Nutrient composition and, identification/quantification of major phenolic compounds in *Sarcocornia ambigua* (Amaranthaceae) using HPLC–ESI-MS/MS

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ABSTRACT

The study systematically investigated the nutritional potential of two different growing populations of *Sarcocornia ambigua* (salt-tolerant) in terms of nutrients, bioactive compounds, and antioxidant activities. The results revealed that the moisture content represented the largest single component (88.15% and 88.57%) in the proximate composition of both samples of *S. ambigua*. The mineral present in highest amounts in both samples (on a fresh matter basis) was sodium, followed by potassium, magnesium and then calcium. The antioxidant activity for samples measured by DPPH ranged from 34.64 to 135.83 μmol TEAC 100 g^{-1} and by FRAP from 31.92 to 170.14 μmol Fe^{+2} 100 g^{-1}. The fifteen phenolic compounds identified in each extract by HPLC–ESI-MS/MS reveal the presence of one coumarin (scopoletin), one phenolic aldehyde (syringaldehyde), eight phenolic acids (p-coumaric, cinnamic, vanillic, ferulic, caffeic, syringic, sinapic, and chlorogenic acids) and five flavonoids (galangin, quercetin, naringin, kaempferol and isoquercitrin). This information can be useful in determining the possible role of the compounds identified which can participate in the prevention of different health disorders. Further studies are needed to evaluate the bioabsorption and bioavailability of the compounds present in *S. ambigua*, as well as the interactions between them, after consumption. In summary, the findings of this study highlight the potential of this halophyte as a valuable source of natural antioxidants and nutrients for use in the food and pharmaceutical industries.

1. Introduction

Plants of the *Sarcocornia* genus (Amaranthaceae) are halophytes (salt-tolerant) that grow in saline areas, usually near the coast and along the shores of salt lakes and marshes (Kadereit, Mucina, & Freitag, 2006). The Amaranthaceae family comprises approximately 160 genera and 2400 species. Five South American species of *Sarcocornia* have been identified and four different morphological types are found in South America and the Mediterranean countries (Alonso & Crespo, 2008). In South America the perennial *Salicornia gaudichaudiana* (Moq.) occurs along the entire coast of Brazil and as far south as Mar del Plata in Argentina (Costa & Davy, 1992; Souza Filho & Paradella, 2002). In Brazil new experimental crops of perennial *Salicornia* and *Sarcocornia* have been grown applying an irrigation system that uses saline effluent from shrimp farms (D'oca et al., 2012). The taxonomy and nomenclature of the taxonomic description of *S. gaudichaudiana* growing in South America has recently been updated and currently this species is referred to as *Sarcocornia ambigua* (Michx.) Alonso and Crespo (2008).

Different species of the *Salicornia* and *Sarcocornia* genera are suitable for crop production and they have received good acceptance from consumers, who are exclusively interested in the young green plant parts that are sold in the market as 'samphire' or 'sea asparagus'. The latter name probably reflects the shape of the shoots, which resemble the tops of green asparagus.

The aerial part of *Salicornia herbacea* has been used as a characteristic food by coastal people and as a folk medicine to treat a variety of diseases (Kim et al., 2011; Zhu & Row, 2010). The *Salicornia* species have been introduced into the European market as a vegetable with leafless shoots resembling green asparagus and, specifically in Italy

and France, they have been used as an ingredient in vinegar. Also, in Korea they are consumed as a seasoned vegetable, salad and fermented food (Kim et al., 2011).

There is currently considerable interest in these plants since they have been shown to be a promising functional food given their high nutritional value in terms of natural minerals, including Mg, Na, Ca, Fe and K, dietary fiber and many bioactive compounds, such as phytosterols, polysaccharides and phenolic compounds, particularly flavonoids and phenolic acids (Jang, Kim, Choi, Woo, & Choi, 2007; Ventura et al., 2011).

A number of studies have been carried out on the nutritional and chemical characterization of the phenolic compounds content and antioxidant activity of different halophytic species (*S. herbacea*, *Salicornia bigelovii*, *Salicornia persica*, *Sarcocornia fruticosa*) that grow in saline areas in several countries (Kim & Lee, 2009; Kim et al., 2009, 2011; Lu et al., 2010; Min, Lee, et al., 2002; Oh, Kim, Lee, Woo, & Choi, 2007; Rhee, Park, & Cho, 2009; Ventura et al., 2011).

The extraction and analysis of the bioactive compounds in *S. herbacea* have attracted attention. Recent studies have been focused on the isolation of bioactive compounds from the aerial parts of *S. herbacea*, such as flavonoid glycosides (isorhamnetin 3-O-β-D-glucoside and quercetin 3-O-β-D-glucoside), phenolic acids (caffeic acid, syringic acid, p-coumaric acid, chlorogenic acid, ferulic acid and sinapic acid) and flavonoids (myricetin, quercetin hesperetin, kampferol, rhamnetin and acacetin) in order to elucidate their potential health benefits (Essaidi et al., 2013; Kim et al., 2011; Kong et al., 2009).

Previous studies have shown that *S. herbacea* has several biological and physiological effects on health, with properties including antidiabetic (Lee et al., 2005; Park, Ko, Choi, & Chung, 2006), antioxidant, anti-inflammatory (Lee et al., 2007; Min, Lee, et al., 2002), antithrombus (Lee et al., 2005) and antihyperlipidemic (Cha et al., 2004) activity.

However, little attention has been given to species found in Brazil, and there have been no reports on the characterization of the nutrient composition, bioactive compounds and antioxidant activity profile of *S. ambigua*.

Thus, a detailed study on the proximate composition of *S. ambigua* will contribute to the generation of data that can be used in food composition tables, as well as to provide better dietary guidance. Additionally, the major phenolic compounds in this species were characterized using HPLC–ESI-MS/MS and the "*in vitro*" antioxidant activity was evaluated.

2. Materials and methods

2.1. Plant material

Sampling of the plant material from two different regions of *S. ambigua* in the state of Santa Catarina (SC), Brazil, was conducted in October 2012. The material was collected from two regions; the first located on a natural tideland near the coast in Palhoça (latitude 27° 40′ 54.76″ S, longitude 48° 38′ 19.63″ O) (Region A), and the second in Barra da Lagoa, Florianopolis (Latitude 27° 34′ 33″ S, Longitude 48° 26′ 33″ O) (Region B) from an experimental crop irrigated once a day with seawater and fertilized with sludge taken from the settling tanks of a shrimp (*Litopenaeus vannamei*) farm.

The material was botanically identified at the Department of Botany of the Regional University of Blumenau. A voucher specimen was deposited in the Dr. Roberto Miguel Klein Herbarium at the Regional University of Blumenau (no. 41346). The plant samples were transported to the Laboratory of Food Chemistry within a maximum of 2 h after collection.

2.2. Chemical reagents

The chemical reagents used were 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and sodium hydroxide purchased from Sigma Chemical Co. (St. Louis, MO). Gallic acid was obtained from Vetec (Rio de Janeiro, RJ,

Brazil). Hydrochloric acid (HCl), anhydrous ferric chloride (FeCl$_3$), methanol (MeOH) and ethanol (EtOH) were purchased from Sigma-Aldrich Chemical S.A. (Madrid, Spain). A commercial mixture of fatty acid methyl esters (FAME): 10 mg mL^{-1} Grain Fatty Acid Methyl Ester Mix (analytical standard) in methylene chloride (CH$_2$Cl$_2$) was purchased from Supelco (Bellefonte, PA, USA). A standard multielement ICP III solution purchased from Perkin Elmer (Shelton CT, USA) and Rh stock solution supplied by Sigma-Aldrich (Buchs, Switzerland) were employed. Argon gas with a purity of 99.996% purchased from White Martins (Sao Paulo, Brazil) was used. All reagents were of analytical grade unless otherwise indicated. Distilled and deionized water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q plus system (Millipore, Bedford, USA). The standards of phenolic compounds used in the HPLC–ESI-MS/MS analysis were obtained from Sigma-Aldrich (Sao Paulo, SP, Brazil) and all of them presented purity above 98%. A stock solution of each standard of phenolic compound was prepared by dissolving 10 mg in 10 mL of methanol (1000 mg L^{-1}), and then, another working solution was prepared by dilution of these 1000 mg L^{-1} stock solution to 10 mg L^{-1} also in methanol. All solutions were stored at 4 °C in a refrigerator.

2.3. Sample preparation

The aerial parts (leaf and steam) of the plants to be studied were washed with deionized water, towel dried and submitted to blanching for 10 min at 85 °C to inactivate the enzymes. Part of each sample was maintained in its natural condition and the other part was dried at 65 °C for 12 h (dry sample — DS). Both samples were stored at a temperature of −20 ± 0.2 °C until use. For subsequent analysis the samples were ground sufficiently in an Ika® Werke A11 (Staufen, Germany) food processor. This sample was utilized for analysis of fatty acids and mineral.

2.4. Chemicals analysis

General parameters were measured following the methods recommended by the Association of Official Analytical Chemicals (AOAC, 2005): moisture content (925.09) was determined by drying the sample in an oven at 105 °C until constant weight; ash content (923.03) was determined by burning the sample in a muffle furnace at 550 °C for 5 h; total lipids (920.85) were determined according to the Soxhlet extraction methodology; and the crude protein (920.87) content was calculated from the total nitrogen content determined by the Kjeldahl method, using a conversion factor of 6.25. Soluble and insoluble dietary fiber content was determined by the enzymatic–gravimetric method (991.43). Available carbohydrate content was obtained by difference, considering 100 g minus the sum (g) of water, protein, lipids, ash and dietary fiber. The chemical analysis was performed in triplicate with the homogeneous fresh sample, derived from the sampling procedure described above, and all results were expressed as fresh matter (% w/w).

2.5. Fatty acid composition

The lipids extracted from the whole plant (DS) by the method described in Section 2.4 were converted to fatty acid methyl esters according to Metcalfe, Schmitz, and Pelka (1966) and Hartman and Lago (1973), with some adaptations (0.5 mol L^{-1} methanolic sodium hydroxide was used for solubilization and saponification; esterification was performed with 3% ammonium chloride and sulfuric acid in methanol and the n-hexane was used for extraction). Fatty acid methyl esters (FAME) were determined using the AOCS Official Method Ce 1f-96 (2002) with appropriate adaptations to the temperature program for the determination of the fatty acid composition. A gas chromatography system (Agilent Technologies model 7890A) equipped with a flame ionization detector (FID), a split/spitless injector (operating with a

split ratio of 1:50) and a capillary column HP-88 (88% cyanopropylaryl 60 m × 0.250 mm ID × 0.20 μm film) was used. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injector and detector temperatures were 250 °C and 300 °C, respectively. The oven temperature was held at 140 ºC for 5 min, ramped at 4 ºC min^{-1} to 240 °C, and held at 240 °C for 10 min. The gas chromatography peaks for FAME were identified by comparison with known standards. The results were expressed as the relative percentage of each fatty acid.

2.6. Mineral analysis

2.6.1. Sample preparation

The concentrations of the major minerals (sodium, potassium, magnesium and calcium) were determined on an inductively coupled plasma mass spectrometer (ICP-MS), Perkin-Elmer SCIEX, model ELAN 6000 (Thornhill, Canada) coupled to a cross-flow nebulizer and a Scott spray chamber. Prior to the determination of the total element concentrations a complete destruction of the organic matrix of homogenized samples is required and during the mineralization process all organic compounds were converted into inorganic elements. In this regard, the samples (0.5 g of DS) were microwave digested (MLS-1200 microwave oven; Milestone, Sorisole, Italy), with 6 mL HNO$_3$ (65% v/v) (Supra pure, Merck, Darmstadt, Germany) and 1 mL H$_2$O$_2$ (30% v/v) (Trace Select Ultra; Sigma-Aldrich) at 250–600 W for 25 min in closed vessels.

Digested samples were diluted appropriately with ultra-pure laboratory Milli-Q water (18.2 MΩ cm quality; Millipore SAS), and to correct for non-spectral interferences, 10 μg L^{-1} Rh was used as the internal standard for all determinations. The concentrations of the standards used for the external calibration, prepared using stock solution Multi-element Calibration Standard 3 (Perkin Elmer, Inc., Shelton, Ct, USA), ranged between 2.0 and 800 μg L^{-1}. In order to verify the trueness of the measurements, certified reference materials of the National Institute of Standards and Technology (Gaithersburg, USA) were used to evaluate the analytical methods for all of the elements studied. The certified samples were of apple leaves (SRM no. 1515) and pine needles (SRM no. 1517a). Each sample, including the certified materials, was digested in triplicate and analysis was carried out once on each digest.

The instrument performance was assessed daily prior to the analysis. Argon (99.996%; White Martins, São Paulo, Brazil) was used as the sample introduction and plasma gas. The instrumental parameters were as follows: RF power, 1200 W; sampler and skimmer cones, Pt; scanning mode, peak hopping; resolution, 0.7 amu; readings per replicate, 50; replicates, 3; sweeps/reading, 20; residence time, 50 s; gas flow rates, main 15.0 L min^{-1}, intermediate 1.0 L min^{-1} and nebulizer 1.0 L min^{-1}; and isotopes ^{23}Na, ^{24}Mg, ^{39}K and ^{43}Ca.

2.7. Isolation and purification phenolic compounds

The aerial parts of the raw plant material (60 g) were treated with methanol (250 mL) and stored at a temperature of 10 ± 0.2 °C for ten days according to Filho and Yunes (1998) with some adaptations. After extraction the extract was filtered through Whatman no. 1 filter paper and then evaporated to give a final volume of 50 mL. The pH of the concentrate obtained was then adjusted to pH 12.0 with 2.0 mol L^{-1} NaOH and the samples were kept in the dark for 12 h at a temperature of 25 ± 2 °C. The pH was then adjusted to 2.0 with HCl solution and partitioned with ethyl ether three times. The ethereal extracts were combined and then evaporated in a rotary evaporator until complete removal of the solvent. Next, the extracts were dissolved in 2 mL of the MeOH/H$_2$O (70:30) mixture, sonicated (1400A ® Unique, Sao Paulo, SP, Brazil) for 2 min at room temperature (25 ± 2 °C), passed through an SPE column (Strata C18-E/Phenomenex previously conditioned with 5 mL MeOH and 5 mL ultrapure H$_2$O) and then eluted with a solution of MeOH/H$_2$O (70:30) with the volume adjusted to 10 mL. Upon injection into the HPLC–ESI-MS/MS, the extracts were further diluted with methanol.

2.7.1. Identification and quantification of phenolic compounds by HPLC–ESI-MS/MS

The HPLC–ESI-MS/MS analysis was performed on a 1200 high-performance liquid chromatography (HPLC) system (Agilent Technologies — Waldbronn, Germany). Separation was performed in a Zorbax Eclipse XDB-C8 column (150 mm, 2.1 mm ID, 3.5 μm particle size). The liquid chromatography analysis was carried out using a mobile phase gradient consisting of acetonitrile:water (95:5) (A) and 0.1% formic acid in water (B) with an initial condition of 15% A. The mobile phase was then linearly increased to 95% A and 5% B over 10 min. This condition was maintained for 5 min, giving a total runtime of 15 min. The initial conditions were returned to 15% A and 85% B and equilibrated for a further 10 min. The column temperature was set at 30 °C, the flow rate was 250 μL min^{-1} and the sample injection volume was 5 μL. The HPLC system was coupled to a mass spectrometer system consisting of a hybrid triplequadrupole/linear ion trap mass spectrometer, model API 3200™ (Applied Biosystems/MDS Sciex, Concord, Canada). Analyst version 1.5.1 was used for the HPLC–ESI-MS/MS system control and data analysis. The mass spectrometry was tuned in negative and positive modes by infusion of a polypropylene glycol solution. The experiments were performed using the TurbolonSpray® source (electrospray ionization—ESI) in positive ion mode. The capillary needle was maintained at 5500 V. The MS/MS parameters were as follows: curtain gas, 10 psi; temperature, 400 °C; nebulizer gas, 45 psi; auxiliary gas, 45 psi; and collision gas, medium. Other parameters for the cone and collision energy are listed in Table 1 and these optimal conditions were obtained by the infusion of the standards of phenolic compounds at concentration of 1 mg L^{-1} using methanol as solvent. The polyphenolic compounds were monitored using the scan mode multiple reaction monitoring (MRM).

2.8. Procedure to determine antioxidant activity (AA)

2.8.1. Extract preparation

The extracts were prepared using 10 g of fresh sample (aerial part) with 25 mL of MeOH in an ultrasonic bath (Unique® 1400A, Sao Paulo, SP, Brazil) at room temperature (25 °C) for 30 min and then centrifuged at 1000 ×g for 10 min using a Fanem® centrifuge, model 280R (Fanem, Sao Paulo, SP, Brazil). The supernatants recovered were used to evaluate the antioxidant activity.

2.8.2. DPPH radical scavenging activity assay

The antioxidant activity against DPPH was determined according to the method Brand-Williams, Cuvelier, and Berset (1995) adapted of Kim, Lee, Lee, and Lee (2002). A methanol solution containing 0.01 mmol L^{-1} DPPH was prepared fresh daily and stored at 20 °C until use. The ethanol solution of the DPPH radical (2.9 mL) was placed in a glass cuvette and the absorbance at 515 nm in $t = 0$ (t_0) was measured using a Hewlett–Packard spectrophotometer, model HP 8452A (Cheadle Heath, Stockport Cheshire, UK). The extract (100 μL) was then added and the mixture was shaken well and incubated in the dark for 30 min (t_{30}) at room temperature (25 °C). The absorbance was taken at 515 nm. Inhibition of DPPH free radicals, in percent terms (I %), was calculated according to the formula:

$$\% \text{ inhibition} = [1 - (\text{absorbance sample}_{t=30min} / \text{absorbance control}_{t=0min})]$$

The antioxidant activity of the samples was expressed as μmol Trolox equivalent antioxidant capacity 100 g^{-1} of fresh matter (μmol TEAC 100 g^{-1} FM), through a calibration curve Trolox 75–1050 μmol·L^{-1}. Each determination was performed in triplicate and was repeated at least three times.

2.8.3. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity was estimated by performing the FRAP assay, following the procedure described in the literature by

Table 1
Operating parameters for analysis in positive MRM mode of the polyphenolic compounds.

Analytes	DP[a]	EP[a]	CEP[a]	CE[a]	CXP[a]
Vanillic acid	26	5.5	10	17/35	4
Chlorogenic acid	26	2.5	18	17/75	6/4
Caffeic acid	21	5	12	11/37	4
Coumarin	36	8.5	10	31/23	4
p-Coumaric acid	21	7.5	14	11/35	4
4-Methylumbelliferone	31	8	10	45/23	4
Coniferaldehyde	31	4	14	13/31	4
Syringaldehyde	21	3.5	10	19/29	4/10
Scopoletin	51	6	12	25/27	4
Ferulic acid	21	3	12	11/17	4
Syringic acid	21	5	12	11/33	4
Sinapaldehyde	31	3.5	16	45/27	4/8
Sinapic acid	16	7	12	13/35	4
Resveratrol	36	3.5	14	31/19	4
Apigenin	21	4.5	16	13/31	4
Naringenina	41	4	12	27	4
Luteolin	46	4	16	113/89	4/30
Kaempferol	51	4.5	14	39/45	4
Aromadendrin	26	5	12	15/19	4
Hispidulin	46	5	14	23/43	4
Quercetin	51	5	14	73/77	4
Apigenin 7-glucoside	51	7	22	29/45	4
Myricetin	26	4	24	19/33	4
Isoquercetin	21	4.5	26	21/43	6/4
Naringin	16	5	16	23/57	4
Rutin	21	5	24	29/19	6
Cinnamic acid	21	4.5	12	11/25	4
Galangin	66	3.5	14	69/67	4
Catechin	31	3.5	14	19/17	4
Epicatechin	31	3.5	14	19/17	4

[a] DP — declustering potential; EP — entrance potential; CEP — collision energy potential; CE — collision energy; CXP — collision cell exit potential.

Benzie and Strain (1996). The extract (200 µL) and 200 µL of $FeCl_3$ (3 mmol L^{-1} in 5 mmol L^{-1} citric acid) were mixed in a tube and incubated for 30 min in a water bath at 37 °C. TPTZ solution (3.6 mL) was then added and the mixture was vortexed. After exactly 10 min the absorbance (620 nm) was read using a Hewlett–Packard spectrophotometer, model HP 8452A (Cheadle Heath, Stockport Cheshire, UK). The results were expressed as µmol Trolox equivalent antioxidant capacity 100 g^{-1} of fresh matter (µmol Fe^{+2} 100 g^{-1} FM), based on a calibration curve (Trolox 75–1050 µmol·L^{-1}). Each determination was performed in triplicate and was repeated at least three times.

2.9. Statistical analysis

All variables were reported as mean ± standard deviation (SD) of three replicates. The results were compared by the one-way analysis of variance (ANOVA) and T-test. To evaluate the relationship between the variables evaluated. The data analysis was performed using the software STATISTICA 7.0 (Statsoft Inc., Tulsa, OK, USA) and differences between the means were considered statistically significant at the 5% level (p < 0.05).

3. Results and discussion

3.1. Chemical analysis

The nutrient composition and mineral content of *S. ambigua* are shown in Table 2. Significant differences (p < 0.05) were revealed between the samples in terms of the contents of ash, insoluble dietary fiber, total carbohydrate and most minerals.

The results revealed that the moisture content represented the largest single component (88.15% and 88.57%) in the proximate composition of both samples of *S. ambigua*. Similar findings have been published for different halophytic species grown in Korea and China, with moisture contents of 88.42 to 90.9% (Lu et al., 2010; Min, Son,

Table 2
Nutrient composition of *Sarcocornia ambigua* collected from two different regions in state Santa Catarina, Brazil.

Proximate composition	Region A	Region B
Moisture (%)	88.15 ± 0.54^a	88.57 ± 0.60^a
Ash (g 100 g^{-1})	2.96 ± 1.31^a	3.64 ± 1.21^b
Crude Protein (g 100 g^{-1})	1.93 ± 0.02^a	2.06 ± 0.01^a
Lipids (g 100 g^{-1})	0.16 ± 0.01^a	0.12 ± 0.09^a
Soluble dietary fiber (g 100 g^{-1})	<0.5	<0.5
Insoluble dietary fiber (g 100 g^{-1})	2.75 ± 0.10^a	1.97 ± 0.03^b
Total carbohydrate (g 100 g^{-1})	3.55^a	3.14^b
Major minerals		
Sodium (mg g^{-1})	10.19 ± 5.3^a	16.57 ± 1.8^b
Potassium (mg g^{-1})	2.90 ± 1.1^a	1.81 ± 0.1^b
Magnesium (mg g^{-1})	0.92 ± 0.5^a	1.30 ± 0.1^b
Calcium (mg g^{-1})	0.54 ± 4.1^a	0.53 ± 0.2^a

Values expressed in fresh matter.
Results expressed as mean ± SD of triplicates.
[a–b] Different superscript letters between samples denote significant differences (ANOVA, p < 0.05).

et al., 2002). The ash content ranged from 2.96% (sample A) to 3.64% (sample B) on a fresh matter basis. These values are two to three times lower than those reported by Min, Son, et al. (2002) and Lu et al. (2010) for *S. herbacea* and *S. bigelovii* (4.36 to 6.2%). Overall, the large oscillations in the ash content may be related to the content of minerals, which varies widely between species of the genus *Salicornia* and *Sarcocornia* (Min, Lee, et al., 2002).

The crude protein content ranged from 1.93% (sample A) to 2.06% (sample B), in agreement with results reported by Min, Son, et al. (2002), Ventura et al. (2011) and Agawu (2012), who observed crude protein contents ranging from 1.7 to 2.0%; 2.0 to 3.59% and 1.20 to 4.66% respectively.

The lipid content was relatively low and ranged from 0.16% to 0.12%. The results were shown to be lower than those obtained in previous studies with *Salicornia europaea* (0.34%) (Guil, Torija, Gimenez, & Rodriguez, 1996), *S. herbacea* (0.20 to 0.30%) (Min, Son, et al., 2002) and *S. bigelovii* (0.37%) (Lu et al., 2010).

The total carbohydrate content ranged from 3.55% (sample A) to 3.14% (sample B), in contrast to the result reported by Lu et al. (2010) (4.48%). The predominant dietary fiber was insoluble and ranged from 2.75% (sample A) to 1.97% (sample B). This content is 2–3 times higher than that reported by Lu et al. (2010) for *S. bigelovii* (0.83%).

Wide variations in the chemical compositions of *Salicornia* and *Sarcocornia* species growing in different geographical locations with different soil conditions (salinity) have been reported (Lu et al., 2010; Min, Son, et al., 2002; Ventura et al., 2011). This study verifies the data presented in these reports, suggesting that the geographical location in which *S. ambigua* grows and the crop system used affect the chemical composition of the plant material.

3.2. Major minerals

The major minerals of *S. ambigua* (on a fresh matter basis) are presented in Table 2. The results revealed that there were significant differences (p < 0.05) with wide variability between samples A and B for sodium, potassium and magnesium. In all samples the mineral present in highest amounts was sodium, followed by potassium, magnesium and then calcium. These results are similar to those reported by Min, Son et al. (2002), Lee, Rhin, and Kim (2009), Rhee et al. (2009), Lu et al. (2010), Parida and Jha (2010) and Ventura et al. (2011), who found that the mineral with the highest concentration in *S. herbacea*, *S. europaea*, *S. bigelovii*, *S. brachiata*, *S. persica* and *S. fruticosa* was sodium, followed by potassium, magnesium and then calcium.

The sodium content ranged from 10.19 mg g^{-1} (sample A) to 16.57 mg g^{-1} (sample B), in agreement with results reported by Min, Son, et al. (2002), Lu et al. (2010) and Ventura et al. (2011), who

observed corresponding sodium values of 10.03 mg g^{-1}, 9.98 mg g^{-1} and 15.57 mg g^{-1}, respectively.

The potassium, magnesium and calcium contents ranged between samples from 2.9 to 1.81 mg g^{-1}; 0.92 to 1.30 mg g^{-1} and 0.54 to 0.53 mg g^{-1}, respectively. Similar findings have been published for different halophytic species with potassium, magnesium and calcium contents ranging, respectively, from 1.76 to 2.71 mg g^{-1}, 0.87 to 1.23 mg g^{-1} and 0.52 to 0.62 mg g^{-1} (Ventura et al., 2011).

In summary, the variation in the mineral content of the *S. ambigua* samples expected, since the plant by accumulation of organic solutes and mineral ions may vary according to the geographic location, crop system, irrigation water salinity, maturity and harvest conditions (Grieve, Shannon, & Poss, 2001; Maggio, De Pascale, Fagnano, & Barbieri, 2011).

In this context, sodium is the main ion accumulated when plants are irrigated with moderately or hyper-saline water and the amounts of other ions, such as K$^+$, Mg^{2+} and Ca^{2+}, which confer nutritional value to the product, typically decrease with increasing irrigation water salinity, predominately as a result of competition with Na$^+$ during uptake (Flowers & Colmer, 2008).

3.3. Fatty acid profile

The results for the fatty acid profile, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *S. ambigua* grown in the different regions are given in Table 3. Significant differences ($p < 0.05$) were revealed between the samples only for the fatty acids that were not identified.

The fatty acid profile of *S. ambigua* showed a predominance of polyunsaturated fatty acids (PUFA) ranging from 60.61 to 61.32%, comprising mainly linolenic acid (44.04 to 44.70%) followed by linoleic acid (16.57 to 16.52%). The saturated fatty acids (SFA) represented 17.82 to 18.27% of the total lipid content, with the main constituent being palmitic acid (13.20 to 13.65%), and the monounsaturated fatty acids (MUFA) represented 4.49% to 4.59% of the total lipid content with the main constituent being oleic acid (3.84 to 3.92%). A similar trend in the fatty acids profile was reported by Ventura et al. (2011) for samples of *S. persica* and *S. fruticosa*, who observed that the most abundant fatty acid was linolenic acid, which ranged from 41.2 to 48.2% of the total fatty acids content, followed by linoleic acid (23.85 to 27.31%) and palmitic acid (20 to 21.13%).

The findings also showed that the lipid content of *S. ambigua* is relatively low (Table 2) and this plant is thus adequate for human consumption as a leafy vegetable, containing a high content of unsaturated fatty acids (65.1% to 65.9%) in its composition. For good nutritional quality, including beneficial health effects, the PUFA/SFA ratios should be higher than 0.45 (Guil et al., 1996), and in the present study the PUFA/SFA ratios ranged from 3.4 to 3.3.

In summary, the fatty acid profile observed for the samples of *S. ambigua* was as expected since, at the cellular level, in plants irrigated with saline water alterations to the membrane fatty acid composition prevent damage to the cellular membranes (Allakhverdiev, Nishiyama, Suzuki, Tasaka, & Murata, 1999). These alterations can lead to a significant amount of unsaturated fatty acids, mainly linolenic acid and linoleic acid (whose benefits to human health are well known), accumulating in the cellular membranes of the leaves of edible plants species, both halophytes and non-halophytes, with culinary uses (Simopoulos, 2004).

3.4. Structural characterization of phenolic compounds isolated by HPLC–ESI-MS/MS

Currently, limited published data are available on the composition and/or content of individual phenolic acids and flavonoids in halophytic species (*S. herbacea*, *S. bigelovii*, *S. persica*, *S. fruticosa*). In previous studies the phenolic acids and flavonoid contents were extracted and isolated by chromatography and the structural characterization of the isolated compounds was carried out by nuclear magnetic resonance (NMR) (Kong et al., 2009; Hwang, Yun, Chun, Chung, & Kim, 2009; Kim et al., 2011; Wang et al., 2013).

The fingerprinting of methanol extracts of *S. ambigua* led to the identification and quantification of phenolic acids and flavonoids based on their molecular formula and fragmentation pattern and by comparison of their retention times with those of commercially available standards.

In this procedure the extracts of *S. ambigua* were injected in order to obtain a qualitative profile and of the 30 analytes (Table 1) included in the method the samples initially presented mass fragmentation corresponding to 22 compounds. However, of these 22 compounds, seven were not confirmed. These compounds showed mass fragmentation similar to umbelliferone, 4-methylumbelliferone, coumarin, coniferaldehyde, sinapaldehyde, catechin and epicatechin but, although the fragmentation profiles were the same, a comparison with the chromatographic column retention times observed for the standards showed considerable differences for these 7 compounds. This may be due to the close structural similarity between some phenolic compounds, such as flavonoids, which have very similar fragmentation patterns.

After identification of phenolic compounds present in the samples, calibration curves were constructed with 7 points for the 15 different analytes identified. It was determined that the limit of quantification was the lowest point of the curve and the mixture of standards with the concentration corresponding to this value for each analyte was injected. Thus, the detection limit was obtained by dividing the lower limit of calibration curves for 3.3. The calibration curves were prepared from stock solutions of analytical standards at a concentration of 1000 mg L^{-1} (approximately) by successive dilution with MeOH: H$_2$O 50% (v/v) until the optimal range of application for each analyte. The calibration curves and the samples were injected in duplicate and the parameters for the linearity, coefficient of determination, detection and quantitation limits, as well as, retention times of the compounds are presented in Table 4.

Table 5 summarizes the 15 phenolic compounds identified in each extract, their retention time, protonation [M + H]$^+$ and phenolic content. The results reveal the presence of one coumarin (scopoletin), one phenolic aldehyde (syringaldehyde), eight phenolic acids (p-coumaric, cinnamic, vanillic, ferulic, caffeic, syringic, sinapic, and chlorogenic acids) and five flavonoids (galangin, quercetin, naringin, kaempferol and isoquercitrin).

The major phenolic acids and flavonoids quantified by HPLC–ESI-MS/MS in the extract of *S. ambigua* samples were ferulic, caffeic, vanillic, p-coumaric acids, kaempferol and galangin (Table 4). Ferulic acid was

Table 3
Fatty acids profile (%) from lipid fraction of *S. ambigua* samples.

Fatty acids	Region A[a]	Region B[a]
Lauric (C12:0)	0.45 ± 0.02 [a]	0.45 ± 0.01 [a]
Myristic (C14:0)	0.59 ± 0.03 [a]	0.58 ± 0.02 [a]
Palmitic (C16:0)	13.20 ± 0.38 [a]	13.65 ± 0.65 [a]
Palmitoleic (C16:1c)	0.65 ± 0.20 [a]	0.67 ± 0.02 [a]
Stearic (C18:0)	1.70 ± 0.23 [a]	1.70 ± 0.18 [a]
Oleic (C18:1c)	3.84 ± 0.16 [a]	3.92 ± 0.16 [a]
Linoleic (C18:2c)	16.57 ± 0.68 [a]	16.62 ± 0.52 [a]
Linolenic (C18:3c)	44.04 ± 1.20 [a]	44.70 ± 1.20 [a]
Arachidic (C20:0)	0.79 ± 0.03 [a]	0.79 ± 0.02 [a]
Behenic (C22:0)	1.08 ± 0.04 [a]	1.09 ± 0.03 [a]
Others	17.09 ± 0.30 [a]	15.83 ± 1.69 [b]
Total SFA	17.82 [a]	18.27 [a]
Total MUFA	4.49 [a]	4.59 [a]
Total PUFA	60.61 [a]	61.31 [a]

Results expressed as mean ± SD of triplicates.
[a–b] Different superscript letters between samples denote significant differences (ANOVA, $p < 0.05$).
[a] Results expressed as percentage.

Table 4
Some figures of merit of the method applied.

Analyte	RT (min)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Analytical range (mg/L)	Eq. of the calibration curves	R^2
Scopoletin	7.19	0.08	0.27	0.27–5.32	y = 295 474x + 54 306	0.9975
Syringaldehyde	7.09	0.11	0.37	0.37–7.36	y = 443 671x + 267 914	0.9944
Galangin	11.32	0.07	0.23	0.23–4.55	y = 155 647x + 19 275	0.9994
Kaempferol	9.72	0.07	0.23	0.23–4.50	y = 101 076x + 7749.3	0.9973
Quercetin	8.93	0.07	0.24	0.24–4.73	y = 35802x − 1838.9	0.9988
Naringin	7.48	0.07	0.24	0.24–4.77	y = 36578x + 6018.7	0.9975
Isoquercetin	6.85	0.06	0.20	0.20–4.09	y = 254117x + 28205	0.9958
Cinnamic acid	9.61	0.07	0.23	0.23–4.59	y = 616682x + 147776	0.9951
p-Coumaric acid	6.90	0.07	0.23	0.23–4.64	y = 308540x + 45202	0.9927
Vanillic acid	4.53	0.07	0.23	0.23–4.55	y = 176087x + 9858.2	0.9959
Caffeic acid	4.25	0.07	0.24	0.24–4.73	y = 209619x + 22547	0.9977
Ferulic acid	7.28	0.07	0.23	0.23–4.59	y = 537574x + 69816	0.9953
Syringic acid	4.53	0.08	0.25	0.25–5.09	y = 130353x + 9729.8	0.9993
Sinapic acid	7.18	0.07	0.23	0.23–4.55	y = 118800x + 120000	0.9896
Chlorogenic acid	2.64	0.07	0.23	0.23–4.64	y = 326704x − 979.78	0.9984

the most abundant phenolic acid in both samples, followed by caffeic acid and the most abundant flavonoid was galangin in both samples. The ferulic acid concentration (μg 100 g^{-1}) ranged from 914.4 (sample A) to 591.79 (sample B) and the caffeic acid concentration (μg 100 g^{-1}) ranged from 318.0 (sample A) to 171.04 (sample B) on a fresh matter basis. The galangin had a concentration of 20.15 μg 100 g^{-1} in sample A and 50.31 μg 100 g^{-1} in sample B, on a fresh matter basis. A similar result was reported for extracts of *S. herbacea* by Oh et al. (2007) and Zhu & Row (2010) who observed that the most abundant phenolic acids were ferulic and caffeic acids.

Previous research conducted on the phenolic composition of *S. herbacea* indicated the presence of chlorogenic, sinapic, ferulic, caffeic, salicylic, syringic, p-coumaric and trans-cinnamic acids, as well as dicaffeoylquinic acid derivatives and other phenolic acids, such as tungtungmadic acid, scopoletin and pentadecyl ferulate (Essaidi et al., 2013; Hwang et al., 2009; Rhee et al., 2009; Wang et al., 2013; Zhu & Row 2010), together with some flavonoids, such as myricetin, quercetin, kaempferol, rhamnetin, isorhamnetin, hesperetin, galangin, acacetin, quercetin-3-b-glucoside, rutin and other flavonoid glucosides (Essaidi et al., 2013; Kim et al., 2011; Lee et al., 2005).

These results indicate that the content of phenols and flavonoids can vary between different species within the same genus and also between different ecotypes. The authors suggest that the different environmental stress conditions (temperature, salinity, water availability, light intensity, nutrient deficiency, ionic stress), under which halophytes species grow, increase the synthesis of small, non-enzymatic molecules with antioxidant properties, such as ascorbate, glutathione, carotenoid and polyphenolic compounds, in order to decrease the production of reactive oxygen species (ROS) (Buhmann & Papenbrock, 2013; Ventura & Sagi, 2013).

According to Buhmann and Papenbrock (2013) the influence of the growth conditions on the biosynthesis of phenolic compounds can be readily tested by cultivating halophytes under different environmental conditions.

The samples collected from the natural marsh (region A) had a higher number of flavonoids (galangin, kampferol, quercetin and isoquercitrin) and phenolic acids (p-coumaric, vanillic, caffeic, ferulic, syringic, sinapic and chlorogenic acid) identified. Ferulic and caffeic acids were the major phenolic compounds in both regions; however, higher concentrations were present in samples collected from the natural marsh. The phenolic profile of the samples under study is reflected in their antioxidant activity, which will be discussed in the next section.

3.5. Antioxidant activity (AA)

The study on the antioxidant activity (AA) of the plant extracts was focused on the phenolic compounds and the two assays used to assess the AA are based on different radicals and mechanisms of reaction. The results for the total phenolics content and the DPPH and FRAP assays are summarized in Table 6.

These variations were to be expected since many factors affect the phenolic content of plants, such as the presence of other constituents and/or of different types of phenols, time of harvest, growth conditions (soil conditions), temperature, light and frequency and duration of inundation by saline water (Ghosh & Scheepens, 2009).

The evaluation of the antioxidant activity of the *S. ambigua* extracts showed large variations in the results obtained, with statistically significant differences (p < 0.05) between the regions, demonstrating that the growing region influences the phenolic compounds and,

Table 5
Identification and quantification of phenolic compounds in *S. ambigua*.

Phenolic compounds	Experimental mass [M-H]$^+$ (m/z)	MS/MS (m/z)	Phenolic content	
			Region A[a]	Region B[a]
Scopoletin	193	133/178	<LOQ	<LOQ
Syringaldehyde	183	123/77	<LOQ	<LOQ
Galangin	271	77/69	20.15	50.31
Kaempferol	287	153/121	41.00	<LOD
Quercetin	69	303/69	30.94	<LOD
Naringin	681	273/153	<LOQ	<LOQ
Isoquercetin	465	319/85	15.11	21.95
Cinnamic acid	149	131/103	<LOQ	<LOQ
p-Coumaric acid	165	147/91	56.84	<LOQ
Vanillic acid	169	93/65	63.31	<LOQ
Caffeic acid	181	163/89	318.00	171.04
Ferulic acid	195	177/145	914.44	591.79
Syringic acid	199	155/77	25.18	<LOQ
Sinapic acid	225	207/91	<LOQ	<LOQ
Chlorogenic acid	355	163/89	<LOQ	<LOQ

ND = not detect; LOQ = limit of quantification; LOD = limit of detection.
[a] Values expressed μg 100 g^{-1} fresh matter.

Table 6
Availability of the antioxidant activity (AA).

Region	DPPH[a] (μmol TEAC 100 g^{-1})[a]	FRAP[a] (μmol Fe^{+2} 100 g^{-1})[a]
A	135.83 ± 5.00 [a]	170.14 ± 3.36 [a]
B	34.64 ± 4.56 [b]	31.92 ± 5.73 [b]

All results expressed as mean ± SD of triplicates.
TEAC, Trolox equivalent antioxidant activity.
[a–b] Different superscript letters for samples in the same column denote significant differences (ANOVA, p < 0.05).
[a] Values expressed in fresh matter.

consequently, the antioxidant activity. The AA for samples measured by DPPH ranged from 34.64 to 135.83 μmol TEAC 100 g^{-1} and by FRAP from 31.92 to 170.14 μmol Fe^{+2} 100 g^{-1}.

The percentage inhibition of DPPH radicals was highest for the extracts of region A with 18.32 ± 0.70%, followed by the extracts of region B (10.26 ± 1.4%), with statistically significant differences ($p < 0.05$).

In summary, the results show that all plant samples have relatively high antioxidant and free radical scavenger activity but sample A had the highest antioxidant activity. The variation in the AA described in this study for the samples of *S. ambigua* can be attributed to the origin of the plant material. The sample collected from region A was growing in natural tideland near the coast, in the presence of high salt concentrations in the soil and the sample collected from region B was grown as an experimental crop irrigated once a day with a seawater effluent and fertilized with sludge collected from settling tanks at a shrimp *L. vannamei* farm. There is a general consensus that salt stress reduces gas exchange, thereby limiting the CO_2 supply to the leaf, and that it causes an over-reduction of the photosynthetic electron transport chain leading to the production of ROS. In response to this type of oxidative damage in halophytes (salt-tolerant vegetation) the synthesis of antioxidants is induced (Ashraf, 2009).

Most studies on plant antioxidants have shown that the production of antioxidants is enhanced in plants in response to salinity to counteract the salt-induced elevated levels of ROS in the cells. However, variations in the production of antioxidants in response to salt stress are evident at the inter-species or intra-species level. The variations in the seawater components, the frequency and duration of inundation by saline water and the salt marsh plants present not only affects the oxygen availability but also influences the production of antioxidants (Ashraf, 2009; Garratt et al., 2002).

4. Conclusions

This paper reports the content of individual phenolic acids and flavonoids in *S. ambigua* for the first time, with 15 phenolic compounds identified in each extract. Ferulic and caffeic acids were the major phenolic acids and galangin, kampferol and quercetin were the major flavonoids quantified in the *S. ambigua* extracts. The results of the current study suggest that the combination of phenolic compounds present in the *S. ambigua* extracts may be responsible for the observed antioxidant activity. This information can be useful in determining the possible role of the compounds identified which can participate in the prevention of different health disorders. Further studies are needed to evaluate the bioabsorption and bioavailability of the compounds present in *S. ambigua*, as well as the interactions between them, after consumption.

However, despite differences in the composition of the plants, the cultivation of this species is viable and can be applied to enhance the production and consequently encourage higher consumption.

In summary, the findings of this study highlight the potential of this halophyte as a valuable source of natural antioxidants and nutrients for use in the food and pharmaceutical industries.

Acknowledgments

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