WILD STRAWBERRIES (*Rubus rosifolius* Sm.) FROM SOUTHERN BRAZIL: CENTESIMAL AND MINERAL COMPOSITION, TOTAL POLYPHENOLS, ANTIOXIDANT, ANTIBACTERIAL AND ANTI-HYPERTENSIVE ACTIVITIES

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ABSTRACT: The objective of the present work was to evaluate polyphenols, trace elements and compounds with antioxidant, antimicrobial and anti-hypertensive activity in wild strawberries cultivated in Southern Brazil. Fruits present high fiber, iron, potassium and sodium concentrations. Polyphenolic content was 242.12 mg of gallic acid equivalent per 100g and monomeric anthocyanin concentration was 100.50 mg of cyanidin 3-glucoside per 100g. Wild strawberries presented the capacity of scavenging 89% of ABTS•+ radical and 61% of DPPH• radicals and chelating power of 3.1, meanwhile the inhibition of the angiotensin-converting enzyme I activity was 12.5%. Fruits presented compounds with capacity to inhibit *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes* and *Aeromonas hydrophyla.* Thus, wild strawberries are important source of functional compounds and their consumption is a way to promote the Brazilian biodiversity and improve human diet.

KEYWORDS: strawberry; phenolic compounds; anti-hypertensive; antimicrobial activity.

MORANGOS SILVESTRES (*Rubus rosifolius* Sm.) DO SUL DO BRASIL: COMPOSIÇÃO CENTESIMAL E MINERAL, POLIFENÓIS, ATIVIDADES ANTIOXIDANTE, ANTIBACTERIANA E ANTI-HIPERTENSIVA

RESUMO: O objetivo do presente trabalho foi avaliar polifenóis, oligoelementos e compostos com atividade antioxidante, antibacteriana e anti-hipertensiva de morangos silvestres cultivados no sul do Brasil. Os frutos apresentam alta concentração de fibras, ferro, potássio e sódio. O conteúdo polifenólico foi de 242,12 mg de equivalente de ácido gálico por 100 g e a concentração de antocianinas monomérica foi de 100,50 mg de cianidina 3-glucosideo por 100 g. Os morangos silvestres apresentaram capacidade de eliminar 89% dos radicais ABTS•+ e 61% dos radicais DPPH• e poder quelante de 3,1, enquanto a inibição da atividade da enzima conversora de angiotensina I foi de 12,5%. Os frutos apresentaram compostos com capacidade de inibir *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* e *Aeromonas hydrophyla*. Assim, os morangos silvestres são importantes fontes de compostos funcionais e seu consumo pode ser importante para valorizar a biodiversidade brasileira e promover a variação da dieta humana.

PALAVRAS CHAVE: morangos; fenólicos; anti-hipertensivo; atividade antibacteriana.

INTRODUCTION

The growing consumers' request for differentiated foods, rich in fiber and bioactive compounds has driven industries and the scientific community to search for foods with such characteristics. Additionally, the valorization of the regional biodiversity is an alternative to the use of conventional foods. Worldwide, there are more than 12,000 plants with potential consumption as food, and Brazil is a great source of vegetable products still little explored for nutritional use (Rapoport et al., 2009; Kinupp and Lorenzi, 2014).

Non-Conventional Edible Plants (NCEP) include all plants with some edible part in its structure and not conventionally used for neither human or animal feeding (Kinupp and Lorenzi, 2014; Brack, 2016). Many NCEP are included in the "plants of the future" in southern Brazil, because they may play an important role in the human nutrition as source of fibers, essential oils, natural dyes, vitamins, among others (Coradin, Siminski and Reis, 2011).

Wild strawberry (*Rubus rosifolius* Sm.) belongs to the Rosaceae family and is a species introduced in southern Brazil (Jacques and Zambiazi, 2011; Donadio, 2014). It has high economical value, being source of antioxidants such as vitamin C and carotenoids, and could be used as dyes, foods and medicines (Jacques and Zambiazi, 2011; Donadio, 2014; Campbell et al., 2017). On the medicinal point of view, strawberries have been used in the popular medicine by presenting antimicrobial activity, in the treatment of diabetes and to reduce pain (Mauro et al., 2002)

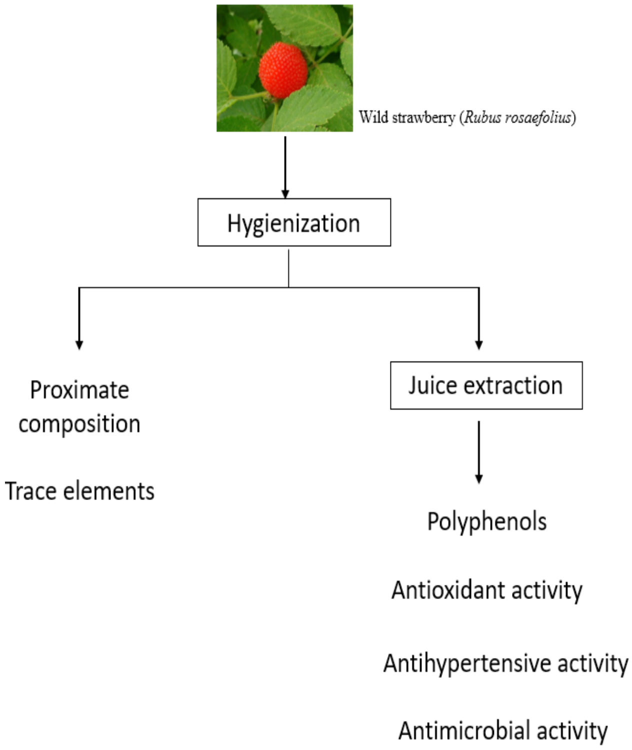
Although some studies have been published about *R. rosifolius*, variations on maturation, cultivation practices, location and harvest conditions may imply on different plant properties. The lack of knowledge about the usefulness and the way of using NCEP, associated to the "modern" tendencies and the dissemination of knowledge about potential health benefits of non-conventional plants may be an interesting strategy for the promotion of human nutrition diversification. Thus, the objective of this project is to characterize wild strawberry fruits harvested in the 'Vale do Taquari' region regarding their centesimal composition, minerals, phenolic compounds and compounds with antioxidant, anti-hypertensive and antimicrobial activity.

MATERIAL AND METHODS

Plant collection and preparation procedure

Wild strawberrys (*Rubus rosifolius*) fruits were harvested in the city of Teutônia (RS, Brazil) in 2017. About of 500 g of fruits were washed and sanitized with sodium hypochlorite at 100 mg L^{-1} for 15 min and washed with tap water. Whole fruits were analyzed for centesimal composition and mineral content. The juice from wild strawberries was extracted in domestic juice maker and analyzed for polyphenols, antioxidant, antibacterial and antihypertensive activities (inhibition of angiotensin-converting enzyme I, ACE-I). Results are presented as the means of triplicates from two independent fruit harvests. Figure 1 shows the analyses performed.

Figure 1. Flowchart of experiments for strawberry analysis.



Proximate Composition

Proximate composition was performed with the fruit pulp by quantifying the content of moisture, ash, total lipids, total soluble solids, proteins, carbohydrates and fibers according to AOAC (2019). Carbohydrate content was calculated by subtracting protein, total lipids, moisture and ash from 100% (AOAC, 2019).

Determination of trace elements

Trace elements were analyzed based on Brazilian Official methods (Brasil, 1991). Iron, sodium, potassium and magnesium contents were analyzed by atomic emission. Zinc concentration was determined by flame atomic. Values were expressed as mg per 100 g of fresh fruit.

Total polyphenols, anthocyanins and antioxidant activity

Total phenolic content (TPC) in extracts was determined by the Folin-Ciocalteau method described by Singleton and Rossi (1965), which involves the reaction of the sample with the Folin-Ciocalteau reagent (**Êxodo** Científica, Brazil) and sodium carbonate saturated solution, followed by absorbance measure at 765 nm. Results were expressed as mg gallic acid equivalent per gram of dry residue (mg GAE g^{-1}).

Monomeric anthocyanins (MA) were determined using the pH differential method (Lee et al., 2008), which involves measuring absorbance at 520 and 700 nm of samples diluted in 0.025 mol L^{-1} potassium chloride buffer pH 1.0 and 0.4 mol L^{-1} sodium carbonate buffer pH 4.5, separately. Extracted MA units were expressed as mg of cyanidin 3-glucoside per gram on dry basis (mg C3G g^{-1} DB).

Antioxidant analysis was performed by the determination of 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS•+) (Re et al., 1999) and DPPH radical (Brand-Williams et al., 1995), scavenging activity. Summarily, ABTS•+ analysis was performed by the reaction of ABTS stock solution and the dried residue extract. The mixture solution absorbance (734 nm) was measured after 6 min. The DPPH• antioxidant activity was evaluated by the reaction of 60 μmol L^{-1} DPPH• alcoholic solution with the extracts and absorbance measurement (517 nm) after 45 min. The results were expressed as: scavenging capacity (%) = [1 − (A/A0)] × 100, where A is the test absorbance and A0 is the control absorbance.

The chelating capacity of Fe_2+ was measured using the method described by Chang et al. (2007), which involves reaction of the extract with $FeSO_4$ (Fe2+) and ferrozine, following by absorbance measurement at 562 nm. The results were expressed as chelating capacity (%) compared to control (distilled water).

Antimicrobial activity

Antibacterial activity was evaluated as described by Kimura et al. (1998). Suspensions of 107 CFU mL^{-1} of *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC14579, *Listeria monocytogenes* ATCC7644, *Salmonella* Enteritidis ATCC13076, *Escherichia coli* ATCC25922, *Aeromonas hydrophila* ATCC7966 (which were kindly provided by the Applied Microbiology and Biochemistry Laboratory from the Food Science and Technology Institute at Federal University of Rio Grande do Sul) were spread onto Plate Count Agar (PCA) (Acumedia, Brazil) with sterile swab and aliquots of 20 μL of the fruit juice were applied. Plates were incubated at 37 °C for 24 h, when the presence of inhibition halos was verified.

Antihypertensive activity

The antihypertensive activity was evaluated by the ACE-I procedure (Cushman and Cheung, 1971), with slight modifications. Aliquots of 20 μL of the fruit juice were added to 200 μL of buffered substrate solution (5 mmol L^{-1} hippuryl-histidyl-leucine in 50 mmol L^{-1} HEPES-HCl buffer containing 300 mmol L^{-1} NaCl, pH 8.3). The reaction started by adding 40 μL of angiotensin I-converting enzyme (0.1 U mL^{-1}) (Sigma Aldrich, USA) to the described system and maintained at 37ºC. Reaction was stopped after 30min with addition of 150 μL of 1 mol L^{-1} HCL. Then, the hippuric acid was extracted with 1 mL of ethyl acetate, and the organic phase was transferred to heat evaporated glass tube. The residue was dissolved with 800 mL of distilled water and spectrophotometrically measured at 228 nm. Fruit inhibitory activity was expressed as percentage according to Equation 1.

$$\%\ inhibitory\ activity = \frac{A - B}{C - A} x100$$

Where, A is the absorbance without the sample, B is the absorbance without the enzyme and C is the absorbance with sample and enzyme.

RESULTS AND DISCUSSION

Proximate composition and trace elements

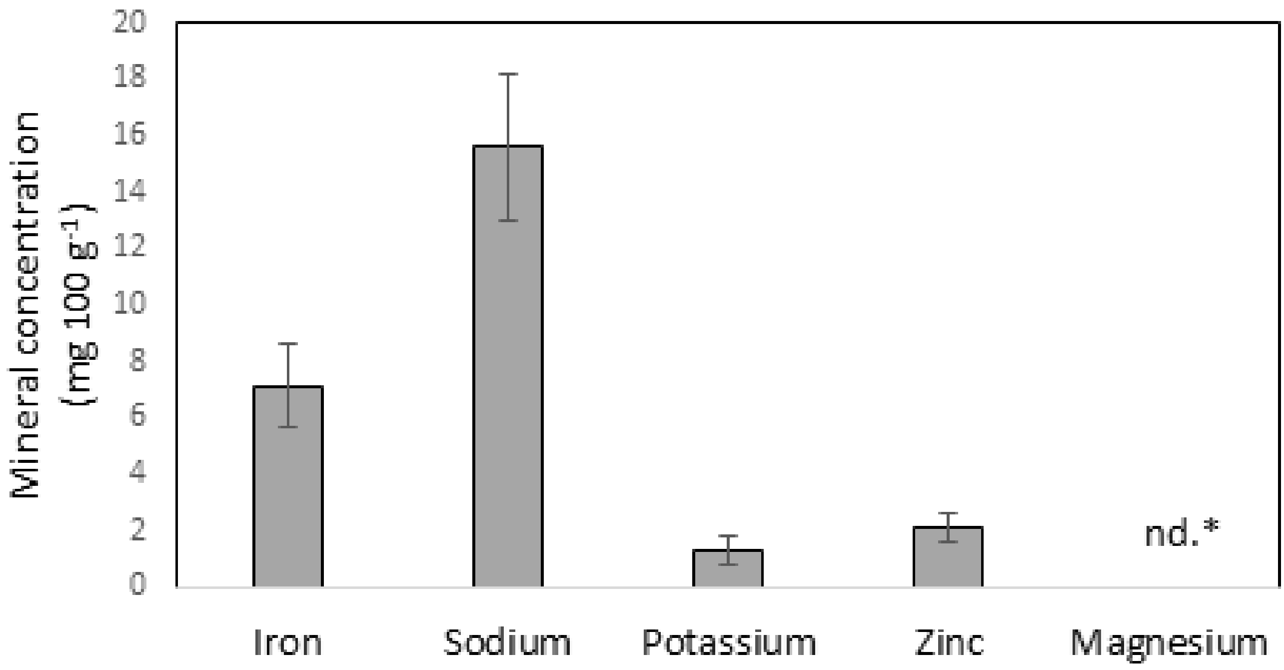
The centesimal composition results of wild strawberries evaluated are presented in Table 1. Fruit moisture content was 85.17±1.56 g per 100 g of fresh fruit and total soluble solutes was 12.5±0.84 **ºBrix,** which could explain the short fruit shelf-life. Current recommendations for dietary fiber intake in the diet vary according to age, sex and energy consumption, with adequate recommendation being 25 g per day (Brasil, 2005). Results have shown protein content of 1.47±0.11 g per 100 g of fresh fruit, lipid content of 0.65±0.02 g per 100 g of fresh fruit and carbohydrate content of 1.80±0.29 g per 100 g of fresh fruit. Protein content and fiber content in fruits were 1.47±0.11 g per 100 g of fresh fruit and 4.97±0.32 g per 100 g of fresh fruit, respectively. Similar results were observed by Oliveira et al. (2016), who studied the pulp from *R. rosifolius* fruits collected in southeastern Brazil, and reported 80 g of moisture, 18 g of carbohydrates, and about 5 g of fibers per 100g of pulp. These results show that wild strawberry is an important source of fibers for human diet. Additionally, the high moisture and carbohydrate content make the fruit susceptible for spoilage br microorganisms and may present short shelf-life.

Table 1. Centesimal composition of wild strawberries (g 100 g-1 of fruit).

Moisture	85.17±1.56
Proteins	1.47±0.11
Lipids	0.65±0.02
Ashes	0.58±0.02
Fibers	4.97±0.32
Total soluble solutes (ºBrix)	12.5±0.84
Carbohydrates	1.80

Wild strawberry presented 7.14±1.15 mg of iron per 100 g of fresh fruit, 15.63±2.60 mg of sodium per 100 g of fresh fruit, 1.3±0.5 mg of potassium per 100 g of fresh fruit and 2.14**±**0.5 mg of zinc per 100 g of fresh fruit. Fruits did not present significant magnesium content (Figure 2). Oliveira et al. (2016) observed in *R.rosaefolius* fruits from Minas Gerais (Ouro Preto, MG, Brazil) 3.9 mg of zinc per 100g of fresh pulp 96.5 mg per 100g of potassium, 15.5 mg per 100g of iron. Kinnup and Barros (2008) found concentrations of 0.0031 mg of zinc, 0.007 mg of sodium, 0.32 mg of calcium per 100g of fresh pulp of *R.rosifolius* from southern Brasil (Porto Alegre, RS, Brazil). Françoso et al. (2007) observed that phosphorus (35 mg per 100 g), sodium (22 mg per 100 g), calcium (20 mg per 100 g) and potassium (19 mg per 100 g) were the minerals with the highest concentrations in strawberries (*Fragraria anassa* Duch.) from Valinhos (SP, Brasil). Results may differ due to climate conditions, soil and methodology of analysis. The daily mean recommendation of the Dietary Reference Intakes (DRI) is 9.5-13 mg of iron, 300-360 mg of magnesium, 8.5-9.5 mg of zinc, 1.3 mg of sodium and 4.6 mg of potassium (Brasil, 2005). Phosphorus acts with the calcium and play the role of keeping healthy bones, teeth, heart, and blood vessels (Nerbass et al., 2010), meanwhile potassium is a mineral that affects the muscles and nerves (Telles and Boita, 2015). Thus, considering the results of the present work, *R. rosefolius* may contribute to human health.

Figure 2. Concentration of trace elements in wild strawberries cultivated in southern Brazil.



Bioactive compounds

Results of polyphenols and compounds with antioxidant and antihypertensive activities are presented in Table 2. Wild strawberries evaluated showed total polyphenolic content of 242.1±3.3 mg GAE per 100 g of fruit, and the monomeric anthocyanin concentration was 100.5±0.3 mg C3G per 100 g.

Table 2. Polyphenols, antioxidant and antihypertensive activity of juice from wild strawberry.

Total polyphenols (mg GAE 100 g^{-1})	242.1±3.3
Anthocyanins (mg C3G 100 g^{-1})	100.5±0.3
ABTS$^{•+}$ (%)	89.0±9.2
DPPH$^{•}$ (%)	61.1**±5.0**
Chelating power	3.1**±0.5**
Antihypertensive (%)	12.5±0.8

These results classify wild strawberries as having medium content of phenolic compounds (Rufino et al., 2007). *R. rosifolius* from southeastern Brazil presented 177.26 mg GAE per 100 g (Oliveira et al., 2016), which reinforce the fruit phenolic classification. Wild strawberries presented capacity to scavenge 89% of ABTS•+ radical and 61% of DPPH• radicals. Its chelating power was 3.1±0.5. Oliveira et al. (2016) observed ABTS•+ activity of 162.4 ± 5.6 μM Trolox equivalent per g of fruit and DPPH• activity of 120.8 ± 1.5 μM Trolox equivalent per g of fruit for *R. rosifolius* pulp. Anthocyanins are the major phenolic compounds in red-purple fruits and vegetables. They are rapidly absorbed in the stomach, providing effective antibacterial, anti-inflammatory and anticarcinogenic activities (Nunes and Novello, 2020). Additionally, antioxidant activity from polyphenolics is linked to reduction of blood glucose, oxidative stress and modulation of a series of events related to proliferation, metastasis and death of cancer cells (Park et al., 2016; Qiu et al., 2017; Nunes and Novello, 2020).

ACE inhibition is currently used for hypertension treatment, since it is a tool to control over activation of the renin angiotensin aldosterone system (RAAS), helping controlling high blood pressure (Hammoud et al., 2007). The antihypertensive activity of wild strawberries was 12.5±0.9%, which is similar to 0.01 mg L^{-1} quercitin or catechin solution (Balasuriya and Rupasinghe, 2012). There is no report in literature about the anti-hypertensive activity of strawberries so far. Extracts rich in flavonoids, mainly quercitin, catechin and epicatechin, are responsible for the high antihypertensive activity in vegetables (Balasuriya and Rupasinghe, 2012) due to their ability to make hydrogen bonds with the active site of the ACE enzyme, and the flat structure of flavonoid molecules has been proposed to play an important role in inhibiting ACE (Kwon et al., 2010).

Antimicrobial activity

Wild strawberries showed antibacterial activity against *S. aureus, B. cereus, L. monocytogenes and A. hydrophyla,* which are important foodborne bacteria. They did not present capacity to inhibit *E. coli and S. enteritidis* (Table 3). Similar results were found by Oliveira et al. (2016), who observed that *R. rosefolius* extract was able to produce halo zones of 10 mm against *A. hydrophila*, 11 mm against *B. cereus* and *L. monocytogenes* and 12 mm against *S. aureus*. The authors, unlike results of the present work, observed that extract from *R. rosefolius* inhibited *E. coli* and *Salmonella* spp, which may be due to the use of different strains and fruits. However, results show great potential of wild strawberry as antimicrobial agent.

Table 3. Antibacterial activity of wild strawberry.

Indicator microorganism	Inhibitory activity*
S. aureus	+
B. cereus	+
L. monocytogenes	++
E. coli	-
S. enteritidis	-
A. hydrophila	+

* Average diameter of inhibition zones: +, 7-10 mm; ++, 11-15 mm; -, no inhibition zone.

The antimicrobial activity of fruits containing anthocyanins is probably caused by multiple mechanisms and synergisms, as they contain several compounds, including anthocyanins, weak organic acids, phenolic acids and their mixtures in different chemical forms (Cisowska et al., 2011). Lacombe et al. (2010) observed that cranberry phenolics and anthocyanins inactivate *E. coli* by disintegrating the cell's outer membrane. In another study, in Gram-positive bacteria, it is believed that tannins can react and inhibit biosynthesis with components of the cell wall (Jones et al., 1994). According to Côté et al. (2011), the antimicrobial effect of red fruits has been associated with the high content of phenolic compounds, including low molecular weight phenolic acids, condensed tannins and flavonoids, such as anthocyanins and flavonols.

Thus, wild strawberry is an important source of polyphenols, mainly anthocyanins, minerals and compounds with antioxidant, anti-hypertensive and antibacterial activities. Studies to evaluate and disseminate the functional properties of non-conventional edible plants are essential to promote the Brazilian biodiversity and improve human diet.

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