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# Nutritional Composition, Phytochemical Performance, Total Content of Polyphenols, Antioxidant Capacity, Bioactive Compounds of Guavijú Fruits (Eugenia Pungens), and Their Potential Health Benefits

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***ABSTRACT***

**Background:** Autochthonous fruits such as Guavijú have beneficial health properties through their bioactive compounds and antioxidant capacity. This study aims to evaluate nutritional composition, phytochemical performance, total content of polyphenols, antioxidant capacity, and bioactive compounds of Guavijú (*Eugenia pungens*) fruits. **Methods:** Macronutrients were analyzed using Anthrona method for carbohydrates, Kjeldahl method for proteins, and Soxhlet method for total fat. Phytochemical screening was performed to detect phenols, flavonoids, saponins, alkaloids, steroids and triterpenoids, as well as leukoanthocyanidins and quinones. The total content of polyphenols was obtained using Folin-Ciocalteu method. Antioxidant capacity was determined by ORAC method, and the identification of bioactive compounds was carried out through LC-QqQ MS/MS. **Results:** Macronutrients were found in proportions of 33.4%, 5.5%, and 4.5% for carbohydrates, proteins, and fats, respectively. Phytochemical screening revealed the presence of phenols and triperthenoids. The total polyphenol content was 46.6 mg/g. Antioxidant capacity was 11394±705 µmol/100 g. **Conclusions:** The identified bioactive compounds were cyanidin, delphinidin, enotein B, quercetin, and myricitrin. The results revealed that beneficial health properties due to the content of bioactive compounds and antioxidant capacity, which constitutes a food, can prevent diseases.

**Keywords:** *Eugenia pungens; Polyphenols; Antioxidant capacity; Phytochemical screening; Bioactive compounds.*

## Introduction

Regular consumption of fruits is widely recommended in dietary guidelines worldwide due to their richness in nutrients. Studies have shown that this consumption can prevent various diseases and disorders due to the presence of bioactive compounds with antioxidant properties (Ahmad *et al.*, 2016, Lenquiste *et al.*, 2015, Plaza *et al.*, 2016). Despite the abundance of fruits in the

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world, there is a large number of underexploited native and exotic species, which may be a possible future source of income for local population, and an opportunity to gain access to special markets where consumers have interest in the presence of compounds potentially capable of preventing diseases (Borges *et al.*, 2014, Haminiuk *et al.*, 2011).

Diets with low nutritional value and little variety have been increasingly consumed, contributing to the growing problems of malnutrition and chronic diseases (Baldermann *et al.*, 2016). Therefore, there is a need for advances in knowledge about the composition and beneficial properties to the health of Brazilian native species that have been neglected and underutilized (Pereira *et al.*, 2013). The use of these species can increase the number of native food types currently used, considering the reduction of problems related to food and nutritional security, as well as the strengthening of conservation and sustainable management of biodiversity (Baldermann *et al.*, 2016, Schulz *et al.*, 2016).

There has been recently a growth in the number of scientific studies on the potential of Brazilian native fruits species. Among these fruits, *Eugenia pungens* can be highlighted. These fruits have potential bioactivity associated with their richness in nutrients (mainly dietary fiber, lipids, potassium, and vitamin C) and phenolic compounds, especially anthocyanins (Bailão *et al.*, 2015, de Araujo *et al.*, 2019, de Paulo Farias *et al.*, 2020, Schulz *et al.*, 2016, Seraglio *et al.*, 2018, Teixeira *et al.*, 2019).

*Eugenia pungens* is a tree widely distributed in South American countries, mainly in Brazil, Argentina, Uruguay, and Paraguay. It is known in South America by various popular names, including “guabiy´u,” “guabij´u,” “guabir´a,” “ibaviy´u,” and “array´an”. Guavijú is a spherical, velvety fruit about 1 to 2 cm in diameter that is purplish when ripe, with a succulent and yellowish pulp). The fruit is edible, and the leaves of this species are used in folk medicine, mainly as an antidiarrheal and a tonic (Cortadi *et al.*, 1996). It has a pleasant sweet taste, and generally consumed fresh and well appreciated (Dalla Nora *et al.*, 2014).

The edible part of the fruits usually contains considerable amounts of nutrients and phytochemicals such as organic acids, sugars, vitamins, polysaccharides, polyphenols, and important minerals (De La Rosa *et al.*, 2009, Donado-Pestana *et al.*, 2015). Meanwhile, it is known that numerous physical, chemical, and biochemical changes occur during fruit ripening (Fawole and Opara, 2013). Given that there are no studies analyzing the nutritional profile and bioactive compounds of this fruit, this was conducted to describe nutritional characteristics and the beneficial potential of the consumption of these fruits in addition to laying the foundations for the nutraceutical industry in terms of the identified bioactive compounds. Thus, this study aims to evaluate the nutritional composition, phytochemical screening, total polyphenol content, antioxidant capacity, and bioactive compounds of Guavijú fruits in order to obtain a profile of its nutritional properties that represent health benefits.

## Materials and Methods

*Collecting, preparing, and storing fruits:* Fruits of Guavijú tree were collected between October and December 2019 after botanical identification, which in this case corresponded to‘*Eugenia pungens*’.. Approximately two kilograms of fruits were collected. Half a kilogram was stored at 4 ºC for analyses that were carried out immediately. The rest were lyophilized as whole fruits, peel and pulp (half kilogram each).

*Nutritional chemical composition:* Carbohydrates were evaluated by Anthrona method. In the presence of concentrated sulfuric acid, carbohydrates are dehydrated to furfurals (or hydroxy-methyl-furfurals) which condense with anthrone (10-keto9,10-dihydroanthracene) forming a blue-green complex. Color intensity was quantified by measuring absorbance at 620 nm). Proteins were evaluated using Kjeldahl method. This test measures nitrogen content of a sample. Protein content is calculated, assuming a protein to nitrogen ratio for the specific food being tested. Lipids were tested by Soxhlet method (gravimetric method consisting of the extraction of fat from the




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sample with solvents, removal of solvent residues by evaporation, and quantification of fat using analytical balances). The results were expressed in grams of macronutrient per 100 grams of sample analyzed (Association of Official Analytical Chemists (AOAC), 1990/2016).

*Phytochemical screening:* To carry out phytochemical screening, 10 g of pulverized sample was weighed and 20 ml of the water-methanol mixture (1:10) and 20 ml of petroleum ether were added in a covered flask isolated from the light. Subsequently, it was placed on a shaker at 150 rpm for 1 h. After that, After this process, the supernatant obtained was placed in a separation funnel to obtain two phases, methano-lwater and oily (ethereal), for the identification of (1) phenols, (2) flavonoids, (3) saponins, (4) alkaloids, (5) steroids and triterpenoids and (6) leukoanthocyanidins and quinones (Hossain *et al.*, 2013).

*Total polyphenol content:* For sample preparation, 5 Guavijú fruits were taken, later they were cut into halves, and the seeds were removed, then the pulp and peel were ground in a glass mortar until obtaining a homogeneous paste (the homogeneous paste will be representative of pulp and peel) for the determination of polyphenols (Hossain *et al.*, 2013). One hundred milliliters of ethanolic solution (50% ethanol) of the crude extract of Guavijú pulp and peel (1 g of sample) was prepared and was taken to sonicator for 30 minutes. Subsequently, the extract was filtered in a 100 ml flask. In different 10 ml volumetric flasks, an aliquot of each of the prepared solutions was taken and 2000 µl of water and 200 µl of the Folin-Ciocalteu 2N reagent (analytical grade, Merck) were added, and then the preparation was shaken and finally left to rest for 5 minutes. It was stirred and then allowed to stand for 5 minutes. Subsequently, 1500 µl of a 20% Na2CO3 aqueous solution was added, subsequently water was added to increase the volume to 10 ml. They were shaken and allowed to stand for 1 hour in dark. After time elapsed, the absorbance at 760 nm was read on a UV Visible spectrophotometer. One thousand microliter of Methanol was used as a blank in parallel in order to correct any interference caused by adding the same. For quantification, they were compared with standard solutions of Gallic acid, which was first prepared with a stock solution of Gallic acid in methanol of 100 $\mu g.ml^{-1}$. From this solution, dilutions of 10, 50, 75, 100, 150, and 200 µl were prepared in different flasks and the samples proceeded in the same way with a final volume of 10 ml. 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). Values are presented as the mean of analyses performed in triplicate ± 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 (Gancel *et al.*, 2011, Ou *et al.*, 2002). The 2,2'-azobis (2-methylpropionamide)-dihydrochloride (AAPH) was a source of peroxyl radicals and induced oxidation assay by measuring fluorescein 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 peroxyl radical generator and fluorescein (61 nM) was used as target; fluorescence decay is an indicator of produced damage by the peroxyl radical. The ORAC results were calculated based on the calibration curves obtained in each run. Results are expressed in micromoles of trolox equivalents (TE) per 100 grams as the average of three replicates for each extract. The 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).

**Identification of bioactive compounds**

*Sample preparation:* Once the samples were received, they were immediately frozen at -80 ° C and subsequently lyophilized to constant weight. The lyophilized samples were individually ground




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in a mortar to a fine powder and kept at –20 ° C until use.

*Extraction of bioactive components:* The extraction of bioactive components was carried out by means of oscillatory shaking at 900 oscillations/min (Vibromatic, Selecta, Spain) for 60 min. Ten milliliter of extractant composed of methanol (77%), water (20%), and formic acid (3%) with 5 ppm of hesperidin was added to 1 g of the dehydrated and pulverized sample, which was used as an internal standard. The solid-liquid system was centrifuged for 5 min at 1480 × g to separate solids from the extract. The operation was carried out three consecutive times to ensure an extraction yield greater than 95%. The extracts obtained from the three extractions were mixed and filtered before analysis by liquid chromatography-mass spectrometry. This extraction was carried out in triplicate for all samples.

*Quantification of bioactive components in extracts by LC–QqQ MS/MS:* Extracts were characterized by liquid chromatography (Agilent Technologies, 1200 series) coupled with mass spectrometry (LC-QqQ MS/MS) using a triple quadrupole detector (Agilent Technologies, model 6460). Chromatographic separation was performed by a C18 reversed phase column (Zorbax Eclipse Plus C18 Rapid HD Resolution 3.0 × 150 mm, 1.8 µm), using water (phase A) and acetonitrile (phase B) as mobile phases, both with 0.1% of formic acid as an ionizing agent. The elution gradient was as follows: 0-1 min, 4% phase B; 1 to 6 min, increased from 4% to 40% phase B; 6-10 min, increased from 40% to 100% phase B; 10-20 min, kept in 100% phase B, to ensure elution of all components of the sample. Subsequently, the column was equilibrated to the initial conditions for 15 min, before the next analysis. The flow of mobile phases was 0.25 ml/min and the injection volume used was 2 µl. Mass spectrometric analysis was performed using an electrospray ionization source.

*Ethical approval and consent to participate:* As it is a species of native fruit, when identifying the species, it was compared with the species found in the herbarium of the Faculty of Chemical Sciences of the National University of Asunción. The identification of the species was in charge of the researcher Antonio Samudio.

## Results

*Nutritional chemical composition:* The determination of the nutritional chemical composition revealed that carbohydrate, protein and fats were 33.4±0.23, 5.5±0.15, 4.48±0.34 g/100 g, respectively.

*Phytochemical screening:* Phytochemical analysis revealed that all fruits presented phenols and triperthenoids, but they did not present saponins, quinones, and alkaloids.

*Total polyphenol content:* Regarding the total content of polyphenols, analysis showed that Guavijú presented an amount of 46.6±0.08 mg/g.

*Antioxidant capacity:* Regarding the determination of the antioxidant capacity, it was found that Guavijú fruit presented (11394±705) µmol/100 g according to the ORAC method.

*Identification of bioactive compounds:* The results of bioactive compounds quantification showed that Guavijú shell samples presented the highest amount of cyanidin 3-O-glucoside (232.2 mg/kg). The amount of Delphinidin 3-glucoside in the shell of Guavijú was 11.3 mg/kg. Quercetin 3-O-rhamnoside in the shell of Guavijú was identified 28mg/kg and 46.3mg/kg myricetin 3-O-rhamnoside was identified in the shell of Guavijú. Finally, oenothein B was detected in the samples of Guavijú shell (569.2 mg/kg, approximately) and 195 mg/kg in the pulp. Mean concentration was expressed in mg of bioactive compound per kg of sample. The results of the quantification of different samples are shown in **Table 1**.


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**Table 1.** Identification of bioactive compounds.

| Fruit (mg/kg of sample) | | Cyanidin 3-O-glucoside | Delphinidin 3-glucoside | Oenothein B | Quercetin 3-O-rhamnoside | Myricetin 3-O-rhamnoside |
|---|---|---|---|---|---|---|
| Guavijú | Shell | 223.5±17.0 | 111.3±12.1 | 195.0±11.8 | 28.0±2.5 | 46,3±2.0 |
| | Pulp | N/D | N/D | 569±23.3 | N/D | N/D |

## Discussion

Given that there was no nutritional chemical composition study on Guavijú fruit species studied in this work, macronutrient values were compared with those of ¨blackberries¨ (Rubus ulmifolius) fruits, since they are considered standard among dark purple fruits. Regarding the amount of carbohydrates, da Silva *et al.* found 26.2 g/100 grams of sample in blackberry fruits, the amount that was found in Guavijú fruits was almost 33,4% (da Silva *et al.*, 2019). It has been seen that the main carbohydrates of this type of fruit are fructose, glucose, and sucrose (Junior *et al.*, 2019). The amount of protein in the study of da Silva *et al.* was 2.4 g/100 grams of sample (Schulz *et al.*, 2016), which was lower than the amount found in Guavijú fruits (5.5 g/100 grams). Regarding the amount of fat, they also observed an amount of 1.22 g/100 grams, an amount lower than the amount of fat in Guavijú (4.5 g/100 grams) (da Silva *et al.*, 2019). It is also important to note that blackberries studied by da Silva were processed in the same way as fruits in this study and in all cases they were evaluated when they were ripe.

Regarding the detection of phenols and triperthenoids in Guavijú fruits, a systematic review on phytochemical components of *Plinia cauliflora* of Brazilian origin, revealed the presence of phenolic compounds, flavonoids, and terpenoids in whole fresh fruit, compounds that were present in fruits of the present study (Apel *et al.*, 2006, Reynertson *et al.*, 2006, Wu *et al.*, 2012). There was no qualitative study of bioactive compounds on Guavijú species; however, the results were compared with Brazilian Yvapurú species. The presence or absence of bioactive compounds can vary based on conditions in which these fruit trees grow, such as temperature and soil composition. Phytochemical screening is one of the initial stages of phytochemical research, which allows for qualitative determination of the main groups of chemical constituents present in a plant and guides the extraction or fractionation of extracts to isolate groups of major interest. This study presents results for future research in the identification and isolation of bioactive compounds.

Souza-Moreira *et al.* found a total content of more than 45 mg/g in Brazilian Yvapurú species, a similar amount compared to Guavijú species (46.6 mg/g) (Souza-Moreira *et al.*, 2010). Phenolic acids are one of the major classes of phenolic compounds, found widely in fruits (Mattila *et al.*, 2006). Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, gentistic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, and salicilic acid are the most common phenolic acids present in fruits (Russell *et al.*, 2009). Phenolic compounds inhibit the viability of pancreatic cancer cells by increasing the percentage of hypodiploids and significantly decreasing mitochondrial transmembrane potential. It has also been found that the proliferation of pancreatic cancer cells can be inhibited in a time- and dose-dependent manner (Liu *et al.*, 2012). The cardiovascular benefits observed with polyphenols may be due to cooperative effects with other bioactive compounds, such as nitrates and fibers. Consuming polyphenol-rich fruits with a Western style meal can attenuate the resulting inflammatory processes associated with high-energy dietary patterns (Joseph *et al.*, 2016). It





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has been shown that polyphenols protect readily oxidized nutrients, in particular, lipids such as polyunsaturated fatty acids, which can enhance their beneficial effects (Warnakulasuriya and Rupasinghe, 2014). In addition, the consumption of polyphenols can prevent or treat obesity and diabetes among other chronic non-communicable diseases (Garcia-Diaz *et al.*, 2019).

Taking into account the study by Joh *et al*., who found antioxidant capacity values between 9,776 and 37,845 $\mu$mol/100g in blackberries, the values of this research regarding the antioxidant capacity of Guavijú fruits (11,39±70.5) µmol/100 g, are relatively high based on the fact that blackberries are a reference point for measuring antioxidant capacity because they are considered to have the highest value. (Joh *et al.*, 2017). The measurement of antioxidant capacity of food is of great interest, since it offers information on the stability of oxidative processes, in addition to the contribution of many phytotherapeutic substances that have antioxidant activity and provide health benefits, such as Guavijú (Rojano *et al.*, 2012).

The five compounds identified in Guavijú fruits included Cyanidin, Delphinidin, Enotein B, Quercetin, and Myricitrin. Cyanidin (Cyanidin 3-O-glucoside) reduces lipid accumulation in adipose tissue via PPARγ and NF-kB, improves dose-dependent insulin sensitivity, improves adiponectin mRNA levels, and suppresses adiponectin production of nitric oxide and inflammatory cytokines in colorectal adenocarcinomas. Its anti-inflammatory activity has been demonstrated both in ulcerative colitis and in the presence of lipopolysaccharide (LPS). It also inhibits oxidative stress and neuroinflammation, improves cell degeneration, and activates neurotrophic factor signaling (Gan *et al.*, 2020, Molonia *et al.*, 2020, Zhang *et al.*, 2019).

Delphinidin (Delphinidin 3-glucoside) inhibits lipid accumulation by restoring fatty acid oxidation genes, suppresses the accumulation of lipids induced by hepatocyte senescence, inhibits the growth of breast tumors, suppresses proliferation induced by oxidized LDL (oxLDL), inhibits endothelial cell apoptosis, attenuates mitochondrial function, decreases the production of reactive oxygen species (ROS), significantly inhibits platelet activation, and attenuates thrombus growth in arterial and venous shear stresses, contributing to its protective functions against thrombosis and CVD (Harada *et al.*, 2018, Jin *et al.*, 2013, Yang *et al.*, 2016, Yang *et al.*, 2012).

Enotein B (Oenothein B) inhibits the proliferation of lung cancer cells, has a high antioxidant capacity, eliminates free radicals, protects macrophages from oxidative damage, increases the production of antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduces neuroinflammation in the brain during systemic inflammation (Li *et al.*, 2020, Okuyama *et al.*, 2013, Pei *et al.*, 2019).

Quercetin (Quercetin 3-O-rhamnoside) reduces cytopathic effects and inhibits the replication of influenza virus in the initial stage of infection and oseltamivir has a relatively lower efficacy compared to Quercetin. Quercetin has been shown to inhibit osteoclastogenesis, osteoblast apoptosis, oxidative stress, and the inflammatory response while promoting osteogenesis, angiogenesis, antioxidant expression, adipocyte apoptosis, and osteoclast apoptosis. Considering important contributions of quercetin in the regulation of bone homeostasis, it can be considered an economical and promising agent for improving bone health. Documented preclinical findings require further validation from human clinical trials (Choi *et al.*, 2009, Wong *et al.*, 2020).

Myricitrin (Myricetin 3-O-rhamnoside) has a potential capacity to accelerate fibroblastic and remodeling phases in wound repair process. It promotes fibroblast migration, demonstrating a twice higher rate of wound closure and can be developed as a possible antibacterial and antibiofilm agent in natural treatment of gastrointestinal disorders, including diarrhea. It is a powerful scavenger of free radicals, an excess of which is harmful to health (Hayder *et*




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*al.*, 2008, Moghadam *et al.*, 2017, Motlhatlego *et al.*, 2020).

There were some limitations in the present study. Additional analyses such as fatty acid and vitamin profile could not be performed due to lack of a sufficient sample. The results could be compared with fruits of the same species, since similar studies were not available. In addition to obtaining extracts of bioactive compounds, further research is required to evaluate their beneficial properties in vitro.

It is the first study to evaluate these parameters in Guavijú fruits, providing possibilities of conducting more studies in order to demonstrate beneficial effects they have on health. Finally, the results obtained in this project offer a wide range of opportunities in terms of food safety, economic possibilities for the agri-food sector, as well as "nutraceuticals".

## Conclusions

Guavijú fruits have a relatively high amount of polyphenols as well as a medium to high antioxidant capacity. In addition, the bioactive compounds identified were Cyanidin, Delphinidin, Enotein B, Quercetin, and Myricitrin. Antioxidant properties and its content of polyphenols and bioactive compounds make Guavijú fruit a healthy option for consumption. In addition, being a leafy tree, its cultivation would make places beautiful. The massive cultivation of this fruit tree could facilitate consuming its fruits in different ways. It is required to conduct cell culture studies and then clinical trials in people with metabolic disorders to see the impact on their health.

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## Conflict of interests

All authors declare that they have no conflict 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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