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RESEARCH ARTICLE

Nutritional, physicochemical and antimicrobial properties of uvaia pulp (*Eugenia pyriformis* Cambess)

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The aim of this work was to evaluate the nutritional, physicochemical and antimicrobial characteristics of Uvaia pulp in aqueous, ethanolic and hydroethanolic extracts. We evaluate the bioactive compounds (phenolics and flavonoid), antioxidant activity by three different methods (DPPH, ABTS and FRAP), and antimicrobial activity using four different bacteria. The results show that uvaia pulp presents a TTA of 0.75 mg of citric acid 100 g⁻¹ and the pH of 3.45, characterizing like an acid fruit. Hydroethanolic extract presented more bioactive compounds (total phenolics: 189.41 mg GAE 100 g⁻¹; flavonoids 0.42 mg QE 100 g⁻¹) and more antioxidant capacity by DPPH (1,600.50 mg TEAC 100 g⁻¹) and ABTS (342.11 mg TEAC 100 g⁻¹) assay and by Pearson's correlation coefficient, they present positive correlation with statistical difference ($p < 0.01$ and $p < 0,05$). But the uvaia extracts did not present antimicrobial activity towards the bacteria tested.

Highlighted Conclusion

Uvaia pulp has great nutritional and physicochemical properties, and presents antioxidant activity being a rich source of natural bioactive compounds, may be applying in processed products as jellies and juices.

Brazilian flora presents a rich biodiversity of fruit trees, and stands out as having a high potential for commercialization and development of new processed products. Along with that, the landscape of Santa Catarina presents a range of native fruits, like feijoa (*Acca sellowiana*), blackberry (*Morus nigra*), uvaia (*Eugenia pyriformis* Cambess), Rio Grande cherry (*Eugenia involucrata*), butia (*Butia eriospatha*), gabiroba (*Campomanesia reitziana*), araçá (*Psidium cattleianum*), blue passion fruit (*Passiflora caerulea*), pitanga (*Eugenia uniflora*) among others.

One of the biggest plant groups present in Brazil belong to Myrtaceae family. It is estimated that more than three thousand species of trees and shrubs belong to this group, being found in almost all the continents, with clear predominance in tropical and subtropical regions (Marchiori and Sobral 1997). The uvaia's tree (known as *uvalheira*) is an example of a plant belonging to the family Myrtaceae, ranging from 6 to 13 meters of height, blooming in August and September, when its fruiting begins. The harvest occurs between the months of September to November (Lorenzi 1998). Uvaia fruit present an acid and sweet taste, velvety texture with a thin skin, spherical appearance with 2 to 4 cm in diameter, fleshy, and yellow-orange color. Native of the Atlantic Forest, it can be found from São Paulo to Rio Grande do Sul.

Researches are important to valorization and conservation of the native species providing possibilities of commercial production and commercialization alternatives to the farmers. Few studies were performed on the nutritional, bioactive and antimicrobial properties of uvaia.

Even so, the consumption of subtropical fruits is increasing in national and international market, mainly because nutritional and therapeutic values (Rufino et al. 2009b, Clerici and Carvalho-Silva 2011). Physicochemical information and nutritional composition knowledge regarding of fruits are important to the formulation of new processed products. As a result, these fruits represent an opportunity for local producers, who can produce them in their orchards and sell the fruit, or produce products with the fruits such as ice cream, jelly and sweets, generating an extra family income.

Fruit consumption provides essential compounds for human metabolism, such as polyphenols, alkaloids and terpenoids, named natural bioactives. These compounds are produced in metabolic routes presents in plants, called as secondary metabolism, and their function in the vegetables are to protect against ultraviolet radiations, bacteria, insects and viruses (Thilakarathna and Rupasinghe 2012, Heleno et al. 2015). Secondary metabolites synthesized by the plant metabolism can be divided into some groups of bioactive compounds, such as carotenoids (alpha and beta carotene, lutein, lycopene, among others), phenolic compounds, alkaloids and organosulfur compounds. In the classification of these groups, phenolic compounds can be subdivided into phenolic acids (gallic acid, chlorogenic acid, p-coumaric acid, caffeic acid, among others), flavonoids (quercetin, epicatechin, campoferol, chrysin, myrhcicin, fisetin, among others), tannins, stilbenes and coumarins (Liu 2004, Veberič 2010).

Polyphenols from vegetables pulp, peels and leaves have attracted the attention of the food industry, pharmaceutical and general population, due to the fact that these compounds have high antioxidant action, being effective against reactive oxygen species, such as peroxy radical, hydroxyl radical, superoxide radical, nitric oxide and peroxy nitrite. Presence of antioxidant molecules in fruits helps cellular defense mechanism and to control the damage caused by free radicals (Halliwell and Gutteridge 1984, Rufino et al. 2009a, Rufino et al. 2010, Thilakarathna and Rupasinghe 2012, Oladeji and Adelowo 2017). Besides that, many research groups have isolated and identified these compounds and related with antifungal, antiviral, antibacterial activity (Oladeji and Adelowo 2017).

The aim of this study was to evaluate the nutritional composition, physicochemical properties, antioxidant and antimicrobial capacity of uvaia pulp from mountain region of Santa Catarina.

MATERIAL AND METHODS

Plant material. Samples were collected at the species maturation point in the community of Morro Grande, rural area of Urupema city, Santa Catarina (Latitude 28°04'34"S, Longitude 49°59 '77' 'W, altitude of 1,114 meters). After the harvest, the seed was manually separated from the pulp, and the pulp was homogenized in domestic multiprocessor, and stored in an industrial freezer (-18±2 °C) until the analyzes were carried out.

Analysis of nutritional composition. The moisture content was determined using an aliquot of 5 grams of samples and was conducted to the forced air circulation dehydrator at 105 °C during 24 hours until constant weight. The resulting fraction was conducted to total ashes analysis, in a muffle at 550 °C during 12 hours (Instituto Adolfo Lutz 2008). Protein quantification was determined according to the methodology of Micro Kjeldahl (AOAC 1996), following the steps of digestion, distillation and titration the sample. Total lipids were determined by cold extraction, using methanol, chloroform and water as solvents (Bligh and Dyer 1959). Total carbohydrates were calculated by the difference among others compounds (100 - (moisture+ashes+proteins+lipids)). Total calories were calculated using coefficients of Atwater and Wood (1896), to proteins and carbohydrates 4 kcal g⁻¹ and lipids 9 kcal g⁻¹.

Physicochemical analyses. The content of Total Soluble Solids (TSS) was expressed in degrees Brix (°Brix) and was determined using a portable digital refractometer (Atago, Pal-3). The pH was determined using a digital pHmeter previously calibrated. Both analyzes were performed in unicate.

The Titratable Total Acidity (TTA) analysis was performed using sodium hydroxide solution (NaOH 0.1 mol L⁻¹) until reaching pH 8.1 and the results were expressed in mg of citric acid per 100 g⁻¹ of pulp (Instituto Adolfo Lutz 2008). TSS/TTA rate was also calculated.

Bioactive compounds extraction. Uvaia extracts were performed using 5 g of homogenized fresh pulp dissolved in three different solvents: distilled water, ethanol and 70 °GL hydroethanolic solution (70% ethanol and 30% distilled water) transferred to volumetric flask (50 mL) and the volume was completed with the respective solvents. The extracts were then kept under refrigeration during one week, filtered on qualitative filter paper after that period and stored in a dark amber bottle. When necessary, the extracts were diluted for the analysis.

Analysis of bioactive compounds. The concentration of total phenolics compounds were determined by Swain and Hillis (1959) with modifications. It was added 104 µL of the sample extract and 1667 µL of distilled water in a test tube. After, it was added 104 µL of Folin-Ciocalteu 0.25 N reagent. Incubated the reaction during 3 minutes, and added 208 µL of sodium carbonate - Na₂CO₃ - 1N. After 2 hours, the absorbance was measured in Spectrophotometer at 725 nm and the standard curve was performed using gallic acid.

Total flavonoids analysis was determined according to Zhishen et al. (1999), in 500 μL of each extract, were added 2 mL of deionized water and 150 μL of sodium nitrite solution 5% (NaNO_2), after 5 minutes was added 150 μL of aluminum chloride aqueous solution 10% (AlCl_3). After more 5 minutes of incubation, was added 1 mL of 1 mol L^{-1} sodium hydroxide (NaOH) and the absorbance measurements were performed using a spectrophotometer (510 nm) and quercetin was used as standard as calibration curve.

Analysis of antioxidant activity. Antioxidant activity through the removal of the radical DPPH (1,1-difenil-2-picrilhidrazil), it was determined according to Brand-Williams et al. (1995), using 515 nm as wavelength. Were used 150 μL of each extract in 1850 μL of DPPH 0.1 mM solution, being the measurement realized after 24 hours, and incubation in a dark environment and ambient temperature. Calibration curve it was accomplished with Trolox solution in different concentrations.

Antioxidant activity through the removal of the radical ABTS (acid 2,2'-azino-bis(3-ethylbenzotiazolin-6-sulfonic), was determined according Rufino et al. (2007). To 30 μL of each extract were added 3000 μL of ABTS+solution and homogenized in a tube shaker. After 6 minutes in the dark, the absorbance lecture of the resultant color it was performed at 734 nm in a spectrophotometer and the standard of Trolox, it was used as calibration curve.

Antioxidant activity through ferric reduction - FRAP (Ferric Reduction Antioxidant Power), was determined according Benzie and Strain (1996) with modifications of Arnous et al. (2002). Aliquots of 100 μL of each extract were added 100 μL of ferric chloride 3 mM and 1800 μL of TPTZ (2.4.6-Tris(2-piridil)-s-triazina) solution, 1 mM. The reaction was maintained 30 minutes in water bath to 37 °C. The absorbance was measured at 620 nm and Trolox was used for the calibration curve.

Analysis of antimicrobial activity. Uvaia extracts were submitted to analysis of antimicrobial activity by disk diffusion. The extracts were tested using four different bacteria: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 25923) and *Shigella dysenteriae*.

The bacteria were recovered in BHI broth (Brain Heart Infusion) and incubated during 24 hours at 37 °C. Filter paper disks of 6 mm in diameter received an aliquot of 15 μL of each uvaia extract and were applied to petri dishes containing MH agar (Mueller-Hinton), previously inoculated with the microorganisms. In addition, discs with their respective solvents were tested on the plates. Positive and negative control with antibiotics were tested with each sensitive and resistant bacteria. Then the plates with the disks were left at 37 °C, and the halos were measured after 24 hours.

Statistical analysis. Bioactive compounds and antioxidant activity results were analyzed by ANOVA and the differences between the means were determined by the Tukey HSD test ($p < 0.05$). Then, a Pearson's correlation coefficients were determined between bioactive compounds and antioxidant capacity by two significance levels ($p < 0.05$ and $p < 0.01$), using Statistica® 7.0 software.

RESULTS AND DISCUSSION

Nutritional composition and physicochemical characteristics of uvaia pulp (Table 1), show that this fruit is composed by a large amount of water (93.29 g 100 g⁻¹). This is usual for Myrtaceae family, which is reflected in highly perishable fruits (Rufino et al. 2009b, Pereira et al. 2012).

The ashes content represents the amount of minerals present in the sample, as calcium, magnesium and iron, and may be associated with the moisture content. In samples with high water content, the ashes are diluted in the food matrix. Due to the fact that this fruit had a high moisture value, the ashes were consequently smaller, 0.44 g 100 g⁻¹. This number was higher than described by Coutinho et al. (2016), where the ash content in uvaia pulp was 0.38 g 100 g⁻¹.

Myrtaceae family fruits presents a high content of protein and lipids (Pereira et al. 2012). The protein content in uvaia pulp according to Coutinho et al. (2016) was 2.14 g 100 g⁻¹ and lipids was 0.55 g 100 g⁻¹. The values obtained in the present study were lower, being 1.69 g 100g⁻¹ for proteins and 0.38 g 100 g⁻¹ for lipids. These differences may be related with the geographic location and genetic variability (Maietti et al. 2012, Olujobi 2012).

The carbohydrate content is determined by the difference method between all the constituents. In this study, the carbohydrate content obtained was low (4.20 g 100g⁻¹), which represent low caloric value (27.00 kcal 100g⁻¹). However, uvaia pulp can present polysaccharides, such as starch (0.35 g 100 g⁻¹) and pectin (0.37 g 100 g⁻¹) (Rufino et al. 2009b).

Table 1. Nutritional and physicochemical characteristics of uvaia pulp.

Determination	Results
Moisture ¹	93.29 ± 0.04
Ashes ¹	0.44 ± 0.06
Lipids ¹	0.38 ± 0.02
Proteins ¹	1.69 ± 0.08
Carbohydrates ¹	4.20 ± 0.01
Calories ²	27.00 ± 0.44
pH	3.45
TSS	4.6
TTA ³	0.75 ± 0.03
TSS/TTA	6.13

The results are expressed in mean ± standard deviation (n=3). pH and TSS were analysed in unicated.

¹ g 100g⁻¹; ² kcal 100g⁻¹; ³ mg of citric acid 100 g⁻¹.

The pH of uvaia pulp, can present values between 2.77 and 3.15 (Rufino et al. 2009b, Zillo et al. 2013), while the value obtained in this study was 3.45. In some studies, performed with uvaia pulp, the TTA can variate between 1.05 and 2.31 mg 100 g⁻¹ of citric acid (Rufino et al. 2009b, Zillo et al. 2013), but in this study, the uvaia pulp presents low value of TTA (0.75 mg 100 g⁻¹ of citric acid) compared with the literature. TSS/TTA ratio is one of the most common indicators of ripeness in fruits for *in natura* consumption or agroindustrial processing (Rufino et al. 2009b). Higher TSS/ATT values, indicate a better maturation point, since it is desired that the TSS content increases with the fruit maturation, and in this study 6.13 was the ratio obtained.

Table 2 shows bioactive compounds (total phenolics and flavonoids) in uvaia extracts using different extraction solvents. Using and mixing solvents in different proportions with opposite polarities influence the extraction rate of phenolic compounds, because they affect in the transference of electrons and hydrogen atoms (Su et al. 2007, Rockenbach et al. 2008, Mira et al. 2008, Ferrareze et al. 2014). In this study, the solvents tested showed that the hydroethanolic solution extracted the highest amount of total phenolic compounds (189.41 mg GAE 100 g⁻¹) and flavonoids (0.42 mg QE 100 g⁻¹). Then, the extract prepared with the solvent ethanol (total phenolics compounds 107.14 mg GAE 100 g⁻¹; flavonoids 0.16 mg QE 100 g⁻¹) obtained an intermediate extraction; and water was the solvent that extracted the least amount of phenolic compounds (34.70 mg GAE 100 g⁻¹) and flavonoids (0.4 mg QE 100 g⁻¹). Generally, the extraction followed this sequence: hydroethanolic > ethanol > aqueous.

Table 2. Bioactive compounds (total phenolics and flavonoids) in uvaia extracts using different extraction solvents.

Extract	Total Phenolics ¹	Total Flavonoids ²
Aqueous	34.70 ± 0.99 ^c	0.04 ± 0.00 ^c
Ethanol	107.14 ± 0.55 ^b	0.16 ± 0.01 ^b
Hydroethanolic	189.41 ± 3.98 ^a	0.42 ± 0.00 ^a

The results are expressed as mean ± standard deviation. All analyzes were performed in triplicates. Different letters in each column after standard deviation represent a significant difference by Tukey test (p <0.05). ¹ mg Gallic Acid Equivalent (GAE) 100 g⁻¹ pulp. ² mg Quercetin Equivalent (QE) 100 g⁻¹ pulp.

Comparing the content of total phenolic compounds in uvaia pulp, extracted with hydroethanolic solution, with other tropical fruits produced in Brazil, it is possible to observe that uvaia presents a higher content of phenolic compounds compared to banana (*Musa x paradisiaca* L.) (117.8 mg GAE 100 g⁻¹), buriti (*Mauritia vinifera*) (108.1 mg GAE 100 g⁻¹), cubiu red (*Solanum sessiliflorum* Dunal) (71.9 mg GAE 100 g⁻¹), cubiu yellow (*Solanum sessiliflorum*) (76.0 mg GAE 100 g⁻¹), jackfruit (*Artocarpus heterophyllus* Lam.) (34.1 mg GAE 100 g⁻¹), physalis (*Physalis angulata*) (39.4 mg GAE 100 g⁻¹), plum variety D'agem (*Prunus domestica* L.) (77.1 mg GAE 100 g⁻¹), plum variety Larri Ann (*Prunus domestica* L.) (105.5 mg GAE 100 g⁻¹) and starfruit (*Averrhoa carambola* L.)

(51.9 mg GAE 100 g⁻¹). However, bacuri (*Platonia insignis* Mart.) (266.8 mg GAE 100 g⁻¹), golden spoon (*Byrsonima crassifolia* L. Kunth) (384.5 mg GAE 100 g⁻¹), pequia (*Caryocar villosum* (Aubl.) Pers.) (4,623.4 mg GAE 100 g⁻¹) and star nut palm (*Astrocaryum aculeatum* G Mey) (456.8 mg GAE 100 g⁻¹) present higher amounts of phenolic compounds (Barreto et al. 2009).

Using the classification according to the criteria of phenolic compounds proposed by Vasco et al. (2008), fruits in the “low” category present less than 100 mg GAE 100 g⁻¹; already the fruits in the “middle” category present between 100 and 500 mg GAE 100 g⁻¹; already the fruits in the “high” category presents more than 500 mg GAE 100 g⁻¹. Therefore, the extracts evaluated in this study, using ethanol and hydroethanolic solution, are classified in the middle category. Already using water as solvent, classified the fruit extract in the low category.

Table 3 presents the antioxidant capacity (DPPH, ABTS and FRAP) in uvaia extracts using different extraction solvents. The methods tested showed a significant difference for the antioxidant activity between different solvents. DPPH and ABTS method, both based on the removal of free radical, the hydroethanolic extract was the one that presented statistically higher antioxidant action ($p < 0.05$), followed by the ethanolic and aqueous extract. However, in the ferric reduction method (FRAP) the extract using ethanol presented the highest antioxidant activity.

Table 3. Antioxidant capacity (DPPH, ABTS and FRAP) in uvaia extracts.

Extract	DPPH ¹	ABTS ¹	FRAP ¹
Aqueous	249.45 ± 62.15 ^c	27.00 ± 0.57 ^c	64.74 ± 1.28 ^c
Ethanol	579.03 ± 6.29 ^b	140.66 ± 6.36 ^b	243.11 ± 2.41 ^a
Hydroethanolic	1600.50 ± 8.39 ^a	342.11 ± 1.82 ^a	227.99 ± 0.00 ^b

The results are expressed as mean ± standard deviation. All analyzes were performed in triplicates. Different letters in each column after standard deviation represent a significant difference by Tukey test ($p < 0.05$).

¹ mg Trolox Equivalent Antioxidant Capacity (TEAC) 100 g⁻¹ pulp.

In addition, it is recommended that at least two antioxidant activity assays must be tested to provide a reliable solution for the quantification of antioxidant activity (Rufino et al. 2009a), and in this work, three different methods were evaluated that determine the antioxidant capacity (DPPH, ABTS and FRAP).

Table 2 and 3 show the antioxidant capacity related with the bioactive compounds, the hydroethanolic extract present more bioactive compounds and more antioxidant capacity (DPPH and ABTS). Statistically, it is possible to verify this fact on the Table 4, using a Pearson's correlation coefficient between total phenolics, flavonoids and antioxidant activity.

Table 4. Pearson's correlation coefficients (r) between bioactive compounds and antioxidant capacity.

Variable	Total Flavonoids	DPPH	ABTS	FRAP
Total Phenolics	0.99*	0.97*	0.99*	0.80
Total Flavonoids		1*	1*	0.69
DPPH			0.99*	0.63
ABTS				0.72

Marked with * presented statistical difference to $p < 0.05$ and $p < 0.01$

In this study, a positive correlation was found between the analysis of bioactive compounds and antioxidant activity, only FRAP assay showed non-significant difference. In general, antioxidant activity by DPPH and ABTS in uvaia extracts is correlated with the presence of phenolics and flavonoids and the correlation coefficient between our results was positive with high magnitude, presenting statistical difference for $p < 0.05$ and $p < 0.01$. A positive correlation coefficient indicates that, for example, the higher content of phenolic compounds results in a higher antioxidant action (Rufino et al. 2010, Zillo et al. 2013).

According to Bhalodia and Shukla (2011), the secondary metabolism in plants, can produce different types of compounds, and these compounds have great antimicrobial properties. Plants products occupy the major part of the antimicrobial compounds discovered until now (Balouiri et al. 2016).

In our study, all the microorganisms tested (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Shigella dysenteriae*) showed resistance to the uvaia extracts, and there was no inhibition halo around the disks and was not possible to determine the Minimum Bactericidal Concentration (MBC) of the extracts.

In conclusion, uvaia pulp has great nutritional and physicochemical properties and may be applied in processed products as jellies and juices. Also, hydroethanolic extract was the best extraction solvent for total phenolics and flavonoids, when compared to ethanol and water. Uvaia pulp presents high antioxidant activity being a rich source of natural bioactive compounds.

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