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CAMPOMANESIA SPP. NATIVE FRUITS AS POTENTIAL SOURCE OF HEALTH-PROMOTING COMPOUNDS

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Abstract

Campomanesia xanthocarpa var. *littoralis*, *Campomanesia xanthocarpa (Berg)*, and *Campomanesia eugenioides* are native fruit plants found in Brazil. Due to the scarce number of controlled scientific studies comparing different native *Campomanesia* species, this study sought to determine their bioactive compounds and antioxidant properties. *C. eugenioides* proved to be a rich source of total phenolic compounds, also showing the best antioxidant capacity by the ABTS, DPPH and molybdenum reduction power methods. On the other hand, *C. xanthocarpa var. littoralis* showed the best results for total flavonoids content, and Iron(II) chelation power. The phenolic compounds contents present in *C. eugenioides* could be responsible for the best antioxidant activity. This study provides key scientific data regarding the use of valuable fruits from different edible *Campomanesia* species to produce bioactive ingredients, as well as natural preservatives for food products. Thus, our results contribute to the discovery of the potential application of these native *Campomanesia* Brazilian fruits, as a natural product with functional and antioxidant properties.

Keywords: Antioxidant Activity. Bioactive Compounds. *Campomanesia xanthocarpa* var. *littoralis. Campomanesia xanthocarpa. Campomanesia eugenioides.* Native Fruits.

1. Introduction

Brazil is one of the world's largest producers of fruit because of its large territory, geographical position, soil composition, and climatic conditions (Barros et al. 2017). In this sense, native tropical fruits have gained consumer preference, especially concerning their unique characteristics (Paz et al. 2015). However, by comparing the quantities of native fruits not yet studied in the world, works on the properties of these fruits are still scarce in the literature. Thus, many relatively unknown fruit species have been recently evaluated as an alternative to their more traditional counterparts (Pereira et al. 2013; Paz et al. 2015; Carvalho et al. 2016).

Among the native plants in Brazil, Myrtaceae are among the ten plant families with the highest

representation among all flora, including approximately 1000 species spread throughout 26 different genera (Donado-Pestana et al. 2018). Among the fruits belonging to the Myrtaceae family is the *Campomanesia* sp genus. The *Campomanesia* genus comprises about 30 species well distributed in tropical and subtropical South America (Landrum and Kawasaki 1997). *Campomanesia xanthocarpa* (Berg) is one of the most popularly known and widespread species among the genus (Barbieri et al. 2017). It presents the botanical synonyms *Campomanesia crenata*, *Campomanesia dusenii*, *Campomanesia malifolia*, and *Campomanesia rhombea*. The fruits are round and green in color when young and yellow and sweet when mature (Vallilo et al. 2008).

Therefore, many other fruits of different *Campomanesia* species are not so well known and studied. Thus, our work presents pioneering results for fruits of other species of *Campomanesia* poorly studied like *Campomanesia xanthocarpa* var. *littoralis* (D. Legrand), and *Campomanesia eugenioides. C. eugenioides* is commonly confused with *C. xanthocarpa* because they have similar characteristics. The fruits are globose and smooth, measuring about 5.5 to 7 mm in diameter (Lima et al. 2011). On the other hand, *C. xanthocarpa* var. *littoralis* (D. Legrand) can be confused with *C. guazumifolia* because of the green color attributed to the fruit when mature. However, in both cases, the trees are easily differentiated concerning their size and other botanical properties. Its leaves differ in size and consistency, and the fruits also differ in size (15 to 40 mm) and color when ripe, from green to orange (Biavatti et al. 2004).

The study of the functionality of native fruits and their components responsible for this effect has become forefront in recent years. Donado-Pestana et al. (2018) state that the functional compounds present in foods can be very varied. Among them are minerals, vitamins, carotenoids, and phenolic compounds (among other secondary metabolites) found in fruits. Moura-Costa et al. (2012) ensure that many fruits of the *Myrtaceae* family have been used throughout history as food and also as traditional medicine for intestinal disorders, especially in tropical and subtropical countries.

Antioxidants obtained from the diet, such as vitamins C, E, and A, flavonoids, and carotenoids are essential for the interception of free radicals. Another mechanism of protection is the repair of the injuries caused by the radicals. This process is related to the removal of DNA molecule damage and the reconstitution of damaged cell membranes (Dröge 2002). Therefore, species of the genus *Campomanesia* have great economic importance as part of the income of numerous families and small producers. Also, the fruits are consumed in nature and are used to produce homemade sweets, sorbets, spirits, soft drinks, and liqueurs by the local population (Vallilo et al. 2005).

Considering the scarcity of studies on the native fruits characterization from *C. eugenioides* and *C. xanthocarpa* var. *littoralis,* the present study aimed to determine the *Campomanesia* fruit bioactive compounds of this species, as well as *C. xanthocarpa* (Berg), evaluating their antioxidant effects; given their future potential application.

2. Material and Methods

Chemicals

Folin-Ciocalteau phenol reagent, gallic acid, (+)-catechin, quercetin, 2,2-diphenyl-1-picrilhydrazil (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt (Ferrozine®), and lutein were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All of the chemicals were of analytical grade.

Location description and collection of samples

Ripe *C. xanthocarpa* (*Campomanesia xanthocarpa (Berg)*), *C. eugenioides* (*Campomanesia eugenioides*), and *C. xanthocarpa var. littoralis* (*Campomanesia xanthocarpa* var. *littoralis (D. Landrum)*) fruits were harvested in the western region of the state of Santa Catarina, Brazil (latitude 27°14'2"S and longitude 52°1'40"W). The specimens were registered at the Herbário do Vale do Taquari (HVAT, Lageado, Rio Grande do Sul, Brazil) and at the Herbário Padre Balduíno Rambo (HPBR, Erechim, Rio Grande do Sul, Brazil) under the codes HPBR 11579, HVAT 2612, and HPBR 11580, respectively. All the samples were

harvested fully mature and were free of any visible injuries, infections and color inconsistency. Furthermore, the fruits were freeze-dried with all the edible parts intact (skin, pulp, and seeds) and then processed in a knife mill (Tecator, Knifetec 1095 model). The powders obtained from the milling process were vacuum sealed in plastic bags and stored at a temperature of −18 ± 0.2 °C until analyzed.

Extract preparation

An extract was prepared for each sample. It was prepared with freeze-dried fruit and ethanol: water 80:20 (v/v) in a mass/solvent ratio of 1:5 to obtain the hydroalcoholic extract by exhaustive maceration with solvent exchange at 24, 48, 72 and 192 hours. The extraction was carried out under agitation in an orbital plate (Etica, 109-2-E model, São Paulo, Brazil) at room temperature without incidence of light. The extracts were filtered and rota-evaporated (40 ± 1°C) until total volume was reduced to 100 mL. All the extracts were stored in the dark at 4 ± 1°C until analyzed.

Determination of total phenolics content (TPC)

Total phenolics were determined by the Folin–Ciocalteu method (Singleton et al. 1999). A volume of 500 µL of a known dilution of the extracts was added to the Folin–Ciocalteu reagent 1:100 (2.5 mL), which then reacted for 5 min. After that followed by the addition of 2.5 mL of a sodium carbonate solution (7.5 %, w/v). Solutions were mixed and allowed to stand for 2 h in a water bath at 25 ± 1ºC. Subsequently, the absorbance was measured at 760 nm. A calibration curve was also prepared, using a gallic acid standard solution (50-200 mg L^{-1}; R^2=0.996). The results were expressed as mg of gallic acid equivalents per 100 g of sample on a fresh weight (fw) basis (mg GAE $100g^{-1}$ fresh fruit weight). Each determination was carried out in triplicate.

Determination of total flavonoids content (TFC)

Total flavonoid content was determined according to Dewanto et al. (2002). A volume of 250 µL of a known dilution of the extracts was added to 75 µL of aqueous solution of sodium nitrite (5%, w/v), and 1.25 mL of deionized water. This mixture was allowed to react for 6 min followed by addition of 150 µL of aluminum chloride solution (10 %, w/v). After 5 minutes, 500 µL of sodium hydroxide 1 M and 2.5 mL of deionized water were added to the mixture. Absorbance was measured at 510 nm and compared to a (+)-catechin calibration curve (50-200 mg L^{-1}; R^2=0.999). Results were expressed as mg of (+)-catechin equivalents per 100 g of sample on a fresh weight (fw) basis (mg CE $100g^{-1}$ fresh fruit weight). The analysis was performed in triplicate.

Evaluation of the antioxidant activity: ABTS method

The ABTS method [2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid] was used in this assay, as described by Re et al. (1999). The ABTS+ radical cation was produced by mixing potassium persulfate 2.45 mM L^{-1} and ABTS 7 mM L^{-1} (1:1), with the mixture being kept in a dark room for 16 h. For the analysis proper, the reagent was diluted in ethanol to an absorption of 0.700 ± 0.02 at 734 nm. A volume of 30 µL of a known dilution of the extracts was mixed to 3 mL of the ABTS solution, which then reacted for 5 min and had its absorbance at 734 nm measured. A calibration curve with Trolox was used (0.5-2.0 mM L^{-1}; R^2=0.991). The results were expressed in TEAC (Trolox equivalent antioxidant activity) in µmol TEAC per 100 g fresh fruit weight (µmol TEAC $100g^{-1}$ fresh fruit weight). Every analysis was performed in triplicate.

Evaluation of the antioxidant activity: DPPH method

The antioxidant activity of the fruit extracts on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was also studied. The method used was previously described by Brand-Williams et al. (1995). An aliquot of 100 µL of the extracts solutions at different concentrations (12.5-250 µg mL^{-1}) was added to 3.9 mL of a methanol DPPH solution (60 µM). The mixture was then homogenized and allowed to stand for 60 minutes at 25 ± 1ºC in the dark. After that, the absorbance was measured at 515 nm. Percentage inhibition values were

calculated from the absorbance using the followed equation: Inhibition (%) = $[(A_{Control} - A_{Sample})/A_{Control}] \times 100$. The result was plotted against extract concentration, and the IC_{50} values (the concentration which provides 50% inhibition) were estimated from the sigmoidal curve equation. Ascorbic acid, gallic acid, (+)-catechin, quercetin and butylated hydroxyanisole (BHA) were used as positive controls. The analyses were carried out in triplicate.

Evaluation of the antioxidant activity: Iron(II) chelation power (ICP)

The extracts' ability to chelate iron(II) was evaluated by using the method described by Dastmalchi et al. (2008). In summary, 500 µL of a known dilution of the extracts was mixed to 50 µL of a Iron(II) chloride solution 2 mM L^{-1}, which was allowed to react for 5 min and then added with 1.850 µL of methanol and 100 µL of a ferrozine solution 5mM L^{-1}. After a 10 min incubation period, the sample's absorbance at 562 nm was checked. A calibration curve with Na_2EDTA was used (2.0-10.0 mg L^{-1}; R^2=0.990). Results were expressed as mg of Na_2EDTA equivalents per 100 g of sample on a fresh weight (fw) basis (mg Na_2EDTAE $100g^{-1}$ fresh fruit weight). All analyses were done in triplicate.

Evaluation of the antioxidant activity: Determination of molybdenum reduction power (MRP)

This assay is based on the reduction of Mo (VI) to Mo(V) by the extracts, and was determined following the methodology described by Prieto et al. (1999). An aliquot of 300 µL of a known dilution of the extracts was mixed to 3 mL of a reagent solution (0.6 M of sulfuric acid, 4 mM of ammonium molybdate, and 28 mM of sodium phosphate) and left still for 90 min in a water bath at 95 ± 1ºC. The mixture was then cooled to room temperature, and its absorbance at 695 nm was then measured. A calibration curve with ascorbic acid was used (0-150 mg L^{-1}; R^2=0.999). The results were expressed as mg of ascorbic acid equivalents per 100 g of sample on a fresh weight (fw) basis (mg AAE $100g^{-1}$ fresh fruit weight). All analyses were carried out in triplicate.

Statistical analysis

The significance of the differences found between the means of each of the samples was determined via analysis of variance (ANOVA) and by Tukey's test ($p>0.095$). The data was also submitted to linear correlation (*R*) from the regression analysis. All statistical analyses were performed using the software STATISTICA v. 13.3 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

Table 1 shows the total phenolic content, flavonoids content, and antioxidant capacity of *C. xanthocarpa (Berg), C. eugenioides*, and *C. xanthocarpa* var. *littoralis* fruits extracts. *C. eugenioides* showed the highest ($P < 0.05$) total phenolics content (TPC), while *C. xanthocarpa* var. *littoralis* displayed the highest ($P < 0.05$) total flavonoids content (TFC), between the three species evaluated. Chen et al. (2014) and Contessa et al. (2013) found lower TPC values (10.32 to 495.12 mg GAE 100 g^{-1} fw) in 50 different fruits species, as, apple, banana, grape, orange, mango, strawberry, raspberry, and blackberry. Therefore, the TPC contents obtained in the present study highlight the bioactive potential of the genus evaluated. However, contrary behavior was verified for the flavonoids contents (TFC). TFC values were also highest ($P < 0.05$) than those found by others authors, as for example, Haminiuk et al. (2011) for others *Campomanesia* species, which ranged from 30.16 mg QE 100 g^{-1} fw to 56.21 mg QE 100 g^{-1} fw; and by Santos et al. (2013) for guabiroba juice (58.94 mg QE 100 mL^{-1}). As for TPC contents supply, *Campomanesia* species evaluated in the present study, also represent excellent flavonoids sources. It is noteworthy that Heim et al. (2002) emphasize that flavonoids, a secondary class of plant phenolics with significant chelating and antioxidant properties, have several biological effects for humans, such as antioxidant activity.

Table 1. Average concentration and standard deviation of total phenolics content (TPC), total flavonoids content (TFC), and antioxidant activity assays of *Campomanesia xanthocarpa* (Berg), *Campomanesia eugenioides*, and *Campomanesia xanthocarpa* var. *littoralis* fruit extracts.

	C. eugenioides	*C. xanthocarpa* (Berg)	*C. xanthocarpa* var. *littoralis*
TPC (mg GAE $100g^{-1}$ fw)	1416.42 ± 48.68^{a}	$604.4.69 \pm 24.99^{c}$	763.35 ± 20.26^{b}
TFC (mg CE $100g^{-1}$ fw)	104.31 ± 4.21^{b}	111.69 ± 3.25^{b}	220.14 ± 9.04^{a}
Antioxidant activity assays			
ABTS (µmol TEAC $100g^{-1}$ fw)	9515.30 ± 639.76^{a}	5342.44 ± 209.99^{c}	6741.14 ± 188.60^{b}
IC_{50} (µg mL^{-1})	23.62 ± 0.63^{c}	53.43 ± 1.73^{a}	27.02 ± 1.42^{b}
MRP (mg AA $100g^{-1}$ fw)	4959.27 ± 53.11^{a}	3504.78 ± 73.36^{b}	3018.95 ± 18.41^{c}
ICP (mg Na_2EDTAE $100g^{-1}$ fw)	5.98 ± 0.37^{c}	30.72 ± 2.04^{b}	39.94 ± 0.43^{a}

Results are presented as mean ± SD, n=3. Identical letters in the same column indicate no significant difference ($P < 0.05$). fw – fresh weight; GAE – gallic acid equivalent; CE – (+)catechin equivalent; TEAC – Antioxidant activity equivalent to Trolox; IC_{50} – inhibitory concentration (50%); AA – ascorbic acid; MRP – molybdenum reducing power; ICP – iron(II) chelation power; Na_2EDTAE – EDTA disodium salt equivalent.

The results from antioxidant activity assays are shown in Table 1, and they demonstrated that the three fruit extracts evaluated had free radical scavenging activity. Pereira et al. (2012) also found higher ($P < 0.05$) DPPH radical scavenging for *Campomanesia* genus in comparison with uvaia and yellow guava fruits, showing that this kind of fruit may have a better antioxidant capacity than those evaluated. The DPPH values reported by Chen et al. (2014) ranged from 6.48 ± 0.16 to 129.71 ± 1.36 µmol AA g^{-1} wet weight for 33 different fruits. Haminiuk et al. (2011) related higher IC_{50} values for two *Campomanesia* genus and araçá fruit (121.24 ± 3.12 µg mL^{-1}, 94.71 ± 1.99 µg mL^{-1} and 94.71 ± 2.15 µg mL^{-1}, respectively) than those data obtained for all the fruits studied in the present work. These results showed that *Campomanesia* fruits evaluated in our work, have a better radical scavenging capacity.

The IC_{50} values showed high negative correlation (R = - 0.821) with the ABTS radical scavenging capacity, i.e., *C. eugenioides* also showed the better antioxidant capacity by the ABTS method. These ABTS results were higher than those obtained by Vasco et al. (2008) who examined 17 fruits from Ecuador and by Chen et al. (2014) for 33 other fruits. In our study, high correlations between TPC and antioxidant capacity methods were also found for DPPH (R = -0.727) and ABTS (R = 0.989) methods. Rufino et al. (2010) verified high correlation between TPC and DPPH (R = -0.720) and ABTS (R = 0.920) for 18 non-traditional Brazilian tropical fruits.

C. eugenioides also showed the best results in relation to the molybdenum reducing power (MRP) method (Table 1). It was observed that the MRP results showed high positive correlation with vitamin C content (R = 0.987), ABTS (R = 0.837), and TPC content (R = 0.909). Similar data was obtained by Banerjee et al. (2005) who found a higher MRP result for black plum skin (17.900 mg AA $100g^{-1}$ DW). These authors stated that these results could be related with vitamin C and phenolic content of the fruit.

Iron(II) chelation power (ICP) method indicated higher value ($P < 0.05$) for *C. xanthocarpa* var. *littoralis* fruit extract (Table 1) when compared with the other two fruits extracts. This method demonstrated high positive correlation between the carotenoid content (R = 0.817) and TFC (R = 0.749). As Dastmalchi et al. (2008) affirmed that ascorbic acid and gallic acid were not good chelators of iron(II), with the behavior observed in our study, we hypothesize that in our case the carotenoids and the flavonoids could have participated in the chelation reactions.

4. Conclusions

In closing, this study was able to provide necessary scientific data to support the use of fruits from three edible *Campomanesia* species in order to produce natural preservatives and bioactive ingredients for food products. The phenolic compounds contents present in *C. eugenioides* could be responsible for the best antioxidant activity. Thus, our results contribute to the discovery of the potential application of these native *Campomanesia* Brazilian fruits, as a natural product with functional and antioxidant properties.

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