



Polyphenol content and antioxidant capacity of the Tarumá (*Vitex cymosa*) tree and the potential health benefits.

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ABSTRACT

Indigenous plants such as the Tarumá tree may have beneficial health properties through their bioactive compounds and antioxidant capacity. The objective of this study was to evaluate the nutritional composition, the phytochemical performance, the total content of polyphenols, the antioxidant capacity and bioactive compounds of the fruits of the Tarumá tree (*Vitex cymosa* Berteo ex Speng) were evaluated for macronutrient content. Phytochemical screening was performed, the total content of polyphenols was obtained and the antioxidant capacity was determined. The macronutrients were in proportions of 11.3%, 9.3% and 16.4% for carbohydrates, proteins and fats respectively. Phytochemical screening showed the presence of phenols and triperthenoids. The total polyphenol content was 23 mg/g and the antioxidant capacity was (6773 ± 107) $\mu\text{mol}/100\text{ g}$. This study shows that the Tarumá fruits may have beneficial health properties based on the amount of polyphenols and antioxidant capacity thus making it likely to be able to prevent diseases caused by nutritional deficiencies.

KEY WORDS: *Vitex cymosa*, polyphenols, antioxidant capacity, phytochemical screening, Tarumá

INTRODUCTION

Several studies have shown that there is a relationship between the occurrence of chronic noncommunicable diseases and the imbalance between levels of free radicals (mainly reactive oxygen species or ROS) and endogenous antioxidant defenses (1). In fact, several studies have shown that populations that eat food rich in fruit and vegetables suffer fewer incidences of chronic degenerative diseases related to aging and oxidative stress (2, 3). In this sense, the knowledge of the contribution of the bioactive compounds to

local diets could allow for a better understanding of the potential of these diets in the prevention of such diseases. Currently, the search for new sources of bioactive compounds has led researchers to becoming more interested in exotic and tropical fruits due to their potential as a source of bioactive compounds with therapeutic values. In this context, native and non-native fruits consumed by the populations in South America, such as Paraguay, are of great interest and have been studied in recent times (4-11).

Paraguay has a variety of climatic conditions with an extensive variety of native and exotic fruit species of potential interest as sources of several nutrients and bioactive compounds. As part of the typical

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vegetation of this region, a nontraditional fruit known as Tarumá (*Vitex cymosa* Berteo ex. Speng) grows. The Tarumá belongs to the genus *Vitex*, family Lamiaceae and is a small tree or shrub growing in tropical and subtropical regions. This plant is endemic to Panama, the Netherlands Antilles, Venezuela, Brazil, Bolivia, Colombia, Ecuador, Peru, Argentina and Paraguay and is threatened due to deforestation. The plant is commonly known as Tarumá in Paraguay, as aceituno in Colombia and as pechiche in Ecuador (12).

Tarumá fruits are harvested between December and February and consist of a fleshy drupe of black or purple in color and they are ovoid between 1.5 and 2 cm in length with a bitter taste. However, when cooked they are converted into a delicious preserve with a very sweet flavor. Present studies related to the chemical composition of the Tarumá and possible beneficial effects on human health are lacking (13). It has been underutilized in Paraguay so there is a potential for economic expansion in agribusiness whether it is consumed in its natural form or manufactured into jams, juices or liqueurs.

Polyphenols are phytochemicals found in fruits, vegetables, grains and are generally classified as flavonoids, phenolic acids, stilbenes, tannins, and lignans with potent biological activity (8). Polyphenols have been recognized *in vitro* for their antioxidant potential. However, *in vivo* they do not reach peripheral tissues in high concentration. Therefore the antioxidant capacities of these phytochemicals seem unlikely to explain their benefits. Although the mechanisms of action are unclear, the hypotheses for the involvement of polyphenols and their metabolites in obesity prevention are (1) induction of satiety, (2) suppression of nutrient absorption, (3) increased energy expenditure, and (4) activation of β -oxidation (7). Recently, some reports have highlighted the composition and potential biological effects of different fruits consumed in Paraguay (14-16). However, these studies are still limited given the country's potential and traditional culture, which has used plants and fruits since ancient times for nutritional and medical purposes (17).

For all the aforementioned, and based on the potential health benefits of the consumption of the Tarumá fruit, this study aimed to evaluate the nutritional composition, phytochemical screening, total polyphenol content and antioxidant capacity of the fruit.

MATERIALS AND METHODS

Collecting, preparing and storing fruits

Fruits of the Tarumá tree were collected between the months of October and December 2019 after botanical identification of *Vitex cymosa* Bertero ex Spreng. Approximately two kilos of fruit were collected and stored at 4° Celsius for the analyses that were carried out immediately. The rest were lyophilized as whole fruits, peel and pulp.

Nutritional chemical composition

The following macronutrients were evaluated in each fruit, for carbohydrates using the Antrona method, for proteins using the Kjeldahl method and for lipids using the Softlex method (18). The results were expressed in grams of macronutrient per 100 grams of sample analyzed.

Phytochemical screening

The phytochemical screening was carried out using the following extraction method where 10 g of the pulverized sample was weighed and 20 mL of a water-methanol mixture (1:10) and 20 mL of petroleum ether were added into a covered flask kept in the dark. Subsequently, it was placed on a shaker at 150 RPM for 1 hour. Then the supernatant was placed in a separation funnel to obtain two phases, methanol-water and oily (ethereal), for the identification of (1) phenols, (2) flavonoids, (3) saponins, (4) alkaloids, (5) steroids and triterpenoids and (6) leucoanthocyanidins and quinones.

Total polyphenol content

To prepare the sample, 5 Tarumá fruits were cut into half and the seeds were removed. The pulp and rind

were crushed in a glass mortar until obtaining 50 grams of a homogeneous paste. The homogeneous paste was representative of the pulp and peel for the determination of polyphenols. 100 ml of a 50% ethanol solution of the crude extract of the Tarumá pulp and peel (1 g of sample) was prepared and then placed in a sonicator for 30 minutes. Subsequently, the extract was filtered into a 100 ml flask. An aliquot was taken from each of the prepared solutions in different 10 mL volumetric flasks, 2000 μ L of water and 200 μ L of the Folin-Ciocalteu 2N reagent (analytical grade, Merck) were added. It was stirred and then allowed to stand for 5 minutes. Subsequently, 1500 μ L of a 20% Na₂CO₃ aqueous solution were added and made up to 10 mL by adding water. They were shaken and allowed to stand for 1 hour in the dark. After the time elapsed, the absorbance at 760 nm was read on a UV Visible spectrophotometer. 1000 μ L of ethanol was used as a blank in parallel in order to correct any interference caused by the addition of the same. For quantification, they were compared with standard solutions of Gallic Acid (Anhydrous, p.a., Biopack, Argentina), which was first prepared a Stock solution of gallic acid in ethanol of 100 μ g mL⁻¹. From this solution, dilutions were prepared taking 10, 50, 75, 100, 150 and 200 μ L in different flasks and proceeded in the same way as in the samples, with a final volume of 10 mL. From the results, a calibration curve was drawn to determine the equivalent concentration of Gallic Acid in the sample tubes. The results were expressed as mg gallic acid equivalents (GAE) g⁻¹ values are presented as the mean of the analyzes performed in triplicate $\pm$ standard deviation (SD).

Antioxidant capacity

The antioxidant capacity in whole fruits was determined through the H-ORAC method. The H-ORAC assay was performed according to (19,20). Briefly, the 2,2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH) is a source of peroxy radicals and induced oxidation assay by measuring the fluorescence signal in a spectrofluorometer (Synergy] HT Multi-Mode Microplate Reader model, Biotek Instruments Inc, Winooski, USA), with a 96-well polypropylene plate. Fluorescence was measured at 565 nm with the

excitation wavelength at 540 nm. AAPH (1.34 mM) was used as peroxy radical generator and fluorescein (61 nM) was used as target. Fluorescence decay is an indicator of damage caused by the peroxy radical. The ORAC results were calculated based on the calibration curves obtained from each run. Results are expressed in micromoles of trolox equivalents (TE) per 100 grams as the average of three replicates for each extract. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis-2-methyl-propion-amidine-dihydrochloride (AAPH), and fluorescein were obtained from Sigma Aldrich (St. Louis, MO, USA).

RESULTS AND DISCUSSION

Nutritional chemical composition

The results are expressed in g/100 grams of sample. Since there are no previous nutritional chemical composition studies of the Tarumá fruit, the macronutrient values were compared with those of elm leaf or thornless blackberries (*Rubus ulmifolius*) as they are considered the standard among dark purple fruits. Regarding the amount of carbohydrates, da Silva *et al.*, (21) found 26.2 g/100 grams of sample in blackberry fruits, almost more than half that was found in Tarumá fruits (11,3%). The main carbohydrates of this type of fruit are fructose, glucose and sucrose (22). The amount of protein in the da Silva *et al.*, study was 2.4 g/100 grams in the sample, lower than that in the Tarumá fruits (9.3 g/100 grams). Regarding the amount of fat, the da Silva's study observed 1.22 g/100 grams, much less fat than in the Tarumá (16,4 g/100 grams) (21). It should be noted that the amount of fat in Tarumá is by far higher than in other fruits, which may be evidence that this fruit could contain fatty acids beneficial to health. This could open the door for future research on the type of fats in the Tarumá fruits. It is also important to note that the blackberries studied by da Silva were processed in the same way as the fruits in this study and that in all cases they were evaluated when they were ripe.

Phytochemical screening

The phytochemical analysis revealed that phenols and

triperthenoids were present in all the fruit. No saponins, quinones, alkaloids, flavonoids and luekoanthocyanidis were found in the fruits. The pH of the fruits was 5.3.

A systematic review of phytochemical components in *Plinia cauliflora* (common name Jaboticaba) originating in Brazil showed the presence of phenolic compounds, flavonoids and terpenoids in whole fresh fruit, that is, the same compounds that were present in this study (23-25). It is important to note that, first there are no qualitative studies of bioactive compounds in the Tarumá fruit, however, the results were compared with what has been observed in the Brazilian Yvapurú species, and second, that the presence or not of bioactive compounds can vary based on the conditions in which these fruit trees grow, such as temperature, soil composition, among others.

Total polyphenol content

Regarding the total content of polyphenols, Souza-Moreira *et al.*, established that the Brazilian Yvapurú species had a total content of >45 mg/g, greater than what was observed in the Tarumá fruits in this study (23.3 ± 0.8 mg/g) (26). A recent study found a total polyphenol content of 22.60 mg/g in the same Tarumá species originating in Ecuador. The value of the phenolic content of the present study corresponds to 23.3 mg/g of sample, a value similar to that observed in the aforementioned study. The amount of polyphenols in these fruits can vary based on environmental conditions such as soil and geographic location. (27).

Antioxidant capacity

Regarding the determination of the antioxidant capacity, it was found that Tarumá fruit presented (6773 ± 107) $\mu\text{mol}/100$ g according to the ORAC method. Taking into account the study by Yoh *et al.*, who found values between 9.776 and 37.845 $\mu\text{mol}/100$ g in blackberries, the values of this research regarding antioxidant capacity are relatively high since the authors evaluated this variable in fermented fruits (28).

CONCLUSIONS

This study shows that the antioxidant properties and its content of polyphenols make the Tarumá fruit a healthy option in terms of consumption. Additionally, being a leafy tree, its cultivation would add beautification to places. The massive cultivation of this fruit tree could also provide the raw material to consume its fruits in different ways. The next step would be to conduct clinical trials in people with metabolic disorders to establish whether the Tarumá fruit will have an impact on their health.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

As the Tarumá is a native fruit, it was compared with the species found in the herbarium of the Faculty of Chemical Sciences of the National University of Asunción for identification. The identification of the species was in charge of the researcher Antonio Samudio.

CONSENT FOR PUBLICATION

Since this study did not involve humans, no consent was required. However, the authors declare that the tables and information will be available to the public free of charge by the decision of all the authors.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study is included in this article. The data obtained are found in the reports of the National Council of Science and Technology of Paraguay, an organization that funded the study.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

All the authors contributed from the conception to the execution of the project and finally in the writing and approval of the manuscript for publication.

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