and pharmaceutical industries.

Keywords: halophyte plants; seaweed; antioxidants; extraction solvents; flavonoids; phenolic compounds

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1. Introduction

Halophyte plants (HAs) can be described as salt-tolerant plants, meaning that they grow and reproduce in high-drought, light, cold, hot and salty environments [1]. The capacity of these plants to thrive in such harsh environments can be attributed to their ability to lead with reactive oxygen species (ROS) through potent antioxidant systems, involving antioxidant enzymes and bioactive secondary metabolites, including some phenolic compounds [2].

Seaweeds are also important for human health, as they contain compounds with proven benefits in the prevention and treatment of various diseases, but also for human nutrition or utilized as fertilizers [3]. Due to their characteristics and diversity of compounds, HAs have different industrial applications, described in the scientific literature. For example, their balanced nutritional profile, which includes fiber and antioxidant vitamins such as

vitamin C, has found applications in the food industry [4]. In addition, they are relevant to the pharmaceutical industry, due to the presence of polyphenols, flavonoids and vitamins. Furthermore, HAs also have environmental benefits. They can remove significant amounts of salt from saline soils and are utilized in the production of food and fodder, as well as in the textile industry. Like other plants, they are also capable of sequestering carbon dioxide, helping to combat climate change [5]. Seaweeds have been described in the literature as having a wealth of bioactive compounds that can be used in the pharmaceutical industry and in food and cosmetic industries. Various seaweed species have been shown to aid in the treatment of different diseases, ranging from neurodegenerative to metabolic disorders, as well as cancer and inflammatory diseases [6]. They are also a source of vitamin B12, polyphenols and proteins, which can be extremely relevant to vegetarian individuals [6].

Different methodologies described in the literature are utilized to extract different relevant compounds from halophytes and seaweed for use in pharmaceutical and food industries [7–14]. Antioxidant compounds are among the most predominant in HAs and seaweed and are consequently extremely relevant for those industries [7–13,15].

The search for new natural antioxidants is of utmost importance worldwide, with HAs and seaweed being among these potential sources [8,9,14]. Oxidative stress is associated with an imbalance between the high production of reactive oxygen species (ROS) and various oxidative defense mechanisms, including enzymatic and non-enzymatic mechanisms [16]. Oxidative stress is implicated in many human diseases, from inflammatory disease to ischemic disease to neurological disorders [17]. Free radicals are produced within the human body due to an inflammatory process but also exercise, ischemia and other factors such as environmental pollutants [17]. Antioxidants help organisms to deal with free radicals by acting as radical scavengers, reducing the potential damage caused by their action [17].

There are endogenous antioxidant mechanisms based on enzymatic mechanisms, such as superoxide dismutase, catalase and glutathione systems, and non-enzymatic mechanisms, such as melatonin and uric acid [18]. However, it is essential that exogenous antioxidants are obtained from one's diet or supplements, such as vitamin C and E and melatonin [17]. Antioxidants act at different levels, starting as a first line of defense to prevent oxidative stress through radical scavenging, repair and finally adaptation.

The number and quantification of antioxidants found in seaweeds around the world have been extensively reported in the literature. However, regarding their levels in halophytes, there is still little information. Nonetheless, some studies have reported antioxidants in HAs. For instance, Stankovic et al. [19] showed high content of phenolic compounds, strongly correlated with antioxidant capacity, in methanolic extracts of several halophyte species (*Mentha pulegium* (L.), *Achielllea collina* (B.), *Statice gmelinii* (W.), *Artemisia santonicum* (L.), *Atriplec littoralis* (L.) and *Aster tripolium* (L.) subsp. *Pannonicus*). Another study, by Bakhouche et al. [20], showed higher antioxidant activities in the methanolic extracts of the roots and leaves of *Limonium delicatulum* (G.) than the aqueous extracts of the same plant. Similar results were reported by Neves et al. [21], which determined the antioxidant capacity in different structures of an HA (*Carpobrotus edulis* (L.)) [21]. The same authors found that the antioxidant capacity of leaves was higher compared to fruits but lower than that determined in flowers. Additionally, they also reported similar trends for total phenolic compounds and ferric reduction antioxidant power (FRAP).

Concerning seaweeds, several studies have shown that, in general, green algae have higher antioxidant capacity than red algae and brown more than green. For example, Farasat et al. [22] reported the antioxidant activity in different species of *Ulva* and also the total phenolic compounds and flavonoid content. In another study, the antioxidant activity and total phenolic compounds of fresh and freeze-dried seaweed were compared, finding that freeze-dried *F. spiralis* (L.) had higher phenolic content and antioxidant capacity than fresh seaweed. Another study, by Alkha-laf [23], reported a higher antioxidant activity determined in methanolic extracts of Chondrus crispus compared to two antioxidant standards, Trolox and BHT (free radical scavenging standard). Another example was

Pinteus et al. [24], where the antioxidant capacities of different seaweeds (green, brown and red) were evaluated, the methanolic fraction of brown seaweeds *Fucales* being the fraction with the highest antioxidant capacity.

Phenolic compounds are extremely important in plants, by defending plants from predators, UV, pathogens or being responsible for plant colors [25]. They also have benefits for humans, as they have a variety of beneficial effects on health (e.g., anti-inflammatory, antidepressant, cytotoxic, anticarcinogenic and antioxidant) [26]. Regarding flavonoids, they are secondary metabolites very abundant in plants, with a role in color, scent, flavor or giving protection against biotic and abiotic stressors [27].

The current literature shows that different extraction solvents have been able to extract different types of molecules; for example, water is good for extracting tannins, saponins and polypeptides, while methanol is able to extract flavones, phenones and polyphenols, and ether is a good solvent for extracting alkaloids and fatty acids [28].

The number of studies in which the antioxidant activity of HAs was studied is limited. Furthermore, the HA species evaluated in the present study are poorly characterized in the available scientific literature. In various studies where the antioxidant activity of algae has been higher, it has been noted that factors such as geographical area, geology and season of collection are relevant. These factors can influence the composition of algae, including antioxidant compounds [29,30]. This study aims to quantify the antioxidant capacity in different parts (stems and leaves) of five different HAs (*Suaeda vera* Forssk. ex J.F.Gmel, *Portulaca oleracea* L., *Inula crithmoides* L., *Salicornia ramosissima* Hook.f. and *Sarcocornia perennis* (Mill.) A.J.Scott) and three different seaweeds (*Gracilaria gracilis* (Stackhouse) Steentoft, L.Irvine and Farnham, *Fucus spiralis* L. and *Ulva rigida* C. Agardh) from the Sado Estuary. Additionally, three different solvents (ethyl acetate, methanol and water) were assessed. The contents of antioxidants determined in the different parts of the halophytes and in seaweeds were compared and critically discussed.

2. Materials and Methods

2.1. Plant and Seaweed Sampling and Processing

HAs (Suaeda vera, Portulaca oleracea, Inula crithmoides, Salicornia ramosissima and Sarcocornia perennis) and three different seaweeds (Gracilaria gracilis, Fucus spiralis and Ulva rigida (Figure 1)) were collected in Sado Estuary Natural Reserve (38°32′41.65″ N, 8°47′17.28″ O). The HAs were divided into leaves and stems, whenever possible. The different parts of HAs and the different seaweeds were freeze-dried (ScanVac CoolSafe 9L, LabGene, Lillerød, Denmark) for 48 h or until fully dried. Subsequently, the different parts were ground with the aid of a blade grinder until obtaining a fine powder.

2.2. Extraction Procedures

Extracts of the different parts of HAs and each seaweed were obtained by using a ratio of 1 g of dry HA or seaweed powder to 40 mL of distilled water or methanol, by adapting previous methods [31–33]). Thus, the powders were mixed with 70% (*v*/*v*) methanol (Honeywell, Seelze, Germany) or distilled water for some minutes in an orbital shaker (Optic Ivymen System, Madrid, Spain) and subsequently subjected to an ultrasound bath (J.P. Selecta, Barcelona, Spain) for 30 min at 40 kHz. Small periods of 5 min were applied to control the temperature. The resulting mixture was shaken for ten minutes and then left in the freezer for 48 h, being shaken for short periods. Then, extracts were filtered with filter paper (Whatman, n°1, Sigma-Aldrich, St. Louis, MO, USA) and stored at −20 °C until use. For the extraction process with ethyl acetate, a double extraction was carried out. Briefly, 1 g of powder was mixed with 40 mL of ethyl acetate and immediately stirred in a vortex. The mixture was then subjected to an ultrasound bath for 30 min, using the same procedure described previously, and then filtered through filter paper. This process was repeated twice, and the resulting filtrates were combined at the end. The ethyl acetate extracts were left to dry and then resuspended in methanol (70% (*v*/*v*)) at a concentration of 10 mg/mL, by adapting a procedure described by Rodrigues et al. [10].

Figure 1. Seaweed species: (**a**) Fucus spiralis, (**b**) Ulva rigida and (**c**) Gracilaria gracilis; halophyte plants: (**d**) Portulaca oleracea, (**e**) Sarcocornia perennis, (**f**) Inula crithmoides, (**g**) Sueda vera and (**h**) Salicornia ramosissima.

A workflow of the extractions procedures is shown in Figure S1 (Supplementary Material).

2.3. Total Soluble Protein

Lowry Method

Total soluble proteins in samples were quantified based on the protocol described by Lowry et al. [34] and adapted to 96-well microplate (Greiner, Bio-one, Kremsmünster, Austria).

In each Eppendorf, 60 µL of sample or standard (Serum Albumin Bovine (BSA, Nzytech, Lisboa, Portugal)) was vortexed with 300 µL of a solution containing sodium carbonate (2% w/v) in sodium hydroxide (0.1 M; Laborspirit, Loures, Portugal; Sigma, Taufkirchen, Germany) and copper sulphate pentahydrate (1% w/v) (Sigma, Taufkirchen, Germany) mixed with sodium potassium tartrate (2% w/v; Sigma, Germany) and left incubating for 10 min in the dark. Subsequently, 60 µL of Folin–Ciocalteu's Phenol reagent (2.2 M; Supelco, Darmstadt, Germany) was added to each microtube and vortexed. After 30 min incubating in the dark, 140 µL was taken from each microtube and added to the wells of a 96-well microplate. The absorbance was read at 680 nm in a microplate reader (Synergy HTX, BioTek, Winooski, VT, USA), and the soluble protein concentration was determined from the calibration curve. Results were expressed as mass of protein per dry weight (mg protein/g DW).

2.4. Antioxidant Assays

2.4.1. ABTS Radical Scavenging Assay

The ABTS radical scavenging assay is based on the reduction of the ABTS radical cation and followed the method described by Re et al. [35], adapted for a 96-well microplate (Greiner, Bio-one).

Initially, 12 h before the beginning of the assay, an ABTS cation solution was prepared by mixing equal volumes of 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid) solution (7 mM; ABTS; Alfa Aesar, Kandal, India) with a potassium persulphate solution (2.45 mM; Carlo Erba, Val de Reuil Cedex, France). The solution was left in the dark overnight. After this period, the $ABTS^{+}$ solution was diluted to achieve an absorbance of 0.7 ± 0.05 at 734 nm. A calibration curve of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich, Moscow, Russia), ranging from 0 to 125 µg, was prepared in the same solvent used for the extraction or reconstitution.

After diluting the $ABTS^+$ solution, 20 µL of sample or standard was added to each well of the microplate, followed by adding 180 µL of $ABTS^+$ solution. After incubating for 6 min in the dark, the microplate was read at 734 nm in a microplate reader (Synergy HTX, BioTek). The results were expressed as Trolox equivalents per dry weight (mg TE/g DW).

2.4.2. DPPH Assay

This assay is based on the reaction of DPPH radical with an antioxidant molecule, following the method described by Blois et al. [36], adapted to a 96-well microplate (Greiner, Bio-one).

Hence, 10 µL of sample or standard was mixed with 190 µL of DPPH solution (0.06 mM in methanol (Sigma); 2,2-diphenyl-1-picrylhydrazyl; Sigma, Steinheim, Germany) and added to each well of the microplate. The mixture was then incubated for 30 min in the dark before being read at 527 nm in a microplate reader (Sinergy HTX, BioTek). A calibration curve using Trolox was prepared in the range between 0 and 0.5 mg/L.

The results were expressed as Trolox equivalents per dry weight (µg TE/g DW).

2.4.3. FRAP Assay

Ferric reduction antioxidant power (FRAP) assay is based on the reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to ferrous iron form (Fe^{2+}). It was performed following the protocol described by Benzie et al. [37], adapted to a 96-well microplate (Greiner, Bio-one).

FRAP reagent was prepared by mixing 50 mL of sodium acetate buffer (0.3 M; pH 3.6) with 5 mL of 2,4,6-tris(2-pyridyl)-s-triazine solution (10 mM; TPTZ; Sigma-Aldrich, Buchs SG, Switzerland) and 5 mL of ferric chloride solution (20 mM; Alfa Aesar, Kandal, Germany). A calibration curve, ranging 0–2000 µM, was prepared with gallic acid (Alfa Aesar, Kandal, Germany).

Then, 20 µL of sample was added to each well, along with 15 µL of milliQ water and 265 µL of FRAP reagent. For blanks, milliQ water was used instead of FRAP reagent. The microplate was then incubated for 30 min, at 37 °C in the dark. Afterwards, the absorbance was measured at 595 nm using a microplate reader (Sinergy HTX, BioTek). The results were expressed as gallic acid equivalents per dry weight (µmol GAE/g DW).

2.4.4. Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin–Ciocalteu method as described by Singleton et al. [38] and adapted to a 96-well microplate (Greiner, Bio-one).

Briefly, 20 µL of sample or standard was added to each well of the microplate, along with 100 µL of Folin–Ciocalteu reagent (10% (*v*/*v*); Supelco, Darmstadt, Germany). After incubating for 5 min in the dark, 80 µL of sodium carbonate (10 mg/mL; Sigma-Aldrich, St. Louis, MO, USA) was added to each well. The microplate was shaken in the dark for one minute and then incubated for an additional 30 min. A calibration curve with gallic acid (Alfa Aesar) was prepared in a range between 0 and 0.2 mg/ mL.

Absorbance was measured at 750 nm in a microplate reader (Synergy HTX, BioTek), and the results were expressed as gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.5. Total Flavonoid Content (TFC)

The total flavonoid content was determined based on a protocol described by Zhishen et al. [39] and adapted to a 96-well microplate (Greiner, Bio-one).

Briefly, 35 µL of sample was mixed with 140 µL of distilled water and 10.5 µL of sodium nitrite (5% (*w*/*v*), $NaNO_2$, Merk, Darmstadt, Germany). After a 5 min incubation period, 10.5 µL of aluminum chloride (10% (*w*/*v*), $AlCl_3$) was added, followed by the addition of 70 µL of sodium hydroxide (1M; NaOH; Laborspirit) to each microplate well. A calibration curve was prepared with quercetin (Sigma, Bengaluru, India), ranging between 0 and 0.175 mg/mL. The absorbance was read at 510 nm using a microplate reader (Synergy

HTX, BioTek), and the results were expressed as milligram quercetin equivalents per gram of dry weight (mg QE/g dry weight).

2.6. Statistical Analysis

To verify statistical differences, a two-way ANOVA was performed, after checking the statistical assumptions. Turkey's post hoc test was used to assess differences between the different samples. Principal Component Analysis was also performed to compare the different assays. All statistical analyses were carried out using the software Prism 9: GraphPad (version 9.5.1), and results were expressed as mean ± SD.

3. Results

3.1. Total Soluble Protein

The results of total soluble proteins in halophytes and seaweeds, according to the different solvents used for extraction, are depicted in Figure 2a–c.

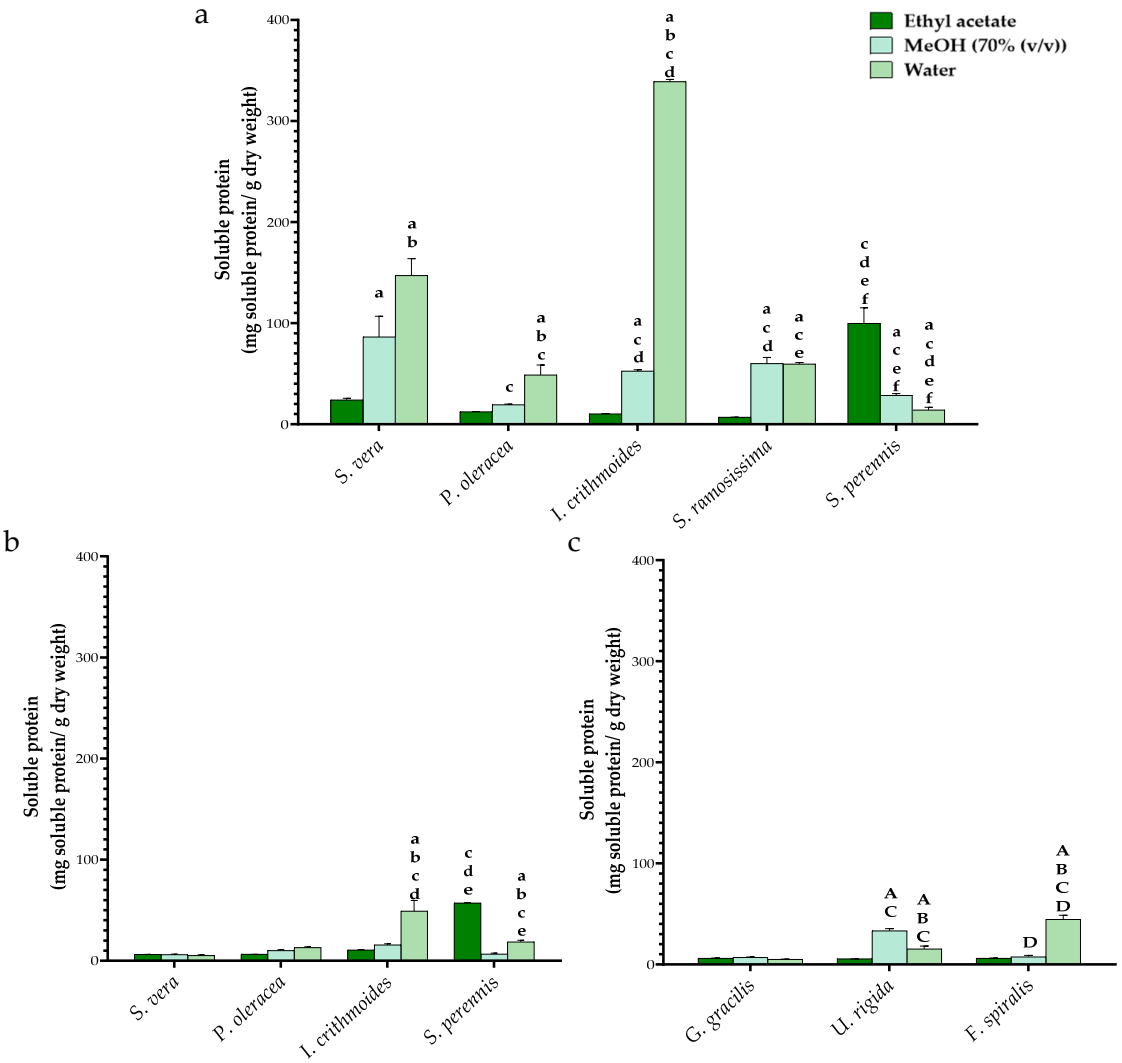

Figure 2. Soluble protein determined in leaves (**a**) and in stems of HAs (**b**) and in seaweed (**c**). For HAs, statistical differences compared with a—ethyl acetate extraction and b—methanol extraction between the same species (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) mean significant differences between the same extraction solvent. For seaweed, statistical differences were compared with A—ethyl acetate extraction and B—methanol extraction in the same species (C—*G. gracilis* and D—*U. rigida*) between the same extraction solvent.

In general, the results for total soluble proteins determined in HAs indicate a higher yield in aqueous extraction followed by methanolic extraction, except for *S. perennis* (stem and leaves), where the yield was higher when extracted with ethyl acetate. Additionally, differences were observed in protein levels among the various parts of the plant, with the leaves showing the highest concentrations (see Table S1), particularly in the aqueous extracts of *I. crithmoides* leaves, which presents the highest soluble protein content among the halophyte plants analyzed.

In relation to the seaweed (*U. rigida* and *F. spiralis*), a higher yield was obtained in the methanol and water extracts, respectively, compared to ethyl acetate extraction ($p < 0.0001$). However, in *G. gracilis*, no differences were observed in the yield obtained between the extraction solvents used.

Regardless of the extraction solvent used, the same trend was observed in the amount of protein determined in the three algae analyzed. The highest were found in *F. spiralis* (44.73 ± 4.00 mg/g DW) extracted with water and in *U. rigida* (15.33 ± 2.77 mg/g DW) and *G. gracilis* (4.987 ± 0.284 mg/g DW), both extracted with methanol.

3.2. Antioxidants Determination

3.2.1. ABTS Radical Scavenging

Figure 3a,b present ABTS values in the leaves and stems of HAs. Overall, methanolic extraction showed higher yields in most species. However, the highest values were found in the leaves (5.525 ± 0.09 mg equivalents of Trolox/g DW) and in the stems (5.18 ± 0.26 mg equivalents of Trolox/g DW) of *S. perennis* after extraction with ethyl acetate. Conversely, the highest values were observed in methanolic extracts from the leaves of *I. crithmoides* (4.664 ± 0.005 mg Trolox equivalents/g DW) and *S. ramosissima* (4.61 ± 0.03 mg Trolox equivalents/g DW).

The quantification of the ABTS radical in seaweed is shown in Figure 2c. In general, methanolic extraction produced the highest yield. *F. spiralis* presented the highest values (3.61 ± 0.10 mg Trolox equivalents/g DW).

3.2.2. DPPH Assay

DPPH results are shown in Figure 4a–c. Overall, in HAs, like in the ABTS assay, methanolic extractions show higher DPPH values, except for the aqueous extraction values obtained from *S. vera* leaves, where the highest values are observed (Figure 3a,b). Generally, leaves have higher DPPH values, except for *P. oleracea* (see Table S1).

In *F. spiralis*, the highest yield was obtained with aqueous extractions, while in *G. gracilis* the highest yield was obtained with ethyl acetate, as depicted in Figure 4c.

3.2.3. Ferric Reduction Antioxidant Power (FRAP) Assay

The results of the FRAP assay are shown in Figure 5a–c. In HAs, the highest FRAP values were found in the leaves (see Table S1), mostly in methanolic extracts, except in *S. vera* and *S. perennis*, where the aqueous and ethyl acetate extracts, respectively, showed higher FRAP values (Figure 5a,b). In seaweed, the yield was higher in aqueous and methanolic extracts, with *U. rigida* and *F. spiralis* showing the highest values (Figure 5c).

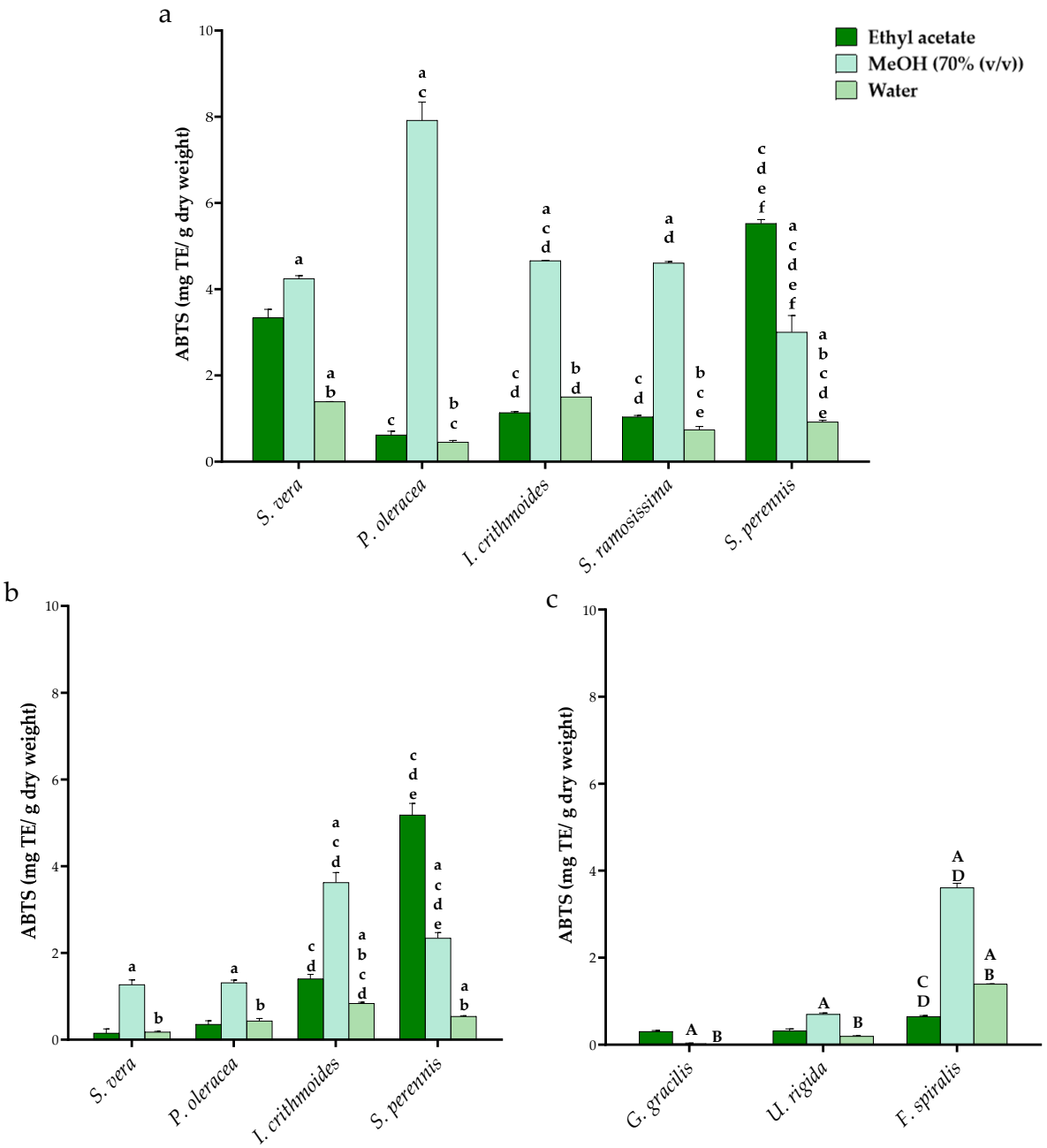

Figure 3. ABTS radical determined in leaves (**a**) and in stems of HAs (**b**) and in seaweed (**c**). For HAs, statistical differences compared with a—ethyl acetate extraction and b—methanol extraction between the same species (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) mean significant differences between the same extraction solvent. For seaweed, statistical differences were compared with A—ethyl acetate extraction and B—methanol extraction in the same species (C—*G. gracilis* and D—*U. rigida*) between the same extraction solvent.

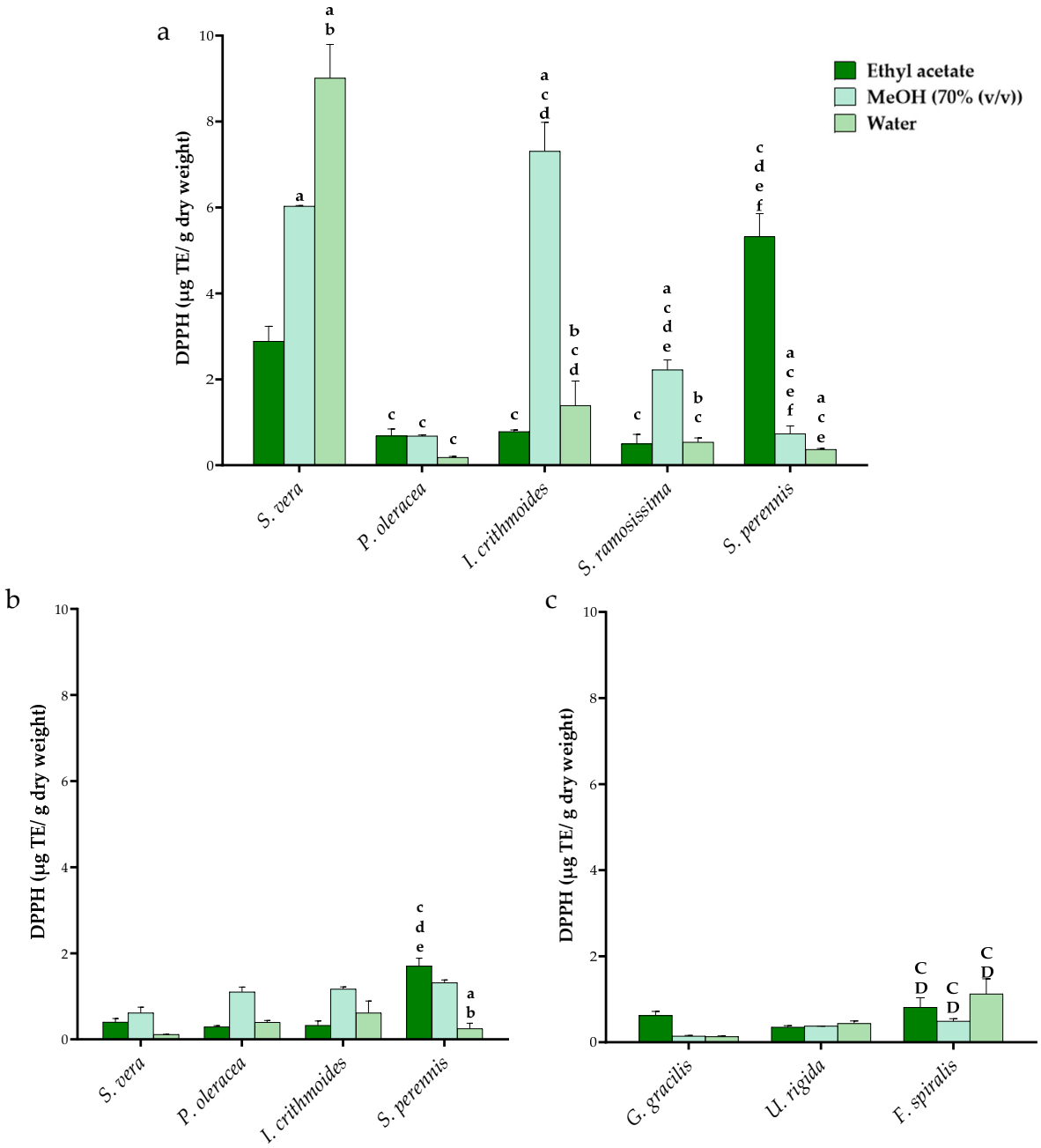

Figure 4. DPPH determined in leaves (**a**) and in stems of HAs (**b**) and in seaweed (**c**). For HAs, statistical differences were compared with a—ethyl acetate extraction and b—methanol extraction between the same HAs (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) between the same extraction solvent. For seaweed, statistical differences were compared with (C—*G. gracilis* and D—*U. rigida*) between the same extraction solvent.

3.2.4. Total Phenolic Content (TPC)

Figure 6a,b shows the total phenolic content in determined HAs, with a higher content being found in the leaves (see Table S1). The highest yield was obtained with methanolic extraction, except for the leaves of *S. vera*, where the highest yield was obtained in the aqueous extracts and for the leaves and stem of *S. perennis*, extracted with ethyl acetate.

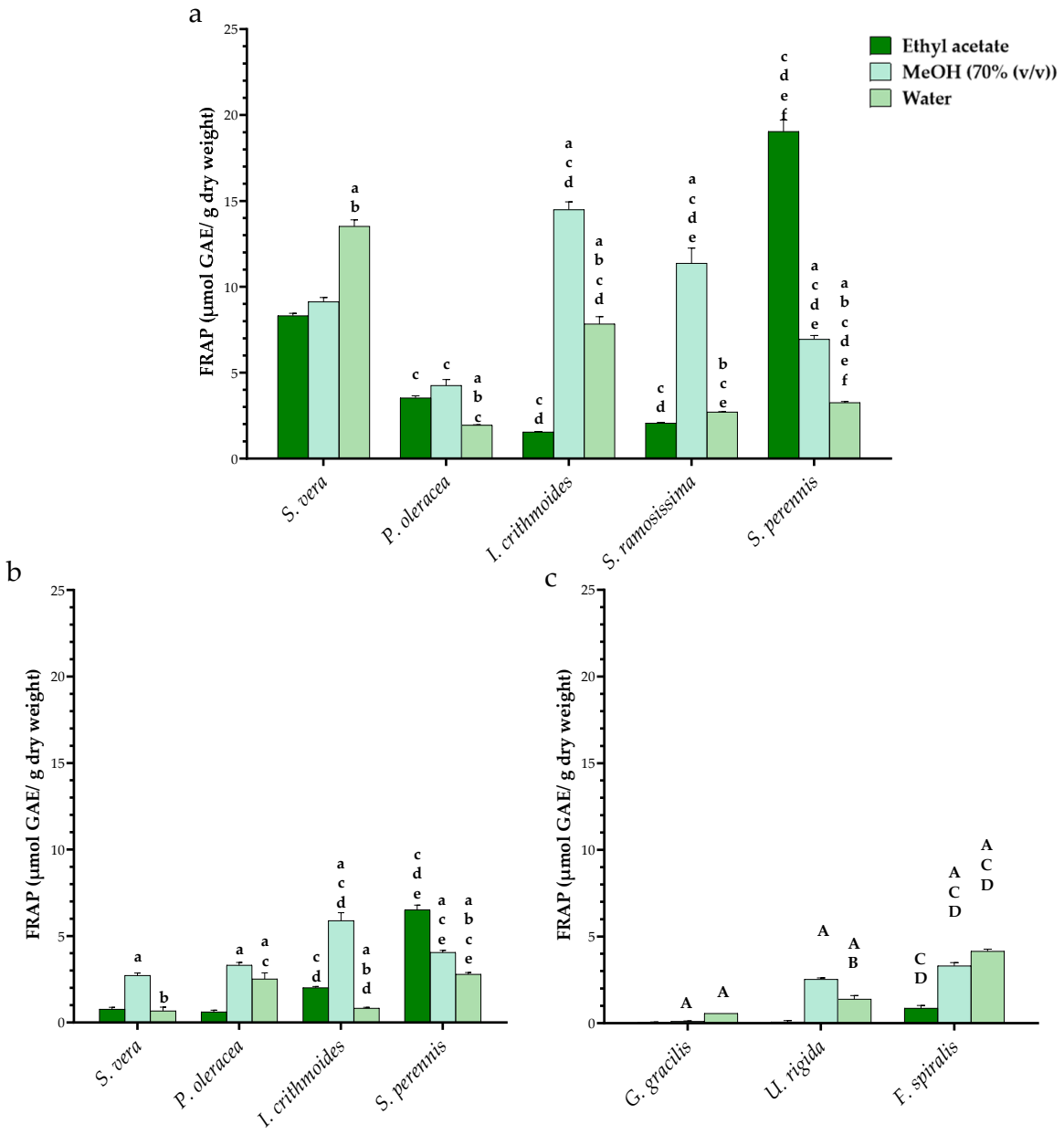

Figure 5. FRAP determined in leaves (**a**) and stems of HAs (**b**) and in seaweed (**c**). For HA, statistical differences were compared with a—ethyl acetate extraction and b—methanol extraction between the same species (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) between the same extraction solvent. For seaweed, statistical differences were compared with A—ethyl acetate extraction and B—methanol extraction in the same species (C—*G. gracilis* and D—*U. rigida*) between the same extraction solvent.

The TPC results determined in seaweeds are shown Figure 5c, where the highest values were observed in the aqueous extracts of *F. spiralis*. Regardless of the extraction solvent, *F. spiralis* presented the highest phenolic content.

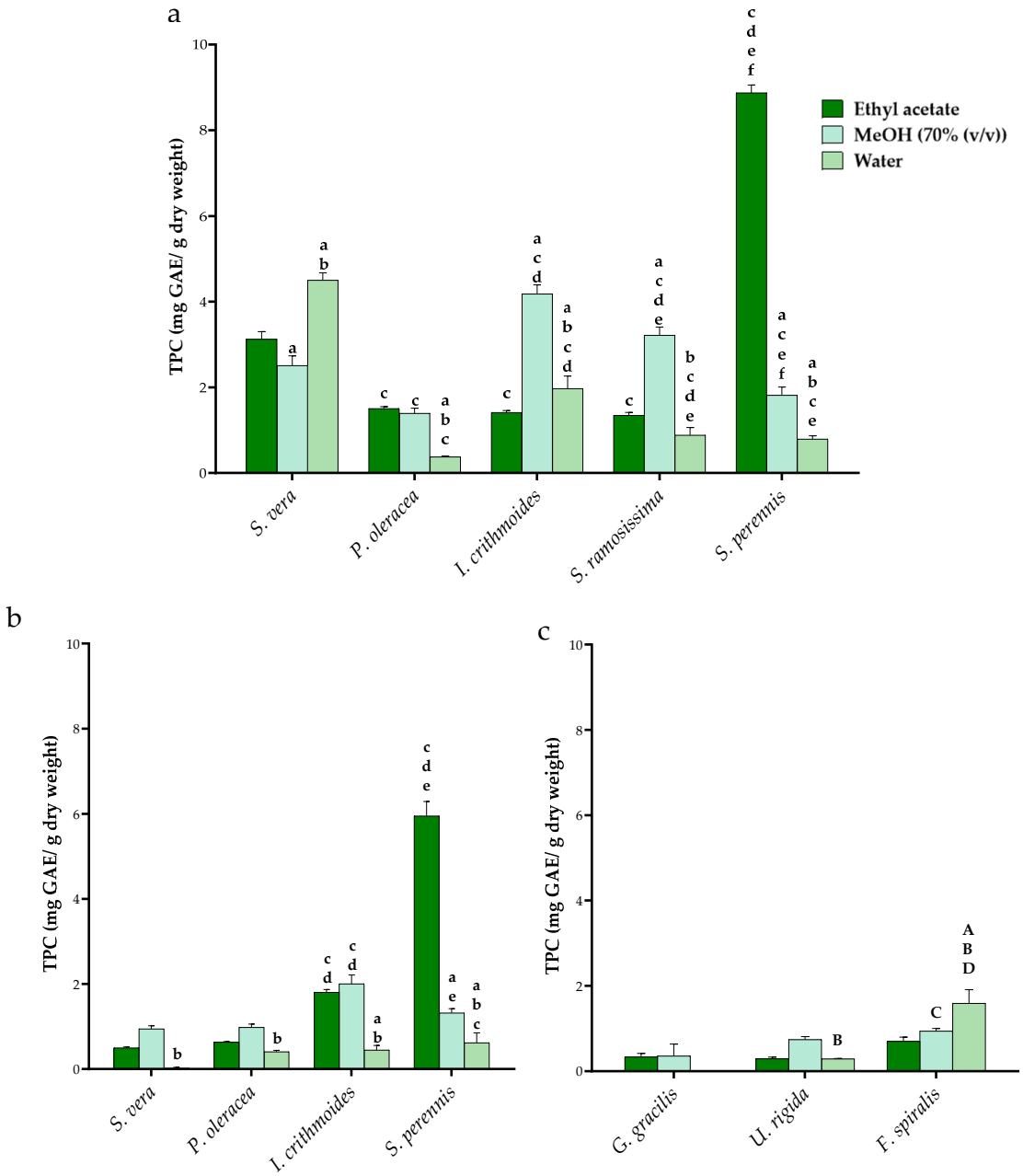

Figure 6. Total phenolic content determined in leaves (**a**) stems of HAs (**b**) and in seaweed (**c**). For HAs, statistical differences were compared with a—ethyl acetate extraction and b—methanol extraction between the same species (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) between the same extraction solvent. For seaweed, statistical differences were compared with A—ethyl acetate extraction and B—methanol extraction in the same species (C—*G. gracilis* and D—*U. rigida*) between the same extraction solvent.

3.3. Total Flavonoid Content (TFC)

The total flavonoid content in HAs (Figure 7a,b) showed that the leaves exhibited the highest flavonoid content (see Table S1). Overall, a higher extraction yield was obtained with methanol, except in *S. perennis* and *P. oleracea*, where the yield was higher with ethyl acetate.

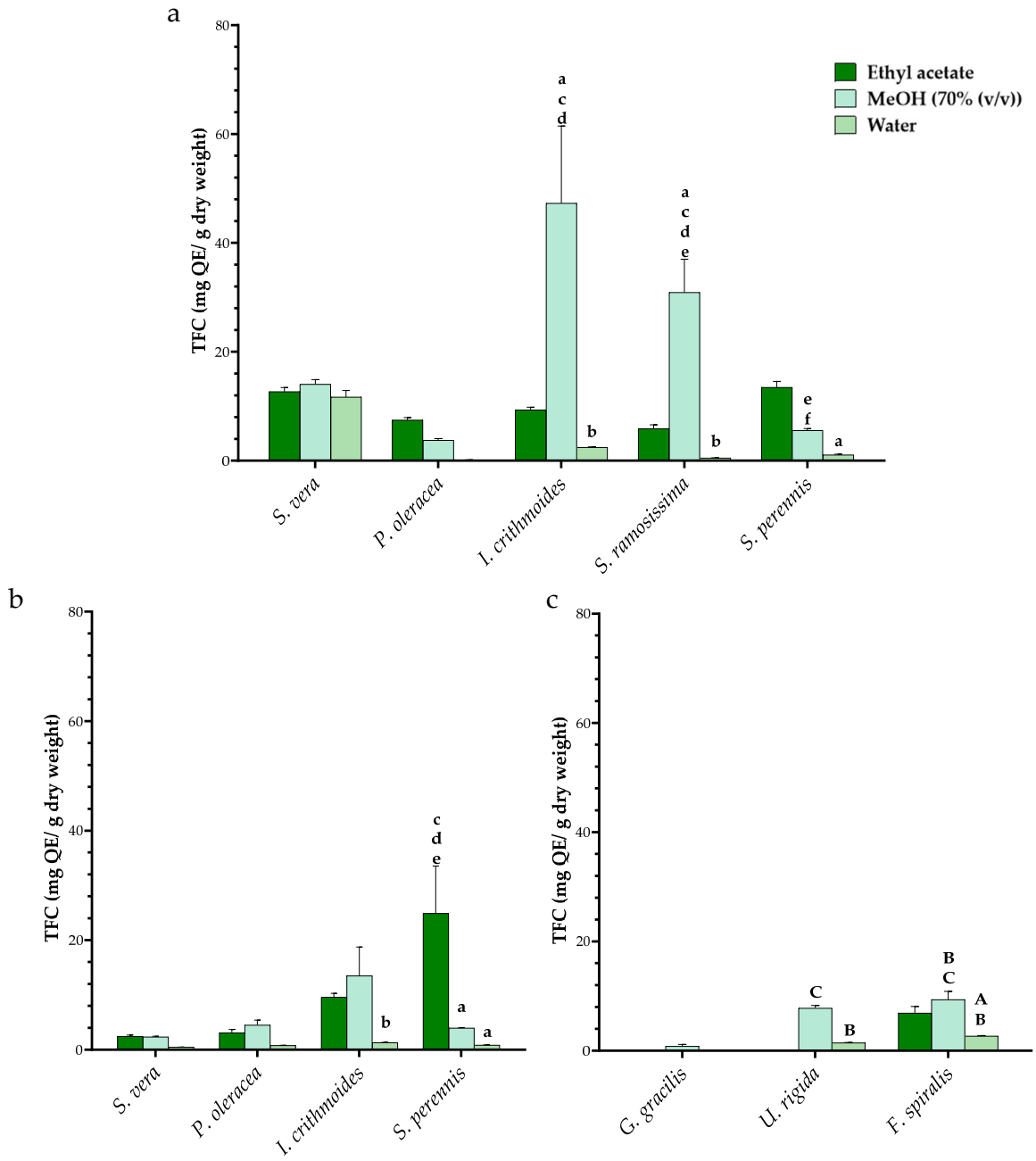

Figure 7. Total flavonoid content determined in leaves (**a**) and stems of HAs (**b**) and in seaweed (**c**). For HAs, statistical differences were compared with a—ethyl acetate extraction and b—methanol extraction between the same species (c—*S. vera*, d—*P. oleracea*, e—*I. crithmoides* and f—*S. ramosissima*) between the same extraction solvent. For seaweed, statistical differences were compared with A—ethyl acetate extraction and B—methanol extraction between the same species, C—*G. gracilis* between the same extraction solvent.

In general, for seaweeds (Figure 7c), methanolic extraction yielded the highest content, with the highest values observed in *F. spiralis* and *U. rigida*.

3.4. Correlation between Diferent Assays

To verify how the different assays were correlated, PCA analysis was performed (Figure 8). Results show that the assays are correlated differently in HAs (Figure 8a) and in seaweed (Figure 8b). In the correlation matrix of HAs, a strong positive correlation was observed between FRAP and TPC (0.88), between DPPH and FRAP (0.84) and between

TPC and ABTS (0.82). Additionally, moderate positive correlations were observed between FRAP and ABTS (0.79) and between ABTS and TFC (0.75). The correlation matrix revealed that in seaweed, there is a moderate positive correlation between ABTS and FRAP (0.77), between TPC and FRAP (0.74), among ABTS and TFC (0.74) and between FRAP and Lowry (0.74). A strong positive correlation was also observed between ABTS and TPC (0.83).

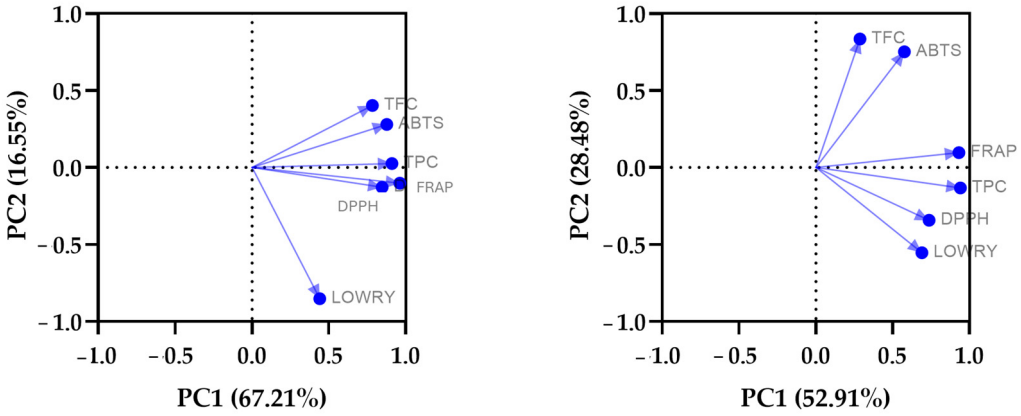

Figure 8. Principal Component Analysis (component loadings) performed on HAs (**a**) and in seaweed (**b**) for the different assays.

Furthermore, the results of HA reveal a strong positive correlation between PC1 and FRAP (0.960) and TPC (0.909). Additionally, PC1 exhibits a moderate positive correlation with TFC (0.782), as well as strong positive correlations with ABTS (0.875) and DPPH (0.845). Seaweed also showed a strong positive correlation between PC1 and TPC (0.939) and FRAP (0.930). Regarding PC2, for HAs, a strong negative correlation was observed between PC2 and Lowry (−0.852), while for seaweed, a strong positive correlation was detected between PC2 and TFC (0.833) and a moderate correlation between PC2 and ABTS (0.750). Indeed, the analysis of the results of HAs indicates that PC1 explains 67.21% of the variance and PC2 explains 16.55%, which means that the two components allowed for an 83.76% distinction between the different assays. On the other hand, the results for seaweed show that PC1 explains 52.91% of the variance in the dataset, whereas PC2 explains 28.48%, which means that the two components were recognized for 81.39% of the separation between different assays.

4. Discussion

The antioxidant capacity of the different parts of HAs and seaweed were evaluated, including phenolic and flavonoid content. The differences found in the yield obtained between the extraction solvents were also relevant to selecting the best approach for these target compounds.

It is recognized that HAs and seaweed can be a valuable source of bioactive compounds [2,23]. Phenolic compounds are crucial for neutralizing and repairing damage caused by ROS [26], while flavonoids, a subgroup of phenolic compounds, act as antioxidant agents by serving as potent donors of hydrogen cations [40].

An important finding in the present study is that seaweeds, in general, have lower protein content than the leaves of HAs, especially *S. vera* and *I. crithmoides*, which could mean that they may be of interest to vegetarians and vegans.

In the scientific literature, HAs are described as sources of phenolic compounds [41], which is in accordance with our findings, namely for *I. crithmoides* and *S. ramosissima*, where flavonoids, phenols and antioxidants were found to be higher in methanolic extracts than in aqueous extracts [2,20].

Regarding the different parts of the HAs analyzed, some studies suggest that leaves have higher values of antioxidant and flavonoids than stems [2]. This was also observed

in our work. However, higher values determined in roots and flowers were also reported [8,20]. In the present work, roots and flowers were not analyzed due to the lack or scarcity of these parts.

Different HAs have different salinity tolerance based on their location and proximity to saltwater. This implies that those close to the water exhibit higher salinity tolerance. Among the five tested, *P. oleracea* shows the lowest salt tolerance [42–45]. Furthermore, our results showed that this species presented the lowest phenolic and flavonoid content, as well as lower antioxidant capacity. This can be attributed to the reduced need for adaption to stressful environments, leading to fewer mechanisms of adaptation and consequently lower levels of antioxidant activity [2].

Different assays were performed to quantify the antioxidant activity because each assay is based on different mechanisms. FRAP has a huge variety of interferents such as polyphenols and amino acids [46], given that this assay was based on the reduction of ferric tripyridyl triazine complexes to ferrous form. Jímenez [47] refers to DPPH showing a greater performance in aqueous extracts, as we could see in aqueous extraction of *S. vera*. On the other hand, ABTS has been described as a satisfactory assay for hydrophobic and hydrophilic solvents [46].

The antioxidant capacity of various vegetables, fruits and drinks has been reported in numerous studies [48,49]. For instance, Floegel et al. [48] studied the antioxidant capacity in methanolic extracts of fruits and vegetables. However, for comparison purposes, the results were converted from vitamin C equivalents to Trolox equivalents [50]. In their study, blueberries exhibited the highest ABTS value (8.31 $\pm$ 0.44 mg TE/g DW), greater than the values obtained in our current work for the analyzed plants and algae, as expected considering that it is known for being rich in antioxidants. Nevertheless, this was one of the few fruits with higher values than the leaves of *S. ramosissima* (4.61 $\pm$ 0.03 mg TE/g DW), *I. crithmoides* (4.66 $\pm$ 0.01 mg TE/g DW) and *P. oleracea* (7.92 $\pm$ 0.42 mg TE/g DW). In terms of the vegetables analyzed in their study, all tested fruit and vegetables exhibited lower ABTS values compared to our results. However, concerning beverages, both green tea (3.18 $\pm$ 0.042 mg TE/g DW) and red wine (3.85 $\pm$ 0.07 mg TE/g DW) showed higher values than the algae and plants analyzed in our study. Arias et al. [49] also reported on the total phenolic content of different fruits and vegetables. Comparing our results with those of different fruits, we can see that most of the fruits tested had a higher phenolic content, with grapefruit being the fruit with the highest phenolic content (77.3 mg GAE/g DW). On the other hand, when comparing the results of our halophyte plants with other vegetables, the amount of phenolic content is more similar, with the artichoke being the vegetable with the highest phenolic content (30.16 mg GAE/g DW). For example, the mushroom has a total phenolic content (4.12 mg GAE/mg DW) comparable to the methanolic extraction of I. crithmoides leaves (4.18 $\pm$ 0.21 mg GAE/g DW) and the aqueous extraction of S. vera (4.50 $\pm$ 0.17 mg GAE/g DW), whereas, for example, carrot or pumpkin (0.58 mg GAE/g DW and 0.22 mg GAE/g DW, respectively) has a lower total phenolic content than almost all of our leaf extractions.

Studies by Pinteus et al. [24] have reported the antioxidant capacity in 27 seaweed species, collected in the Portuguese coast, with samples extracted using different solvents. As reported by other studies, *F. spiralis* generally exhibited higher antioxidant capacity among the three seaweeds studied. It has also been reported [24] in the literature that green seaweeds generally have higher antioxidant capacity, as well as phenolic and flavonoid contents, compared to red seaweed [24]. This agrees with our results, as *U. rigida* showed higher antioxidant capacity than *G. gracilis*. Our results contrast with those by Chaires-Martínez et al. [51], who reported higher antioxidant capacity in aqueous extracts (DPPH assay) than in methanolic extracts, along with a higher content of phenols and flavonoids in the same extracts. However, our findings are consistent with other previous studies showing higher extraction efficiencies with methanol extraction [52,53]. Our results show higher phenolic content in methanolic extracts, consistent with findings by Pinteus et al. [24]. The same study found a strong correlation between DPPH and TPC results. However, in

our study, a high positive correlation was detected but between ABTS and TPC, both in HAs and seaweeds. Furthermore, we found high positive correlation between ABTS and TPC and TFC in HAs, which agrees with other studies [19,54]. These results suggest that phenols, but also flavonoids, are extremely relevant for the antioxidant system in HAs. Similarly, lower antioxidant capacity, phenolic compounds and flavonoids were observed in seaweed compared to the leaves of these two HAs. The differences found between seaweed and the leaves of these two halophytes can be partially explained by the difference in collection times. HAs were collected in spring, while algae were collected in late autumn. The seasonal variations in the antioxidant capacity and phenolic compounds of seaweeds are reported in the literature [30]. On the other hand, HAs (terrestrial) and seaweed (marine) have different physiological and structural characteristics, adapted to different environments and conditions. Therefore, it is natural that they present different profiles of the compounds analyzed. Nonetheless, the results indicate that HAs and seaweed have great potential to be introduced into human diets as sources of antioxidants through food and supplements.

5. Conclusions

Seaweed and HAs have been described in the literature as relevant sources of antioxidants and phenolic compounds. As expected, different extraction solvents resulted in different yields of the target compounds.

Furthermore, this study shows that HAs have more contents of antioxidants, phenols and flavonoids than seaweed. While selecting a specific HA with the highest concentration of these compounds is challenging, it is evident that leaves contain the highest concentration of phenolic and flavonoid compounds.

Regarding the seaweed *F. spiralis*, it exhibited the highest antioxidant capacity and a notable content of phenolic and flavonoid compounds.

As a final remark, both seaweed and HAs hold significant potential for the food industry, as well as pharmaceutical and supplement industries, owing to their rich content of antioxidant compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/oxygen4020011/s1, Figure S1: Workflow of the processing and extraction methodologies followed for halophytes and seaweeds; Table S1: Results (mean ± s.d.) of the different assays performed (Lowry, ABTS, DPPH, FRAP, TPC and TFC) on halophyte plants; Table S2: Results (mean ± s.d.) of the different assays performed (Lowry, ABTS, DPPH, FRAP, TPC and TFC) on seaweed.

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