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Seasonal Biochemical Variations in Mediterranean Halophytes and Salt-Tolerant Plants: Targeting Sustainable Innovations in Ruminant Health

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Abstract

Climate change intensifies water scarcity and soil salinization, threatening agriculture and livestock systems, especially in arid Mediterranean regions. Halophytes and salt-tolerant plants offer sustainable alternatives to support ruminant health and productivity where traditional crops fail, helping mitigate climate impacts. This work evaluated seasonality effects on the biochemical properties, including proximate composition, minerals, antioxidant properties, and the phenolic composition of the aerial organs of halophytes and salt-tolerant species, aiming at their future exploitation in ruminant production as novel nutraceutical or phytotherapeutic products. Target species included four halophytic species according to the eHaloph database (*Calystegia soldanella* (L.) R. Br. 1810, *Medicago marina* L. 1753, *Plantago coronopus* L. 1753, and *Limoniastrum monopetalum* (L.) Boiss. 1848) and five salt-tolerant plants (*Pistacia lentiscus* L. 1753, *Cladium mariscus* (L.) Pohl 1809, *Inula crithmoides* L. (*syn. Limbarda crithmoides* Dumort. 1827), *Helichrysum italicum* subsp. *picardii* (Boiss. & Reut.) Franco 1984, and *Crucianella maritima* L. 1753). *H. italicum*, *M. marina,* and *C. soldanella* appear well-suited for nutraceutical applications, while *P. lentiscus*, *L. monopetalum*, and *C. mariscus* hold promise for the development of, for example, phytotherapeutic products. This research underscores the significance of seasonal and species-specific variations in nutrient and phytochemical composition, displaying a range of opportunities for novel, sustainable, and tailored solutions to ruminant production systems in arid environments.

Keywords: halophytes; bioactive plants; phenolics; antioxidants; nutraceuticals; phytotherapeutics; plant extracts

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1. Introduction

The Mediterranean area faces a critical challenge, with the expected worsening of freshwater shortages and increased soil and water salinization due to climate change [1,2]. The stress on freshwater resources is intensified by the region's demographic and economic pressures, demanding comprehensive adaptation and mitigation strategies [3]. In this region, small ruminants have a substantial economic, cultural, and ecological impact, providing meat, milk, and wool [3,4]. These resilient animals are well-adapted to the harsh Mediterranean conditions, taking advantage of mountainous, degraded, and marginal lands [4]. However, climate change will similarly challenge ruminant farming systems by impairing animal performance and reproduction, limiting feed and forage quality and freshwater availability, and altering disease transmission patterns [5]; thus, novel resources that can aid in mitigating these issues need to be discovered. To such ends, bioactive plants play a role in the development of innovative integrated strategies to improve the ruminant nutrition, health, and quality of their derived food products, either as feed or nutraceutical or phytotherapeutic options.

Our research posits that salt-tolerant plants indigenous to the Mediterranean, given their resilience to aridity and saline soils, hold promise as bioactive plants or as sources of nutraceutical or phytotherapeutic products targeting animal health improvement. Their robustness under Mediterranean climatic stresses may ensure their status as a reliable resource during the semi-arid and dry seasons, which are notorious for water and feed shortages, thereby supporting more sustainable farming practices [6,7]. The traditional utilization of various species in food and feed practices, such as *Atriplex* L. sp. and *Plantago* L. sp., underscores their suitability for animal maintenance and productivity [7–10]. Furthermore, this heterogeneous group of plants is recognized for their abundance of antioxidants, such as phenolic compounds [11] and minerals [12–14], contributing to improved animal health, well-being, and quality of the animal products for human health. In veterinary parasitology, nutraceutical plants are "*a livestock feed which combines nutritional value with beneficial effects on animal health*", whereas phytotherapeutics are plant-derived products administered to the animals for short-term periods mainly for treatment purposes [15]. Both concepts converge on the premise that the plants under study are endowed with bioactive metabolites in adequate concentrations to exert beneficial effects, and polyphenols have been in the spotlight for their numerous biological properties, including their antioxidant, anti-inflammatory, and anthelmintic properties [15,16]. The inclusion of polyphenols in the diets of small ruminants has proven to be beneficial not only for the animals' health and productivity but also in enhancing the quality of dairy and meat [17–19], aligning with the principles of the Mediterranean diet and focusing on improved health outcomes for both animals and humans.

A challenge that is encountered whenever wild plants are explored is that fluctuations in the biomass composition and metabolites' concentration, driven by plant-specific and environmental factors, consequently influence their chemical and biological properties [20,21]. Unveiling these changes is crucial for a rational exploitation and valorization of these plants, particularly in defining optimal harvesting periods, either to ensure nutritional quality or to maximize the extraction of the bioactive metabolites of interest (e.g., polyphenols). Although the chemical profile and bioactivities of many of these salt-tolerant species have been previously reported, works addressing their seasonal variability remain scarce.

In this sense, this work goes beyond existing research by providing a comprehensive assessment of the seasonality of nutrient compositions and the phenolic content of salt-tolerant species. By integrating this data, this study aims to establish optimal collection periods for selected species, each collected in four timepoints in the Algarve region, Southern Portugal. To achieve this, each sample was assessed for its nutrient and phytochemical

profiles and antioxidant activity, and subsequently, the most promising samples were subjected to a detailed phenolic identification and quantification using HPLC-DAD analyses.

2. Materials and Methods

2.1. Plant Collection and Processing

This work targeted four halophytic species included in the eHaloph database, namely, *Calystegia soldanella* (L.) R. Br. 1810 (Convolvulaceae), *Medicago marina* L. 1753 (Fabaceae), *Plantago coronopus* L. 1753 (Plantaginaceae), and *Limoniastrum monopetalum* (L.) Boiss. 1848) (Plumbaginaceae). It also focused on the following five salt-tolerant plants: *Pistacia lentiscus* L. 1753 (Anacardiaceae), *Cladium mariscus* (L.) Pohl 1809 (Cyperaceae), *Inula crithmoides* L. (syn. *Limbarda crithmoides* Dumort. 1827; Asteraceae), *Helichrysum italicum* subsp. *picardii* (Boiss. & Reut.) Franco 1984 (Asteraceae), and *Crucianella maritima* L. 1753) (Rubiaceae) [22]. From an initial list of plants identified along the Algarve coastline in previous projects (XtremeBio, PTDC/MAR-EST/4346/2012), these nine selected species were selected based on combined available knowledge on their (1) traditional veterinary uses [10], (2) phytochemical available data, and (3) abundance in wild settings during the harvesting years, aiming at their nutraceutical or phytotherapeutical application within the context of gastrointestinal nematode infections in small ruminants [23].

Fresh aboveground plant organs, including leaves, stems, flowers, and fruits, whenever present, were harvested by hand in spring (Sp), summer (Su), autumn (Au) and winter (Wi) seasons during 2017 and 2018 (Appendix A, Table A1), covering four districts of Algarve, Southern Portugal (Figure 1). The voucher specimens of each plant species were identified based on morphological features by Dr. Luísa Custódio (CCMAR), and a record was maintained at the herbarium of the XtremeBio group (CCMAR; Appendix A, Table A1). Before solvent extraction, the biomass was reduced to dry powder after lyophilization for three days (Lyoalfa 15, Telstar, Barcelona, Spain) by firstly using a coffee grinder and, afterwards, using a ball miller to reduce particle size (Retsch PM 100, VWR, Carnaxide, Portugal). To avoid compound degradation by light, samples were stored in the dark in a desiccator. This protocol was followed in compliance with the standard procedures recommended by "Instituto da Conservação da Natureza e das Florestas (ICNF)", the national regulatory body, and mandatory licenses for wild collections were obtained appropriately.

2.2. Nutritional Assessments

The nutritional evaluations were performed as follows: First, fresh biomass was dried in a ventilated oven at 105 °C for 16 h, and in agreement to the AOAC guidelines [24], the dry samples were ignited in a muffle furnace at 600 °C for 2 h—thereof, dry matter (DM) and ash contents were estimated by gravimetry. Ash residue was used for the mineral assessments, including Ca, K, Na, Mg, Cu, Zn, Mn, and Fe, which were determined using a microwave plasma-atomic emission spectrometer (MP-AES; Agilent 4200 MP-AES, Agilent Victoria, Australia), as detailed elsewhere [25]. Then, using a CHN Elemental Analyzer (Vario EL III, Bruker, Karlsruhe, Germany) for the quantification of the total nitrogen (N) of dry samples allowed the estimation of the crude protein (CP) content, which is given by the formula N x 6.25, while total lipids (TLs) were measured using the method described in [25,26]. The total carbohydrate (CHO) was estimated as the residue upon the subtraction of CP, TL, and ash values, whilst gross energy (GE; Mcal kg^{-1}) was calculated using Equation [26]:

$$GE = (CP \times 0.056) + (TL \times 0.094) + [(100\text{-}CP\text{-}TL\text{-}A) \times 0.042] \quad (1)$$

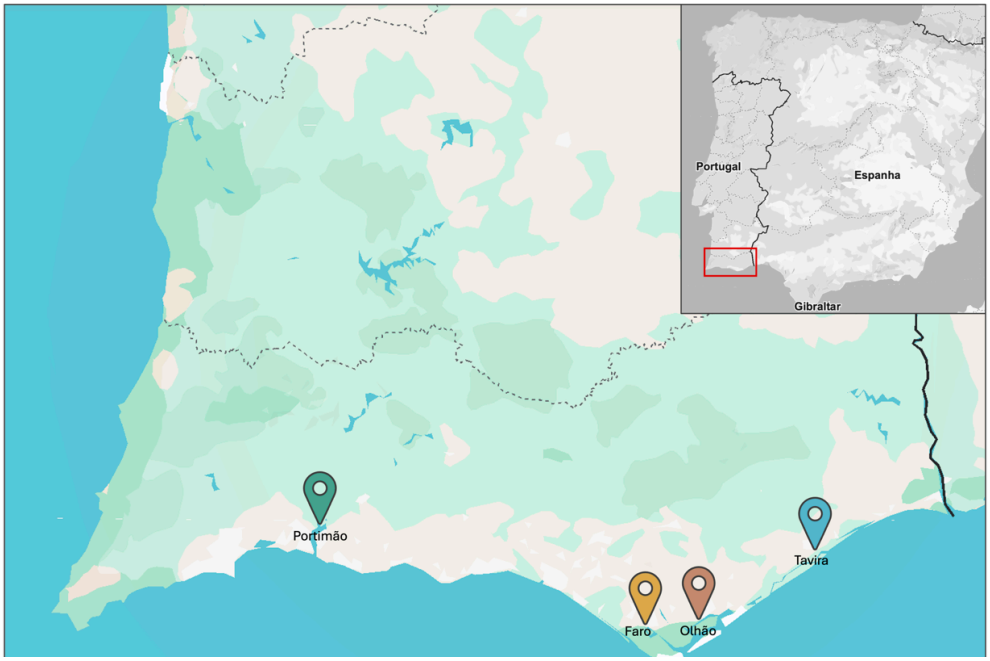

Figure 1. Harvesting locations of the target species along the Algarve coast, Southern Portugal, namely, Portimão (green), Faro (yellow), Olhão (red), and Tavira (blue). Adapted from Instituto Geográfico Português ® 2024 using Google Maps.

2.3. Preparation of the Extracts

Dried ground biomass was extracted with an 80% water-based acetone solution (1:40, *w/v*) at 20–25 °C for 16 h under constant stirring, aiming at an effective extraction of phenolics and enhanced antioxidant properties, which was based on the results obtained by others working with halophyte plants [27,28]. Then, extracts were filtered, concentrated in a rotary evaporator under reduced pressure and temperature (40 °C), dissolved in dimethyl sulfoxide (DMSO), and stored at −20 °C until use. For the high-performance liquid chromatography diode array (HPLC-DAD) analysis, dry extracts were dissolved in a mixture of ultrapure water (90%) and methanol (10%) at a concentration of 10 mg mL^{-1}.

2.4. Total Phenolic (TPC), Flavonoid (TFC), and Condensed Tannin Contents (CTCs) of the Extracts

Total phenolics, flavonoids, and condensed tannins were assessed as fully described in [25]. The total phenolic content (TPC) was estimated using the Folin–Ciocalteu (F-C) method; total flavonoid content (TFC) was estimated by the aluminum chloride ($AlCl_3$) method; condensed tannin content (CTC) was evaluated using the 4-dimethylaminocinnamaldehyde-hydrochloric acid (DMACA-HCl) colorimetric protocol, adapted to 96-well microplates. Calibration curves were prepared using gallic acid, quercetin, and catechin, and the concentrations of TPC, TFC, and CTC were expressed as gallic acid equivalents (GAEs), quercetin equivalents (QEs), and catechin equivalents (CEs), respectively, in mg per gram of extract dry weight (mg eq. g^{-1} extract DW).

2.5. Phenolics Profiling by High-Performance Liquid Chromatography–Diode Array (HPLC-DAD)

The HPLC-DAD (Agilent 1100 Series LC system, Agilent Technologies, Waldbronn, Germany) consists of a vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), and thermostatic column compartment (G1316A) coupled with a DAD detector (G1315B). The diluted extracts were injected (40 µL; flow 0.35 mL min^{-1}; draw speed 200 µL min^{-1}) on a Mediterranea sea18 column, (15 × 0.21 cm, 5 µm particle size; Teknokroma, Spain) using methanol (A) and a 2.5% acetic acid aqueous solution with

the following gradient: 0–5 min: 10% A; 5–10 min: 10–30% A; 10–40 min: 30–90% A; 40–45 min: 90% A; 45–55 min: 90–10% A; and 55–60 min: 10% A. Detection was performed at 210, 280 (quantification), 320, and 350 nm. The retention parameters were compared with those of the standards, and peak purity was compared with the UV-visible spectral reference data. For this, thirty-two standards were prepared in methanol (1 mg mL^{-1}) and further diluted using ultrapure water, namely gallic, vanillic, chlorogenic, neochlorogenic, p-hidroxybenzoic, 3-4-dihdroxybenzoic, 3-hydroxybenzoic, 4-*O*-caffeoylquinic, caffeic, syringic, coumaric, ferulic, salicylic, rosmarinic, ellagic, and cinnamic acids and catechin hydrate, 4-hydroxybenzaldehyde, epigallocatechin gallate, epicatechin, ourateacatechin, umbeliferone, taxifolin, coumarin, naringenin-7-glucoside, luteolin-7-*O*-glucoside, rutin, quercetin, morin, flavone, and chrysin. The concentrations of the reference compounds in samples were extrapolated from the prepared calibration curves. The software LC3D ChemStation (version Rev.A.10.02[1757], Agilent Technologies) allowed data acquisition and instrumental control throughout the analyses.

2.6. In Vitro Antioxidant Properties

Five methods related to antioxidant effects were utilized: Namely, two focused on the scavenging of free radicals (DPPH and ABTS assays) and the Ferric Reducing Antioxidant Power (FRAP), and two targeted metal chelation, either of copper (CCA) or iron (ICA), using the protocols exhaustively described in [25]. The dissolved extracts were tested at various concentrations (from 10 to 0.078 mg mL^{-1}), absorbance was measured in a multiplate spectrophotometer reader (Biotek Synergy 4, Biotek, San Diego, CA, USA), and the results were expressed as half inhibitory concentrations (IC_{50} values; mg/mL) if applicable. Butylated hydroxytoluene (BHT), ethylenediamine tetraacetic acid (EDTA), and ascorbic acid were used as positive controls, respectively, in the free radical scavenging assays, metal chelation assays, and FRAP.

2.7. Statistical Analysis

All experiments were performed at least in duplicate. Spectrophotometric data was expressed as the mean ± standard error of the mean (SEM), and antioxidant results are presented as IC_{50} values, inferred by the sigmoidal fitting of the transformed data (GraphPad Prism © Software v.5.0). Differences between means ($p \leq 0.05$) were assessed by analysis of variance (SPSS Statistics © v.20.0 software), aiming at addressing seasonality effects on phenolic contents and antioxidant properties, for each salt-tolerant plant species in this study. To rank the extracts in descending order of their antioxidant effects, we applied the Relative Antioxidant Capacity Index (RACI), which is based on standardized score values derived from the raw data of the DPPH, ABTS, FRAP, CCA, and TPC assays, as described in [29]. Principal component analysis (PCA) was performed using the PAST 5 software [30], whilst Spearman's correlations were calculated in GraphPad Prism © software v.8.0.

3. Results

3.1. Nutritional Assessments

The results for crude protein, ash, and total lipids are summarized in Figure 2 and fully detailed in Appendix A, Table A2. Crude protein levels ranged between 39.4 to 189.4 g kg^{-1} DM across seasons, being frequently lower in the dry periods (Su/Au), with most species barely meeting the limit established for animal maintenance (70 g kg^{-1} DM) [7]. In contrast, during Sp, most species had adequate levels of protein (87.3–132.7 g kg^{-1} DM), with the exceptions including *P. lentiscus* and *C. maritima*, while in Wi, the *I. crithmoides*, *L. monopetalum*, *P. coronopus*, *M. marina*, and *C. soldanella* samples exceeded this threshold (91.4–189.4 g kg^{-1} DM). Overall, the highest protein values were observed in *M. marina*

during Wi/Sp (132.7–139.4 g kg^{-1} DM) and, exceptionally, in *C. soldanella* during Wi (189.4 g kg^{-1} DM). Total lipid contents differed among species: *L. monopetalum* recorded the lowest levels (34.4–44.1 g kg^{-1} DM), followed by *C. mariscus* (48.9–53.3 g kg^{-1} DM) and *M. marina* (45.9–55.1 g kg^{-1} DM). Conversely, the highest lipid contents were registered in *C. soldanella* (141.9–250.1 g kg^{-1} DM), showing levels declining in dry seasons, and *H. italicum* (90.9–142.7 g kg^{-1} DM), which peaked in Au/Wi. Ash contents showed a broad range from 49.3 to 326.6 g kg^{-1} DM, depending on the species and season. While *H. italicum*, *P. lentiscus*, *C. mariscus*, and *M. marina* exhibited adequate ash levels, *L. monopetalum* and *I. crithmoides* stood out for their remarkably high ash content (148.3–326.6 g kg^{-1} DM), accompanied by lower GE values (3.3–3.8 Mcal g^{-1}; Appendix A, Table A2). As illustrated by Weiss and Tebbe (2019) [26], GE is highly dependent on ash, lipid, and protein concentrations, and therefore, it decreases with substantial increases in ash. In agreement, the highest GE values were recorded for the *C. soldanella*, particularly in Sp (GE = 5.2 Mcal kg^{-1}), and *H. italicum* (GE = 4.5–4.7 Mcal kg^{-1}) samples, following the trend of higher lipid contents.

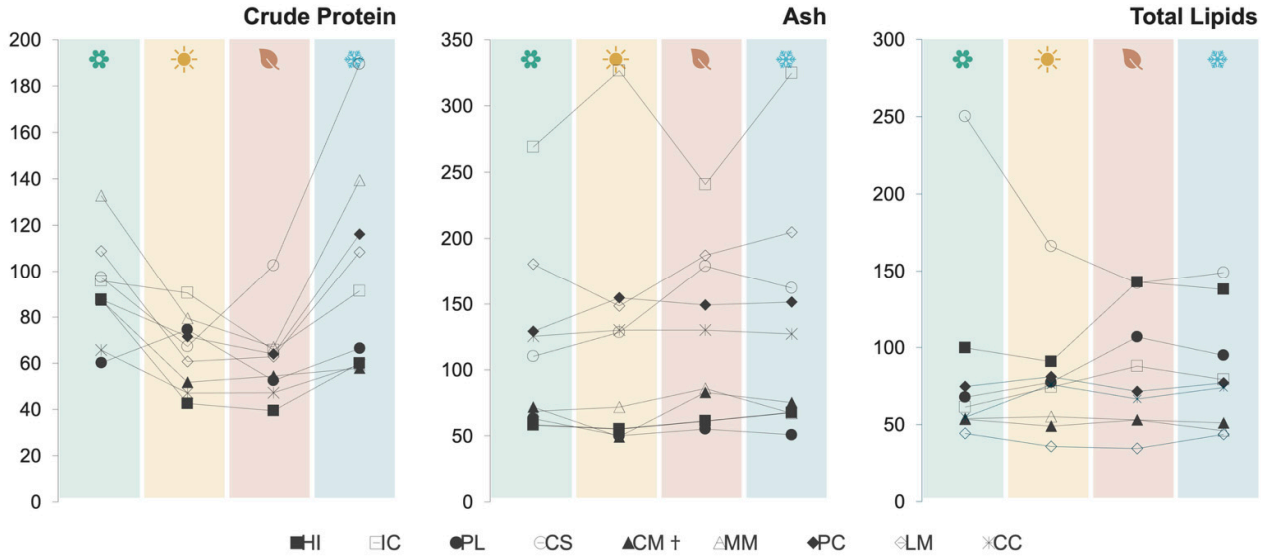

Figure 2. Seasonal variations of the crude protein, ash, and total lipid contents (g kg^{-1} DM) of halophytes and salt-tolerant species biomass. †, data published in [25]. Different symbols and colors represent the seasons as follows: spring (Sp), green flower; summer (Su), yellow sun; autumn (Au), brown leaf; winter (Wi), blue snowflake. HI, *H. italicum picardii*; IC, *I. crithmoides*; PL, *P. lentiscus*; CS, *C. soldanella*; CM, *C. mariscus*; MM, *M. marina*; PC, *P. coronopus*; LM, *L. monopetalum*; CC, *C. maritima*.

Macromineral analysis revealed high levels throughout the year for most samples, with some exceptions: *C. mariscus* had reduced potassium (K) and Mg levels, while *M. marina* exhibited lower K content during Au (Figure 3). Regarding trace minerals, except for *C. mariscus* in Wi, all samples surpassed Fe dietary recommendations (40 mg kg^{-1} DM), remaining within maximum tolerable levels (MTL) [31]. Similarly, the Zn content of most samples was adequate, except *L. monopetalum* in Su, whilst *H. italicum* was the only species with high levels of copper (Cu) and manganese (Mn) for all seasons. *H. italicum* stood out since its macromineral and trace mineral profiles were consistently greater throughout seasons for every element tested. *I. crithmoides*, *C. mariscus*, *P. coronopus*, and *C. maritima* exhibited Cu contents adequate for animal maintenance, whereas *C. soldanella* had increased levels in Au/Wi. Following *H. italicum*, *C. maritima* and *C. mariscus* showed high levels of Mn in the four sampled seasons, along with *C. soldanella* collected in Su and Au.

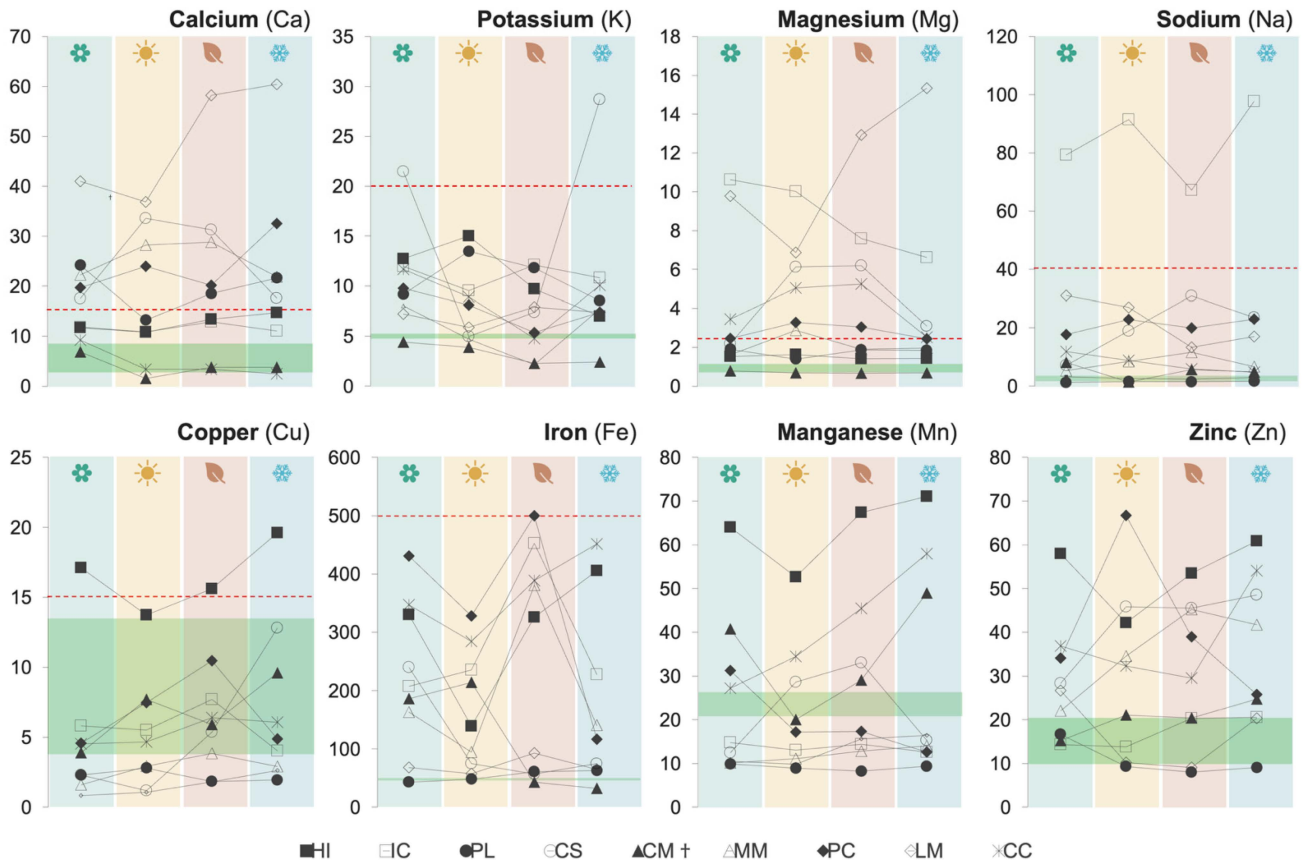

Figure 3. Seasonal profile of macrominerals (Ca, K, Mg, and Na; g kg^{-1} DM) and trace minerals (Fe, Cu, Mn, Zn; mg kg^{-1} DM) evaluated in the dry biomass of halophytes and salt-tolerant species. Ca, Calcium; K, Potassium; Mg, Magnesium; Na, Sodium; Fe, Iron; Cu, Copper; Mn, Manganese; Zn, Zinc. Dietary requirement (DR) limits are represented as green bars: The lower limit refers to the minimum concentration required for animal maintenance, while the upper limit refers to reproduction, lactation, and rapidly growing animals [32]. DR: Ca, 1.4–7 g kg^{-1} DM; K, 5 g kg^{-1} DM; Mg, 0.9–1.2 g kg^{-1} DM; Na, 0.7–1.0 g kg^{-1} DM; Fe, 40 mg kg^{-1}; Cu, 4–14 mg kg^{-1} DM; Mn, 20–25 mg kg^{-1} DM; Zn, 9–20 mg kg^{-1} DM. Maximum tolerable levels (MTLs) are represented as a dashed red line [31], above which dietary concentrations will have negative impacts on animal health. MTL (sheep): Ca, 15 g kg^{-1} DM; K, 20 g kg^{-1} DM; Mg, 6 g kg^{-1} DM; Na, 40 g kg^{-1} DM; Fe, 500 mg kg^{-1} DM; Cu, 15 g kg^{-1} DM; Mn, 2000 mg kg^{-1} DM; Zn, 300 mg kg^{-1} DM. †, data published in [25]. Different symbols and colors represent the seasons as follows: spring (Sp), green flower; summer (Su), yellow sun; autumn (Au), brown leaf; winter (Wi), blue snowflake. HI, *H. italicum picardii*; IC, *I. crithmoides*; PL, *P. lentiscus*; CS, *C. soldanella*; CM, *C. mariscus*; MM, *M. marina*; PC, *P. coronopus*; LM, *L. monopetalum*; CC, *C. maritima*.

3.2. Phenolic Profiling and Antioxidant Properties

The accumulation of total phenolics, total flavonoids, and condensed tannins was assessed in all samples (Figure 4). Except for *M. marina* and *I. crithmoides*, a high TPC was noted for all samples (>20 mg g^{-1} DW; Figure 4) [33], ranging from 23 up to 230 mg GA eq. g^{-1} extract DW. Seasonal patterns varied widely among salt-tolerant species: *H. italicum* and *I. crithmoides* had higher TPC in Au (105.6 and 27.2 mg GA eq. g^{-1} extract DW, respectively), while *L. monopetalum* and *C. soldanella* phenolic contents increased in Sp (144.8 and 73.2 mg GA eq. g^{-1} extract DW). The *P. lentiscus* and *C. maritima* samples reached the lowest values in Su (195.2 and 19.3 mg GA eq. g^{-1} extract DW), while no differences were observed for other seasons.

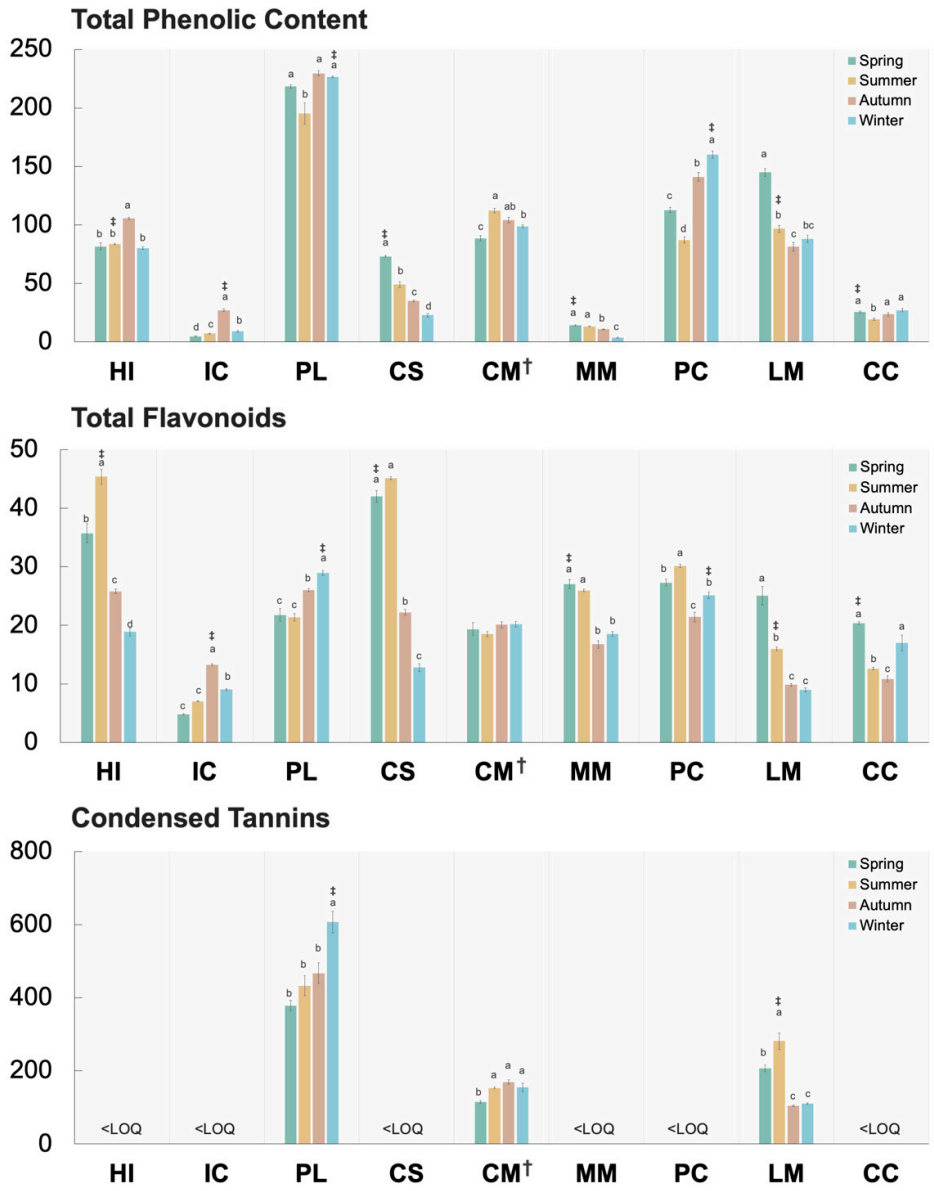

Figure 4. Seasonal variations of the total phenolic (TPC), total flavonoids (TFCs), and condensed tannin contents (CTCs) of the 80% acetone extracts of halophytes and salt-tolerant species. Results are expressed as mg gallic acid eq. g^{-1} extract DW (TPC), mg quercetin eq. g^{-1} extract DW (TFC), and mg catechin eq. g^{-1} extract DW (CTC). Different letters refer to significant differences for each species across seasons per species ($p < 0.05$). LOQ, Limit of quantification; HI, *H. italicum picardii*; IC, *I. crithmoides*; PL, *P. lentiscus*; CS, *C. soldanella*; CM, *C. mariscus*; MM, *M. marina*; PC, *P. coronopus*; LM, *L. monopetalum*; CC, *C. maritima*. LOQ (CTC) = 0.026 mg mL^{-1}. †: Data published in [28]; ‡: data published in [25].

P. coronopus had higher contents during Wi (160 mg GAE g^{-1} extract DW), while *M. marina* exhibited a marked increase in Sp/Su (13.3–14.2 mg GA eq. g^{-1} extract DW). *C. mariscus's* best results were achieved in the dry periods (Su/Au; 104.3–112.3 mg GAE g^{-1} extract DW) in contrast to Sp (86 mg GAE g^{-1} extract DW) [25]. Total flavonoid levels ranged between 4.8 and 45.4 mg QE g^{-1} extract DW. *I. crithmoides* consistently showed the lowest TFC values (4.8–13.3 mg QE g^{-1} extract DW), while *C. soldanella* and *H. italicum* had the highest levels, particularly in Sp/Su samples (42–45.2 and 35.8–45.4 mg QE g^{-1} extract DW, respectively). In general, the highest amounts of flavonoids were recorded in Sp/Su samples, except for *I. crithmoides* and *P. lentiscus*, which had increased contents in Au (13.3 mg QE g^{-1} extract DW) and Wi (28.9 mg QE g^{-1} extract DW), respectively.

Condensed tannins were only detected in three species: *P. lentiscus* > *L. monopetalum* > *C. mariscus* (Figure 4). Compared to TPC, seasonal variations did not follow a trend, showing species-specific features. An increased accumulation of these metabolites in *P. lentiscus* was detected in Wi, *L. monopetalum* peaked in Su, and *C. mariscus* was consistently a rich source of these metabolites in Su, Au, and Wi.

Regarding antioxidant properties, only five species exhibited IC_{50} values below 1 mg mL^{-1} in at least two antioxidant assays (Appendix A, Table A3). Notably, none of the samples demonstrated iron chelating capacity, and only *P. lentiscus* and *L. monopetalum* consistently presented IC_{50} values below 1 mg mL^{-1} for copper chelation. In the latter, seasonal variations were negligible for *P. lentiscus*, while *L. monopetalum* showed consistent activity during Sp, Su, and Wi (IC_{50} = 0.58–0.66 mg mL^{-1}). In the DPPH assay, *C. mariscus* and *P. lentiscus* displayed stable antiradical activity across all seasons within each respective species ($p < 0.05$). Conversely, the other three species exhibited significant seasonal variability: *H. italicum* samples were mostly active during Su/Au (IC_{50} = 0.27–0.29 mg mL^{-1}); *L. monopetalum* showed a decrease in activity only during Au (IC_{50} = 0.18 mg mL^{-1}); *P. coronopus* consistently demonstrated lower IC_{50} values in Sp, Wi, and Au (IC_{50} = 0.17–0.25 mg mL^{-1}; Appendix A, Table A3).

3.3. Principal Component Analysis (PCA) and Relative Antioxidant Capacity Index (RACI)

A principal component analysis (PCA) was conducted for an overview of interactions and distribution of the studied parameters across different species and seasons. Temperature, total rainfall, crude protein, lipids, and K were excluded from the analysis due to their low contributions to variance (Figure 5A). The first two principal components (PC) accounted for 59.4% of total variation, with PC1 (34.8%) primarily differentiating samples based on ash, Na, phenolics (TPC, TFC, and CTC) and antioxidant assays (DPPH, ABTS, CCA, and FRAP), while PC2 (24.6%) was driven by ash, minerals (Ca, Mg, Na, Fe, Zn, Cu, and Mn), and CTC. As previously noticed, samples clustered more distinctly by species rather than seasons. Species with higher phenolic contents and lower IC_{50} values in antioxidant assays, namely, *P. lentiscus*, *L. monopetalum*, *C. mariscus*, *H. italicum picardii*, and *P. coronopus*, were positioned on the left side of the biplot. Significant positive correlations were verified between phenolic contents, while negative associations were registered between these species and antioxidant activity (Figure 5B). *H. italicum picardii* samples, located in the top-left quadrant, were characterized by high flavonoid and micromineral (Mn, Zn, and Cu) levels, whilst *I. crithmoides* samples were on the opposite side, influenced by their high ash and Na levels, along with low phenolic contents and weaker antioxidant effects. The high ash values of samples were largely attributed to elevated Na and Mg levels, as evidenced by positive correlations between ash and these elements (Figure 5B). Significant positive correlations between Mn, Cu, and Zn were also noted (Figure 5B), highlighting potential interactions that may influence their nutritional contributions. However, weaker associations between TFC, TPC, ash, and Na were observed (Figure 5B), suggesting that phenolic accumulation was more pronounced in plants with lower Na levels, which may be linked to species-specific salinity tolerance mechanisms. Indeed, the findings of other works sustain these results [34,35], as they suggest that highly salt-tolerant plants, being well-adapted to high Na levels, do not experience oxidative stress that would trigger an antioxidant defense response, leading to lower phenolic metabolite production.

In addition to PCA, the Relative Antioxidant Capacity Index (RACI), previously used to rank the antioxidant capacity of foods and algae [29,36,37], combined the results obtained in TPC, CCA, DPPH, CCA, ABTS, and FRAP for each sample, providing higher ratings for the most promising extracts, regardless of species or season (Figure 6). Altogether, these combined and individual results allowed the selection of *P. lentiscus* and *P. coronopus* Wi

samples, as well as *L. monopetalum*, *C. mariscus*, and *H. italicum* harvested during Su, to be further characterized by HPLC-DAD analysis for their phenolic profiles.

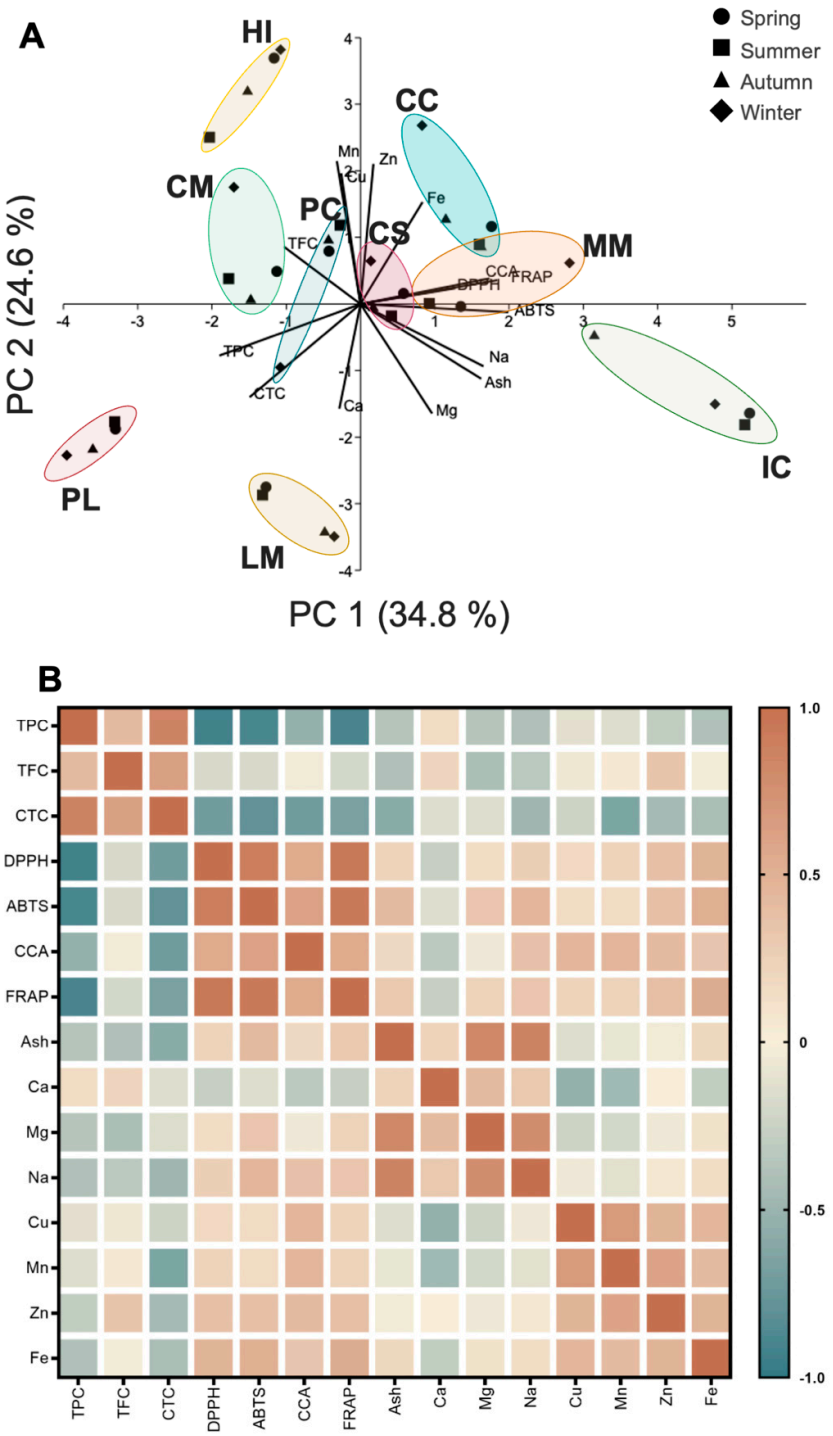

Figure 5. (**A**) Scatterplot of the principal component analysis (PCA) conducted using PAST 5 software, including data scores for each sample analyzed, represented by distinctive geometric shapes according to harvesting season, and loadings of the analyzed parameters on PC1 (24.2%) and PC2 (39.3%). Samples corresponding to the same species are identified in the oval shapes of different colors: PL, *P. lentiscus*; HI, *H. italicum picardii*; LM, *L. monopetalum*; CM, *C. mariscus*; CS, *C. soldanella*; PC, *P. coronopus*; CC, *C. maritima*; MM, *M. marina*; IC, *I. crithmoides*. (**B**) Spearman's correlation matrix of the studied parameters: dark blue and dark yellow, respectively, correspond to stronger negative and positive correlations between investigated factors.

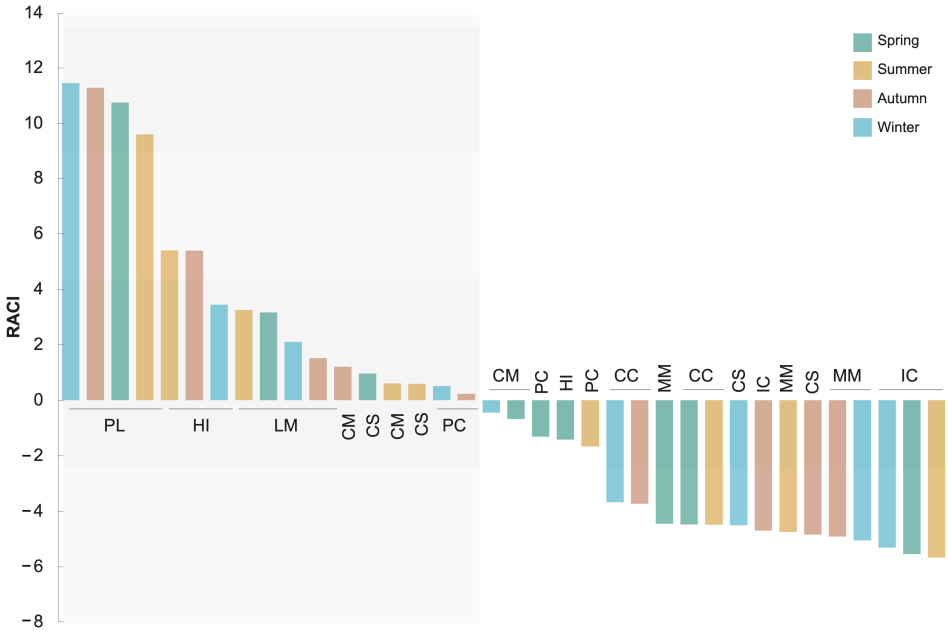

Figure 6. Sample ranking using the Relative Antioxidant Capacity Index (RACI) for each sample, in descending order, based on calculating average standard scores from the data collected in TPC, DPPH, ABTS, CCA, and FRAP assays. PL, *P. lentiscus*; HI, *H. italicum picardii*; LM, *L. monopetalum*; CM, *C. mariscus*; CS, *C. soldanella*; PC, *P. coronopus*; CC, *C. maritima*; MM, *M. marina*; IC, *I. crithmoides*.

3.4. Individual Phenolic Composition

The individual chromatograms revealed notable differences, reflecting the extracts' chemical complexity and species-specific variations using a sum of 32 standards (Figure 7; Table 1). In total, 19 compounds were identified in *P. lentiscus* (Wi; 138.91 mg phenolics g^{-1} extract DW), 23 in *H. italicum picardii* (Su; 27.91 mg phenolics g^{-1} extract DW), 14 in *C. mariscus* (Su; 9.22 mg phenolics g^{-1} extract DW) [28], 22 in *P. coronopus* (Wi; 23.03 mg phenolics g^{-1} extract DW), and 21 in *L. monopetalum* (Su; 25.43 mg phenolics g^{-1} extract DW).

Table 1. Identification and quantification of individual phenolic metabolites (mg g^{-1} extract) on *P. lentiscus*, *H. italicum picardii*, *C. mariscus*, *P. coronopus*, and *L. monopetalum* with selected 80% acetone extracts using HPLC-DAD detection. Compounds are numbered according to their retention time (Rt; min).

Rt (min)	No.	Compound	*P. lentiscus* Wi	*H. italicum* Su	*C. mariscus* Su †	*P. coronopus* Wi	*L. monopetalum* Su
2.6	1	Gallic acid	13.81	<0.01	0.01	<0.01	3.79
4.6	2	3.4-Dihydroxybenzoic acid	0.22	0.36	n.d.	0.33	0.27
5.4	3	Neochlorogenic acid	0.39	2.52	n.d.	0.07	0.52
8.1	4	Gentisic acid	33.80			5.53	3.79
8.4	5	*p*-Hidroxybenzoic acid		0.17	<0.01		
9.9	6	Catechin hydrate	2.43	1.92	0.77	0.12	
10.0	7	4-Hydroxybenzaldehyde			<0.01		
10.7	8	3-Hydroxybenzoic acid	0.84	0.19	n.d.	0.14	1.24
11.6	9	Vanillic acid	12.62	0.14		0.05	0.41
12.2	10	Chlorogenic acid	1.29	9.12	2.96		2.12
12.3	11	4-*O*-Caffeoylquinic acid			n.d.		1.76
12.6	12	Caffeic acid	0.74	0.89	0.73	0.76	6.79
13.5	13	Syringic acid	2.62		0.35		
13.6	14	Epigallocatechin gallate		0.05		<0.01	0.72

Table 1. *Cont.*

Rt (min)	No.	Compound	*P. lentiscus* Wi	*H. italicum* Su	*C. mariscus* Su †	*P. coronopus* Wi	*L. monopetalum* Su
13.9	15	Epicatechin	25.59	2.38	0.88	0.51	0.57
14.9	16	Ourateacatechin	34.72	1.76	n.d.	0.41	0.95
15.2	17	Umbelliferone		0.15	n.d.	0.41	0.05
15.9	18	Coumaric acid	<0.01	0.16	<0.01	1.19	0.40
16.8	19	Taxifolin			n.d.		0.36
16.9	20	Coumarin		0.01	n.d.		
17.0	21	Ferulic acid		0.51	1.38	0.81	0.06
17.6	22	Salicylic acid		2.59	1.64	5.36	0.48
19.1	23	Naringenin-7-glucoside	2.78	0.82	0.01	1.18	
20.3	24	Luteolin-7-*O*-glucoside	5.47	3.58	0.46	5.36	0.23
21.3	25	Rosmarinic acid	0.60			0.12	0.58
21.4	26	Rutin	0.54			0.09	
22.2	27	Ellagic acid	0.24			0.26	<0.01
22.6	28	Cinnamic acid	0.21	0.38	n.d.	0.26	0.34
25.9	29	Quercetin		0.06	0.03	0.07	
28.8	30	Morin		0.03	n.d.		
31.7	31	Flavone					
32.6	32	Chrysin		0.12	n.d.		
		∑Phenolics	**138.91**	**27.91**	**9.22**	**23.03**	**25.43**

†: Data published in [25]; retention times may differ for some compounds.

The predominant metabolites in *C. mariscus* (Su) were phenolic acids, including chlorogenic acid (**10**; 2.96 mg g^{-1} extract DW), ferulic acid (**21**; 1.38 mg g^{-1} extract DW), and salicylic acid (**22**; 1.64 mg g^{-1} extract DW) [25]. *P. lentiscus* stood out as a rich source of ourateacatechin (**16**; 34.72 mg g^{-1} extract DW), gentisic acid (**4**; 33.80 mg g^{-1} extract DW), epicatechin (**15**; 25.59 mg g^{-1} extract DW), gallic acid (**1**; 13.81 mg g^{-1} extract DW), and vanillic acid (**9**; 12.62 mg g^{-1} extract DW), together contributing 120.54 mg out of 138.91 mg phenolics g^{-1} extract DW. In the *H. italicum picardii* (Su) sample, prominent phenolics included chlorogenic acid (**10**; 9.12 mg g^{-1} extract DW), neochlorogenic acid (**3**; 2.52 mg g^{-1} extract DW), epicatechin (**15**; 2.38 mg g^{-1} extract DW), ourateacatechin (**16**; 1.76 mg g^{-1} extract DW), salicylic acid (**22**; 2.59 mg g^{-1} extract DW), and luteolin-7-*O*-glucoside (**24**; 3.58 mg g^{-1} extract DW), accounting for 21.95 mg of the total 27.91 mg phenolics g^{-1} extract DW. In *P. coronopus* (Wi), dominant compounds were gentisic acid (**4**; 5.53 mg g^{-1} extract DW), salicylic acid (**22**; 5.36 mg g^{-1} extract DW), and luteolin-7-*O*-glucoside (**24**; 5.36 mg g^{-1} extract DW), which together made up 16.25 mg of the total 23.03 mg phenolics g^{-1} extract DW. The *L. monopetalum* (Su) extract was rich in caffeic acid (**12**; 6.79 mg g^{-1} extract DW), gallic acid (**1**; 3.79 mg g^{-1} extract DW), gentisic acid (**4**; 3.79 mg g^{-1} extract DW), chlorogenic acid (**10**; 2.12 mg g^{-1} extract DW), 3-hydroxybenzoic acid (**8**; 1.24 mg g^{-1} extract DW), and 4-*O*-caffeoylquinic acid (**11**; 1.76 mg g^{-1} extract DW).

Interestingly, certain compounds displayed species-specific occurrences. For instance, 4-*O*-caffeoylquinic acid (**11**) was unique to *L. monopetalum*, while morin (**30**) and chrysin (**32**) were exclusively detected in *H. italicum picardii* samples. Conversely, luteo-lin-7-*O*-glucoside (**24**), epicatechin (**15**), caffeic acid (**12**), and gallic acid (**1**) were common across all species, albeit in varying concentrations. These findings highlight the distinct phenolic profiles of each species, offering opportunities for their targeted exploitation.

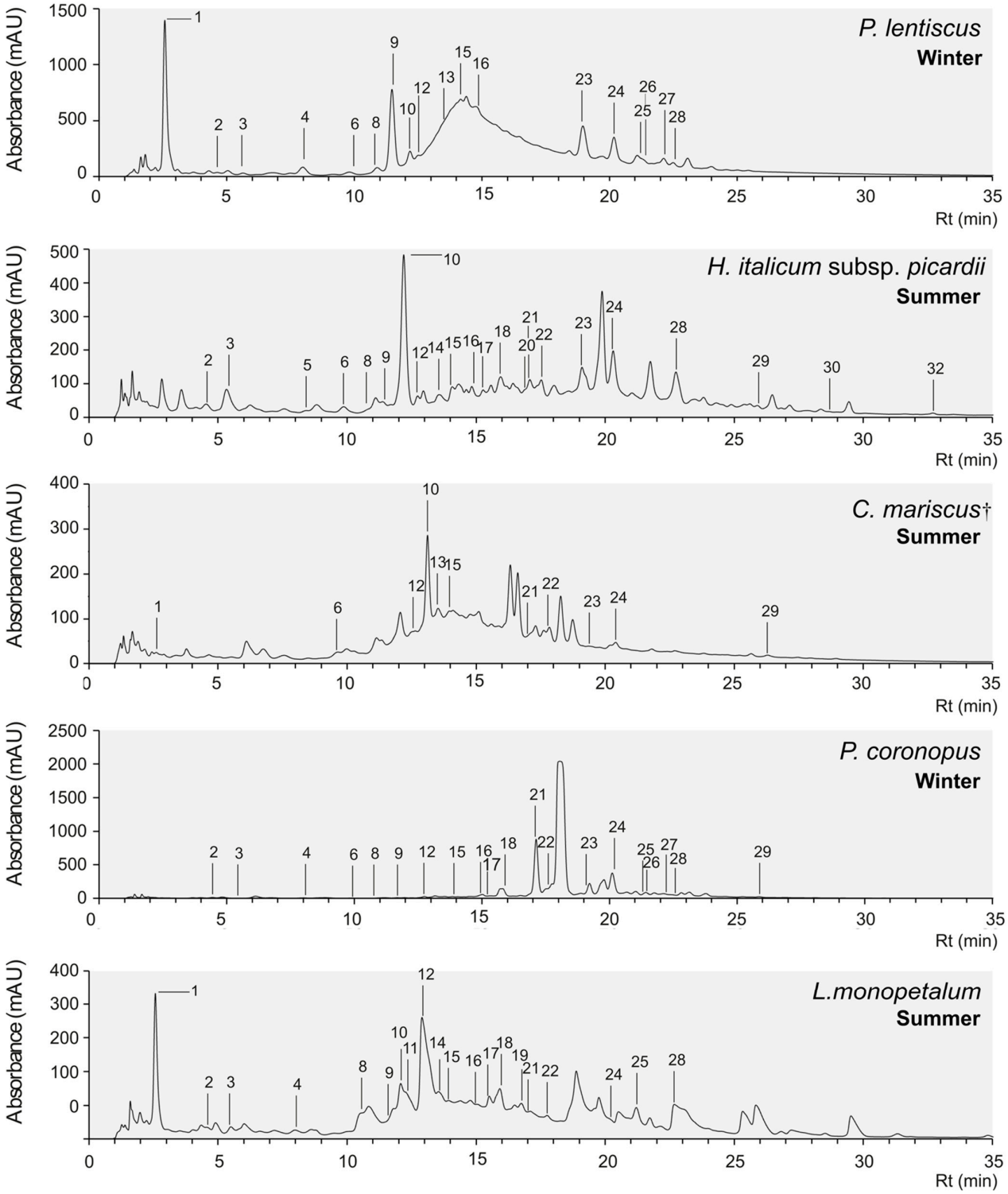

Figure 7. Peak chromatograms of *P. lentiscus*, *H. italicum picardii*, *C. mariscus*, *P. coronopus*, and *L. monopetalum* with selected 80% acetone extracts using HPLC-DAD. Peaks are numbered according to their retention time (Rt). †: Data published in [25].

4. Discussion

The prospection of plants intended for nutraceutical uses must first focus on gathering nutritional evidence [15,38]. Crude protein values are often a decisive factor in establishing the nutritional quality of botanical resources, since they influence biomass intake and rumen function, and a sharp decline occurs when their levels are below 70 g kg^{-1} DM [7].

In this work, protein levels were frequently low in the dry periods (Su/Au), and this is possibly linked to phenological or environmental aspects, with most species barely reaching the 70 g kg^{-1} DM mark, consistent with other non-traditional salt-tolerant fodder species of the Mediterranean basin [39,40]. Whilst most samples sustain animal maintenance, higher values would be required for the growing or lactating stages of animal production systems (140–180 g kg^{-1} DM) [7]. Still, further work on their amino acid profile may unravel the additional biochemical assets of these plants for exploitation, as some essential and non-essential amino acids are often found in high amounts in this plant group (e.g., leucine, valine, glycine, proline, alanine, glutamic, and aspartic acids), which is associated with their defensive role in salt stress [41,42].

Considering the importance of protein on animal growth, reproduction, and lactation [32], the low values reported for dry periods (average for Su = 65 g kg^{-1} DM and Au = 61.7 g kg^{-1} DM) may constrain its use in times when there is a manifest need for alternatives. Furthermore, the anticipated climate shifts in the Mediterranean region, characterized by prolonged droughts, higher temperatures, and shorter rainfall periods, will likely extend the duration of the dry season [43,44]. It is crucial to acknowledge that while local flora is adapted to such conditions, these environmental changes may still influence their growth cycles and, by extension, their nutritional profiles during what are currently considered wetter seasons. Despite these challenges, the scarcity of plants that can thrive in these increasingly arid conditions underscores the invaluable role of salt-tolerant species, either as a feed complement or as a source of specific nutrients [45].

A constraint often mentioned as a limiting factor for halophytes and salt-tolerant species' feeding value is their high ash content [7,45,46]. Although it has no energy value, minerals are essential for ruminant health, productivity, and performance, and many pastures fail to provide enough amounts to sustain high levels of animal productivity year-round [13]. While all species demonstrated adequate macromineral levels for maintenance purposes, most proved to be rich sources of microminerals throughout the year, capable of supporting various levels of production (Figure 3). Copper (Cu), manganese (Mn), zinc (Zn), selenium (Se), and sulfur (S) are particularly valuable due to their roles in antioxidant defense mechanisms [12], and significant positive correlations between these elements were identified. Trace minerals like Cu, Mn, Se, and Zn, alongside bioactive molecules such as phenolics, offer added value by mitigating oxidative stress-related disorders commonly experienced during ruminant production, such as reproduction, lactation, heat stress, and gastrointestinal parasitic infections [39,47]. These benefits extend to enhancing the quality of meat and dairy products [12,19,48], positioning these salt-tolerant species as functional resources for improved nutrition and production sustainability. As a main point, this study underscores the opportunity to exploit these plants as dietary supplements or nutraceutical products, addressing mineral imbalances across seasons.

A main strength of this work was the evaluation of the phenolic profiles across species and seasons, since these are molecules of antioxidant [11,47] and anthelmintic interest [15,16,23,49] for ruminant animals. The seasonal dynamics assessment of the phenolic profile and antioxidant properties presented herein targets a bidirectional approach: It complements the nutraceutical evaluation of these plants, aiming at their use as bioactive plants, and prospects extracts as phytotherapeutic products while identifying the optimal time of harvesting for maximizing their production. As evidenced in the individual and combined data (PCA and RACI, Figures 5 and 6), phenolic accumulation and antioxidant effects varied in a species-specific manner. Indeed, based on these results, Wi was identified as the optimal harvesting period for *P. lentiscus* and *P. coronopus*, whilst Su samples were the best performing regarding *L. monopetalum*, *C. mariscus*, and *H. italicum*.

The individual chromatograms of these five most promising samples obtained through HPLC-DAD analysis were quite distinct due to the chemical complexity of the extracts and, again, species-related differences. *P. lentiscus* was rich in ourateacatechin (**16**), gentisic acid (**4**), epicatechin (**15**), gallic acid (**1**), and vanillic acid (**9**), consistent with the findings comprehensively reviewed by Sehaki et al. [50] in different works, which summarized a diverse range of phenolics in this species, encompassing various classes such as flavonoids, flavonols, flavanols, flavones, flavonoid glycosides, myricetin derivatives, anthocyanins, catechins, and phenolic acids, along with their derivatives [50]. The *H. italicum picardii* (Su) sample had higher contents of chlorogenic acid (**10**), neochlorogenic acid (**3**), epicatechin (**15**), ourateacatechin (**16**), salicylic acid (**22**), and luteolin-7-*O*-glucoside (**24**). Using LC-MS, Pereira and colleagues (2017a) mainly detected quinic and chlorogenic acids, syringic acid, caffeic acid, astragalin, hyperin, and oleanolic acids in the decoctions and infusions of aerial organs and the flowers of *H. italicum picardii* collected in the same location during June 2013 [51]. *L. monopetalum* (Su) accumulated caffeic acid (**12**), gallic acid (**1**), gentisic acid (**4**), chlorogenic acid (**10**), 3-hydroxybenzoic acid (**8**), and 4-*O*-caffeoylquinic acid (**11**). Working with the same species, Trabelsi et al. (2012) [52] quantified eleven phenolics (gallic, p-hydroxybenzoic, chlorogenic, syringic, vanillic, ferulic, and trans-cinnamic acids, quercetin, apigenin, amentoflavone, and flavone) in leaf extracts after acidic hydrolysis from Tunisian plants collected in May 2006. The samples from the species *P. coronopus* (Wi) mainly contained gentisic acid (**4**), salicylic acid (**22**), and luteolin-7-*O*-glucoside (**24**). Compounds 2–4, 8, 14, 16–17, 23, and 26–28 were not detected previously in the work of Pereira and colleagues (2017b), while, in reverse verbascoside, apigenin and the aglycone luteolin were not identified herein (Table 1) [53].

In ruminant production, oxidative stress, i.e., imbalances in the production of reactive oxygen species, is mostly associated with inflammatory disorders, parasitic infections, reproductive events, dietary imbalances, and environmental challenges (e.g., heat stress [47], impairing animal health, welfare, and productivity and being particularly impactful at vulnerable stages (e.g., young animals). It is recognized that dietary antioxidants improve the animal's overall antioxidant status, thereby supporting immune functions, bolstering resilience to stressful events, and helping prevent diseases, ultimately resulting in the improved growth and quality of their derived products [54]. In this context, identifying and characterizing novel plant resources that are naturally rich in antioxidants are of utmost relevance, as these can offer significant nutritional and health benefits for ruminants while contributing to more sustainable farming practices. Specifically, regarding phenolics, increasing evidence has been gathered on the positive outcomes of the dietary inclusion of individual molecules (e.g., gallic acid, quercetin [55,56]) and phenolic-rich plants or extracts for ruminant animals [57–59]. For example, adding gallic acid (0.5–1 g kg^{-1}) to the starter diet of preweaning dairy calves improved growth, antioxidant function, rumen fermentation, and microbial communities [56]. Chlorogenic acid-enriched plant extracts improved the antioxidant status of lambs under transport-induced stress [57,58] and dairy cows in heat stress [59].

Besides antioxidant credit, polyphenols have long been at the core of investigations concerning natural anthelmintic options. Likewise, the potent anthelmintic properties of plant extracts have been attributed to different phenolic molecules, some of which are identified and quantified herein, such as hydroxycinnamic acids (e.g., gallic, chlorogenic, ferulic acids) and flavonoids (e.g., quercetin) alone or in combination [49,60,61]. In alignment with these findings, we previously reported that the acetone aqueous extracts of these five species have anthelmintic activity on larvae exsheathment and/or the egg hatching stages of the life cycle of two gastrointestinal nematode species [23], strengthening the possibility

of using these plants as novel sustainable options for fighting GIN infections in ruminant production systems, either as nutraceutical plants or phytotherapeuticals.

5. Conclusions

This study demonstrates that the optimal harvesting periods of halophytes and salt-tolerant plants vary depending on the target species and sought-after biotechnological applications, whether for maximizing specific nutrients, extracting valuable metabolites, or combining both. *M. marina* and *C. soldanella* exhibit a more balanced nutrient profile across seasons, characterized by higher protein and lower ash contents, making them promising candidates for feed or nutraceutical applications; *I. crithmoides*, *L. monopetalum*, and *P. coronopus* may serve as viable alternatives, particularly in seasons when ash content is lower; *H. italicum picardii* is notable for its high mineral and antioxidant content, indicating its potential as a feed supplement or nutraceutical resource; *P. lentiscus* (Wi) and *L. monopetalum* and *C. mariscus* (Su) stand out due to their elevated polyphenol levels and abundance of identified antioxidant and anthelmintic metabolites, presenting opportunities for their exploitation as nutraceutical or phytotherapeutic products or as sources of novel bioactive molecules. Overall, this work increases our understanding of how seasonal variations influence the suitability of salt-tolerant plant biomass for diverse applications in ruminant production systems and underscores the value of these species as year-round resources, maximizing their use for feed, nutraceuticals, and phytotherapeutics or as sources of added-value individual metabolites.

Both intrinsic factors, like plant organs, evolutionary adaptations, and ecological requirements, and the simultaneous influence of multiple abiotic and biotic pressures in the wild environment trigger the synthesis of primary and secondary metabolites in each plant species. Although left outside of this work, unravelling the ecological configurations that shape each plant species' chemical profile is of utmost significance and should be addressed in future work. Either way, by integrating seasonal dynamics into the decision-making process, this study provides a framework for optimizing the utilization of halophytes and salt-tolerant plants alone or in complementary strategies, supporting sustainable ruminant production in the Mediterranean area.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Sp	Spring;
Su	Summer;
Au	Autumn;
Wi	Winter;
CCMAR	Centre of Marine Sciences;
UAlg	University of Algarve;
ICNF	Instituto da Conservação da Natureza e das Florestas;
DM	Dry matter;
N	Total nitrogen;
CP	Crude protein;
TL	Total lipid;
Ca	Calcium;
K	Potassium;
Na	Sodium;
Mg	Magnesium;
Cu	Copper;
Zn	Zinc;
Mn	Manganese;
Fe	Iron;
Se	Selenium;
MP-AES	Microwave plasma atomic emission spectrometer;
CHO	Total carbohydrate;
GE	Gross energy;
DMSO	Dimethyl sulfoxide;
TPC	Total phenolic content;
TFC	Total flavonoid content;
CTC	Condensed tannin content;
DMACA-HCl	4-Dimethylaminocinnamaldehyde-hydrochloric acid;
GAE	Gallic acid equivalent;
QE	Quercetin equivalent;
CE	Catechin equivalent;
DW	Dry weight;
RSA	Radical scavenging activity;
CCA	Copper chelation assay;
ICA	Iron chelation assay;
BHT	Butylated hydroxytoluene;
EDTA	Ethylenediamine tetraacetic acid;
FRAP	Ferric reducing antioxidant power;
SEM	Standard error of the mean;
IC50	Half inhibitory concentration;
RACI	Relative antioxidant capacity index;
PCA	Principal component analysis;
HI	*Helichrysum italicum picardii*;
PL	*Pistacia lentiscus*;
LM	*Limoniastrum monopetalum*;
CM	*Cladium mariscus*;
CS	*Calystegia soldanella*;

PC *Plantago coronopus*;
CC *Crucianella maritima*;
MM *Medicago marina*;
IC *Inula crithmoides*.

Appendix A

Table A1. Plant harvesting details, including voucher number, collection dates, organs, and climacteric data, expressed as monthly mean values (source: IPMA).

Species (Family) (Voucher No.)	Location	Season	Date	Organs	$\bar{x}$ Min. Temp. (°C)	$\bar{x}$ Max. Temp. (°C)	$\bar{x}$ Total Rainfall (mm)
Helichrysum italicum subsp. *picardii* (Asteraceae) (XBH32)	Tavira 37°07′51.8″ N, 7°36′37.6″ W	Sp	Apr/2017	L/S	14	24	25
		Su	Jul/2017	L/S/FL	18	30	1
		Au	Oct/2017	L/S/FL	14	26	25
		Wi	Jan/2018	L/S	6	16	50
Inula crithmoides (Asteraceae) (XBH04)	Olhão 37°01′11.7″ N, 7°53′04.8″ W	Sp	Apr/2017	L/S	14	24	25
		Su	Jul/2017	L/S	18	30	1
		Au	Oct/2017	L/S/FL	16	26	25
		Wi	Jan/2018	L/S	6	16	50
Pistacia lentiscus (Anacardiaceae) (XBH06)	Portimão 37°07′34.7″ N, 8°36′02.3″ W	Sp	Apr/2017	L/S	10	24	10
		Su	Jul/2017	L/S	14	30	1
		Au	Oct/2017	L/S/FR	12	26	10
		Wi	Jan/2018	L/S/FR	2	16	100
Cladium mariscus (Cyperaceae) (XBH03)	Faro 37°01′03.3″ N, 7°59′18.1″ W	Sp	Apr/2017	L	14	22	25
		Su	Jul/2017	L/I	18	30	1
		Au	Oct/2017	L/I	16	26	25
		Wi	Jan/2018	L	6	16	50
Calystegia soldanella (Convolvulaceae) (XBH07)	Portimão 37°07′23.1″ N, 8°36′10.7″ W	Sp	Apr/2018	L/S/FL	10	20	10
		Su	Jul/2018	L/S	12	26	1
		Au	Oct/2017	L/S	12	26	10
		Wi	Jan/2018	L/S/FL	2	16	100
Medicago marina (Fabaceae) (XBH41)	Portimão 37°07′23.1″ N, 8°36′10.7″ W	Sp	Apr/2018	L/S/FL	10	20	10
		Su	Jul/2018	L/S/FR	12	26	1
		Au	Oct/2017	L/S	12	26	10
		Wi	Jan/2018	L/S	2	16	100
Plantago coronopus (Plantaginaceae) (XBH02)	Olhão 37°01′32.8″ N, 7°53′04.4″ W	Sp	Apr/2018	L/S/FL	12	20	100
		Su	Jul/2018	L/S/FL	16	26	1
		Au	Oct/2017	L/S/FL	16	26	25
		Wi	Jan/2018	L	6	16	50
Limoniastrum monopetalum (Plumbaginaceae) (XBH05)	Portimão 37°07′34.7″ N, 8°36′02.3″ W	Sp	Apr/2017	L/S	10	24	10
		Su	Jul/2017	L/S/FL	14	30	1
		Au	Oct/2017	L/S	12	26	10
		Wi	Jan/2018	L/S	2	16	100
Crucianella maritima (Rubiaceae) (XBH40)	Portimão 37°07′23.2″ N, 8°36′12.3″ W	Sp	Apr/2017	L/S	10	24	10
		Su	Jul/2017	L/S/FL	14	30	1
		Au	Oct/2017	L/S	12	26	10
		Wi	Jan/2018	L/S	2	16	100

Sp, Spring; **Su**, summer; **Au**, autumn; **Wi**, winter; **L**, leaves; **S**, stems; **FR**, fruits; **FL**, flowers; **I**, inflorescences.

Table A2. Seasonal assessments of the chemical composition and mineral content of aerial parts of halophytes and salt-tolerant plants. Nutritional data (DM, CP, A, TL, and CHO) and macrominerals (Ca, K, Mg, and Na) are expressed in g kg^{-1} DM, trace minerals (Fe, Cu, Mn, and Zn) in mg kg^{-1} DM, and GE as Mcal kg^{-1}. **Sp**, Spring; **Su**, summer; **Au**, autumn; **Wi**, winter. † Data published in [25].

Species	Season	Nutritional Parameters					Energy	Mineral Content							
		DM	CP	A	TL	CHO	GE	Ca	K	Mg	Na	Cu	Fe	Mn	Zn
Helichrysum italicum subsp. *picardi* (Asteraceae)	Sp	314.6	87.6	58.1	99.6	754.6	4.6	11.7	12.7	1.5	3.2	17.1	330.2	64.0	58.0
	Su	458.6	42.5	55.1	90.9	811.5	4.5	10.8	15.0	1.6	2.7	13.7	138.8	52.7	42.2
	Au	549.4	39.4	61.2	142.7	756.7	4.7	13.4	9.7	1.4	2.4	15.6	325.9	67.4	53.5
	Wi	496.3	60.0	67.6	137.8	734.7	4.7	14.7	7.0	1.4	3.0	19.6	405.2	71.0	60.9

Table A2. *Cont.*

Species	Season	Nutritional Parameters					Energy	Mineral Content							
		DM	CP	A	TL	CHO	GE	Ca	K	Mg	Na	Cu	Fe	Mn	Zn
Inula crithmoides (Asteraceae)	Sp	97	95.7	269.0	61.1	574.2	3.5	11.8	12.0	10.6	79.4	5.8	207.3	14.8	14.3
	Su	161.8	90.5	326.6	74.2	508.7	3.3	10.8	9.5	10.0	91.5	5.5	235.5	13.0	13.8
	Au	124.6	65.6	240.6	87.7	606.0	3.7	12.9	12.1	7.6	67.2	7.7	452.9	14.4	20.3
	Wi	104.3	91.4	324.9	79.0	504.7	3.4	11.0	10.8	6.6	97.7	4.0	227.8	12.6	20.5
Pistacia lentiscus (Anacardiaceae)	Sp	484.5	60.1	63.0	67.5	809.4	4.4	24.2	9.2	1.9	1.2	2.3	43.0	9.9	16.7
	Su	494.6	74.5	50.2	77.3	798.1	4.5	13.2	13.5	1.4	1.5	2.8	48.0	8.9	9.3
	Au	479.9	52.5	55.1	106.7	785.7	4.6	18.5	11.8	1.9	1.5	1.8	61.0	8.2	8.0
	Wi	504.3	66.4	50.8	94.9	787.9	4.6	21.6	8.5	1.8	1.6	1.9	62.9	9.4	9.1
Calystegia soldanela (Convolvulaceae)	Sp	121.4	97.3	110.2	250.1	542.5	5.2	17.5	21.5	2.2	7.1	2.3	239.8	12.4	28.3
	Su	84.3	67.2	128.4	166.0	638.4	4.6	33.6	5.0	6.1	19.0	1.2	75.1	28.6	45.8
	Au	163.3	102.5	178.9	141.9	576.7	4.3	31.3	7.4	6.2	30.9	5.4	57.0	33.1	45.5
	Wi	224.6	189.4	161.9	148.8	499.8	4.6	17.6	28.7	3.1	23.5	12.8	74.8	15.3	48.5
Cladium mariscus (Cyperaceae) †	Sp	449.1	87.3	71.7	53.3	787.8	4.3	6.9	4.4	0.8	8.1	3.9	186.0	40.7	15.2
	Su	585.5	51.8	49.4	48.9	850.0	4.3	1.6	3.9	0.7	1.4	7.7	214.0	20.0	21.1
	Au	559.1	54.5	82.9	52.9	809.8	4.2	3.8	2.3	0.7	5.7	5.9	42.8	29.1	20.4
	Wi	469.4	57.8	75.1	51.1	816.0	4.2	3.8	2.4	0.7	4.9	9.6	32.3	49.0	24.7
Medicago marina (Fabaceae)	Sp	376.6	132.7	68.5	53.8	745.1	4.4	22.3	8.0	1.6	5.3	1.6	162.7	10.2	22.0
	Su	307	79.4	71.6	55.1	793.9	4.3	28.2	4.7	2.9	8.5	2.9	94.3	11.1	34.5
	Au	271.8	66.9	85.7	53.0	794.5	4.2	28.8	2.1	1.9	11.7	3.8	380.6	12.8	45.3
	Wi	400.2	139.4	67.1	45.9	747.7	4.4	21.9	7.7	2.0	6.7	2.9	140.3	14.0	41.7
Plantago coronopus (Plantaginaceae)	Sp	251.6	87.6	129.1	74.6	708.7	4.2	19.7	9.8	2.5	17.6	4.6	430.8	31.3	34.1
	Su	127.6	71.5	154.7	81.0	692.8	4.1	23.9	8.1	3.3	22.8	7.5	328.2	17.1	66.7
	Au	152.5	63.9	149.1	71.5	715.5	4.0	20.2	5.3	3.1	19.9	10.5	499.9	17.3	38.9
	Wi	280.9	116.1	151.3	76.9	655.6	4.1	32.5	7.4	2.4	23.0	4.9	116.3	12.6	25.8
Limoniastrum monopetalum (Plumbaginaceae)	Sp	291.3	108.7	180.5	44.1	666.7	3.8	41.0	7.2	9.8	31.0	0.8	68.2	10.5	26.7
	Su	374.2	60.6	148.3	35.7	755.4	3.8	36.8	5.9	6.9	27.0	1.1	57.6	9.9	10.2
	Au	495.5	62.8	187.1	34.4	715.8	3.7	58.2	7.9	12.9	13.3	1.8	92.9	15.6	9.1
	Wi	322.8	108.3	204.8	43.7	643.2	3.7	60.4	7.4	15.3	17.0	2.6	64.7	16.4	20.3
Crucianella maritima (Rubiaceae)	Sp	174.8	65.6	125.3	54.7	754.4	4.1	9.2	11.7	3.4	11.8	4.5	346.9	27.2	36.9
	Su	473.3	47.0	130.0	75.9	747.0	4.1	3.4	8.9	5.0	8.8	4.7	284.4	34.5	32.3
	Au	573.9	47.2	130.0	66.6	756.2	4.1	3.4	4.8	5.3	5.9	6.4	388.7	45.4	29.5
	Wi	359.9	59.2	127.1	74.0	739.7	4.1	2.4	10.1	2.5	4.8	6.1	451.5	58.0	54.0

† Data published in [25]. **DM**, Dry matter; **CP**, crude protein; **TL**, total lipids; **CHO,** total carbohydrates; **GE**, gross energy; **Ca**, calcium; **K**, potassium; **Mg**, magnesium; **Na**, sodium; **Cu**, copper; **Fe,** iron; **Mn,** manganese; **Zn,** zinc.

Table A3. Antioxidant capacity of 80% acetone extracts prepared from selected halophytes and salt-tolerant plant species collected across seasons. Results are expressed as the concentration that inhibits 50% of the radical (IC_{50} values) in mg mL^{-1}. Different letters correspond to significant differences for each season in each assay ($p < 0.05$; Tukey's test). **Sp**, Spring; **Su**, summer; **Au**, autumn; **Wi**, winter.

Species	Season	CCA			DPPH			FRAP			ABTS		
Helichrysum italicum subsp. *picardi* (Asteraceae)	Sp	>1			0.43	±	0.01 ^{c}	0.74	±	0.11 ^{b}	0.63	±	0.13 ^{a,b}
	Su	>1			0.29	±	0.01 ^{a,b}	0.32	±	0.02 ^{a}	0.30	±	0.02 ^{a}
	Au	>1			0.27	±	0.00 ^{a}	0.45	±	0.04 ^{a}	0.30	±	0.06 ^{a}
	Wi	>1			0.33	±	0.01 ^{b}	0.40	±	0.03 ^{a}	0.86	±	0.14 ^{b}
Pistacia lentiscus (Anacardiaceae)	Sp	0.18	±	0.02 ^{a}	0.03	±	0.01 ^{a}	0.07	±	0.00 ^{a,b}	0.03	±	0.00 ^{a}
	Su	0.13	±	0.02 ^{a}	0.04	±	0.01 ^{a}	0.07	±	0.00 ^{a,b}	0.04	±	0.00 ^{a,b}
	Au	0.18	±	0.02 ^{a}	0.04	±	0.00 ^{a}	0.09	±	0.01 ^{b}	0.04	±	0.01 ^{b}
	Wi	0.17	±	0.01 ^{a}	0.03	±	0.00 ^{a}	0.05	±	0.00 ^{a}	0.03	±	0.00 ^{a}
Cladium mariscus † (Cyperaceae)	Sp	>1			0.30	±	0.00 ^{a}	0.25	±	0.02 ^{a}	0.29	±	0.03 ^{b}
	Su	>1			0.24	±	0.02 ^{a}	0.21	±	0.03 ^{a}	0.12	±	0.01 ^{a}
	Au	>1			0.25	±	0.01^{a}	0.18	±	0.05 ^{a}	0.20	±	0.02 ^{a,b}
	Wi	>1			0.26	±	0.03 ^{a}	0.27	±	0.01 ^{a}	0.23	±	0.03 ^{b}
Plantago coronopus (Plantaginaceae)	Sp	>1			0.25	±	0.02 ^{a,b}	0.23	±	0.01 ^{a}	0.47	±	0.06 ^{a}
	Su	>1			0.30	±	0.03 ^{b}	0.29	±	0.01 ^{a}	0.55	±	0.13 ^{a}
	Au	>1			0.20	±	0.03 ^{a}	0.32	±	0.05 ^{a}	0.40	±	0.10 ^{a}
	Wi	>1			0.17	±	0.02 ^{a}	0.20	±	0.01 ^{a}	0.33	±	0.04 ^{a}

Table A3. *Cont.*

Species	Season	CCA			DPPH			FRAP			ABTS		
Limoniastrum monopetalum (Plumbaginaceae)	Sp	0.65	±	0.03 ^{a}	0.12	±	0.01 ^{a}	0.09	±	0.01 ^{a}	0.11	±	0.02 ^{a,b}
	Su	0.58	±	0.03 ^{a}	0.12	±	0.01 ^{a}	0.13	±	0.01 ^{a,b}	0.06	±	0.01 ^{a}
	Au	0.92	±	0.11 ^{b}	0.18	±	0.00 ^{b}	0.18	±	0.03 ^{b}	0.15	±	0.02 ^{a,b}
	Wi	0.66	±	0.04 ^{a,b}	0.14	±	0.01 ^{a}	0.11	±	0.01 ^{a,b}	0.16	±	0.03 ^{b}

† Data published in [25]. EDTA was used as a positive control for CCA (IC_{50} = 0.08 ± 0.01 mg mL^{-1}), ascorbic acid for FRAP (IC_{50} = 0.03 ± 0.01 mg mL^{-1}), and BHT for DPPH (IC_{50} = 0.14 ± 0.00 mg mL^{-1}) and ABTS (IC_{50} = 0.11 ± 0.01 mg mL^{-1}).

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