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Antioxidant Potential of Polyphenols from Colombian Melicoccus Bijugatus fruit

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Abstract

Applications related to tropical fruits call the attention of research groups worldwide. Ethnobotanical information on Melicoccus bijugatus comes mainly from the Orinoco region (covering Colombia and Venezuela). In Venezuela, roasted seeds are powdered and mixed with honey for anti-diarrhea syrup or tea or for dietary purposes. Indigenous people in the Orinoco used them in flour to make bread. In Nicaragua, seed milk, or "horchata," is used to treat parasites. Roasted seeds appear to reduce their toxicity and improve digestibility; other parts of the fruit also have biological and medical purposes. This work focused on quantifying phenolic compounds and antioxidant capacity in Melicoccus bijugatus. Total polyphenol content in M. bijugatus matrices was determined spectrophotometrically following the Folin-Ciocalteu reaction. The total flavonoid content in different parts of the fruit was evaluated with the aluminum chloride colorimetric assay. The DPPH assay for radical scavenging was used to quantify antioxidant activity. Antioxidant capacity was highest in pulp, followed by lower values in pericarp, seed, and peel. Results here suggest that a higher amount of polyphenols in a matrix does not necessarily provide a higher antioxidant capacity. Further studies are recommended to test other potential nutritional and biological activities in the edible and non-edible parts of the fruit.

Keywords: Antioxidants, Melicoccus bijugatus, Non-Edible Parts, Pulp.

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1. Introduction

In recent years, interest in the assessment of polyphenol content in tropical and exotic fruits and plants has grown. Several studies show that polyphenol consumption is closely associated with a lower risk of developing cardiovascular and inflammatory diseases, as well as cancer. These effects are due to the anti-inflammatory, antioxidant, and anti-proliferative properties found in polyphenols from multiple natural sources [1-3]. In addition, the increasing resistance to antibiotics, human immunodeficiency virus (HIV) treatments, and the effects of low-grade inflammation present in chronic non-communicable diseases such as obesity, diabetes mellitus, and others require research to find new therapeutic alternatives. There is a current interest in the search for natural remedies from plants, considering the worldwide variety of species (400,000–500,000) with their multiple structural components that have pharmaceutical potential [4].

More than 8,000 phenolic compounds have been described, some of which are present in foods (fruits and vegetables). These components are secondary metabolites that play a series of metabolic functions in plants, such as growth, reproduction, and protection from ultraviolet light and pests. Epidemiological information supports studies advising high consumption of polyphenol-rich fruits and vegetables to help lower the risk of chronic diseases. In addition, these foods contain vitamins, minerals, and dietary fiber connected to medicinal properties and benefits for human health [2, 5]. Figure 1 shows several healthy contributions of polyphenols in fruits.

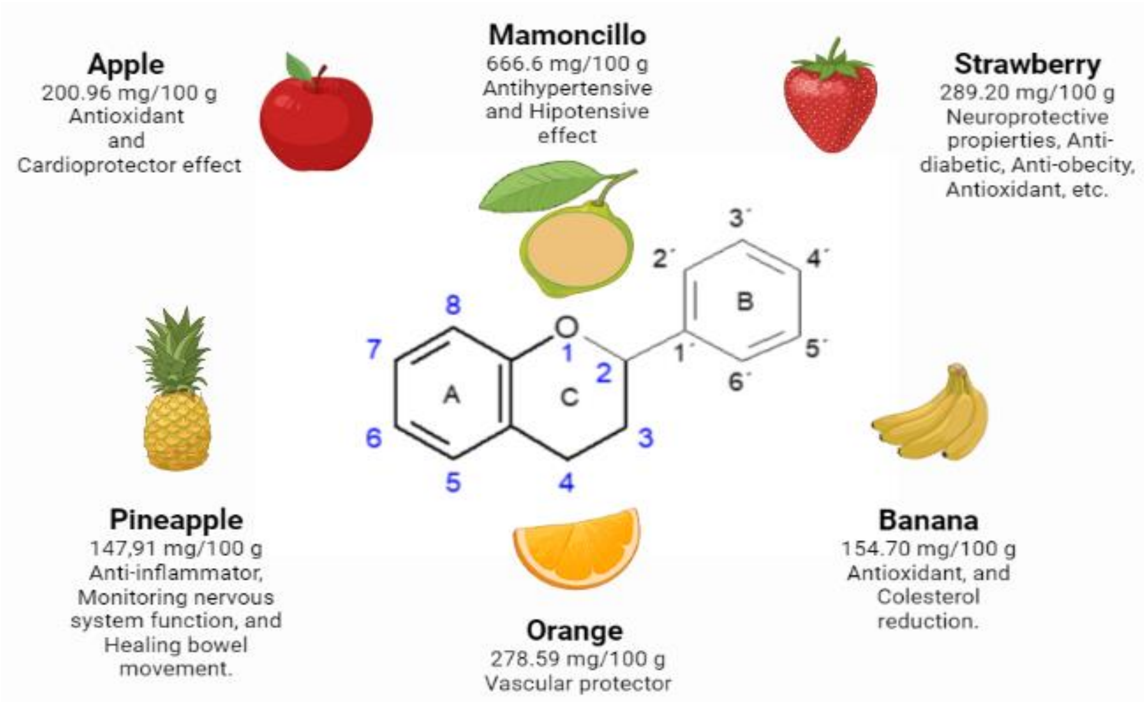

Figure 1.
Examples of polyphenol therapeutic contributions (*Melicoccus bijugatus* – mamoncillo).

A wide variety of plants, as well as fruits, have been investigated considering each of their parts separately, seeking to find the chemical compounds they contain and their possible biological properties [4, 6, 7]. Studies report that polyphenols have antimicrobial, xenobiotic, detoxifying, and immune system-stimulating effects. Table 1 summarizes several biological properties and benefits from fruit consumption and from the use of other non-edible components from plants and fruits.

Table 1.
Polyphenol health benefits (biological activity) from different parts of plants/fruits.

Fruit/Component	Biological Activity	Plant source	Reference
Mango/Pulp	Antiglycemic	Fresh fruit	Pinneo, et al. [8]
Apple/ Seeds	Antioxidant/ antiglycemic	Food additive	Beltrão Martins, et al. [9] and López-Fernández, et al. [10]
Strawberry/ Leaves	Anti-inflammatory	Fermented leaves	Lee, et al. [11]
Wild blueberry/ fruit	Antitumor	Dehydrated pulp	Al Hasani, et al. [12]
Physalis angulate/ Leaves and fruit	Antibacterial/ antioxidant	Leaves and fruit extracts	Ramakrishna Pillai, et al. [13]

The species M. bijugatus is a woody fruit tree from the Sapindaceae family, native to northern South America, especially Colombia and Venezuela. It is known colloquially in Colombia (South America) as manmoncillo. Other common names for M. bijugatus are mamón, guaya, guinep, and quenepa [14, 15]. Its cultivation has extended to the rest of the continent in countries such as Mexico, Puerto Rico, Cuba, and the United States. The fruit of M. bijugatus has a thin, brittle, leathery green rind. It contains a white seed, two of them in some varieties, occupying most of the fruit's volume. A salmon-colored, gelatinous, juicy pulp, with a sweet and sour flavor, and a slight winey flavor, surrounds it. These fruits ripen normally between the summer months, generally from July to September [16]. Figure 2 depicts fresh Colombian M. bijugatus (mamoncillo) fruits and their inner parts.

Figure 2.
Colombian *Melicoccus bijugatus* (mamoncillo), peels (exocarp), b) fresh pulp (mesocarp), c) dry endocarp (whole seed), d) endocarp layers, and inner peeled seed.

In the Dominican Republic and Cuba, possible toxic effects in adolescents were reported. Its pulp is irritating to the throat when consumed in large quantities; however, this effect disappears after macerating the seeds with the teeth [17]. In previous research, positive health effects are focused on the types of phenolic compounds found in the different parts of the *M. bijugatus* (mamoncillo) plant [14, 17, 18]**.** In the seeds, effective treatment of gastrointestinal problems was related to the presence and activity of epicatechin, catechin, and procyanidin B2. This part of mamoncillo is also used as an antiparasitic and its positive effects are attributed to naringenin [14, 17-19].

Medicinal properties have been attributed to this fruit for the treatment of hypertension, diabetes mellitus, asthma, diarrhea, and constipation [20, 21]. The effect of *M. bijugatus* on the treatment of hypertension has been associated with its content of caffeic and coumaric acid derivatives, as well as some saponins with antioxidant and antiplatelet activity in the pulp of the fruit. The fruit is also used for the treatment of diarrhea offering a positive antimicrobial effect [17]. In terms of toxicity, excessive consumption of the pulp could irritate the mucous membranes and the respiratory tract; these symptoms would be produced by the astringency of its high polyphenol content [14].

This fruit has been investigated in different regions of the world with emphasis on its phenolic content and its possible health benefits. In Mexico (Yucatan), polyphenols from peels and pulp have been tested for antioxidant capacity and antiviral effect [22, 23]. In the USA (Montgomery) *M. bijugatus* (mamoncillo) fruits have also been evaluated, specifically pulp, seed, and pericarp. In Caracas (Venezuela) polyphenols from pericarp and seed were studied previously [23]. To the best of our knowledge, no study reports polyphenol content and specific biological activity on the different parts of the fruit of Colombian *M. bijugatus.* Previous research reported considerable amounts of bioactive compounds in different parts and tissues of multiple fruits [24-26]. Regularly the shell and seeds of the fruits, which can cover 49% of the entire volume of the fruit, are discarded. In some cases, the seeds are roasted and eaten [14]. The present work focuses on quantifying the phenolic and flavonoid compounds, as well as their antioxidant activity, in each part of the Colombian *M. bijugatus* (mamoncillo) fruit. The nonedible parts of this fruit can be useful, relating their polyphenol content, with possible applications in the food or pharmaceutical industry.

2. Materials and Methods

Reagents and chemicals: Ethanol, sodium carbonate, and Folin-Ciocalteu reagent were obtained from PanReac AppliChem, ITW Reagents (Darmstadt, Germany). Sodium nitrite and aluminum chloride were obtained from LOBA Chemie (Mumbai, India), and sodium hydroxide was sourced from EMSURE Merck (Darmstadt, Germany). The reference standard quercetin was purchased from MP Biomedicals (California, USA). Ascorbic acid standard and DPPH (2,2-Diphenyl-1-picrylhydrazyl, Sigma-Aldrich) were obtained from Merck KGaA (Darmstadt, Germany).

Plant material and sample preparation for the extraction of phenolics. Approximately 50 grams of fresh Colombian M. bijugatus (mamoncillo) were purchased at the main Farmers Market in Manizales (Colombia). All fruits were cleaned with deionized water and dried prior to the separation of fruit components. The fruits were peeled, and the fresh pulp was separated from the seeds. Dry seeds were treated to remove the covering layers (pericarp) from the inner seeds. From the dried and separated components of the fruit, 5 g of the different parts (peels, fresh pulp, pericarp, and seeds) were mixed with 20 mL of aqueous ethanol (80%), stirred (DRAGON LAB, Beijing – China) for 20 minutes at 500 rpm, and allowed to sit for 24 hours in the dark. After the extraction process was completed, 2 mL of each sample were centrifuged (Hermle Z 206 A, Wehingen– Germany) at 3500 rpm for 10 minutes at room temperature. Supernatants were used to test polyphenol and flavonoid content and antioxidant activity.

Total Polyphenol Content (TPC). The polyphenol content, in all *M. bijugatus* matrices (Figures 2 and 3), was determined spectrophotometrically following the Folin-Ciocalteu (F-C) (Pan Reac Appli Chem, ITW Reagents, Darmstadt–Germany) reaction based on tests assayed before with a few modifications [27]. From the supernatants 500 µL of each sample was mixed with 500 µL of deionized water and 1 mL of the Folin-Ciocalteu reagent (10%), after 1 minute 2 mL of sodium carbonate (Na_2CO_3) at 3.5% were added to each sample and mixed [28]. The reaction lasted 90 minutes in the dark. All assays were done in triplicates. The evaluation of absorbance for TPC was measured at 655 nm (UV/VIS Optizen POP®, Daejeon-South Korea). All data were calculated based on a gallic acid standard calibration curve with range of 0-4.0 mg/L. TPC is expressed as milligrams gallic acid equivalent (GAE) per 100 grams of fresh or dehydrated sample (mg GAE/100 g).

Total flavonoid content (TFC). For comparison of total flavonoid content in the different parts of *M. bijugatus* fruit, the aluminum chloride ($AlCl_3$) colorimetric assay was used with minor modifications [28]. Amounts of 0.5 mL of each extract, were added into test tubes with 2.0 mL of distilled water. To each solution 150 µL of sodium nitrite ($NaNO_2$) (5%) were added followed by a 5-second vortex after 5 minutes of reaction 150 µL of $AlCl_3$ (10%) were added to each solution following the same agitation and reaction time. Finally, 1 mL of sodium hydroxide (NaOH) (1M) and 1.2 mL of distilled water were added followed by vortexing and a 5-minute reaction time [29]. Total flavonoid content is measured spectrophotometrically at 510 nm and reported as milligram of quercetin (reference standard compound) equivalents (QE) per 100 grams of fresh pulp or sample (mg QE/ 100 g sample). All experimental runs were done in triplicates.

DPPH assay for radical scavenging - antioxidant activity. The DPPH radical scavenging test is one of the most useful technique to evaluate the antioxidant activity in polyphenols extracted from natural products. The DPPH compound is a stable free radical in methanol. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was done following previous work by the research group [30]. Volumes of 1900 µL of DPPH (100 µM) prepared in pure methanol were mixed with 100 µL of each diluted (1:5) extract and let react in the dark at room temperature for 30 minutes. The antioxidant activity from phenolics in fresh and dehydrated parts of *M. bijugatus* was measured via spectrophotometry at 517 nm comparing against a methanol blank and using a positive control of ascorbic acid based on a calibration curve. The control curve was prepared with concentrations of comparable reference ascorbic acid (Merck KGaA, Darmstadt, Germany) in a concentration range from 50 to 600 µg/mL. All dilutions followed the same DPPH reaction conditions for the antioxidant activity evaluated in fruit extracts. The slope taken from the calibration curve served for the calculation of the inhibition concentration (IC50%) when 50% of the antioxidant component is reduced. Results for IC 50% were determined based on the equation:

% scavenging DPPH free radical = $(ABS_{Control} - ABS_{Extracts}/ABS_{Control}) * 100\%$

The antioxidant activity of phenolics from *M. bijugatus* is expressed as mg of ascorbic acid equivalents per 100 g of fresh or dehydrated pulp or seeds (mg AAE/100 g). All experimental runs were done in triplicate.

Statistical Analysis. Results were tested based on initial conditions of normality and homogeneity. All data were statistically evaluated for analysis of variance (ANOVA) followed by a Tukey test. The statistical significance was set considering values for $p<0.05$ [25]. IBM SPSS Statistics software version v.20 (Armonk, NY – USA) was used for all data evaluation. All analyses ran in triplicates; TPC, TFC, and DPPH values are expressed as mean ± standard deviation (SD).

3. Results and Discussion

Total Polyphenol Content (TPC). Total polyphenol content, in different parts of *M. bijugatus* as shown in Figure 3, result in higher content in the non-edible parts. TPC in peel, pericarp and seeds triple the content with respect to polyphenol content in fresh pulp. TPC in pulp and pericarp yielded values of 398.47 and 776.35 mg GAE/100 g of fresh fruit.

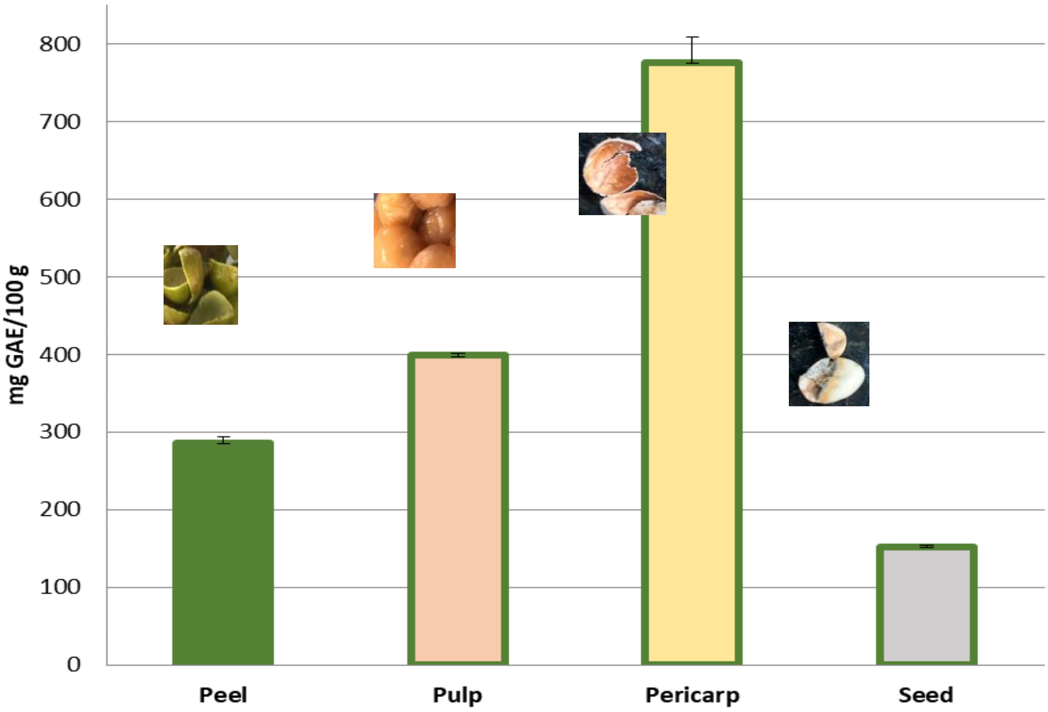

Figure 3.
TPC *Melicoccus bijugatus* (mamoncillo). Data represent means, in mgGAE/100g of sample, ± sd based on ANOVA followed by Tukey test.

Lowest and highest TPC for Colombian mamoncillo are found in seed and pericarp (non-edible parts of the fruit).

Total flavonoid content (TFC). The trend for total content, comparing polyphenols and flavonoids as shown in Figure 4, is similar. Non-edible parts of M. bijugatus have the highest flavonoid content. Flavonoids in the peel and seeds yielded 32.84 and 38.06 mg QE/100 g of fresh fruit, which are the lowest values. The other non-edible part of the M. bijugatus fruit, the pericarp, registered the highest TFC at 85.26 mg QE/100 g, while the only edible component in mamoncillo, the pulp, recorded 51.22 mg QE/100 g of the whole fresh fruit.

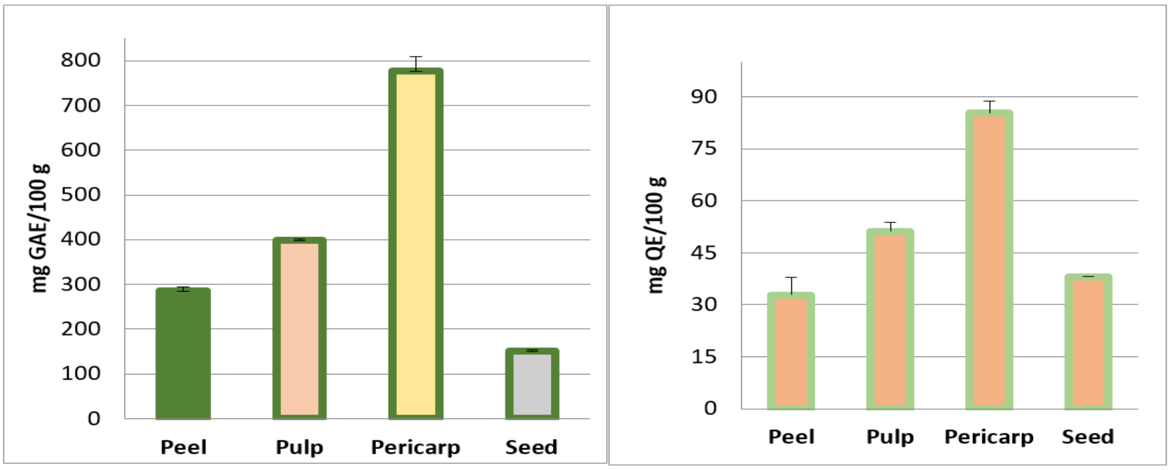

Figure 4.
TFC *Melicoccus bijugatus* (mamoncillo). Results represent means ± sd based on ANOVA followed by Tukey test.

With respect to the approximate proportion of total polyphenols and total flavonoids, in the different parts of the mamoncillo fruit, the ratio for TPC:TFC was about 10:1 for peel, pulp and pericarp. For polyphenols and flavonoids in seed the ratio was approximately 3:1. This comparative approach can help in the elucidation of specific antioxidant activity based on total polyphenol and flavonoid content for Colombian *M. bijugatus*. Figure 4 registers antioxidant activity, based on DPPH assays, from phenolics in the different parts of the fruit.

DPPH assay for radical scavenging - antioxidant activity. Different to the pattern for total phenolics and total flavonoids in *M. bijugatus* peel, pulp, pericarp and seed, antioxidant activity based on DPPH tests (IC50%), yielded a different trend. The highest antioxidant activity was registered in the direct edible part of *M. bijugatus* (pulp) with 88.22%.

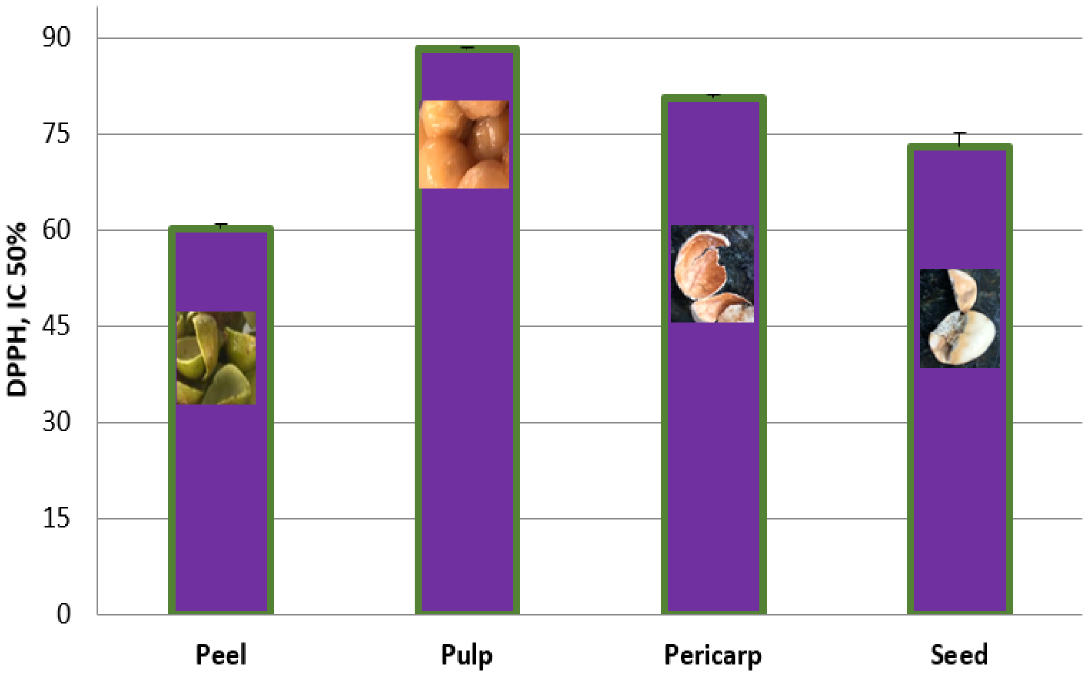

Figure 5.
Antioxidant activity (IC50%) from polyphenols in *Melicoccus bijugatus* (mamoncillo). Results, (inhibitory concentration) are expressed as means ± sd based on ANOVA and Tukey test.

The highest DPPH value registered in the pericarp (a non-edible part of the mamoncillo fruit) was 80.55%. The lowest antioxidant activity was recorded from polyphenols and flavonoids extracted from the peels.

Results in the present assays show a distinction with respect to polyphenol and flavonoid concentration among the different studied parts of *M. bijugatus*, (mamoncillo) cultivated in Colombia. Patterns were similar, with respect to content, in the four evaluated components highlighting higher concentration in a non-edible part (pericarp) as shown in Figures. 4 and 5. These quantification coincides with previous work already reported in in the Phenol-Explorer database Pérez-Jiménez, et al. [31] where total polyphenol content in mamoncillo, from highest to lowest, ordered as pericarp, pulp (edible part), peel and seed. In addition, the composition of results from the present study with those of rosemary and black elderberry classified in this international database showed that *M. bijugatus* was among the top 12 dietary products rich in natural bioactive substances [31].

It is known that there are variations in the total content of phenols and flavonoids and in the antioxidant, antiproliferative, and anti-inflammatory potential of different varieties of the same plant product. This is due to their relationship with genetic composition and meteorological factors such as temperature, sunshine hours, humidity, and radiation, which are related to the number of secondary metabolites and their antioxidant capacities [32, 33]. However, in this case, studies, evaluating polyphenol content done by Gómez-Maqueo, et al. [34] and Moo-Huchin, et al. [23] report similar total phenolic concentrations coinciding with results obtained in the present work [20, 23]. On the other hand, the highest polyphenols and flavonoid content needs more evaluation for proportionality, with respect to the highest antioxidant capacity. This approach would specify if whether a higher amount of polyphenols and flavonoids lead to a direct proportionality to higher antioxidant capacity [35].

Information related to the total polyphenol content and its specific antioxidant activity, in the different components of this tropical fruit, can be useful for further studies leading to find a direct application in the pharmaceutical or commercial fields [14, 36]. Other potential benefits from antioxidant polyphenols from edible and non-edible parts of Colombian in *M. bijugatus* and other Tropical fruits, can be investigated not only for medical applications, but also for areas of food engineering, nutraceuticals, industrial, and human and animal nutrition [23, 37].

3. Conclusions

Each component of the fruit may contain different amounts of bioactive compounds, and in a large majority of cases, the peel and seeds of the fruit, which can constitute up to 49% of the entire volume of the fruit, are considered inedible and therefore are normally discarded. This act leads to the wasting of high amounts of functional compounds with different functionalities, such as the antioxidant and anti-inflammatory potential of food by-products. In Colombia, no studies of this fruit (M. bijugatus) have been found in which the content of total polyphenols and flavonoids, as well as their antioxidant potential, in all parts of this fruit have been evaluated.

The limitations of the present study could arise from having evaluated fruit from the Andean region of Colombia, and as mentioned above, the fruit from this region could differ in the content of bioactive substances according to the multiplicity of thermal floors in the country. On the other hand, the strengths may derive from its novelty, since there are no previous studies of the fruit regarding the content of polyphenols and flavonoids in the Colombian variety, which can now serve as a point of comparison with varieties from other regions and other countries in which the plant is cultivated. Results in the present study suggest that a higher amount of polyphenols in a matrix does not necessarily provide a higher antioxidant capacity. Thus, further studies are required to explore more potential biological or nutritional capacities of tropical and exotic fruits in all edible and non-edible parts.

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