

Salicornia emerici Duval-Jouve and Sarcocornia alpini (Lag.) Rivas Mart. from Southern Tunisia: Potential Sources of Functional Ingredients with Nutritional and Health Promoting Benefits

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Abstract

Aerial biomass of the edible halophyte species *Salicornia emerici* Duval-Jouve and *Sarcocornia alpini* (Lag.) Rivas Mart. (Amaranthaceae), collected from southern Tunisia, were investigated as potential sources of functional ingredients. For that purpose, dried biomass was profiled for proximate and mineral composition, and its hydroethanolic extracts were analyzed for phenolics contents by spectrophotometric and chromatographic (HPLC-ESI-MS) methods. The extracts were then profiled for *in vitro* antioxidant properties (radical scavenging activity (RSA) of DPPH and ABTS radicals, and ferric reducing power (FRAP)), cytotoxicity, and enzymatic inhibition toward enzymes related with human diseases, namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) (Alzheimer's disease: AD), α -amylase and α -glucosidase (type 2 Diabetes Mellitus: T2DM), and tyrosinase (skin hyperpigmentation disorders). Biomass had relevant levels of minerals, especially sodium, potassium and iron. The extracts were not toxic, contained various bioactive phenolic compounds, such as naringin and quinic acid and exhibited relevant FRAP and inhibition properties against α -glucosidase and tyrosinase. Our findings suggest that there is potential to further explore the biomass from *S. alpini* and *S. emerici* as safe and sustainable sources of nutrients and of natural products with antioxidant, anti-diabetic and anti-tyrosinase properties, which might yield significant economic benefits for the southeastern region of Tunisia.

1 Introduction

Over the past few years, consumers' dietary preferences have undergone a significant transformation as they increasingly opt for natural and healthier food products that not only provide essential nutrients but also offer health advantages, including improved well-being, disease prevention, and potential cost savings in healthcare [1]. This change in dietary habits, amplified by the impact of the COVID-19 pandemic, contributed to the heightened interest in dietary supplements, nutraceuticals, and functional foods, thereby fueling the expansion of their worldwide market [2–4].

Functional foods, a concept firstly mentioned in Japan in the 1980s, can be described as dietary components that may provide health-enhancing or disease-preventing benefits beyond their basic nutritional value [5, 6]. While an ultimate definition of functional foods is still lacking, its concept generally includes both conventional foods that are naturally rich in bioactive compounds like minerals, vitamins, and antioxidants, as well as fortified, enriched foods with specific components such as omega-3 fatty acids, vitamins and probiotics which provide numerous health benefits, such as reducing the risk or delaying the onset of major diseases like cardiovascular issues, neurodegeneration, and cancer [7–9].

Due to the growing interest and the acceptance of functional foods, the number of studies aiming to find novel sources of products with beneficial properties has increased. In this context, halophytes stand out as intriguing plants due to their abundance and untapped potential for both nutrition and functionality [10]. In fact, halophytic species manage to grow and thrive in extreme conditions of drought, high salinity, and UV exposure by means of various morphological, physiological, and biochemical adaptation

mechanisms [11]. One of these mechanisms is the production and accumulation of secondary metabolites, including phenolic acids, flavonoids, and tannins which display important biological activities such as antioxidant, anti-inflammatory, anti-diabetic, and anti-cancer [12, 13]. Several studies have highlighted halophytes' potential as novel sources of nutrients including minerals, vitamins, and polyunsaturated fatty acids [14–16]. Besides, halophytes are becoming increasingly attractive in modern agriculture due to their ability to thrive in challenging conditions of high salinity and water scarcity, which is particularly relevant in the Mediterranean region, especially in Tunisia, where climate change in recent years has exacerbated these issues [17–19]. The *Salicornioideae* subfamily (*Amaranthaceae*) encompasses approximately 100 succulent halophytic plant species found in coastal and inland saline regions worldwide including *Sarcocornia* and *Salicornia* species which are morphologically and taxonomically similar and can only be distinguished by inflorescence characters and life form with *Sarcocornia* being perennial and *Salicornia* being annual [20, 21]. Their edible succulent, crunchy, and salty shoots are commonly known as “glasswort”, “samphire” or “sea asparagus” and are highly appreciated in gourmet cuisine either raw or cooked, especially when combined with fish or sea food [18, 22, 23]. *Sarcocornia* and *Salicornia* species are considered good sources of fiber, minerals (such as calcium, and iron), vitamins (including vit C and A), polyunsaturated fatty acids (e.g., stearic and linolenic acids), and polyphenolic compounds (e.g., chlorogenic acid and rutin) [12, 18, 24–27]. These components have been linked to health-promoting properties, such as antioxidant and anti-inflammatory effects, thereby enhancing the potential use of such species to functionalize food matrices like bread, pasta, and savory snacks [28, 29].

From a broader perspective, increasing water and soil salinization represents a serious threat to agricultural lands, affecting traditional crops growth and by consequence food security. Hence, exploring the potential of using medicinal halophytes, such as *Salicornia* and *Sarcocornia*, as sustainable solution to mitigate food scarcity by providing both nutrition and health benefits, could present a promising alternative. Introducing biosaline agriculture systems would allow for the direct harvesting and consumption of fresh, edible tips as food. Simultaneously, the non-food-grade biomass can be repurposed through biorefinery processes resulting in bio-products with high-value applications, such as nutraceuticals and functional ingredients [19, 30].

Our continuous endeavors to enhance the sustainable use of halophyte biodiversity in southern Tunisia have led us to assess the nutritional and functional qualities of two lesser-known plant species, namely *Salicornia emerici* Duval-Jouve and *Sarcocornia alpini* (Lag.) Rivas Mart. reported for the first time in southern Tunisia [31]. Previous studies primarily focused on the phylogenetic and morphological characteristics of such species as well as forage quality parameters [31, 32]. This study focused on the commonly underutilized biomass of the two species, which has been underestimated in food and medicinal applications, aiming to explore its potential as a source of nutrients and natural bioactive compounds. To the best of our knowledge, this is the first time the nutritional content, phytochemical profile and potential health benefits of *Salicornia emerici* are reported. To this end, wild *S. alpini* and *S. emerici* biomass collected from southeastern Tunisia, was profiled for proximate and mineral composition, and used to produce food-grade hydroethanolic extracts. The extracts were then evaluated

for their polyphenolic profiles using colorimetric methods and high-performance liquid chromatography (HPLC-ESI-MS). Furthermore, these extracts were appraised for *in vitro* acute toxicity towards a panel of tumoral and non-tumoral mammal cell lines, and assessed for *in vitro* antioxidant properties by measuring radical scavenging ability (DPPH and ABTS) and ferric reducing power (FRAP). Finally, extracts were examined for inhibitory properties toward enzymes implicated on the onset of human diseases, namely AD (AChE and BuChE), T2DM (α -amylase and α -glucosidase), and skin hyperpigmentation disorders (tyrosinase).

2 Results and discussion

2.1 Nutritional profile

Collected biomass of *S. alpini* and *S. emerici* were appraised for proximate composition and mineral contents and results are showcased in Table 1. Dried biomass from both species presented moisture contents of 6.32 and 8% DW, for *S. alpini* and *S. emerici*, respectively. These values were lower than the results reported for *Sarcocornia alpini*, *Sarcocornia perennis*, and *Sarcocornia pruinoso* from the Iberian Peninsula where values were between 10.18 and 14.78% [33]. This parameter reflects the drying process's ability (ambient air drying in our case) to remove residual water from dried plants, hence extending plant shelf life.

Table 1
Proximate and mineral compositions of *Sarcocornia alpini* and *Salicornia emerici* biomass

Parameters	Species	
	<i>Sarcocornia alpini</i>	<i>Salicornia emerici</i>
Moisture (%)	6.32 ± 0.01 ^a	8 ± 0.06 ^b
Total ash (%)	42.54 ± 0.05 ^b	53.84 ± 0.24 ^a
Total protein (%)	10.42 ± 0.23 ^a	5.77 ± 0.08 ^b
Macro-elements (mg/g)		
Na	157.17 ± 1.29 ^b	235.47 ± 1.20 ^a
K	36.42 ± 2.98 ^b	49.61 ± 3.57 ^a
Ca	17.09 ± 1.63 ^b	30.96 ± 2.27 ^a
Mg	15.13 ± 0.74 ^a	13.83 ± 0.18 ^a
Micro-elements (µg/g)		
Cu	5.95 ± 0.61 ^b	22.63 ± 0.18 ^a
Zn	14.50 ± 0.29 ^b	85.9 ± 1.45 ^a
Fe	205.9 ± 3.06 ^b	381.53 ± 1.64 ^a
Mn	14.55 ± 0.66 ^a	15.60 ± 0.29 ^a
Data are presented as mean ± standard error of the mean (SEM), (n = 3). In the same row, values followed by different letters are significantly different ($p < 0.05$).		

The ash content is directly related to the amount of minerals present in the biomass [34], and reached 42.5 and 53.8% for *S. alpini* and *S. emerici*, accordingly, which were like the findings of Hayder et al. for the same species [32], as well as those for *S. ramosissima* collected from northern Portugal [35]. Castañeda-Loaiza et al. obtained lower levels for *S. fruticosa* collected from the wild in Spain and Portugal (28.9 and 37.3 g/100g, respectively) [36], and the same was observed for *S. perennis alpini*, *Sarcocornia perennis perennis*, and *Salicornia ramosissima* from Portugal [10]. Halophytes commonly have a higher ash content than other edible glycophytes which can be attributed to their ability to retain minerals, which is correlated with their adaptation mechanisms to the high levels of salinity in their natural habitat [37, 38].

S. alpini and *S. emerici* presented low protein levels of 10.4 and 5.7% accordingly, which were comparable to the values described by Hayder et al. (11.87 and 8.58% DW, for *S. alpini* and *S. emerici*, respectively) [32]. They were also akin to those reported for dried *S. ramosissima* [35]. The plant's

protein content is influenced by different factors namely species, fluctuations in temperature, salinity, and UV radiation [39], and can be boosted as a result of stress response [40].

Both species presented a high mineral content (Table 1) which could be related to their tolerance and capacity to survive in extreme conditions of high salinity and drought [10, 41, 42]. Overall, *S. emerici* presented higher mineral content which could be directly correlated to its higher ash content. The values disclosed by Hayder et al. for the same species showed lower Na, K, Ca, Mg, and Fe contents for *S. emerici* and higher amounts of Na, Ca, Mg, and Fe in *S. alpini* [32]. The macro-nutrients contents in *S. alpini* and *S. emerici* were higher than those reported for other edible *Salicorniaceae*, whereas Fe and Mn levels were lower [10]. *S. alpini*'s mineral content was lower than the values reported for wild *S. fruticosa* [36]. The same was observed for *S. emerici* except for Na, K, Ca, Cu, and Zn, where it presented similar or higher values. The mineral content in halophytes can be influenced not only by maturation stage and the studied part of the plant but also by environmental conditions related to water and soil characteristics, pollutants, and climate [38, 43]. Therefore, the elevated mineral levels in *S. emerici* might be attributed, at least in part, to the continuous immersion of the plant in seawater and its maturation stage, as the salt content in halophytes tends to increase with age [44].

Like previously reported for different *Salicornia* and *Sarcocornia* species, Na was the prevalent macro-element in both *S. alpini* and *S. emerici* (157.17 and 235.47 mg/g, respectively). This mineral is deemed essential for maintaining various physiological functions, including heart and nerve functions, muscle contraction, and the regulation of the body's water balance [45]. However, as high sodium intake has been positively associated with increased blood pressure and proved to be a major risk factor of cardiovascular diseases

[46], numerous studies proposed the substitution of table salt with halophytes to prevent such health repercussions. This recommendation is primarily based on their potassium and magnesium content, contributing to vascular protection. Knowing that replacing salt by the same amount of dried *Sarcocornia perennis* resulted in a decrease of more than 70% of Na content, *S. alpini* and *S. emerici* could be used as salt substitute while providing other beneficial elements like K, Mg, and Fe [28]. Besides, they could be considered flavor enhancers with various health benefits attributes.

Iron was the predominant micro-element in both species with values of 205.9 and 381.53 µg/g for *S. alpini* and *S. emerici*, respectively. Thus, their biomass could be further investigated as potential source of iron to be incorporated in functional food as part of the diet in case of anemia, a common blood disorder resulting from iron deficiency [47].

The presence of all identified essential minerals that are vital for the maintenance of various biochemical and physiological processes in the human body in relevant amounts, adds nutritional value to both halophytic species and enhances their value as functional compounds reservoir [48, 49].

2.2 Chemical profile

To adapt to harsh environmental conditions, particularly high levels of salinity and UV exposure, halophytes produce significant amounts of bioactive secondary metabolites such as phenolic acids, flavonoids, and tannins [18, 50, 51]. These compounds exhibit a wide range of health benefits, including antioxidant, anti-inflammatory, anti-cancer, and anti-diabetic activities [50, 52–55]. Having this in mind, the total phenolics, flavonoids, and condensed tannins contents of *S. alpini* and *S. emerici* extracts were quantified and results are presented in Table 2.

Table 2

Total phenolics (TPC), flavonoids (TFC) and condensed tannin contents (CTC) of aqueous ethanolic extracts of *Sarcocornia alpini* and *Salicornia emerici*

Extracts	TPC mg GAE/g DW	TFC mg QE/g DW	CTC mg CE/g DW
<i>Sarcocornia alpini</i>	15.4 ± 0.13 ^a	3.81 ± 0.07 ^a	0.94 ± 0.18 ^a
<i>Salicornia emerici</i>	4.7 ± 0.13 ^b	0.91 ± 0.03 ^b	1.45 ± 0.13 ^a

Data are presented as mean ± standard error of the mean (SEM), (n ≥ 5). In the same column, values followed by different letters are significantly different (*p* < 0.05).

Overall, *S. alpini* had higher TPC and TFC (15.4 mg GAE/g DW and 3.81 mg QE/g DW, respectively) while *S. emerici* presented higher CTC (1.45 mg CE/g DW). The TPC of *S. alpini* exceeded the levels previously reported in methanolic extracts of *S. alpini* gathered from different locations across the Iberian Peninsula, which ranged from 3.43 to 3.77 mg GAE/g DW [33]. For the same species collected from Portugal, Lopes et al. (2023) reported higher values (38.2 mg GAE/g DW) [19]. *S. alpini* exhibited higher TPC, similar TFC, and lower CTC than wild *S. fruticosa* [36]. The TPC of *S. emerici* (4.7 mg GAE/g DW) was comparable to the value described for *Salicornia patula* extracts collected in Spain [16], but lower than that reported for *S. ramosissima* extracts [35]. Hayder et al. relayed lower TFC and CTC contents for the same species from southern Tunisia [32]. Such variations can be due to several factors related to species, sites of collections, drying processes, and extraction methods [13, 35].

2.3 HPLC-ESI-MS analysis

The results of the phenolic compositions of *S. alpini* and *S. emerici* extracts identified with HPLC-ESI-MS method are summarized in Fig. 1, 2 and Table 3. Among the 31 tested standards, 11 compounds were identified in *S. alpini*: six phenolic acids (quinic, chlorogenic, 4-*O*-caffeoylquinic, syringic, *p*-coumaric and trans-ferulic acids) and five flavonoids (rutin, hyperoside (quercetin-3-*O*-galactoside), naringin, quercetin and cirsiol). Whereas in *S. emerici*, only five phenolic compounds were detected (quinic, *p*-coumaric and trans-ferulic acids, naringin and cirsiol). Even though several studies have described the phytochemical profiles of *Salicornia* and *Sarcocornia* species, data concerning the phenolic composition of *S. alpini* and *S. emerici* remains limited. As far as we know, this is the first time the phenolic profile of *S. emerici* has been disclosed.

Table 3

Phenolic compounds (mg/kg DW) identified in aqueous ethanolic extracts of *Sarcocornia alpini* and *Salicornia emerici*, by HPLC-ESI-MS

Compound	Formula	RT (min)	m/z	<i>Sarcocornia alpini</i>	<i>Salicornia emerici</i>
Quinic acid	C ₇ H ₁₂ O ₆	2.129	191	148.46	229.15
Chlorogenic acid	C ₁₆ H ₁₈ O ₉	14.730	353	128.23	-
4- <i>O</i> -caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	15.397	353	159.22	-
Syringic acid	C ₉ H ₁₀ O ₅	19.825	197	41.55	-
<i>p</i> -coumaric acid	C ₉ H ₈ O ₃	24.376	163	6.02	8.17
Transferulic acid	C ₁₀ H ₁₀ O ₄	26.974	193	6.93	1.72
Rutin	C ₂₇ H ₃₀ O ₁₆	28.439	609	87.70	-
Hyperoside	C ₂₁ H ₂₀ O ₁₂	28.900	463	53.22	-
Naringin	C ₂₇ H ₃₂ O ₁₄	30.448	579	212.43	46.28
Quercetin	C ₁₆ H ₁₄ O ₆	35.975	301	0.81	-
Cirsiliol	C ₁₇ H ₁₄ O ₇	39.290	329	98.25	51.30

Naringin, a flavanone glycoside known for its numerous biological activities such as anti-inflammatory, antioxidant, and anticancer [56], was the major compound in *S. alpini* (212.43 mg/kg) while quinic acid (cyclitol) was the dominant molecule in *S. emerici* (229.15 mg/kg). In fact, several studies described the multiple biological properties of quinic acid namely antioxidant [57], antidiabetic [58], anti-aging [59], and antitumor [60]. Chlorogenic and 4-*O*-caffeoylquinic acids were also prevalent in *S. alpini* alongside quinic acid. Previous studies reported the wide range of medicinal benefits of chlorogenic acid such as antioxidant, anti-cancer, anti-diabetic, and anti-inflammatory properties [61]. Plus, caffeoylquinic acids have been reported to exhibit antioxidant, antibacterial, antidiabetic, and anti-Alzheimer activities amongst others [62]. The levels of chlorogenic and *p*-coumaric acids, rutin and quercetin were lower than those reported for *Salicornia ramosissima* and *S. perennis alpini* [19]. The same was observed for chlorogenic acid and quercetin contents in *S. alpini* when compared to the results of wild *S. fruticosa* extracts from Spain and Portugal, while its rutin content was higher [36]. The observed variation in *Salicornia* and *Sarcocornia* species is noteworthy, as studies have suggested that the tolerance mechanism in halophytes is specific to each species [63]. This variability could elucidate the differential expression of phenolics as a protective mechanism against stressful environments. Variations in the collection sites and natural habitats of the two plants may also partially influence the production of bioactive phytochemicals as adaptive responses to challenging conditions. This could help to explain the observed differences, considering that *S. emerici* plants were submerged in seawater, while *S. alpini*

biomass was collected at a distance from the tides [50, 64–67]. Furthermore, the harsh environmental conditions could, in part, account for the reduced diversity and quantities of phenolic compounds detected in *S. emerici*. In fact, studies indicate that beyond a specific threshold of high salinity, *Salicornia* and *Sarcocornia* species may either lose the ability to produce these compounds or shift to alternative protective mechanisms [25].

All the identified compounds are associated with diverse biological activities, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, and cardiovascular-protective properties [12, 13, 35]. Since halophytes present a rich source of polyphenols manifesting protective potential against various metabolic syndromes, including T2DM, hyperlipidemia, and cardiovascular disorders [68], our results underscore the significance of the edible *S. alpini* and *S. emerici* as sources of bioactive natural products.

2.4 In vitro toxicological evaluation

Because of their complex chemical profile, plants are prone to contain potentially harmful compounds. Therefore, it is advisable to assess potential toxicity before investigating their therapeutic properties to guarantee their safety for further nutritional and medicinal applications. Moreover, there is a lack of available data regarding the potential toxicity of *S. alpini* and to our knowledge, this is the first toxicity evaluation of *S. emerici*. Considering that these two species are being documented for the first time in southern Tunisia, it is crucial to investigate their toxicological characteristics. Different methods are used for this purpose including the assessment of acute toxic effects *in vivo* on *Artemia salina* and *in vitro* on mammalian cell lines. In this work, the toxicity of the tested extracts was evaluated towards three mammalian cell lines, namely murine RAW 264.7 macrophages, murine bone marrow stromal (S17), and human hepatocellular HepG2 carcinoma cells. No toxicity was observed after the application of the extracts (Fig. 3). In general, the application of the extracts resulted in cell viability near to 100% or higher in RAW 264.7 and HepG2 cells, and above 85% in S17 cells. The lack of cytotoxicity observed in *in vitro* models employing mammalian cell lines is regarded as an indicator of the low toxicity associated with a natural extract [69, 70]. Besides, various *Salicornia* and *Sarcocornia* species are not only edible but also traditionally consumed in fresh, boiled, or pickled forms. As such, it is logical to hypothesize that extracts from *S. alpini* and *S. emerici* could be deemed safe for consumption. This suggests a need for further exploration as potential novel and secure sources of functional ingredients with noteworthy biological attributes.

2.5 Antioxidant properties

In their native environment, halophytes encounter challenging abiotic conditions, including severe drought and salinity, leading to the generation of substantial quantities of reactive oxygen species (ROS) which are responsible for cellular damage [18]. To counteract ROS, halophytes have evolved effective antioxidant defense mechanisms, including the synthesis of enzymes and various categories of antioxidant compounds, particularly polyphenolics [71, 72]. To assess the antioxidant capacity of the *S. alpini* and *S. emerici* extracts, complementary methods were used based on their radical scavenging

ability (RSA) towards ABTS and DPPH free radicals and iron reducing power (Table 4). In general, *S. alpini*, at 12.5 mg/mL, showed higher RSA against both DPPH (45.89%) and ABTS (73.44%) radicals and higher FRAP (EC_{50} = 1.32 mg/mL). *S. emerici* had no significant RSA (16.80 and 26.79% on DPPH and ABTS at 12.5 mg/mL, respectively) and showed a moderate capacity to reduce iron (EC_{50} = 7.26 mg/mL). To our best knowledge, there are no reports on the antioxidant properties of *S. emerici*. When compared to *Sarcocornia perennis alpini*, *Sarcocornia perennis perennis* and *Salicornia ramosissima* from Portugal, these three species exhibited higher DPPH radical scavenging ability and ferric reducing potential [10]. Wild *Sarcocornia fruticosa* collected from Spain and Portugal exhibited higher DPPH scavenging activity than both of our extracts with IC_{50} values of 6.10 and 8.19 mg/mL, respectively [36]. Typically, there is a correlation established between the phenolic and flavonoid content of extracts and their antioxidant activity. This correlation could explain the higher antioxidant activity of *S. alpini*, as it exhibited higher TPC and TFC than *S. emerici*. Furthermore, its individual phenolic profile displayed greater diversity, and the identified phenolic compounds are likely individually or in synergy responsible for the observed antioxidant effect [73, 77]. In fact, numerous identified phenolic acids such as quinic, chlorogenic and 4-*O*-caffeoylquinic acids, and flavonoids namely rutin and cirsiol are renowned antioxidants [62, 75, 76]. Given that the extracts are a complex mixture of various phytochemicals, it is often difficult to predict exactly which compounds are responsible for the detected activity. These compounds can exhibit varying reactions to individual antioxidant mechanisms, contingent on their respective affinities, thus yielding distinct activity patterns [77, 78]. Hence, the use of several assays to evaluate the antioxidant activity of natural extracts is recommended [19, 79, 80]. Nevertheless, one should not exclude the potential effect of non-identified phytochemicals present in the extracts.

Table 4

Radical scavenging activity (RSA, %) on DPPH and ABTS free radicals and ferric reducing activity power (FRAP) (EC_{50} , mg/mL) of aqueous ethanolic extracts of *Sarcocornia alpini* and *Salicornia emerici*

Extracts	RSA		FRAP
	DPPH	ABTS	
<i>Sarcocornia alpini</i>	45.89 ± 5.18 ^b	73.44 ± 2.08 ^b	1.32 ± 0.03 ^b
<i>Salicornia emerici</i>	16.80 ± 0.70 ^c	26.79 ± 1.68 ^c	7.26 ± 0.33 ^c
GA†	80.96 ± 0.22 ^a	93.62 ± 0.10 ^a	-
BHA†	-	-	0.16 ± 0.01 ^a
Data are presented as mean ± standard error of the mean (SEM), (n = 5).			
In the same column, values followed by different letters are significantly different at $p < 0.05$ (Tuckey HSD test).			
† Positive control at 1 mg/mL; GA: Gallic acid, BHA: Butylated hydroxyanisole.			

Table 5

Inhibitory activity (%) of aqueous ethanolic extracts of *Sarcocornia alpini* and *Salicornia emerici* on AChE, BuChE, α -amylase, α -glucosidase and tyrosinase

Extracts	AChE	BuChE	α -amylase	α -glucosidase	Tyrosinase
<i>Sarcocornia alpini</i>	14.19 $\pm$ 0.24 ^c	15.80 $\pm$ 0.54 ^b	10.05 $\pm$ 0.02 ^b	57.00 $\pm$ 1.04 ^b	51.3 $\pm$ 0.31 ^b
<i>Salicornia emerici</i>	26.87 $\pm$ 4.24 ^b	8.00 $\pm$ 2.07 ^c	4.17 $\pm$ 0.29 ^c	62.82 $\pm$ 3.67 ^b	31.4 $\pm$ 0.06 ^c
Galanthamine†	85.48 $\pm$ 0.95 ^a	74.99 $\pm$ 0.34 ^a	-	-	-
Acarbose†	-	-	69.03 $\pm$ 0.19 ^a	78.12 $\pm$ 0.04 ^a	-
Arbutin†	-	-	-	-	90.1 $\pm$ 0.0 ^a
Data are presented as mean $\pm$ standard error of the mean (SEM), (n = 3).					
In the same column, values followed by different letters are significantly different at $p < 0.05$ (Tuckey HSD test).					
† Positive control at 1 mg/mL.					

The findings from the *in vitro* antioxidant tests offered initial insights into the potential of the bioactive compounds within *S. alpini* and *S. emerici* extracts to function as scavengers of radicals and reducers of iron. Hence, it is advisable to conduct further investigations into the antioxidant attributes of both halophytes through supplementary *in vitro* assays, including metal chelating and cell-based methods, as well as *in vivo* studies. The outcomes of our study indicate that both species hold potential as rich and novel sources of natural antioxidants highlighting their consumption either in a traditional way or as functional ingredients sources in nutraceuticals and functional food.

2.6 Enzymatic inhibitory properties

This work assessed the inhibitory effects of *S. alpini* and *S. emerici* extracts against enzymes associated with various human disorders, namely AChE and BuChE (Alzheimer's disease), α -amylase and α -glucosidase (type 2 diabetes mellitus, T2DM), and tyrosinase (hyperpigmentation disorders). Both extracts had low inhibitory activity toward AChE and BuChE, nil activity against α -amylase but relevant inhibition against α -glucosidase and tyrosinase (Table 4). To our best knowledge, there is no previous information of these enzymatic inhibitory properties for the two species.

Alpha-amylase and α -glucosidase are enzymes involved in carbohydrate digestion, therefore, their inhibition has the potential to curb the postprandial surge in blood glucose levels, consequently mitigating the hyperglycemia associated with T2DM which is a significant public health concern, given its substantial impact on patients' well-being, quality of life, and life expectancy [81, 82]. The extracts of

S. alpini and *S. emerici* have shown potential as sources of α -glucosidase inhibitors, with inhibition rates of 57% and 62.82% at a concentration of 12.5 mg/mL, respectively. Both extracts were more active against α -glucosidase than α -amylase, in accordance with previous reports concerning plant extracts anti-diabetic effect [83]. *S. ramosissima* ethanolic extract at 10 mg/mL exhibited similar results (68.63%) but its raw water extracts showed lower α -glucosidase inhibition [30]. The observed enzymatic inhibition is likely attributed to the presence of various identified phenolic compounds and it's worth noting that the involvement of unidentified metabolites cannot be ruled out [84]. Further investigation is therefore required to fully explore the potential antidiabetic activity of both species, as products with a mild amylase inhibitory effect and strong glucosidase inhibitory action are sought after [85].

Tyrosinase is recognized as the key enzyme accountable for both mammalian melanogenesis and food oxidation. Hence, its inhibitors are sought after in medicine to treat skin hyperpigmentation disorders and cancers or as anti-browning compounds in agri-food industry [86, 87]. Given the interest of its diverse applications, the inhibitory activity of both *S. alpini* and *S. emerici* extracts against tyrosinase was evaluated and both extracts exhibited enzymatic inhibitory potential of 51.3 and 31.4%, respectively. Hulkko et al. reported higher tyrosinase inhibition activity (71.85%) for *S. ramosissima* EtOH extract at 10 mg/mL [30]. Considering that plant extracts with high TPC have a higher probability yielding tyrosinase inhibitors, this could explain in part the higher tyrosinase inhibition exhibited by *S. alpini* extract [85]. Furthermore, many identified phenolic compounds namely quinic and chlorogenic acids could individually or in synergism be behind melanogenesis suppression as previously disclosed [88].

This study reported the nutritional and phytochemical profiles, as well as the biological properties of the halophytes *S. alpini* and *S. emerici* species' biomass collected from southern Tunisia. *S. alpini* presented higher phenolic contents and antioxidant activity, while *S. emerici* showed significant amounts of essential minerals, including Na, K, Mg, and Fe. Both species had no capacity to inhibit AChE and BuChE, exhibited similar capacity to inhibit α -glucosidase, while *S. alpini* was more active towards tyrosinase. Naringin was the major compound in *S. alpini* and quinic acid was the dominant molecule in *S. emerici*. The findings show that both species are valuable sources of nutrients, especially minerals, and underscore the potential of their use as a source of functional ingredients with beneficial biological attributes.

The sustainable harvesting and use of *S. alpini* and *S. emerici* biomass could offer economic advantages in Tunisia. This might be realized by either gathering this biomass from natural habitats or by developing saline agriculture systems with tailored growth conditions to improve their phenolic content and nutritional value. Additional research is necessary to elucidate the connection between the phytochemical composition of these plants and their health-promoting attributes, as well as to validate their consumption in a traditional way or as potential sources of functional ingredients with future applications in nutraceuticals and functional food.

3 Materials and methods

3.1 Chemicals, cells, and reagents

Sigma-Aldrich (Germany) provided the following chemicals: catechin, quercetin, Folin-Ciocalteu reagent, aluminium chloride (AlCl_3), sodium carbonate (Na_2CO_3), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), electric eel AChE (type-VI-S, EC 3.1.1.7), horse serum BuChE (EC 3.1.1.8), glucosidase (EC 3.2.1.20, from *Saccharomyces cerevisiae*), amylase (EC 3.2.1.1, from porcine pancreas), tyrosinase (EC 1.14.18.1), acetylthiocholine iodide, butyrylthiocholine chloride, galanthamine, acarbose, arbutin, gallic acid and butylated hydroxyanisole (BHA). The human hepatocarcinoma (HepG2) and murine bone marrow stromal (S17) cell lines were both kindly provided by the Center for Biomedical Research (CBMR), University of Algarve, Portugal while the murine leukemic macrophage (RAW264.7) cell line was purchased from CLS Cell Line Service GmbH (Germany). Cell culture media, supplements, additional solvents, and chemicals were acquired from VWR International (Belgium).

3.2 Plant samples collection and processing

Aerial parts from twenty individual per species were collected from two neighboring regions from the governorate of Medenine in the Southeast of Tunisia. *S. alpini* was gathered from El Alandaya, Zarzis (33°25'49.4"N-11°05'40.7"E) and *S. emerici* from Sidi Jmour, Djerba (33°51'59"N-10°47'31"E) during the month of November 2020. Dr. Mohamed Tarhouni, Professor at the Arid Regions Institute, Medenine, Tunisia, authenticated the species' identities, which were documented in a publication as their first identification in Tunisia [31]. Voucher specimens were deposited in the herbarium of the Rangeland Ecosystems and Valorization of Spontaneous Plants and Associated Microorganisms Laboratory at the Arid Regions Institute (*S. alpini*, voucher code: IRAPL015; *S. emerici*, voucher code: IRAPL020). Since *S. alpini* and *S. emerici* are not protected species, no collecting permission is needed as their gathering focused on public rights of way. Our study was conducted in accordance with relevant institutional, national, and international regulations. Because of their geographic proximity, the two locations presented similar environmental conditions marked by their arid climate (average rainfall of 100–200 mm/year). Both collection sites were characterized by their greater or lesser proximity to the sea: *S. emerici* was constantly submerged by the tides while *S. alpini* was collected from soils that were far away from the tidal influence. Biomass was air-dried in the shade at room temperature (RT, 20–25°C) for three weeks until they were completely dry, ground into a fine powder with a particle size of 0.5 mm and stored in a dark environment at RT until needed.

3.3 Proximate and mineral composition

Moisture, total protein, ash, and minerals were analyzed according to the Association of Official Analytical Chemists methods [89]. Dried samples were weighed and placed at 105°C for 24 h for humidity determination. Crude protein content was determined according to the Kjeldahl method by multiplying the total nitrogen by the factor 6.25. Ash content was determined by incineration at 550°C in a muffle furnace for 4 h. The obtained ashes (1g) were digested with HCl (20%, 5 mL) and later diluted in ultrapure water. The resulting solutions were used to estimate the contents of Na, K, Ca, Mg, Co, Zn, Fe

and Mn using a Flame Atomic Absorption Spectrometry (FAAS), (Shimadzu AA6800, Japan). A similar protocol was carried out for the blanks.

3.4 Extraction

Dried biomass was extracted with 70% aqueous ethanol (1:7, w/v) by maceration at RT for 24 h under stirring (IKA Labortechnik, Germany). The extract was recovered, and the residue was extracted 4 more times. The obtained extracts were combined and filtered using a standard filter paper (Ref: ST60F4252), and the solvent was removed under reduced pressure at 40°C using a rotary evaporator (Dragon Lab, RE 100-Pro, China). The concentrated extracts were later dried using a freeze-dryer (Christ Alpha 1–2 LD plus, Germany) for 48 h. Lyophilized extracts were resuspended in 70% ethanol at the concentration of 12.5 mg/mL and stored in the dark at 4°C until further analysis.

3.5 Phytochemical composition

3.5.1 Total contents of phenolics (TPC), flavonoids (TFC) and condensed Tannins (CTC)

TPC, TFC and CTC were determined in the extracts at the concentration of 12.5 mg/mL using the following colorimetric methods: Folin Ciocalteu (F-C) reagent [90], aluminum chloride [91], and 4-dimethyl amino cinnamaldehyde-hydrochloric acid (DCAMA) adapted from vanillin test [92], respectively. The absorbance was measured in a microplate reader (Biotek Synergy 4, USA) and results of TPC, TFC and CTC were expressed in mg gallic acid equivalents per gram of dry weight (mg GAE/g DW), mg of quercetin equivalents per gram of dry weight (mg QE/g DW) and in mg of catechin equivalents per gram of dry weight (mg CE/g DW), respectively. All the assays are detailed in Rodrigues et al. [93].

3.5.2 HPLC-ESI-MS analysis

The phenolic profiling of the extracts was carried out using a Shimadzu UFLC XR tandem (Kyoto, Japan) equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadripole 2020 detector system. The separations were performed on a Inertsil ODS-4 C18 column (150mm x 3mm, 3µm) maintained at 40°C. Mobile phases A (95% Water + 5% Methanol + 0.2% Acetic acid) and B (50% Water + 50% Acetonitrile + 0.2% Acetic acid) were used in the following linear gradient program: 0,01–14 min: 10–20% B; 14–27 min: 20–55% B; 27–37 min: 55–100% B; 37–45 min: 100% B; 45–50 min: 10% B. The flow rate of the mobile phase was 0.5 mL/min and the injection volume was 20 µL. ESI-MS conditions were as follows: dissolving line temperature was 275°C, nebulizing gas flow 1.5L/min, the drying gas was set at 15 L/min and temperature of Heat block was 450°C. LC-ESI (-)-MS mass spectra [M-H]⁻ were acquired using LabSolutions software, Shimadzu.

The following thirty one commercial standards were used as reference solutions: quinic, gallic, protocatechuic, caffeic, syringic, chlorogenic, 4-*O*-caffeoylquinic, *p*-coumaric, trans-ferulic, 3,4-di-*O*-caffeoylquinic, rosmarinic, 4,5-di-*O*-caffeoylquinic, *O*-coumaric, salvianolic and trans-cinnamic acids, (+)-catechin, (-)-epicatechin, rutin, hyperoside (quercetin-3-*O*-galactoside), cynaroside (luteolin-7-*O*-

glucoside), naringin, quercitrin (quercetin-3-*O*-rhamonoside), apigenin-7-*O*-glucoside, kaempferol, quercetin, naringenin, apigenin, luteolin, cirsiol, cirsilinol and acacetin. The peaks of phenolic compounds present in the extracts were identified by comparing their retention parameters to those of the standards. External standard method, based on the calibration curves of these standards, was used to quantify the identified compounds.

3.6 Bioassays

3.6.1 In vitro toxicological evaluation

Three mammalian cell lines were used, namely RAW 264.7 (murine leukemic macrophage), S17 (murine bone marrow stromal) and HepG2 (human hepatocarcinoma). The RAW 264.7 cells were cultured in RPMI 1640 media, while S17 and HepG2 cell lines were maintained in DMEM media, both complemented with 1% L-glutamine (2 mM), 10% heat-inactivated FBS and 1% penicillin (50 U/mL)/streptomycin (50 µg/mL). Cells were kept in a humidified atmosphere at 37°C with 5% CO₂. To evaluate possible toxic effects of the extracts, cells were firstly seeded in 96-well tissue plates at a density of 1×10⁴ cells/well (RAW 264.7) and 5×10³ cells/well (S17 and HepG2). After 24 h of incubation, the extracts were applied at the concentration of 200 µg/mL for 72 h. Ethanol 70% was used as control. Cell viability was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method and absorbances were read at λ = 590 nm using a microplate reader (Biotek Synergy 4), as described previously [94]. Results were expressed in terms of cellular viability (%).

3.6.2 In vitro antioxidant properties

The capacity of the extracts to scavenge the DPPH and ABTS radicals was assayed as described by Rodrigues et al. [93], on samples at the concentrations of 12.5 mg/mL. Gallic acid (1 mg/mL) was used as positive control. Results were expressed as a percentage of radical scavenging activity (% RSA) in relation to negative control (70% ethanol).

The ability of the samples (12.5 to 0.098 mg/mL) to reduce iron was determined by the FRAP assay, as described by Rodrigues et al. [93]. Absorbances were measured at 700 nm (Biotek Synergy 4) and results were expressed as percentage relative to butylated hydroxyanisole (BHA) (1 mg/mL) used as positive control and as medium effective concentration, EC₅₀ (mg/mL) whenever possible.

3.6.3 Enzyme inhibition

The enzymatic inhibitory property of the extracts, at a concentration of 12.5 mg/mL, was assessed toward different enzymes, as detailed below. The results were expressed as percentage of inhibitory activity relative to a negative control containing 70% ethanol.

The neuroprotective activity of the extracts was assessed towards AChE and BuChE according to the Ellman's method as detailed previously [95]. Absorbances were read at 412 nm. Galantamine (1 mg/mL) was used as the standard.

The α -amylase and α -glucosidase inhibition of the extracts was evaluated according to Uysal et al. [96]. Absorbances were read at a wavelength of 580 and 405 nm respectively. Acarbose (1mg/mL) was used as positive control.

The tyrosinase inhibition was carried out as described by [95]. Absorbances were recorded at 492 nm and arbutin (1mg/mL) was used as the standard inhibitor.

3.7 Statistical Analysis

The results were presented as mean $\pm$ standard error of the mean (SEM). Statistical analysis was carried out with SPSS 27.0.1 software (SPSS Inc., Chicago, IL, USA). Significant differences were assessed by Student's t-test for cytotoxicity, nutritional and phenolic profiles, and by one-way analysis of variance (ANOVA) using the Tukey HSD (honestly significant differences) test for antioxidant and enzymatic properties ($p < 0.05$). EC_{50} values were estimated by data sigmoidal fitting using GraphPad Prism V.8.1 software.

Declarations

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Competing interests The authors declare no competing interests

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

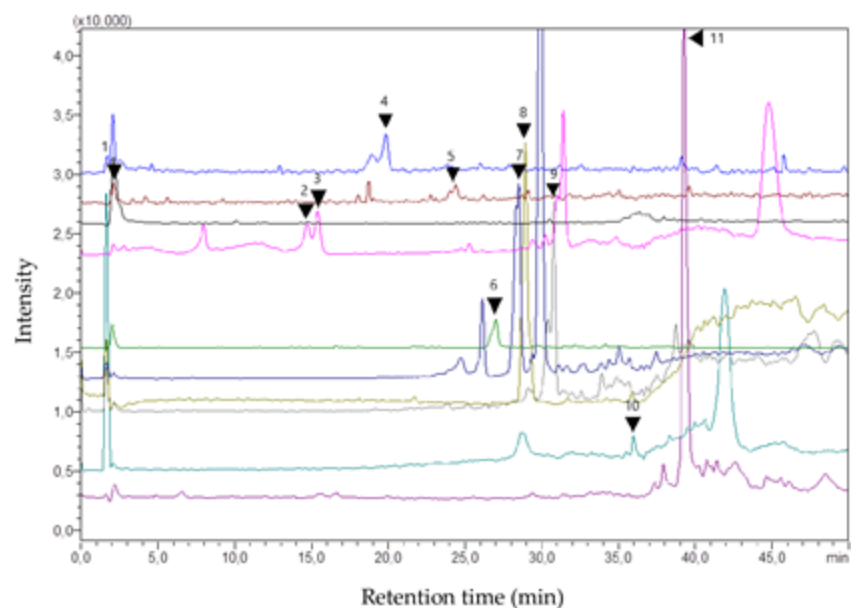


Figure 1

Chromatograms of the identified compounds in *Sarcocornia alpinia* extract obtained by HPLC-ESI-MS (1: quinic acid; 2: Chlorogenic acid; 3: 4-*O*-caffeoylquinic acid; 4: syringic acid; 5: *p*-coumaric acid; 6: trans-ferulic acid; 7: rutin; 8: hyperoside; 9: naringin; 10: quercetin and 11: cirsiolol).

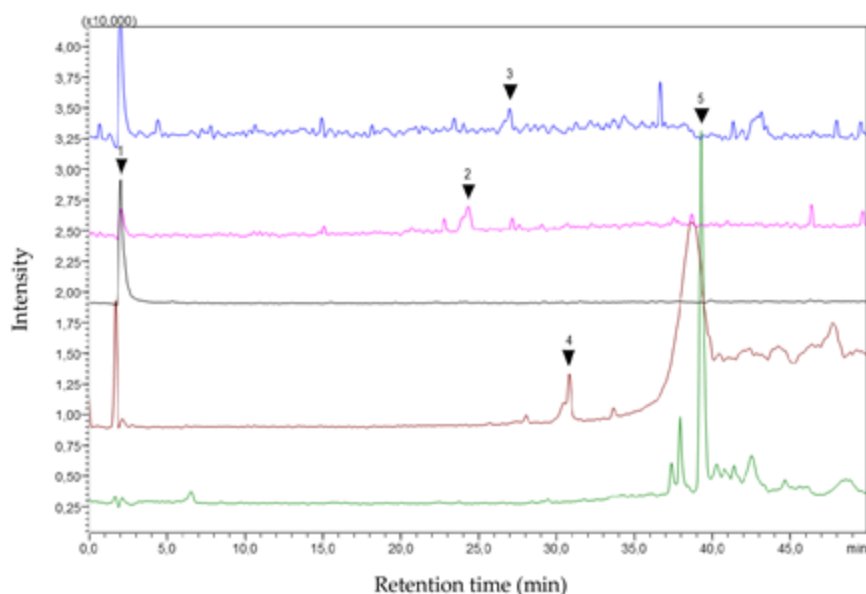


Figure 2

Chromatograms of the identified compounds in *Salicornia emerici* extract obtained by HPLC-ESI-MS (1: quinic acid; 2: *p*-coumaric acid; 3: trans-ferulic acid; 4: naringin and 5: cirsiolol).

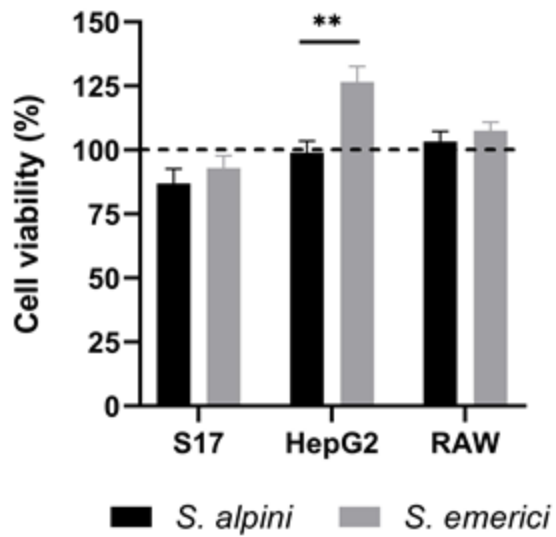


Figure 3

Cellular viability of HepG2, S17 and RAW 264.7 cells after the application of 200 µg/mL of *S. alpini* and *S. emerici* extracts. Values represent the mean of the % of viability relative to a control containing ethanol 70% ± SEM, (n ≥ 5). (**) represent the existence of statistical difference between HepG2 cell viability after application of *S. alpini* and *S. emerici* extracts ($p < 0.05$).