Rosaceae Rubus Rosifolius Smith: Nutritional, Bioactive And Antioxidant Potential Of Unconventional Fruits

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Short Report

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Abstract

Porpuse: *Rubus* are economically important fruits, being highly valued for their taste and medicinal properties, for which it is estimated that there are between 400 to 700 species but many of them are still unknown by the population.

Methods: The literature does not present much information regarding the physicochemical, bioactive composition and antioxidant activity of *Rosaceae Rubus rosifolius Smith* and for this reason this research was carried out.

Results: The fruits presented low content of lipids (7.42%) and ash (0.27%), intermediate values of protein (11.54%), and with considerable content fiber (23.72%) and carbohydrates (59.18%), low acidity, and interesting values of soluble solids. Regarding bioactive compounds, good concentrations of phenolic compounds (289.4 (mg. g^{-1} EAG), flavonoids (155 $mg100g^{-1}$), anthocyanins (120.11 mg cyanidin-3-glucoside $100g^{-1}$) and phytoene (517.3 $mg100g^{-1}$).

Conclusion: The wild strawberry showed good antioxidant capacity verified by different methods of analysis. Given the findings of this research, it is observed that this fruit has nutritional and phytochemical potential to be inserted in the human diet and therefore its consumption should be encouraged.

Declarations

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Ethics approval - Not applicable

Availability of data and material- Not applicable

Code availability - Not applicable

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1. Introduction

Fruits have high nutritional value, with vitamins, minerals, antioxidants, in addition to several other substances that help in preventing and fighting diseases. The blackberry belongs to the family *Rosaceae*, genus *Rubus*, forming a diverse and widespread group, for which it is estimated that there are between 400 to 700 species, known as berries, whose term has been commonly used to describe any small fruit, with a sweet flavor and rounded shape [1].

Fruits play important roles in the health of human beings, since they are a source of essential nutrients and phytochemicals that help to reduce or act in the prevention of diseases [2]. *Rosaceae* is a plant with pharmacological potential traditionally used to treat diarrhea, stomach diseases, and as an analgesic, antimicrobial, antihypertensive, and as well as other pharmacological properties [3]. Various lipophilic and hydrophilic compounds are found in berries, whose biological properties have been attributed to the high levels and wide diversity of phenolic compounds.

The species *Rubus rosaefolius* originates from the Himalayas, Western Asia and Australia, belonging to the raspberry and blackberry group. A new record of *Rubus (Rosaceae*) for Ecuador is described [4]. *Rosaceae Rubus rosifolius Smith,* popularly known also as blackberry and redberry, is a shrub plant present in several continents and in the Brazilian territory, mainly in the south and southeast region. Rubus genus is a part of berries plants, which are small, soft, commonly edible, colorful and have important nutritional content. The blackberry has a thin, rounded skin, with color ranging from purple to dark red [5]. The blackberry is a wild plant, and although its composition contains several bioactive compounds, it has not been the object of relevant studies and its potential for application is untapped.

Because it is a little-known species and not found at fairs and supermarkets, it is called an unconventional food plant (UFPs). UFPs are among the food sources that develop in natural environments without the need for inputs or new areas [6]. The fact that many of these plants are in areas managed by farmers becomes a fundamental strategy for strengthening the food sovereignty of many families [7]. Food biodiversity presents one of the most significant opportunities to enhance food and nutrition security today. The lack of data on many plants, however, limits our understanding of their potential and the possibility of building a research agenda focused on them [8].

There is a wide variety of Rubus species, many of them of unknown composition, as is the case of *Rosaceae Rubus rosifolius Smith*, which leaves a gap in the literature and for this reason this area has called our attention to better investigate bioactivities and their chemical components. Given the above, this work aimed to carry out the physical-chemical characterization of the fruits, as well as to evaluate the bioactive composition and the antioxidant potential of *Rosaceae Rubus rosifolius Smith* from the southern region of Brazil.

2. Materials And Methods

2.1. Samples

The fruits of wild strawberry were purchased in Canguçu / RS (latitude **31º 23' 42"** and longitude **52º 40' 32"),** in the southern west region of Rio Grande do Sul (Supplementary Information).

When obtained, the fruits were selected with respect to health, physical integrity, color uniformity and degree of maturity.

2.2. *Physical and chemical analyzes*

The protein, pH, titratable acidity, moisture, ash content, fiber, the total soluble solids (TSS) content was determined by AOAC [9] and lipids concentration were determined by Bligh-Dyer [10]. The carbohydrate content was determined by subtracting the total protein, fat, moisture, and ash contents present in 100 g of fresh fruits from 100. The total calorie content (TCC) was determined by multiplying the percent protein and carbohydrate contents by 4 and that of the fat content by 9. The sum of the resulting values was then used to determine the TCC of the fruits in kcal/100 g. The color was evaluated using Minolta CR-300 colorimeter, with standard D65 illuminant and observation angle of 2°. The readings were obtained in five distinct positions, such that practically the entire surface of the fruit was sampled. Vitamin C was determined by the method described by Otero et al., [11]. For this, fruit juice was extracted, filtered and the quantification of ascorbic acid was performed by titration, using standard solution of iodine and sodium thiosulphate and starch solution as indicator. The results were determined by equation 1, expressing the results in mg of ascorbic acid.100 mL^{-1} of juice.

$$\text{Vit C} = (V_1 \times F_1) - (V_2 \times F_2) \qquad (1)$$

Where: V1= Volume of iodine spent in titration; V2 = Volume of thiosulfate spent in the titration; F = solution correction factor.

2.3. Determination of bioactive compounds

2.3.1Preparation of the aqueous extract

The extracts were prepared as described by Tan et al. [12]. Three g of the fruit was used separately in 20 mL of water and extraction for 1 h at 45°C using stirring. The extracts were then centrifuged at 4350 *x*g for 10 min at 10 ° C, and the supernatant from each sample was filtered on filter paper. The final extract was used for the analysis of phenolic compounds, flavonoids and antioxidant activities. All extractions were performed in triplicate.

2.3.2 Assay of phenolic compounds content

The phenolic compounds were determined by the Folin-Ciocalteu method [13], the absorvance was measured at 725 nm in a spectrophotometer (JENWAY 6705 UV / Vis). The standard curve with gallic acid at concentrations of 30 to 500 $\mu g.mL^{-1}$ was used as the calibration curve. The results were expressed in mg equivalent of gallic acid per 100 g of dry matter (mg EAG.$100g^{-1}$).

2.3.3 Assay of flavonoids content

The total flavonoid content was determined using the Otero [11] adapted method, where 500 µl of 2% aluminum chloride in methanol were mixed with the same volume of the sample solution. The reading was made at 425 nm, and the results were expressed as milligram equivalents of quercetin per 100 grams of dry matter (mg EQ $100g^{-1}$).

2.3.4 Assay of carotenoids content

The total carotenoid content was determined by Rodriguez-Amaya [14]. The readings were performed at the absorbances of 450 nm (β-carotene), 445 nm (α-carotene), 449 nm (zeaxanthin) and 470 nm (lycopene). Quantifications were performed through Equation 2 and the results expressed in milligrams per 1g of dry matter.

$$\text{Carotenoids } (\mu g.g^{-1}) = \frac{\text{Absorvance x extract volume (mL) x } 10^6}{\text{Absorption coefficient x 100 x sample weight (g)}} \quad (2)$$

2.3.5 Assay of anthocyanins content

Ethanol acidified with HCl was added in 1 g of sample, homogenized for 1 hour, filtered, swollen into a 50 mL flask and reading on a spectrophotometer at 520 nm [15]. The results were expressed in mg cyanidin-3-glycoside $100g^{-1}$.

2.4 Antioxidant activity

2.4.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ability

The antioxidant activity of the extracts, based on the elimination activity of the stable DPPH, was determined by the method described by Braca et al. [16]. The aqueous extract (0.1 mL) was added to 3 mL of a 0.001 M DPPH in methanol. The absorvance at 517 nm was determined after 30 min and the % inhibition of activity was calculated by equation 3:

$$\% \text{ inibition} = 100 - \frac{(Abs_{sample} - Abs_{white}) \times 100}{Abs_{control}} \quad (3)$$

2.4.2 Iron reducing capacity (FRAP)

The ferric reducing antioxidant power was determined by Silva et al. [17]. 100 µL of the aqueous extract at 1 mg. mL^{-1} concentration in 300 µL of ultra-pure water was mixed, then 3.0 mL of the FRAP reagent was added, homogenized at 37 °C for 30 minutes. The reduction of the Fe^{3+} to Fe^{2+} complex was obtained by reading the wavelength absorvance at 595 nm in a spectrophotometer (JENWAY 6705 UV / Vis.). The procedure was carried out in triplicate and the values of Fe^{+2} found by means of the linear regression equation, through the construction of the analytical curve of the ferrous sulphate.

2.5 Statistical analysis

The data was expressed as means ± standard deviation, and all analyzes were performed in triplicate, and then submitted to analysis of variance (ANOVA) using SAS 9.1 (Cary, NC).

3. Results And Discussion

The physical-chemical parameters in food are very important both from a technological point of view, as well as from a microbiological and nutritional point of view. Some of these parameters influence microbiological, chemical, physical, enzymatic and food stability changes [7], therefore, the physicochemical characterization of blackberry fruits was carried out and the results are exposed in the Table 1.

Table 1. Physico-chemical characterization of wild strawberry from Rio Grande do Sul, Brazil.

Mean ± standard deviation of three repetitions. * Estimated by difference (100%).

The wild strawberry presented a high percentage of moisture (81.20%), the literature reports different moisture contents for fruits, Guedes et al. [18] describes that the blackberry presented 78.74%; Rambaran and Bowen-Forbes [19] determined the levels of 87.7 and 83.4% of moisture for *Rubus rosifolius Red* and *Rubus rosifolius Wine red*, respectively.

The blackberry can be considered a fruit with a low content of lipids (7.42%) and ash (0.27%), intermediate values of protein (11.54%), and with considerable content fiber (23.72%) and carbohydrates (59.18%). Fruits generally contain sugar and fibers, such as pectin, with a high-water content and less amount of fat and protein. Dietary fiber from fruits helps reduce heart disease risk and blood cholesterol levels [20], and therefore its consumption should be encouraged. Ahmad et al. [21] reported that the fiber content of some *Rubus* varied from 1.66% (*R. ulmifolius*) to 5.90 (*R. niveus*). Surya et al. [22] report higher values for this component where 7.10% of fibers are described for *R. rosifolius* and 8.43% for *R. chrysophyllus*.

Carbohydrates are the most abundant constituents in fruits, representing 50% to 80% of the total dry weight. The contents of carbohydrate total of the fruits varied among species of *Rubus* was evaluated by Surya et al.,[22]; the contents were between 6.84 g to 11.48 g. In these samples, the fiber content was also evaluated and the concentration varied from 3.36% to 8.43%. As for ash analysis, a concentration of 0.27% was found in this study, Guedes et al. [18] obtained 0.78% and Rambaram et al. [19] found 0.4%. The micro and macro nutrients content in a vegetable is associated with soil conditions, climate and agricultural management [7], and that's why they have variations.

Acidity is an important parameter in the quality of products of plant origin, as it confers the advantage of greater microbiological stability and conservation period. Curi et al. [23] found higher values of acidity, 1.6

Analyzes	Results (wet basis)	Results (dry basis)
Moisture (%)	81.2 ± 0.47	-
Proteins (%)	2.17 ± 0.07	11.54 ±0.12
Fibers (%)	4.46 ± 0.66	23.72 ± 1.20
Lipids (%)	1.35±0.88	7.42 ± 1.4
Ashes (%)	0.05±0.02	0.27 ± 0.10
Carbohydrates (%) *	10.77	59.18
Total calories (Kcal/100 g)	63.91	-
Titratable acidity (mg citric acid $100g^{-1}$)	0.73±0.00	-
Total Soluble Solids - SST (ºBrix)	12.70±0.06	-
pH	3.93±0.03	-
Ascorbic acid (mg $100g^{-1}$)	2.30 ±0.10	-
Ratio (TSS/TA)	17.30±0.01	-
Diameter (cm)	1.12±0.19	-
Length (cm)	2.20±0.18	-
Color parameters		
L*	37.38±2.44	-
a*	13.81±2.53	-
b*	7.72±1.54	-

mg acid. citric acid $100g^{-1}$ in the blackberry than found in this work (0.73 mg citric acid 100g-1). Regarding the pH value, the wild strawberry showed a pH of 3.93, being considered an acidic fruit, found similar values when analyzing the pH of the blackberry (3.40).

The TSS is used as an indirect measure of the sugar content, being related to the sweetness of the fruit. Curi et al. [23] obtained 9.7 ºBrix and 6.1 ratio (TSS/TA) in red bramble, while in the present study we obtained a lower ratio (1.1) and a higher soluble solids content (12.7 ºBrix). The TSS/TA ratio is used as a parameter to define the flavor, also considered an indicator of fruit maturity or quality. The fruit had an average TSS/TA ratio of 17.30, due to the high content of soluble solids present in blackberry (12.70 ºBrix), which demonstrates that the fruit has a sweet taste. Environmental factors such as sunlight incidence, temperature, soil type, as well as the cultivation techniques employed can influence the sugar content and acidity of foods and, therefore, the TSS/TA ratio [19]. The content of soluble solids can vary between cultivars and environmental conditions, in addition to being an indication of the sweetness of the fresh fruit [24].

In sensorial terms, colour is thought of as a threedimensional characteristic of appearance which consists of a lightness attribute (L∗), which differentiates between light and dark colours, and two chromatic parameters a∗ which is the red/green coordinate and b∗ which is the yellow/blue coordinate. By specifying these three attributes, colours can be distinguished [19]. The fruit had an average lightness of 37.38, indicating its dark color, since the parameter L varies from 0 (black) to 100 (white). In parameter a*, 13.81 was found, while in b*, it was 7.72 indicating that the fruit tends to reddish in color. The selection of nutritious and healthy foods is often carried out by consumers through visual perception, with color being an important factor. The coloring of these products can be used as an indicator of quality and even defects in the products. In addition, there is a recommendation for a colorful diet to obtain micro and macro nutrients [2].

It is worth remembering that variations in the contents of chemical compounds may occur to the detriment of the place where they are grown, due to differences in the intensity of solar radiation and thermal amplitude, which influence the organoleptic characteristics of the fruits [25].

Anthocyanins, flavonoids, phenolics, and carotenoids are the phytochemicals that normally presented in berries, known to possess anticancer, anti-inflammatory, antioxidant, antimutagenic, antineurodegenerative, antihypertension and other bioactivities [26]. It is well established that the nutritional value and bioactive potential of plants are determined by production and accumulation of primary and secondary metabolites, which may differ among plants of the same species due to differing environmental conditions [27]. Therefore, the occurrence of the anthocyanin, flavonoid, total phenolic and carotenoid content in the *Rubus rosaefolius Sm* was investigated (Table 2).

Table 2. Determination of bioactive compounds in wild strawberry

Analyzes	Results
Total phenolic (mg. g^{-1} EAG)	289.04 ± 0.19^a
Flavonoid (mg. $100g^{-1}$)	155.01 ± 0.11^b
Anthocyanin (mg cyanidin-3-glucoside 100 g^{-1})	120. 11 ± 0.20^b
Individual carotenoids	
Lutein (mg. $100g^{-1}$)	8.6 ± 0.09 ^{b}
Zeaxanthin (mg. $100g^{-1}$)	7.3 ±0.08 ^{b}
Lycopene (mg. $100g^{-1}$)	6.6 ±0.04^b
Phytoene (mg. $100g^{-1}$)	517.3 ±0.27^a

Mean ± standard deviation of three repetitions. Same letters in the column indicate that there is no significant difference between samples ($p< 0.5$).

A wide range of phytochemicals are found in *Rubus*, with phenolics being the most commonly reported. Anthocyanins are found in these fruits, giving attractive colors ranging from red to black and polyphenols are the main cause of astringency in these.

Rambaran and Bowen-Forbes [19] studied the composition of two species of *Rubus* regarding the content of anthocyanins and phenolic compounds. The anthocyanin content for the Red variety was 163.4 mg $100g^{-1}$ whereas for wine red the concentration was 287.4 mg $100g^{-1}$. The total phenolic contents of the fruits ranged from 1.0 to 252.0mg GA 100 g^{-1} FW, depending on the extracting solvent. Dujmović Purgar et al. [28], who examined the total phenolic content of wild *R. idaeus* grown in Croatia, and obtained values ranging from 35.4 to 48.3mg GAE 100 g^{-1} FW, using an 80% etanol extract.

Among the anthocyanins present in *Rubus* fruits, the compounds in greater concentration are cy-3-glu, pel-3-glu, and pel-3-rut and cyanidin and pelargonidin aglyclones are found in small concentrations in *Rubus species*, the higher the concentration of anthocyanins in fruits, the darker their color [19,29] Although the concentration of total anthocyanins in the samples was not so high, it is believed that the consumption of these fruits is another alternative to be incorporated into human food and thus the wild strawberry contribution, along with other vegetables, the benefits of a diet rich in bioactive compounds.

Anthocyanin values were considered low, 12.11 mg of cyanidin-3-glycoside.$100g^{-1}$. The literature indicates that the variation in the content of anthocyanins between cultivars can be quite marked, ranging from 12.70 to 197.34 mg.$100g^{-1}$ fruit. This wide variation in the content of anthocyanins in blackberries is due to possible effects of climatic conditions in the cultivated region, maturation stage, species and cultivar.

It has been reported in *Rubus* species that there are phenolic compounds such as ellagic acid, gallic acid, chlorogenic acid, and caffeic acid. Abu Bakar et al. [30] evaluated biocompounds in wild berries, the results showed that *R. alpestris* contained the highest total phenolic [24.25 mg gallic acid equivalent (GAE)/g] and carotenoid content [21.86 mg β-carotene equivalents (BC)/100g]. The highest total flavonoid [18.17 mg catechin equivalents (CE)/g] and anthocyanin content [36.96 mg cyanidin-3-glucoside equivalents (c-3-gE)/g] have been shown by *R. moluccanus*.

Information on the composition of carotenoids in blackberries (*Rubus spp.*) is still scarce, when compared to studies of fruits of the same species, such as blackberry. Phytoene, a precursor to carotenoids, was the compound that stood out in the analysis, presenting 517.3 mg.$100g^{-1}$ of fruit, followed by lutein, zeaxanthin and finally lycopene.

Fruit consumption is encouraged by epidemiologists to prevent illness. The defense mechanisms provided by fruits against some diseases are related to the presence of antioxidant compounds [31]. The antioxidant capacity of the fruits is believed to have a correlation with the color presented [2] can be

measured through different methods, where each one of them acts through different mechanisms of action and interact in different ways with the samples, therefore evaluating the antioxidant activity, by different methods becomes interesting to search for the result that most reflects reality [11]. For this reason, three different methods were tested and the results are described in the research. The wild strawberry showed a high percentage of inhibition (99.02% ± 0.1 %) through DPPH and intermediate antioxidant capacity by FRAP
(224.5 ± 59.3 mmol Trolox g^{-1}).

An antioxidant substance is one that, when available in low concentrations when compared to an oxidizing compound, can significantly impede the oxidation process. Therefore, since oxidative species and other radicals are responsible for oxidative stress, resulting in several long-term diseases, such as cardiovascular disease, cancer, and diabetes, the antioxidant capacity of phytochemicals is the primary mechanism in preventing such diseases and promoting human health [32].

Abu Bakar et al. [30] describe the of DPPH radical scavenging activity is as follows: ascorbic acid (10.00 ± 0.58 - 86.00 ± 3.65 µg/mL) and the reducing ability (FRAP) (70.93 ± 6.26 -26.34 ± 4.79 mM Fe^{2+}/g). Two *Rubus rosifolius* raspberry varieties were analyzed for their antioxidant properties by Campbell et al. [33] the antioxidant activity was evaluated by the radical ABTS using three different extracts and the values found were considered low for the extracts with hexane (3.0 and 6.4 mg ascorbic acid equivalents/100 g) and ethyl acetate (4.9 and 4.8 mg ascorbic acid equivalents/100 g) and high for the extract obtained by extraction with methanol (208.9 and 366.2 mg ascorbic acid equivalents/100 g) respectively.

Comparing the results obtained with those described in the literature, it can be seen that for blueberries, which are known for their high antioxidant activity, the value found by the FRAP method was 0.663 µmol of trolox eq/g [34]. This shows a good antioxidant capacity for wild strawberries, regardless of the method used in the determination. Despite having a lower antioxidant capacity than blueberries, wild strawberries have an antioxidant capacity superior to that of fruits such as pitaya and shelled pineapple.

No food by itself is capable of providing all the micro and macronutrients essential to human health, as is the case with bioactive compounds and antioxidant activity. A varied and colorful diet is recommended so that the body receives all the necessary nutrients, in view of the results found in this research, the wild strawberry has the potential to be included in the diet and complement a healthy diet.

Conclusion

In this research, valuable information about the physical chemical characterization, bioactive compounds and antioxidant capacity of *Rubus rosaefolius Sm* were described. The characteristics of wild strawberries, such as the high content of phenolic compounds, considerable content of carotenoids and good antioxidant action, demonstrate the importance of promoting their commercialization and introduction into the population's diet. In addition, its physical-chemical composition is similar to that of other blackberry cultivars, and for this reason, it is believed that this unconventional food plant has great potential for both fresh consumption and technological processing. This fruit had an important nutritional composition, which shows that the wild strawberry can contribute to reduce food insecurity, and for that its consumption should be encouraged.

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Supplementary

Supplementary Information is not available with this version.

Figures

Figure 1

Map of Rio Grande do Sul indicating the place where the samples were collected

Supplementary Files

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