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PHYSICOCHEMICAL ATTRIBUTES AND BIOACTIVE PROPERTIES OF STATICE LEAVES

Atributos fisico-químicos e propriedades bioativas de folhas de statice

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Abstract: Some ornamental plants have been receiving special attention, not only for their use in landscaping, but also because of their bioactive, aromatic and spicy properties. The objective of this study was to characterize physical and chemical attributes and antioxidant activity in leaves of three cultivars of statice (*Limonium sinuatum*). The cultivars used were QIS White, QIS Blue Dark, and QIS Yellow. The content of total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio, pH, vitamin C, anthocyanin, flavonoids, total phenolic compounds (TPC), total antioxidant activity (TAA) and the relationship between TPC and flavonoids and TAA were analyzed for each cultivar. The results showed that the leaves of the QIS White cultivar have a higher TSS/TTA ratio, indicating better flavor. The QIS White cultivar also showed higher vitamin C and flavonoid content. The cultivars QIS White and QIS Blue Dark showed higher TPC contents, but there were no differences in TTA and anthocyanin content. The average TAA values were 94.3%, indicating that all cultivars have a high capacity to sequester the DPPH radical. There was a positive relationship between TPC with flavonoids and TAA (R^2=0.6168 and 0.8826, respectively). The leaves of statice plants, traditionally used in landscaping and as a cut flower, have potential for use in human food.
Keywords: *Limonium sinuatum* (L.) Mill. Cut flowers. Phenolic compounds. Antioxidant activity.

Resumo: *Algumas plantas ornamentais vêm recebendo atenção especial, não apenas por seu uso no paisagismo, mas também devido a suas propriedades bioativas, aromáticas e condimentares. O objetivo desse estudo foi caracterizar atributos físicos, químicos e a atividade antioxidante em folhas de três cultivares de statice (Limonium sinuatum). As cultivares utilizadas foram QIS White, QIS Blue Dark e QIS Yellow. Foram analisados, o conteúdo de sólidos solúveis totais (SST), acidez total titulável (ATT), relação SST/ATT, pH, vitamina C, antocianinas, flavonoides, compostos fenólicos totais (CFT), atividade antioxidante total (AAT) para cada cultivar, além da relação entre os CFT e flavonoides e AAT das plantas. Os resultados mostraram que as folhas da cultivar QIS White tem maior relação entre SST/ATT, indicando melhor sabor. A cultivar QIS White também apresentou maior conteúdo de vitamina C e flavonoides. As cultivares QIS White e QIS Blue Dark apresentaram maiores teores de CFT, porém não houve diferenças para ATT e o conteúdo de antocianina. Os valores médios da AAT foram de 94,3%, indicando que todas as cultivares apresentam elevada capacidade de sequestrar o radical DPPH. Houve relação positiva entre os CFT com os flavonoides e AAT (*R^2*=0,6168 e 0,8826, respectivamente). As folhas das plantas de statice, tradicionalmente empregadas no paisagismo e como flor de corte, apresentam potencial de uso na alimentação humana.*
Palavras-chave: *Limonium sinuatum (L.) Mill. Flores de corte. Compostos fenólicos. Atividade antioxidante.*

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1 INTRODUCTION

The search for a healthy diet has increased the demand for new foods and forms of production, keeping in mind their functionality and sustainability. In this sense, in the last decades, there has been a growing insertion in the human diet of plants that are little known or that have more than one purpose. This makes edible leaves a good material for culinary innovation[1]. In the context of multifunctionality, besides having ornamental value, countless plants also have characteristics that make them attractive for human food, raising interest in cultivation not only for ornamental purposes but also for alimentary ones[2-3].

Among the plants that have been receiving increased attention in recent years are those of the genus *Limonium*, family Plumbaginaceae, with more than 600 reported species distributed in different areas, including steppes, grasslands and ponds worldwide[4]. Many of these plants are traditionally used as ornamentals, in food, and in folk medicine[5]. Among them is *Limonium sinuatum*, which in recent years has been receiving attention in Brazil as a cut flower, especially due to the beauty and durability of the flowers after harvest[6]. Commonly known as statice, evergreen, limonio, and sea lavender, the leaves are part of the Mediterranean diet, for which wild foods are an important element. The leaves can be eaten raw as a salad or in the preparation of various dishes, especially stewed[5].

In recent years, studies on the use of statice leaves in human food, their nutritional characteristics and health benefits have been conducted. Some results found in the literature report characteristics in the leaves of different statice species as high contents of flavonoids, vitamin C, total phenolic compounds and antioxidant activity[6-7]. The plant exhibits anti-inflammatory and antitumor action[8-9], and is employed in the treatment of fever, menstrual disorders and colic[5].

However, the use of the plant for food purposes in Brazil is restricted, attributed to the lack of knowledge of its bioactive properties being commonly used in gardens and as a cut flower. Given the bioactive potential of statice leaves, the objective of this study is to generate information about the physical, chemical bioactive properties in the leaves of three statice cultivars produced in Brazil as a cut flower. Our hypothesis is that the leaves of the three statice cultivars have bioactive properties with the potential for use in human food.

2 MATERIAL AND METHODS

The statice plants (*Limomium sinuatum*) of the cultivars QIS White, QIS Yellow and QIS Blue Dark were grown in the experimental area in Rio do Sul, SC (latitude 27º11'16", longitude 49º39'22", and altitude 673 m). The climate is Cfa, subtropical humid with hot summers, annual mean temperature of 18°C, and soil classified as Haplic Cambisol. The seeds (Ball Seeds®) were sown in polyethylene trays filled with Maxfertil® commercial vegetable substrate. After sowing, the trays were kept for five days in the dark in a cool environment until emergence. They were then placed in the greenhouse until they had between six and eight leaves.

The plants were transplanted to previously prepared beds and fertilized with 2 kg/m² of chicken manure. The spacing used was 0.5 m between rows and 0.30 m between plants. Each repetition consisted of eight plants, of which five were evaluated. The cultivation of the plants took place without the use of pesticides.

The statistical design used was entirely randomized blocks, with three treatments (cultivars) and three repetitions, each repetition consisting of five plants. The data were submitted to statistical analysis, considering the premises of normality and homogeneity variance of the data. The analysis of variance considered the Tukey test to identify statistical differences between treatments at the level of 5% of error probability[10].

The leaves were harvested manually in the early morning when the plants presented the first inflorescence with visible calyx color (phenological stage R3)[6], and immediately transported to the Plant Physiology and Postharvest Laboratory. Five fully expanded leaves were harvested from each plant (Figure 1).

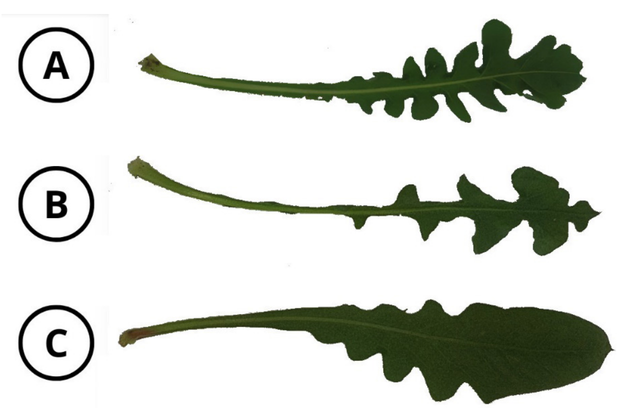

Figure 1 – Fully expanded statice leaves harvested at the R3 phenological stage from QIS White (A), QIS Blue Dark (B) and QIS Yellow (C) cultivars. Rio do Sul, 2021.

In the leaves, the attributes of total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio, pH, vitamin C content, anthocyanin, flavonoids, total phenolic compounds (TPC) and total antioxidant activity (TAA) were analyzed. The analyses were performed from juice extracted from the leaves of each treatment with the aid of a mixer (Arno-Turbomix model).

The TSS content of the juice was measured in a digital refractometer and the results were expressed as a percentage. The TTA levels were obtained by titration of 10 mL of leaf juice diluted in 95 mL of distilled water with 0.1N sodium hydroxide until the turning point[11], and the results were expressed as a percentage of citric acid. The TSS/TTA ratio was calculated by dividing the TSS by the TTA. The pH in the juice extracted from the leaveswas determined with a benchtop pH meter (model mPA210)[11].

Vitamin C content was determined by the spectrophotometric method using 2,4-denitrophenylhydrazine[12]. It was used 1 g of the sample macerated in 50 mL of oxalic acid (0.5%, m/v). After filtration, 1 mL of the sample was used and 3 mL of oxalic acid (0.5%, m/v), 5 drops of the oxidizing agent 2,6 dichlorophenol-indofenol (2,6-DCFI) (0.25%, m/v), 1 mL of 2,4 dinitrophenylhydrazine (2,4-DNPH) (2%, m/v), one drop of thiourea (10%, m/v) and 5 mL of sulfuric acid (85%, v/v). Readings were taken in a spectrophotometer at 520 nm and the results expressed as mg ascorbic acid 100 g^{-1} fresh mass (FM).

To determine the contents of anthocyanins and total flavonoids, the leaves were grind and homogenized with extraction solution (ethanol 95%: HCl 1.5 N – 85:15, v/v) and stored for 12 hours at 4°C. Samples were filtered with filter paper and absorbance was measured in a spectrophotometer at an electromagnetic wavelength (λ) of 535 nm for anthocyanins and 374 nm for flavonoids[12].

The results were expressed in mg 100 g^{-1} of FM, calculated by the equation 1 and 2:

$$Anthocyanins = \frac{Absorbance * dilutionfactor}{98.2} \qquad (1)$$

$$Flavonoids = \frac{Absorbance * dilutionfactor}{76.6} \qquad (2)$$

The hydroalcoholic extract was obtaining for the quantification of TPC and TAA[12]. For extraction, 10 g of the processed leaves and 50% methanol (v/v) solution were used for one hour. The material was centrifuged at 5000 rpm for 30 minutes in a refrigerated centrifuge at 4°C. The supernatant was stored and the residue was further extracted with 70% acetone (v/v). After one hour, the material was centrifuged again, with the addition of the supernatant to the previous one, and the volume was made up to 100 mL with distilled water.

Quantification of TPC was performed by the Folin-Ciocalteau colorimetric method, which involves the reduction of the reagent by the phenolic compounds in the sample, with the formation of a blue complex that linearly increases the absorbance at λ of 760 nm[12]. Gallic acid was used as a standard for TPC. Were used 2.5 mL of the extract and 7.5 mL of distilled water. In a dark environment, 1 mL of the diluted extract was taken and 1 mL of Folin-Ciocalteau (1:3, v/v, Folin-Ciocalteau:water), 2 mL of 20% sodium carbonate (20%, m/v) and 2 mL of distilled water were added. Readings were taken in triplicate, after 30 minutes, in a spectrophotometer, λ of 760 nm. Water was used as a blank. The TPC content of the leaves was expressed as gallic acid equivalent (GAE; mg GAE g^{-1} FM), using the straight-line equation obtained from the calibration of the curve with gallic acid.

TAA was determined using the methodology based on the ability of the extract to sequester the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH method)[13]. In a dark environment, 0.1 mL of the extract was used with 3.9 mL of the DPPH radical (v/v) (in triplicate). The mixture was stirred in a Vortex and left to stand. Readings were taken in a spectrophotometer at λ of 515 nm after 30 minutes. Methyl alcohol was used as a blank. The DPPH radical inhibition rate was calculated using the equation 3:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100\% \qquad (3)$$

in which A_{blank} is the absorbance of the control reaction (contains all reagents except the tested sample) and A_{sample} is the absorbance of the tested sample.

3 RESULTS AND DISCUSSION

Statice leaves showed differences in TSS contents among cultivars, with average values of 4.0%. TSS values were higher in QIS White and QIS Yellow, which did not differ, and lower for QIS Blue Dark (Table 1). These values are similar to the 4.47%, 3.14%, 4.65% and 5.20% found in leaves of purslane (*Portulaca oleracea*) and malabar spinach (*Basella rubra*) (Viana et al., 2015), respectively, and lower than the 8.2% of piscine (*Stachis lanata*) and 17.49% of slender amaranth (*Amaranthus viridis*)[14], indicating that statice leaves have reduced sweetness.

The average TTA value found in statice leaves was 0.8%, with the lowest acidity found in the QIS White cultivar (0.6%) and the highest in QIS Yellow (0.9%) (Table 1). The TTA results are higher than the 0.14; 0.22; 0.27; 0.34; and 0.17% reported for purslane, malabar spinach, slender amaranth, piscine and nasturtium (*Trapaeolum majus*) leaves, respectively[14-15]. Thus, statice leaves can be considered acid vegetable.

The leaves showed average TSS/TTA values of 5.1 with highest values for the cultivar QIS White, followed by QIS Blue Dark and QIS Yellow (Table 1). The higher TSS/TTA ratio of the QIS White cultivar can be attributed to the lower TTA and higher TSS values. In a study conducted with arugula cv. Rococo, mean values of 6.74 were reported[16], close to those of statice leaves of this study.

Variations in TSS and TTA contents can cause changes in the sensory characteristics of foods and the TSS/TTA ratio is an indicator of food flavor[17], and the higher the ratio values (which occurs because of higher sugar or lower acid contents), the better the flavor. Thus, the leaves of the QIS White cultivar have better flavor characteristics compared to other cultivars. However, the perception of flavor depends on a number of factors, since it is perceived by the human receptors which includes appearance, size, color, shape and texture.

The pH did not differ among the statice cultivars, with average values of 4.0 (Table 1).

Lower values have been reported for leaves of purslane, malabar spinach, slender amaranth, piscine and nasturtium[14-15]. Acidity is an attribute that directly influences the flavor and according to the results in this study, statice leaves can be considered acidic, attributing a slightly acidic or bitter taste[15].

Table 1 – Content of total soluble solids (SS; %), total titratable acidity (TA; %), SS/TA ratio, and pH in leaves of different statice (*Limonium sinuatum*) cultivars.

Attributes	QIS White	QIS Yellow	QIS Blue Dark
SS	4.2 ±0.07a	4.1 ±0.01a	3.9 ±0.01b
TA	0.6 ±0.02c	0.9 ±0.01a	0.8 ±0.03b
SS/TA	6.2 ±0.06a	4.3 ±0.01c	4.8 ±0.05b
pH	4.1 ±0.00a	4.1 ±0.01a	4.0 ±0.01a

Note: Values followed by the same letter in the rows do not differ by Tukey's test ($p<0.05$).

There was a difference in vitamin C content in statice leaves between the different cultivars, with average values of 5.8 mg 100 g^{-1} FM. The highest values were observed in the cultivar QIS White, with 8.7 mg 100 g^{-1} of FM and the lowest in QIS Yellow, with 3.6 mg 100 g^{-1} of FM (Table 2). These values are lower than the 13.48 and 25.0 mg 100 g^{-1} FM reported for leaves of different statice species produced in Tunisia and the 72.45 mg 100 g^{-1} FM for leaves of common sorrel (*Rumex acetosa*)[14,18]. However, similar contents were presented for purslane leaves (5.62 mg 100 g^{-1} FM)[19].

Table 2 – Vitamin C, anthocyanin, flavonoid (mg 100 g^{-1} FM), total phenolic compounds (TPC; mg EAG g^{-1} FM)* and total antioxidant activity (TAA; %) contents in leaves of different statice (*Limonium sinuatum*) cultivars.

Attributes	QIS White	QIS Yellow	QIS Blue Dark
Vitamin C	8.7 ±0.4a	3.6 ±0.2c	5.0 ±0.1b
Anthocyanin	1.1 ±0.08a	1.0 ±0.03a	1.0 ±0.03a
Flavonoids	21.1 ±0.8a	17.5 ±0.3c	18.3 ±0.2b
TPC	149.5 ±0.09a	136.4 ±1.31b	149.1 ±0.1a
TAA	94.5 ±0.05a	94.2 ±0.05a	94.4 ±0.05a

Note: Values followed by the same letter in the rows do not differ by Tukey's test ($p<0.05$). *EAG g^{-1} of FM: Gallic Acid Equivalent per gram of fresh mass.

Vitamin C is important in maintaining the immune system of the human body, in the health of the skin, and circulatory system, in the prevention of scurvy, and as an important antioxidant[20]. Since it is not produced by the human body, it needs to be ingested, and vegetables are the main sources of this vitamin. The recommended daily intake (RDI) of vitamin C established for adults in Brazil is 45 mg[21]. Thus, the daily intake of 100 g of statice leaves, supplies 12% of the RDI recommendation of the vitamin in Brazil, indicating that they are important sources in the human diet.

There was no difference in anthocyanin content between cultivars, with average values of 1.0 mg $100g^{-1}$ FM (Table 2). In leaves of different lettuce varieties anthocyanin contents ranging from 0.21 to 3.07 mg $100g^{-1}$ FM have been reported[22]. Low anthocyanin values are expected in leafy greens, with higher contents commonly found in fruits and other vegetables ranging in color from pink, red, purple and blue[23].

The average total flavonoid content was 18.9 mg $100g^{-1}$ FM, with the highest values for QIS White (21.1 mg $100g^{-1}$ FM), followed by QIS Blue Dark (18.3 mg $100g^{-1}$ FM) and QIS Yellow (17.5 mg $100g^{-1}$ FM) (Table 2). Contents of 48.72 and 1.60 mg g^{-1} FM in statice leaves and root, respectively, and 14.49 mg g^{-1} dry mass in statice flowers have been reported[2,24], indicating that the cultivars evaluated in this study have average contents of flavonoids.

Plants with higher flavonoid and anthocyanin content have superior functional properties. Flavonoids cannot be synthesized by humans and animals[25]. In Brazil, the average daily consumption of flavonoids by Brazilians is 138.92 mg[26]. An adequate daily intake recommendation has not been established, but studies point out the importance of regular flavonoid consumption, as these are related to a wide variety of biological activities, highlighting antioxidant, anti-inflammatory, antitumor, antiallergic, antiviral action, in addition to protecting the skin from the effects of ultraviolet radiation, among others[27]. Among the main sources of flavonoids described in the literature are vegetables, fruits and plant-derived beverages) and it is, therefore, essential to consider alternative sources such as statice leaves[25].

The leaves showed high TPC contents with average values of 145.0 mg GAE g^{-1} of FM (Table 2). There were differences in TPC among cultivars, with QIS White and QIS Blue Dark standing out with the highest values (149.5 and 149.1 mg GAE g^{-1} FM, respectively), followed by QIS Yellow with the lowest (136.4 mg GAE g^{-1} FM). Similar values were reported in statice leaves with 145.96 mg GAE g^{-1} of FM[2]. In another work conducted in Tunisia, TPC values ranged from 85.56 to 135.07 mg GAE g^{-1} of MF in leaves of eight wild-occurring statice species[18].

The TPC values obtained in this study were higher than those of other edible leafy vegetables such as common sorrel type I (5.56 mg GAE g^{-1} FM) and II (2.62 mg GAE g^{-1} FM), slender amaranth (1.64 mg GAE g^{-1} FM)[14], and purslane (51.46 mg GAE g^{-1} FM)[28], but lower than those of nasturtium leaves (378,1 GAE g^{-1} FM)[15].

The results of this study, demonstrate that statice leaves are a potential source of TPC, considered phytochemicals with multiple functions, including antioxidant activity.

There was no difference in TAA (DPPH method) among the statice cultivars, with average values of 94.3% (Table 2), and they can be considered plants with high antioxidant activity. The TAA of statice leaves was similar than the 95,1% reported for leaves of moringa (*Moringa oleifera*)[29].

The data presented show that flavonoids contribute to the high TPC contents, as can be observed in the cultivars QIS White and QIS Blue Dark, which showed the highest values of TPC and total flavonoids, while QIS Yellow showed lower values of flavonoids and TPC, resulting in a significant positive linear relationship between TPC and flavonoids (R^2=0.6168) (Figure 2A).

The antioxidant activity is largely attributed to the presence of TPC. Many works show the positive relationship between TAA and TPC contents in various vegetables[3,12]. In this study, statice leaves (regardless of cultivar) showed, on average values, a significant positive linear relationship (R^2=0.8826) between TPC and TAA (Figure 2B). These results indicate that phenolic components may be the main contributor in the antioxidant activities of statice leaves.

Despite the advancement of research, little is still known about the functional potential of many edible leaves, which are considered non-traditional foods[15]. Therefore, the knowledge generated in this study are essential to understand that the edible leaves of statice can be characterized by high content of flavonoids, phenolic compounds, and natural antioxidants. The plants that are currently cultivated for ornamental purposes, due to the beauty and durability of the flowers, can also be cultivated for use in human food, in order to be a potential nutraceutical and bioactive food that should also be explored for the prevention of problems arising from oxidative stress in cells.

Since it is an annual plant of indeterminate growth, that is, it continues to produce leaves after the appearance of the floral stems and produces a large number of leaves until senescence (50 to 417 according to the cultivar)[6], there is the possibility of cultivating the species with the purpose of producing leaves for food and floral stems for ornamentation. It is important to emphasize the importance of growing the plants without the use of agrochemicals and future studies to verify the impact of leaf harvesting on the development and production of the floral stems.

The results obtained in this work, in addition to adding nutritional value to food, can boost the production and consumption of statice leaves and constitute an alternative in promoting the expansion of production and diversification on family farms.

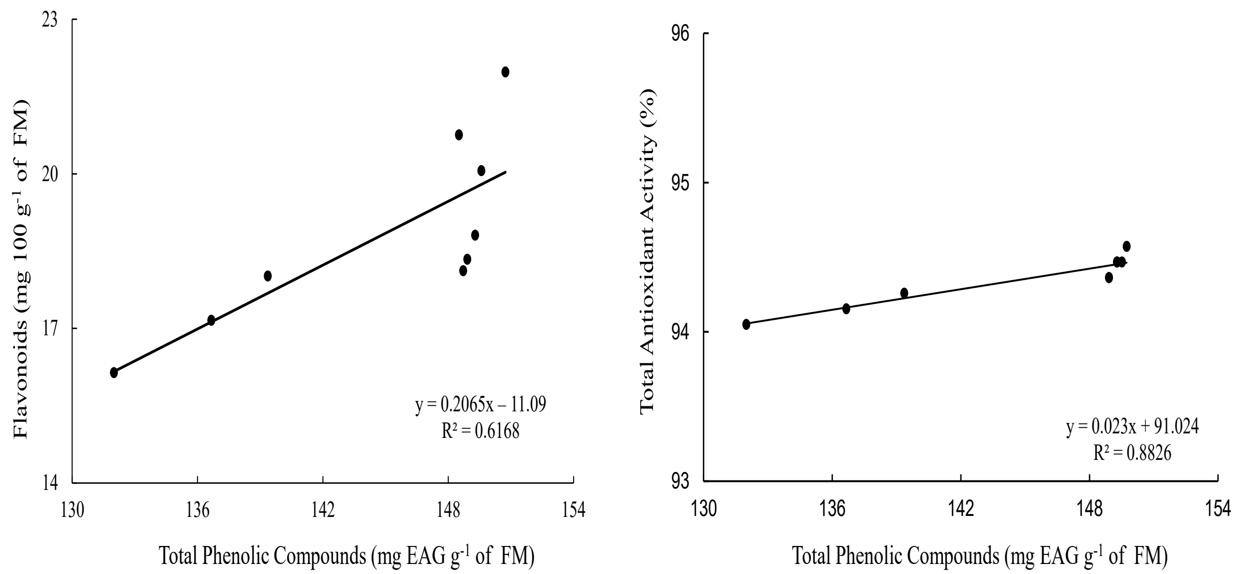

Figure 2 – Relationship of total phenolic compounds with total flavonoid content (A) and total antioxidant activity (DPPH radical inhibition capacity) (B) in statice (*Limonium sinuatum*) leaves produced in Rio do Sul, SC.

4 **CONCLUSION**

Statice leaves can be considered a functional food because they have high acidity and total phenolic compounds and average flavonoids content. In addition, the leaves show high antioxidant activity, attributed to a high capacity to scavenge the DPPH radical, indicating that they are plants with beneficial bioactive properties for use in human food. However, as it is little known in Brazil as a food plant, more studies on the nutritional and bioactive characteristics of statice leaves are necessary.

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