

Elemental uptake and antioxidant activity in edible Mediterranean halophytes from Tinto River

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Abstract

A complete survey is presented on elemental composition, selected amino acids and antioxidant capacity in edible halophytes from Spain. We include materials from the Iberian Peninsula: littoral-coastal Tinto River basin areas (SW Spain: Huelva province) and mainland territories (NW and central Spain: Zaragoza). The aim of this contribution is to characterize the relation between nutritional content compounds and their key role in antioxidant capacity in stress environments. Using analytical techniques such as ICP-MS, DPPH, FRAP and TBARS our results revealed the importance of different antioxidant responses in edible halophytes and their relations between elemental inorganic nutrients and stronger accumulation of some bioactive compounds like phenolic acids, flavonoids and amino acids. These molecules may be the key to the survival of these species in extreme saline environments located in Spain.

Keywords: antioxidants; compounds; halophytes; heavy metals; proline

Introduction

Halophytes are defined as plants capable of developing and completing their biological cycle in natural saline environments with concentrations greater than 200 mM NaCl (Flowers *et al.*, 2008).

The species of the subfamily Salicornioideae (Chenopodiaceae) constitute a highly specialized flora adapted to hypersaline environments. Their survival in these stressful conditions is ensured by osmotic adjustments and morpho-anatomical and physiological adaptations. They are considered euhalophytes, or obligate halophytes, as they thrive in environments with high salt concentrations. All are succulent plants, with large amounts of water in their tissues, and have the ability to accumulate salts and ions to maintain the osmotic balance (Grigore *et al.*, 2017).

Spain and Portugal present a great diversity of halophilic species, among which are, amongst which, the following genera stand out: *Arthrocnemum* Moq., *Salicornia* L. and *Sarcocornia* A. J. Scott. Previous studies improved the definition of each of these genera in these Mediterranean and Atlantic areas, providing keys for the correct differentiation at the species level (Fuente *et al.*, 2008; Rufo *et al.*, 2021). In recent years, the nutritional interest of these genera has led to an increase in research articles on elemental composition, minerals, bioactive compounds, polyphenols, fatty acids, and flavonoids (Loconsole *et al.*, 2019). These compounds have been of interest in recent decades due to their therapeutic effect in different conditions,

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including a protective role in ischemia, anti-thrombotic effects and other health-promoting neurovascular benefits (Lima *et al.*, 2020).

In the Iberian Peninsula, there are annual biotypes (terophytes) with an annual life cycle, for example the genus *Salicornia* L. The perennial biotypes include camephytes and nannofanerophytes (50 to 150 cm tall) with prostrate, erect and branched habits, as are the species of the genera *Sarcocornia* A.J. Scott, and *Arthrocnemum* Moq (Fuente *et al.*, 2010) as we show in Figure 1 (A, B, C).

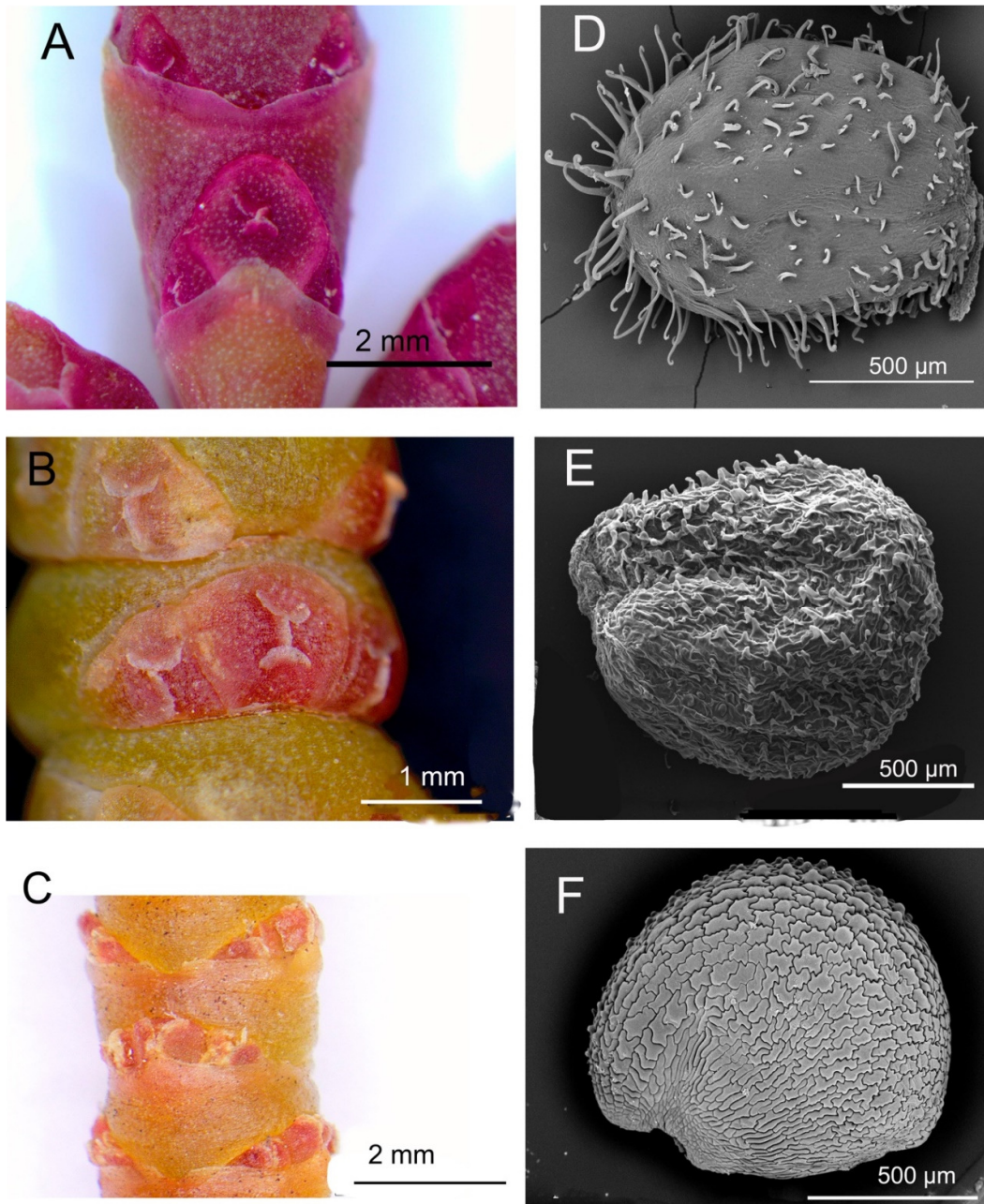


Figure 1. Studied material. A and D. *Salicornia patula*. B and E. *Sarcocornia pruinosa*. C and F. *Arthrocnemum macrostachyum*.

In some cases, samples are sold with the names *Salicornia ramosissima*, *S. herbacea*, without checking whether these names are synonyms of *S. europaea* accepted from the taxonomic point of view in the different floras of the world. It is of interest to know the samples because behind the name of a species there may be varieties with different compounds depending on the origin.

Salicornia taxa accumulate inorganic salts and water in their stems. The most common elements found are Na, Ca, K and Mg, among others, and are present in stem, leaves and roots (Sánchez-Gavilán *et al.*, 2021). Like other chenopods such as *Sarcocornia perennis* Mill. and *Arthrocnemum macrostachyum* (Moric.) Moris, recently introduced for human consumption, *Salicornia patula* is also an excellent candidate due to its mineral content.

Other studies also report new uses of *Salicornia patula* with a long history of gathering from the wild as a source of food (Urbano *et al.*, 2017).

The production of different active metabolites is a mechanism of defense against oxidative stress in preventing the toxicity of reactive oxygen species (ROS). The antioxidant activity due to enzyme systems such as catalase or superoxide dismutase, among others, have been widely studied in plants in response to the harmful effects of oxidative stress. Extracts of *Salicornia fragilis* have been reported to have antioxidant properties (Roberto *et al.*, 2021). *S. europaea* extracts ameliorate hypertension and vascular dysfunction induced by high salt intake in vivo as well as reduce hyperplasia during vascular remodelling. Recently, polyphenols from *Salicornia ramosissima* extracts have been evaluated on the deleterious effects induced by hypoxia and cerebral ischemia (García-Rodríguez *et al.*, 2021).

The aim of this work is to explore the relationship between elemental uptake and antioxidant and toxicological capacity in halophytes from Río Tinto in South Iberian Peninsula. Río Tinto is a 92 km long extreme acidic environment, which is the product of the metabolic activity of chemolithotrophic microorganisms thriving in the high concentration of metal sulfidic minerals existing in the Iberian Pyrite Belt (Amils *et al.*, 2020).

Materials and Methods

Table 1 and Figure 1, show the material studied from *Salicornia patula*, *Sarcocornia alpini*, *Sarcocornia pruinoso*, *Sarcocornia perennis* and *Arthrocnemum macrostachyum* in the Iberian Peninsula.

Table 1. Information of collected and studied taxa

Specimen	Locality	Date
<i>Salicornia patula</i>	Spain, Huelva, La Rábida	24.10.17
<i>Sarcocornia alpini</i>	Spain, Huelva, San Juan del Puerto	14.12.17
<i>Sarcocornia pruinoso</i>	Spain, Huelva, Tinto river estuary	14.12.17
<i>Sarcocornia perennis</i>	Portugal, marismas de Tavira, Santa Luzia	18.07.18
<i>Arthrocnemum macrostachyum</i>	Spain, Zaragoza, Belchite	19.01.17

CEM microwave digestion (US) at medium pressure.

Elemental analysis

The elemental content was quantified by inductively coupled plasma mass spectrometry (ICP-MS), a highly sensitive technique for the determination of multiple elements. Soil samples (500 mg) were digested with a mixture of HCl and HNO₃ at 60 °C in an MSD-2000. Plant samples were analysed using the protocol

described by Zuluaga *et al.* (2010), which optimizes the digestion process of plant matter and allows the optimal recovery of a large number of elements in a single semi-quantitative analysis.

Aliquots of the solutions obtained from both soil and vegetable samples were analysed for Na, Mg, K, Fe, Cu, Zn by ICP-MS using an ICPELAN-600 PE Sciex Instrument (Toronto, Ontario, Canada).

This technique determined the concentrations in mg/kg, based on the dry weight of the digested sample. The ICP-MS technique has an inherent error of 15%.

Quantification of proline and glycine betaine

Radical method 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH radical scavenging was determined following the protocol (Barros *et al.*, 2008) using ELX800 microplate reader (Bio-Tek Instruments, Inc.) with different 5 ml aliquots. The reaction is made up of the different concentrations of the extracts (30 μ l) and an aqueous methanolic solution (80:20 v/v, 270 μ l) with a radical concentration of 6×10^5 mol/l. The mixture was allowed to stand for 60 min in the dark. The reduction of the 2,2-diphenyl-1-picrylhydrazyl radical was determined by measuring the absorption at 515 nm. Radical scavenging activity (RSA) was calculated as percentage discoloration of the 2,2-diphenyl-1-picrylhydrazyl radical using the equation: % RSA = [(ADPPH - AS) / ADPPH] x 100, where AS is the absorbance of solution, and ADPPH is the solution absorbance of the 2,2-diphenyl-1-picrylhydrazyl radical. The Concentration of extract providing 50% of radicals. The scavenging activity (EC50) was calculated from the graph of percentage RSA versus extract concentration. Trolox was used as standard. The unit in which we express this inhibitory activity will be according to the concentration, in mg/ml.

Reducing power method (FRAP)

Different concentrations of the extracts (0.5 ml) mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 ml) and potassium ferricyanide (1% w/v, 0.5 ml). The mixture was incubated at 50 °C for 20 min in order to stop the reaction and trichloroacetic acid (10% w/v, 0.5 ml) was added. The resulting mixture (0.8 ml) was poured into 48 wells, with deionized water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance was measured at 690 nm. in the microplate reader.

Inhibition of lipid peroxidation by the TBARS method

Pig brains (*Sus scrofa*) weighing 150 kg were obtained, dissected and homogenized with a Polytron in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate), which was centrifuged at 3000 g for 10 min.

An aliquot (0.1 ml) of the supernatant was incubated with the different concentrations of the extracts (0.2 ml) in the presence of FeSO₄ (10 μ M; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2% w/v, 0.38 mL). The mixture was then heated at 80 °C for 20 min. After centrifugation at 3,000 g for 10 min to remove precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: % Inhibition = [(AxB)/A] x 100%, where A and B were the absorbances of the control and the solution extract, respectively. The concentration of the extract that provides 50% inhibition of lipid peroxidation (EC50) was calculated from the plot of percentage TBARS inhibition against the concentration of the extract. Trolox was used as standard.

Quantification of apigenin-7-glucoside in plant extracts

It has been performed at the Interdepartmental Research Service (SIdI) of the UAM by injection in Agilent 1100 HPLC (California, USA) with a C18 column at 35 °C. The solvent system used was a gradient of solvent A and B starting at 85% A and 15% B up to 85% A and 15% B. Solvent A was 0.1% formic acid in

water, and solvent B was 0.1% formic acid in acetonitrile (). The flow rate was 0.5 ml/min, using a photodiode array detector set at 280. The total elution time was 50 min. 20 µL injection volume was used.

Kirby-Bauer method

In Kirby-Bauer testing, bacteria are placed on a plate of solid growth medium and wafers of antibiotics (white disks, shown) are added to the plate. After allowing the bacteria to grow overnight, areas of clear media surrounding the disks indicate that the antibiotic inhibits bacterial growth.

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey test was performed with Statgraphics Plus 5.1. Software to analyze data at 95% confidence level. Values were expressed as means of triplicate analysis and corresponding standard deviations.

Results and Discussion

Elemental analysis

As shown in Table 2, sodium is the element with the highest concentration in all samples, although the average values obtained are variable.

Table 2. Elemental composition of selected halophytes analyzed by ICP-MS. Expressed in mg/kg d.w. n=3.

Species	Na	Fe	Cu	Zn	K	Mg
<i>S. patula</i>	94518	1087	185	48.65	13505	14031
<i>S. alpini</i>	22831	1030	29.81	96.94	11120	6954
<i>S. pruinosa</i>	49057	185	73.86	25.89	8651	8210
<i>S. perennis</i>	7904	190	60.7	27.4	8514	7904
<i>A. macrostachyum</i>	14109	1067	105	90	7540	4257

Sodium content is above 25 g/kg d.w. (dry weight) in all cases, with average values of more than 94 g/kg d.w. *S. patula* and *A. macrostachyum* contents highest values of this element.

After sodium, the elements with the highest concentration are K and Mg with very variable amounts between 4 and 14 g/kg, highlighting *S. patula* values.

Iron has average values considered high for vascular angiosperm plants – although a high variability is also observed among samples from different localities.

S. patula and *S. alpini* shows the highest values with more than 1 g/kg d.w.

The mean concentrations of this element differ significantly, but no relationship was found with the phenological state.

High values of Cu and Zn were recorded in samples from Huelva salt marshes, in particular in *S. patula* samples from Tinto River, with higher average values of Cu and Zn than in the rest of analyzed halophytes.

These elements present statistical differences between inland and saltmarsh samples, highlighting La Rábida locality with the highest values.

The pattern of accumulation of the elements in all analyzed halophytes is very similar with the following order:

Na > K > Mg > Fe > Cu > Zn.

Antioxidant properties

The antioxidant properties of the samples studied were assessed by different in vitro methods: Radical 2,2 diphenyl-1-picrylhydrazyl, iron reducing power and Inhibition of lipid peroxidation.

Analyzed samples present around 80% inhibitory activity at different concentrations. Kinetic curve was made for each species since different specimens from different populations but from the same species presented the same antioxidant activity values.

S. patula was the species that showed the highest antiradical activity (RSA), obtained by this method, presenting saturation kinetics at a concentration of 6 mg/ml. On the other hand, *S. alpini*, presents a concentration of 6.25 mg/ml; while *S. pruinosa* and *S. perennis* did not reach the same level of inhibition up to 8 and 8.25 mg/ml, respectively. *A. macrostachyum* achieved saturation at 7 mg/ml, showing slower kinetic curves.

In relation to the iron reducing power method, the analyzed samples of *S. patula* presented saturation kinetics at concentrations of 2 mg/ml. *S. alpini*, *S. pruinosa*, *S. perennis* reached their plateau or saturation point at a concentration of 2 mg/ml, 3.2 mg/ml and 3.1 mg/ml, respectively. *A. macrostachyum* also obtained saturation kinetics at concentrations of 2.1 mg/ml.

For the lipid peroxidation inhibition method (TBARS), a greater variability of results is observed, where with 0.125 mg/ml of extract (maximum concentration studied for this method) only *S. patula* presented percentages of inhibition greater than 78% for said concentration; while *S. pruinosa*, *S. alpini* and *S. perennis* achieved percentages of 65%, 60% and 51% inhibition, respectively. Finally, *A. macrostachyum* obtained a lower percentage with 55% for the active concentration by this method.

The highest levels of proline and glycine betaine are observed in *S. patula* and *A. macrostachyum*. These two species contain more than 1.5 mol/g ms (Figure 2).

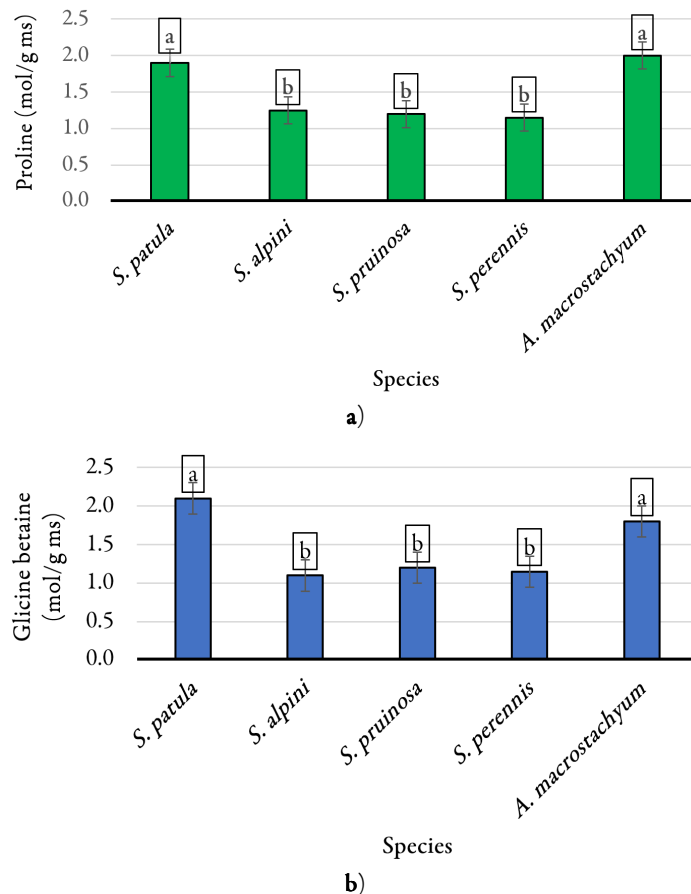


Figure 2. Concentration of proline (a) and glycine betaine (b) in studied halophytes, as mean \pm SD. Means followed by the same letter do not differ by the Tukey test ($p \leq 0.05$)

S. pruinosa shows very similar values with the two metabolites with more than 1.2 mol/g ms.

The lowest values are found in *S. perennis* for proline and *S. alpini* for glycine betaine.

One of the most important bioactive compounds describe in *S. patula* is Apigenin-7-glucoside.

The compound apigenin-7-glucoside was also found to be an inhibitor of *S. aureus* with a disk containing 1.5 μ g. Apigenin-7-glucoside inhibition halos measured 16 mm. The inhibition halos of the selected antibiotics (nalidixic acid, streptomycin and ampicillin) had a diameter of 18 mm.

As shown in Figure 3, in *Salicornia* sample, a concentration of 6.83 ng/mg was obtained, which corresponds to 0.00068%(p/p) of apigenin-7-glucoside with respect to the dry extract.

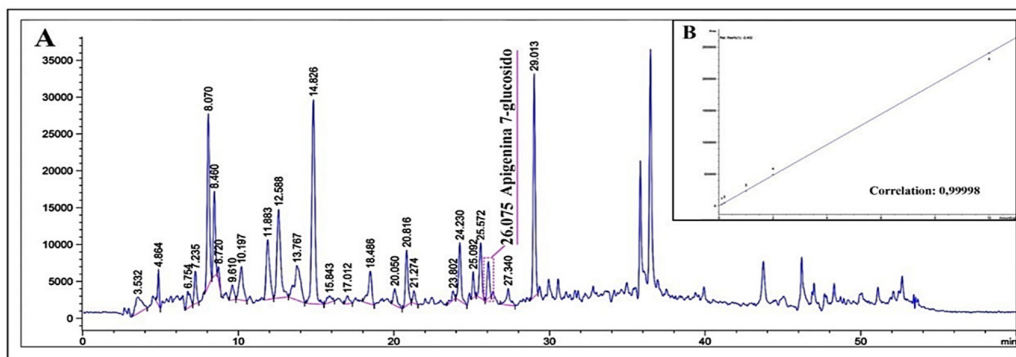


Figure 3. Chromatogram and calibration curve of apigenin-7-glucoside in *S. patula*

This material has been previously analyzed to determine its content of phenolic acids, flavonoids and fatty acids. Phenolic compounds have been described as important components of plants with redox properties responsible for antioxidant activity and have been related to the content of phenolic compounds and fatty acids as other authors (Rodrigues *et al.*, 2014; Barreira *et al.*, 2017; Sánchez-Gavilán *et al.*, 2021 b, c). Limongelli *et al.*, (2022), suggest the beneficial effects of *Salicornia*, a halophytic plant of the Mediterranean basin on gastrointestinal disorders, diabetes, hypertension, inflammation, vascular diseases, and oxidative stress (Limongelli *et al.*, 2022)

In this study, the combination of several mechanisms that act against different oxidative systems by different mechanisms has been used.

Hydrophilic antioxidants have been evaluated by electron transfer mechanisms, using free radical scavenging (DPPH) and metal ion reduction (FRAP) as oxidative systems.

In the 2,2 diphenyl-1-picrylhydrazyl radical assay, *S. patula* presented the highest antioxidant capacity at a concentration of 6 mg/ml. The antioxidant capacity has been studied as *S. ramosissima* with an active concentration with values between 10 mg/ml and 5.69 mg/ml of antioxidant capacity (Barreira *et al.*, 2017). *S. alpini* showed high activity, similar to *S. aff. europaea* with 6.25 mg/ml, while in *S. perennis* it was lower with 8 mg/ml (Gargouri *et al.*, 2013). The antioxidant activity of *S. pruinosa* is measured in this work for the first time by the 2,2 diphenyl-1-picrylhydrazyl radical method with an active concentration of 7.5 mg/ml. *S. perennis*, showed the same activity with 8.04 mg/ml, while in specimens collected in Korba (Tunisia) they obtained a higher antioxidant activity of 0.43 mg/ml. For *A. macrostachyum*, our results show greater antioxidant activity with 7 mg/ml, compared to the 2,2 diphenyl-1-picrylhydrazyl radical assays described in this species by other authors with values of 2.54 mg/ml and 3.4 mg/ml (El Amier *et al.*, 2022; Ramírez *et al.*, 2022). The high antioxidant capacity of *A. macrostachyum* in ecotypes from Egypt has recently been confirmed against other halophytes, with activity data of 71% at concentrations of 50 mg/ml (Fuente *et al.*, 2018).

In addition, it has been observed that the content of total polyphenols and other bioactive compounds with antioxidant properties in *A. macrostachyum* increases significantly when treated at moderate and high salinity levels (200 and 400 mM NaCl), which would improve the nutritional properties of *Arthrocnemum* crops through irrigation with saline water (Oliviera- Alvés *et al.*, 2023).

In the iron reducing power test, the active concentration of the studied material obtained higher values than those described by other authors. In *S. ramosissima* and *A. macrostachyum*, in ecotypes from Portugal, they report a concentration of 10 mg/ml and 0.84 mg/ml (Barreira *et al.*, 2017). In the case of the genus *Sarcocornia*, values lower than those obtained here have been described, with 6.55 mg/ml and 4.77 mg/ml for ecotypes of these species in Portugal (Barreira *et al.*, 2017). The antioxidant capacity of these halophytes in this test may be due to the high content of metallic elements, among which iron stands out, with values of 2571 mg/kg in *S.*

patula stems, 245 mg/kg in *S. pruinoso* or 1067 mg/kg. kg in *A. macrostachyum*. Iron is the majority element in the Rio Tinto marshes where the collected material comes from (Sánchez-Gavilán *et al.*, 2021a; Lopes *et al.*, 2023).

Due to their antioxidant properties, flavonoids play an important role in the antioxidant defense mechanisms of biological systems. This group of polyphenolic compounds, well represented in plant species, inhibits the start of the oxidation chain and prevents the formation of free radicals. *S. ramosissima* presented a higher proportion of phenolic acids than flavonoids, whereas in the *S. perennis alpini* extract flavonoids were the principal polyphenols quantified. The results revealed that p-coumaric acid, chlorogenic acid, ferulic and caffeic acid were the most abundant phenolic acids in both extracts (Sánchez-Gavilán *et al.*, 2021b). This study doesn't detect Salicylic acid, that it is one of the most important compounds in *S. patula* from Spain material.

Lopes *et al.*, 2022; identify quercetin and rutin as the most abundant flavonoids. *S. ramosissima* extract also contained high amounts of kaempferol when compared with *S. perennis alpini* extract. In addition, these authors detected as well, luteolin and apigenin. Rutin and luteolin are the flavonoids found in all samples, as well as other glycosylated compounds such as apigenin-7-glucoside, luteolin-7-glucoside, quercetin-3-o-rutinoside, among others (Sánchez-Gavilán *et al.*, 2021b, c). The latter are in the form of glycosides with monosaccharides and disaccharides as parts of their structure. Glycosylated flavonoids might play roles in response to salinity and functions as important potential ROS scavengers, including quercetin and its derivatives (Duan *et al.*, 2023).

Other authors suggest polyphenol-rich *S. ramosissima* extracts protects ischemia brain damage, using a *Drosophila* model of hypoxia regeneration. They highlight many different compounds that can be responsible for the therapeutic effect of S-EE, mainly including caffeoylquinic acid derivatives, like tungtunamic acid, flavonoids, and fatty acids (Nájar *et al.*, 2023). These studies related some compounds like tungstunamic acid, chlorogenic acid, sinapic acid and coloneic acid identified by HPLC and compared with retention times and molecular mass.

Among the phenolic acids, the one with the highest content in the material of *S. patula* and *A. macrostachyum* is salicylic acid. The accumulation of salicylic acid has been linked to the mitigation of the effects of salt stress in plants. This compound is involved in the regulation of water loss and osmotic conditions during saline exposure, acting as a phytohormone in the modulation of defense reactions under salinity (Singh *et al.*, 2023).

Lipophilic antioxidants have been evaluated by hydrogen ion transfer mechanisms, using lipid substrate oxidation inhibition (TBARS) as the oxidative system.

In the case of the lipid peroxidation test, the data provided here represent the first report on material from the Iberian Peninsula. *S. patula* presented a high antioxidant capacity with 78%. *S. pruinoso* presented 65% and *S. alpini* 60%. *S. perennis*, 51%, while *A. macrostachyum* obtained 55%. We agree with Rozenvest *et al.* (2020), in the lipid composition values, where palmitic acid is the majority for euhalophytes, *S. perennans* with 24% and *H. strobilaceum* with 27% studied by these authors in regions of Russia.

Curiously, these species have been mainly collected in spring and summer, when there is greater saline stress, with higher concentrations of osmolytes. The fact that the highest accumulations occur in these periods suggests that it is at this period when the plant needs the greatest amount of water, as during these months it flowers and produces fruits, which large amounts of water. In summer plants reduce their metabolic necessities, requiring less water and thus not requiring such high values of osmolytes.

No clear pattern of accumulation has been detected in proline levels. This reinforces other studies that have already shown the role of proline to be very complex and may be related to other environmental factors. Therefore, different climatic conditions, especially related to water stress (not analyzed here) can affect proline levels, giving unexpected results. Therefore, in this study it has not been possible to observe a clear trend of

proline accumulation, therefore we consider that the role of this osmolyte continues to be complex and difficult to explain.

Osmolyte contents increased to levels high enough to contribute significantly to osmotic balance when plants were exposed with higher salt concentrations than those they would encounter in their natural habitats with normal sodium concentrations (González-Orenga *et al.*, 2019).

In addition to their strong tolerance to salinity, some halophytes have also adapted to drought and waterlogged conditions. In such habitats, synergistic effects of salinity with drought or flooding amplify the magnitude of applied stress, which in turn increased the synthesis of antioxidant compounds. Increasing sodium concentrations led to a significant and progressive proline and glycine accumulation in the aerial part of *S. europaea* (Calone *et al.*, 2023). When comparing all halophyte species, the more salt-tolerant *S. patula* and *A. macrostachyum* accumulated relatively higher proline levels in response to salt stress.

In other previous studies (Rufo *et al.*, 2021; Sánchez-Gavilán *et al.*, 2021^a) some endemic Spanish halophytes accumulated iron as the major metal ion, followed by Cu, Zn, and Mn, while the accumulation patterns significantly differed between species and tissues. This indicated that high salinity positively influenced the mobility of all four elements, and species-specific translocation mechanisms depend largely on the main adaptation mechanisms of halophytes.

The presence of different bioactive compounds in the halophytes studied is clearly advantageous to prevent the damage caused by the high salinity of their substrates, providing them with greater antioxidant activity.

Halophytes naturally grow and complete their life cycle in harsh saline environments. Under these conditions, the production of ROS is enhanced many folds which necessitates the role of an efficient antioxidant system. As a result, tolerant plants tend to synthesize bioactive compounds including polyphenolic antioxidants in order to protect their vital metabolic functions from oxidative damage.

Conclusions

From our results it is clear that studied halophytes shoots have a strong antioxidant potential, an interesting mineral profile and can be considered as non-toxic novel food in view of the preliminary toxicological assessment with *in vitro* models. These findings confirm that Spanish Halophytes may be a potential source of bioactive molecules and/or products for the food industry, as for example antioxidants and minerals. Elemental composition is biologically linked with antioxidant capacity. The high presence of sodium, magnesium and iron confers strong response to salt stress. All halophytes have a very similar elemental pattern accumulation.

The studied material shows a wide antioxidant capacity. *S. patula* and *A. macrostachyum* presented the best antioxidant capacity, as shown by the 2,2 diphenyl-1-picrylhydrazyl radical assays and reducing power. This activity could be attributed to difference of osmolytes in these species, like proline and glycine betaine. Higher phytochemical composition in halophytes suggest that applying eco-physiological approach in medicinal plant studies would help to find promising candidates with high bioactive properties.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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