Nutritional and Functional Potential of Selliera radicans Cav., a Chilean Native Halophyte

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ABSTRACT

Background: Selliera radicans was recognized as one of the foods consumed by the oldest human settlement in America (Monte Verde, Chile) that had a diet with a high component of plants. Objective: This study aims at investigating nutritional and functional characteristics of S. radicans, a native halophyte from Chile. Materials and Methods: An analysis of total protein, carbohydrate, ash, and moisture from S. radicans leaves was performed, using standard methods. The content of macro and micronutrients was quantified by atomic absorption spectrometry. The inulin content was carried out based on the Seliwanoff reactions. S. radicans leaves were extracted with methanol and the total content of phenolic and flavonoids and antioxidant activity were evaluated by spectroscopic method. Results: Leaves from cultivated plants proved to be a suitable source of proteins (75 % on DW), ash (6.8 % on DW), and a wide range of macro and micronutrients, where Ca, K, and Na had the highest values. In addition, inulin (2.3 % on DW), total phenolics (63.4 GAE/g LDW) and flavonoids (21.8 QE/g LDW), and antioxidant capacity (10 TE/g LDW) were noted. Conclusions: According to the results, cultivated S. radicans leaves are promising sources of food with beneficial health properties.

Key words: Antioxidant activity, Goodeniaceae, Inulin, Macro and Micronutrients, Total Phenolics, Total Flavonoids.

INTRODUCTION

The wide range of edaphoclimatic variables that characterize Chile is reflected in its large and specific vegetal biodiversity. Only a small group of native plant species has been explored and studied. Therefore, there are still few scientific papers published about Chile’s typical flora. Among the little studied species are the halophytes. Halophytes represent roughly 1 % of the world’s flora (both dicots and monocots) and are recognized for their ability to survive in saline environments. Plants are widely distributed in arid, semi-arid, and wetland regions, throughout the tropical and temperate areas of the planet. Traditionally, halophytes have been used as food and source of medicinal substances during the last centuries. Nowadays, halophytes are used as source of vegetable fibers, bio-fuel, as stabilizers or phytoextracts of heavy metals in bioremediation processes, as landscape plants and others purposes. According to eHALOPH data, in Chile there are 138 halophyte species distributed in 31 families, and more than 80 % of these species are herbaceuous and about 55 % are exotic.

Goodeniaceae is a Eudicot family in the order Asterales comprising more than twelve genera and typical metabolites of Goodeniaceae species, Weber (1955) reported the presence of inulin, a group of naturally occurring polysaccharides considered as a probiotic, in samples of Selliera radicans Cav. Selliera radicans Cav. is a halophyte native to Australia, Chile and New Zealand. According to archaeological studies, fragments of edible seeds and stems of S. radicans together with a diverse range of other plant specimens, including wood artifacts, were found near Puerto Montt, in a site that would correspond to a habitat dating back 14 500 thousand years, the oldest in its genre discovered in Chile.

In Chile, Selliera radicans grows naturally from region Atacama to region Aysén del General Carlos Ibáñez del Campo, in riparian zones near rivers, lakes, and the sea. Local inhabitants know this creeping herbaceous plant as “hierba de las marismas”. It is characterized by stolons that hold nodal fibrous roots, succulent green, shiny leaves, and small white flowers. It is easy to propagate, grows relatively quickly, and survives temporary flooding. In addition, it is used in landscaping as a groundcover in gardens and green roofs in houses.
It has been observed that rabbit, black-necked swans, and other animals, consume the leaves of *Selliera radicans*. Thus, a chemical study was performed related to the nutritional and functional properties of *Selliera radicans* leaves, obtained from a controlled crop. There are no nutritional or phytochemical published studies involving *Selliera radicans*.

**MATERIALS AND METHODS**

**Collection of Plant material**

Leaves were collected from cultivated *Selliera radicans* in a polyethylene-covered greenhouse, located at the Universidad de Talca, Talca, Chile, and maintained at environmental temperature. The *Selliera radicans* plants grown in this greenhouse are originated from wild samples of this species collected from Vichuquén (Maule Region, 34°51’ SL-72°00’ WL), Chile. The botanist Dr. Patricio Peñailillo identified this species and a voucher specimen (No. 3367) was deposited at the Herbarium Universidad de Talca.

**Nutrition and functional characterization**

Leaves of *Selliera radicans* were washed with tap water, dried in oven (Memmert ULM 700), grounded (Wiley® Mini-Mill, “Thomas”), and stored in dark bags at -18°C. Moisture content was measured according to the AOAC method. An empty container and the lid were dried in an oven at 106°C for 3 h and transferred to desiccators until cool and weight was recorded. Then, a sample of *S. radicans* was placed in the container and oven dried at 40°C until constant weight. At the end of drying, the container with partially covered lid was transferred to a desiccator for cooling and the container weight was recorded as above. Ash content was calculated according to AOAC method. Crucible and the lid were left in the furnace until fumes were no longer produced. Then, the crucible was heated at 555°C for 12 h. The crucible was cooled in a desiccator and the weight was recorded. Weight of a sample of *S. radicans* and crucible was then recorded. Crucible with the sample was heated over low Bunsen flame with half covered lid and placed in a muffle furnace until fumes were no longer produced. Then, the crucible was heated at 555°C for 12 h, cooled in a desiccator and the weight was recorded. Total carbohydrates were analyzed by ultraviolet spectrophotometry, using UV-1800 Shimadzu Spectrophotometer, and following the method described by Morse. A 0.2% anhydrous solution was prepared in concentrated sulfuric acid and then stored in a dark bottle in the refrigerator. Standard solutions were prepared by serial dilutions of a glucose stock solution of 200 mg L⁻¹. Methanol extract of 100 mg of dried and ground leaves from *S. radicans* were obtained by sonication (30 minutes). Subsequently the methanol extract was centrifuged at 800 rpm. The absorbance of the solution was measured in a spectrophotometer at a wavelength of 630 nm, against a target prepared under the same conditions of the sample. The total nitrogen content in *S. radicans* leaves was determined following the Dumas method using the analyzer equipment TruSpec micro CN (LECO). Crude protein was calculated by multiplying the evaluated nitrogen by 6.25. The content of Ca, Na, K, Mg, Fe, Zn, Cu and Mn was spectrophotometrically determined using an atomic absorption equipment (Agilent 280FS AA). For the analysis of inorganic substances, samples of *Selliera radicans* leaves were washed with bidistilled water. 1.000 g of leaves was calcined in a muffle for 6 hours at 550°C for 1 h and subsequent extraction with 0.36 N H₂SO₄. Then, 5 ml of leaves extract were mixed with 2 ml of ammoniac acetate buffer (pH 5.5), and 2 ml of 0.02 M EDTA in a B-free test tube and vortexed. Then to the resulting mixture was added 1 ml of azomethine-H reagent (0.9% azomethine-H + 2% ascorbic acid solution). It was vortexed gently and the resulting solution was allowed to stand for 1 h at room temperature, and vortexed again, and the readings were taken at 420 nm using the spectrophotometer. Phosphorus content was determined in 2 ml of each one of the freshly prepared solutions, reading the absorbance at 650 nm using ammonium molybdate, hydroquinone and sodium sulphide solutions according to the AOAC method. *S. radicans* leaves from the greenhouse were collected at three different times (July and September 2015 and March 2016). The inulin content in these leaves was determined by spectrophotometry, measuring total fructose content, based on the Seliwanoff reaction. Dry samples were dissolved in 80% ethanol solution and the extraction procedure was performed by ultrasound with a water bath for 25 minutes. Then, they were left at rest for six hours previously covered so that the solvent did not evaporate. After this time, the extracts were filtered on filter paper and the liquid extracts were evaporated in a rotavaporator. The liquid extracts that did not evaporate were frozen and then lyophilized, providing extracts rich in inulin. Then, 500 mg of each of the dry extracts rich in inulin were dissolved in 20 ml of distilled water, and placed in a water bath (90°C) for about 10 minutes. Then, 70 ml of distilled water were added to each one of the flasks and again in a water bath for 30 minutes with occasional shaking. After the time, they were allowed to cool and adjusted to 100 ml. Next, the hydrolysis of the inulin contained in each of the aqueous extracts was carried out using 2 ml of each one of the freshly prepared EDTA in a B-free test tube with 2 ml of 0.02 M EDTA, reading the absorbance at 650 nm using ammonium molybdate, hydroquinone and sodium sulphide solutions according to the AOAC method. *S. radicans* leaves from dry ashing in a muffle furnace at 550°C. Dry leaves were dissolved in concentrated sulfuric acid and then stored in a dark bag at -18°C. Moisture content was measured according to the AOAC method. It has been observed that rabbit, black-necked swans, and other animals, consume the leaves of *Selliera radicans*. Thus, a chemical study was performed related to the nutritional and functional properties of *Selliera radicans* leaves, obtained from a controlled crop. There are no nutritional or phytochemical published studies involving *Selliera radicans*.
equivalents (GAE) per g of dry leaves based on a calibration curve of concentrations with known gallic acid (1-4 μg gallic acid mL⁻¹). The calibration curve had a correlation coefficient of 0.9999. The total flavonoids were determined by the AlCl₃ method. The methodology was based on the reaction between the OH groups of flavonoids and aluminum (Al³⁺) which forms a pink complex that can be measured spectrophotometrically at 415 nm. The methanol extract of the leaves of S. radicans was obtained following the same methodology used in the analysis of total phenolics. Then, 500 μL of centrifuged methanolic extract were mixed with 1.5 mL of ethanol, 100 μL of AlCl₃, 10%, 100 μL of potassium acetate 1M and 2.8 μL of distilled water. The solution obtained was incubated at room temperature for 30 minutes. The results were expressed at μmol of gallic acid equivalent (GAE) per g of dry leaves based on a calibration curve of concentrations with known quercetin (25-100 μg quercetin mL⁻¹). The calibration curve had a correlation coefficient of 0.9953. The antioxidant activity of methanolic extracts of the S. radicans leaves samples was determined by Free Radical Scavenging Assay (DPPH) (2,2-diphenyl-1-picrylhydrazyl) as described in the literature with some modifications. It was prepared a methanolic solution of DPPH at 20 mg L⁻¹. The standard used was Trolox and methanolic solutions were prepared in a concentration range of 0.1 to 2 μg mL⁻¹. A calibration curve was created with a correlation coefficient value of 0.9946. The reaction was initiated by directly incorporating into test tubes, 2940 μL of DPPH and 60 μL of methanolic extract. The reaction mixtures were incubated for 30 minutes in the dark, and then the absorbances were measured at 517 nm in a spectrophotometer. The results were expressed as μmol trolox equivalent (TE) per g of dry leaves. Aqueous extracts [decoction, infusion and cold (0 oC)] were used for comparison between groups, and post analysis post hoc Dunnett’s was used to compare with the control group or post hoc Bonferroni comparisons between all groups. Differences in p<0.05 were considered statistically significant. Data were analyzed and plotted using the computational statistical software GraphPad Prism 4.0.

RESULTS

The results obtained in the analysis of nutritional and functional characteristics from Selliera radicans leaves are shown in Table 1 and 2. The results displayed that S. radicans leaves have similar water, ash, protein, macro and microelements contents to some raw vegetables (Table 3), except for the sodium content which is higher.

DISCUSSION/CONCLUSION

The nutritional characteristics of cultivated Selliera radicans leaves are shown in Table 1. Its moisture content is comparable to edible plants species such as broccoli, parsley, dandelion, basil, and others. This value is within the range for halophytes, for instance, moisture content of Chilean Sarcocornia neei aerial succulent shoots is 86.96%, almost equal to the leaves of cultivated Selliera radicans. Ash content in Selliera radicans is similar to broccoli and Chinese cabbage. In general, the percentage of ash in halophytes is characteristically high (2-36% on dried sample). According to the literature, the percentage of proteins in halophytes comprises values ranging from 6-21% on a dry weight basis. The protein content in Selliera radicans is within this range and comparable to vegetables consumed daily. Macro and micronutrients contents of halophytes depend on several factors, such as the plant species, stage of growth, season, and others.

Content of macro and micronutrients in cultivated Selliera radicans leaves are in Table 2 and all these values are lower than cultivated S. neei. For instance, in 100 g of fresh S. neei plants collected in coastal marsh and irrigated with sea water there were 8.07 g of Na, while in S. radicans there were only 0.66 g of Na, irrigated with tap water containing 17 mg L⁻¹ sodium. The leaves from cultivated Selliera radicans are a good source of carbohydrates, total phenolics and flavonoids, and antioxidant activity (Table 1). Likewise, the results indicate that leaves are a source of inulin, a prebiotic fiber with powerful health properties. This value is similar to values found in artichokes (1.7%) and asparagus (2.5%), species cultivated worldwide as food source. In Table 3 the nutrition content from Selliera radicans leaves was compared with some vegetables commonly consumed in the world. In comparison to asparagus (Asparagus officinalis), broccoli (Brassica oleracea var. italica), Chinese cabbage (Brassica rapa subsp. pekinensis), alfalfa sprouts (Medicago sativa), and spinach (Spinacia oleracea), Selliera radicans has higher values for Ca, Mg and Na.

Table 1: Main physicochemical data related to nutritional and functional characteristics of cultivated Selliera radicans leaves.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content</th>
</tr>
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<tbody>
<tr>
<td>Proximate</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>87.8 ± 3.2 %</td>
</tr>
<tr>
<td>Dry matter</td>
<td>12.2 ± 3.2 %</td>
</tr>
<tr>
<td>Ash</td>
<td>6.8 ± 0.1 % DW</td>
</tr>
<tr>
<td>Protein</td>
<td>7.5 ± 0.3 % DW</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>14.2 ± 1.3 %</td>
</tr>
<tr>
<td>Health promoting substances</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>2.3 ± 1.0 (% DW)</td>
</tr>
<tr>
<td>Total phenolics</td>
<td>63.4 ± 1.0 (GAE/g LDW)</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>21.8 ± 0.3 (QE/g LDW)</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>10.0 ± 0.2 (TE/g LDW)</td>
</tr>
</tbody>
</table>
| DME | Dry methanolic extract; DW = Dry weight; GAE = Gallic acid equivalent; QE = Quercetin equivalent; LDW = Leaves in dry weight; TE = Trolox Equivalent.

Table 2: Macro and micronutrients of cultivated Selliera radicans leaves.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>%</th>
<th>Micronutrients</th>
<th>mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.92</td>
<td>B</td>
<td>68</td>
</tr>
<tr>
<td>K</td>
<td>2.62</td>
<td>Cu</td>
<td>8</td>
</tr>
<tr>
<td>Na</td>
<td>5.42</td>
<td>Fe</td>
<td>75</td>
</tr>
<tr>
<td>Mg</td>
<td>0.82</td>
<td>Mn</td>
<td>41</td>
</tr>
<tr>
<td>P</td>
<td>0.20</td>
<td>Zn</td>
<td>29</td>
</tr>
</tbody>
</table>
content was the only mineral that had a lower value compared to other vegetables.

Among the three aqueous extracts prepared, the one obtained by decoction was chosen to perform the toxicity test because it had the lowest IC₅₀ value. The cell viability was not affected by the extract, even at the highest concentration at 488 µg mL⁻¹. The toxicity test helped to confirm that this plant does not harm mammalian cells and supports the thesis that the earlier inhabitants of Monte Verde consumed this plant.

Under the global warming scenario, new sources of food are needed to ensure food availability.⁴ The edible plants species that can cope with environmental stress factors could be a great approach. According to the results, Selliera radicans may be a potential new source of food with health benefits. This is the first report about nutritional and functional study of Selliera radicans.

The nutritional and functional preliminary analysis revealed that cultivated Selliera radicans leaves are an important source of ash, proteins, B, Ca, Cu, Fe, K, Na, Mg, Mn, P, Zn, inulin, total phenolics, and antioxidant capacity. Given the nutritional and functional characteristics of Selliera radicans leaves, it may be considered a promising source of food with healthy beneficial properties.

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CONFLICTS OF INTEREST

No potential conflict of interest was reported by the authors.

REFERENCES

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