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Characterization and evaluation of antioxidant capacity in mari-mari fruit (*Cassia leiandra* Benth)

Caracterização e avaliação da capacidade antioxidante do fruto mari-mari (*Cassia leiandra* Benth)

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The Amazon has a wide variety of exotic and commercially underutilized fruits with high nutritional value and high content of bioactive compounds that are still little explored. The *Cassia leiandra* Benth is a species native to the Amazon that has the fruit widely consumed in natura, being popularly known as mari-mari. The objective of this study was to evaluate the biometric characteristics of mari-mari fruit, its chemical and phytochemical composition, as well as the evaluation of the antioxidant capacity of its different parts. The fruit can be described as long, thin and with low pulp yield, with the seed as its main constituent. The pulp had higher moisture content, the protein seed and the ash and carbohydrate peel. Peel had the highest phenolic content and the almond the highest flavonoid content, however the peel showed greater antioxidant capacity for DPPH, ABTS and FRAP methods. Thus, the mari-mari fruit can be a promising raw material for the development of nutraceutical and food products.
Keywords: Amazon, bioactive compounds, extraction.

A Amazônia possui uma grande variedade de frutas exóticas e comercialmente subutilizadas com alto valor nutricional e alto teor de compostos bioativos que ainda são pouco exploradas na região amazônica. A *Cassia leiandra* Benth é uma espécie nativa da Amazônia que tem o fruto bastante consumido in natura, este fruto é conhecido popularmente como mari-mari, ingá-mari e marimarizeiro. O objetivo desse estudo foi avaliar as características biométricas do fruto de mari-mari, a sua composição centesimal e fitoquímica, bem como a avaliação da capacidade antioxidante de suas diferentes partes. O fruto pode ser descrito como comprido, fino e com baixo rendimento de polpa, tendo como o seu maior constituinte a semente. A polpa apresentou maior conteúdo de umidade, a semente de proteínas e a casca de cinzas e carboidratos. A casca apresentou o maior teor de fenólicos e a amêndoa o maior teor de flavonoides. A casca apresentou maior capacidade antioxidante frente aos métodos do DPPH, ABTS e FRAP. O fruto mari-mari se apresentou neste estudo como uma matéria-prima promissora para o desenvolvimento de produtos nutracêuticos e alimentícios.
Palavras-chave: Amazônia, compostos bioativos, extração.

1. INTRODUCTION

The Brazilian territory presents a great diversity of fruit species, many of which have aroused growing interest in the agro-industrial market due to the high economic potential added to the fruits, which can be sold *in natura* or processed as sweets, juices, jellies and pulps [1]. The Amazon has a wide variety of native fruits with high nutritional value and high content of bioactive compounds [2] that can contribute to a better nutritional quality diet and to the prevention of chronic non-communicable diseases in the population that consume them.

In the central Amazon, flooded floodplain and igapó ecosystems constitute important means for the use and preservation of natural resources, being exploited in a sustainable [3]. Many trees

bear fruit during the flooding period and some fruits when they fall into the water remain floating, favoring the dispersion of the seeds. Among the fruit species native to the Amazon is the *Cassia leiandra* Benth which has as its edible part the fruit, popularly known as mari-mari, ingá-mari, seruaia and marimarizeiro, which besides being consumed by the population is also consumed by several species of fish [4].

This tree belonging to the Leguminosae family, one of the three most numerous families among the angiosperms being classified into three sub-families (Caesalpinioideae, Mimosoideae and Papilionoideae), where Caesalpinioideae is subdivided into four tribes (Cercideae, Detarieae, Cassieae and Caesalpea), with 2,250 species [5]. From the Cassieae tribe, the genus Cassia has pharmacological and medicinal properties studied worldwide, especially in tropical countries [6].

This enormous biological potential is associated with hepatoprotective, anti-inflammatory, antigenotoxic, hypolipidemic, spasmogenic and antinociceptive, antiproliferative, hypotensive, purgative, antidiabetic, antiulcer, antioxidant, antifungal, etc. These biological activities are related to the various chemical compounds isolated from the roots, stem peel, seeds and fruits of species of the genus Cassia, such as glycosylated anthraquinone, glycosylated naphthopyrone, flavonoids and other phenolic compounds [6].

Species of the genus Cassia have relevant biological properties and the genus Cassia has many species that have not yet been studied and explored from a pharmacological and nutritional point of view, especially endemic species in the Amazon. Thus, the present study aims at the physical, chemical and nutritional characterization, as well as the evaluation of the antioxidant potential of different parts of the *Cassia leiandra* Benth. fruit endemic to the central Amazon region.

2. MATERIALS AND METHODS

2.1 Sample collection and processing

The mari-mari fruits were collected at random in the municipal market of Santarém-Pará-Brazil, in the month of May of the year 2019. Then, they were compared with authentic exsiccates existing in the herbarium of the National Institute of Research of the Amazon - INPA, deposited under registration number 54017. Fruits were selected by the olive-green color of the peel (Figure 1), washed in running water and dried at room temperature for 24 hours. After this period, the fruits were evaluated for their biometrics and manually fractionated in peel, seed, pulp and endocarp. The fractions were crushed individually in a knife mill and separated into two parts. The first was packed in polypropylene bags and frozen for moisture analysis and the second was frozen, lyophilized and stored at -20 °C in an amber glass bottle, until the analyzes were carried out.

(A) (B)

Figure 1: Mari-mari fruit: (A) whole pod and (B) seed with pulp.

2.2 Biometric Assessment

Mari-mari fruits were evaluated according to de Carvalho and Muller (2005) [7]. The weight of the fruit, seed, peel and pulp of 10 fruits were measured with the aid of a semi-analytical balance; and the length, width and thickness of the fruit were measured with the aid of a caliper.

2.3 Centesimal composition

The centesimal composition was carried out according to AOAC (2018) [8]. The moisture was determined by the gravimetric method, subjecting the sample to heating of 105 °C in an oven, until reaching constant weight. The ashes were determined by incinerating the samples in a muffle furnace at 550 °C. To obtain the ether extract, the dry samples were extracted with hexane using the Soxhlet extractor for 5 hours. The crude protein was determined by the Kjeldahl method, using the factor of 6.25 to convert nitrogen into protein. The carbohydrate was obtained by theoretical calculation, reducing the sum of proteins, lipids, ash and moisture by 100. All results were expressed in percentage values (%).

2.4 Phytochemical analysis

To evaluate the phenolic compounds present in the mari-mari fruit (pulp, peel and seed), different extraction methods were performed in order to verify the best method to extract the phenolic compounds from samples of pulp, peel, seed (tegument and almond).

2.4.1 Extraction of phenolic compounds

For the extraction of phenolic compounds, the seed was divided into tegument and almond. Thus, the lyophilized samples analyzed were: pulp, peel, tegument and almond. The samples were submitted to 3 different extraction methods, the assisted ultrasound method - AU, by decoction in a water bath - DWB and by maceration at room temperature - MRT, to find the best method of extracting phenolic compounds for these samples.

The phenolic compounds were extracted by adding 0.25 g of dry sample and 12.5 mL of hydroalcoholic solution containing ethyl alcohol and distilled water in the proportion of 1:1, in a 50 mL amber glass bottle. The flask was incubated in an ultrasound bath at 37 °C for 30 min (AU); or incubated in a water bath at 60 °C for 30 min (DWB); or incubated at room temperature with shaking at 100 rpm, with the aid of a magnetic shaker, for 30 min (MRT). After the incubation period, the supernatant extract was removed and filtered on qualitative filter paper. The extraction process was repeated once again by adding 12.5 mL of the hydroalcoholic solution, to obtain an extract with a concentration of 10 mg/mL. This extract was used to analyze the content of phenolic compounds, flavonoids and tannins, and the antioxidant capacity.

2.4.2 Total phenolic

For the quantification of total phenolic, the Folin-Ciocalteau method was used, according to Singleton and Rossi (1965) [9]. The test was carried out in a test tube, adding 0.5 mL of sample and 2.5 mL of the reagent Folin-ciocalteau 5%. The mixture was homogenized and remained at rest for 5 minutes. Then, 2.0 mL of 4% sodium carbonate were added and the samples were vortexed and kept in the dark for 2 hours. After this period, readings were taken at 740 nm in a spectrophotometer. The results were calculated from an analytical curve of gallic acid and the result expressed in micrograms equivalent to gallic acid per milligram of sample ($GAE.mg^{-1}$).

2.4.3 Total flavonoids

Total flavonoids were determined using the aluminum chloride method according to the procedure described by Zhishen Zhishen, Mengcheng (1999) [10] with some modifications. For the test, 0.6 mL of sample and 2.4 mL of 0.1% aluminum chloride solution were added to a test tube, vortexed and kept at rest for 30 min in the absence of light. After this period, readings were taken at 420 nm in a spectrophotometer. The results were calculated from an analytical curve of rutin and expressed in micrograms of rutin equivalents per milligram of sample ($RE.mg^{-1}$).

2.4.4 Condensed tannins

Condensed tannins were determined by the vanillin method according to Broadhurst Broadhurst and Jones (1978) [11]. For the reaction, 1.5 mL of 4% methanolic vanillin, 0.25 mL of extract in the concentration of 1 mg/mL and 0.75 mL of concentrated hydrochloric acid were added to glass test tubes. The reaction mixture remained at rest for fifteen minutes at room temperature and in the absence of light. After this period, readings were taken at 500 nm in a spectrophotometer (LGI-VS-721N, Brazil). The results were calculated from an analytical curve of catechin and expressed in micrograms of catechin equivalents per milligram of sample ($CE.mg^{-1}$).

2.4.5 Anthocyanin

The determination of anthocyanins was carried out according to Fuleki and Francis (1968) [12] with modifications. To perform the test, 0.1 g of dry sample and 10 mL of acidified alcoholic solution (85: 15 v/v of 95% Ethanol and 1.5 M HCl) were added to a glass test tube. Then the samples were vortexed for 5 minutes, centrifuged (5000 rpm /5 min) and readings of the supernatant in a spectrophotometer with a wavelength of 535 nm. The concentrations of total anthocyanins were calculated using the coefficient 1% /1 cm = 98.2, referring to the average of the anthocyanins molar absorptivities: cyanidin 3-glycoside, cyanidin 3-arabinoside, peonidine 3-galactoside and peonidine 3-arabinoside in alcoholic solution Ethanol/HCl, and the results expressed in mg of anthocyanins per 100 g of sample.

2.5 Evaluation of antioxidant capacity

Considering the high content of phenolic compounds in the different parts of the mari-mari fruit and the recommendation that at least two antioxidant methods be combined to provide reliable results of the total antioxidant capacity of a food [13], the antioxidant capacity was evaluated against the DPPH, ABTS and FRAP methods.

2.5.1 Determination of antioxidant activity by the DPPH radical scavenging method

The analysis using the DPPH method was carried out according to Brand-Williams, Cuvelier (1995) [14] with some modifications. The reaction mixture was composed by the addition of 2.4 mL of DPPH ethanolic solution (2,2-Diphenyl-1-picryl-hydrazil) (29 µg/mL) and 0.6 mL of extract or ethanol to perform the control. The reaction mixture was homogenized and the readings were taken on a spectrophotometer (LGI-VS-721N, Brazil) at 516 nm until the absorbance remained constant. The results were calculated from an analytical curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and expressed in µmol $TEAC.mg^{-1}$ of sample. (TEAC - Trolox equivalent antioxidant capacity).

2.5.2 Determination of antioxidant activity by the ABTS method

The antioxidant capacity by the ABTS method was determined according to the methodology described by Re, Pellegrini (1999) [15] and modified by Rufino, Alves (2007) [16]. The stock solution of the ABTS radical (2.2-Azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid)) was composed of 7 mM ABTS with 140 mM potassium persulfate diluted in water, and the mixture was kept in the absence of light and at room temperature for 16 h. Subsequently, the radical was diluted with ethyl alcohol to obtain an absorbance of 0.80 ± 0.05 at 734 nm. For the assay, 15 µL of extracts or ethanol was added to prepare the negative control, and 1500 µL of the ABTS radical solution, homogenized in a tube shaker and the reading performed on a spectrophotometer (LGI-VS-721N, Brazil) at 734 nm after 6 min of reaction. The results were calculated from an analytical curve of Trolox and expressed in µmol TEAC.mg^{-1} of sample.

2.5.3 Determination of antioxidant activity by the FRAP method

The antioxidant activity by iron reduction (FRAP) was determined according to the Rufino, Alves (2006) [17] methodology. The FRAP Reagent consisted of 25 mL of sodium acetate buffer and 0.3 M acetic acid pH 3.6, 2.5 mL of TPTZ (2,4,6-Tris-(2-pyridyl)-s-triazine solution) 10 nM and 2.5 mL of 20 mM ferric chloride solution. The test was carried out in 2.0 mL microtubes, by adding 30 µL of extract or water to perform the negative control, 90 µL of distilled water and 900 µL of the FRAP reagent. The mixture was homogenized on a tube shaker, maintained at room temperature for 15 min, and the spectrophotometer read at 595 nm. The results were calculated from an analytical curve of Trolox and expressed in µmol TEAC.mg^{-1} of sample.

2.6 Statistical analysis

Tests were performed in triplicate, and the results were submitted to analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$). The correlations between variables were quantified by the correlation coefficient "r", considering $p < 0.05$. For this, the free access software PAST [18].

3. RESULTS AND DISCUSSION

3.1 Biometric characterization

The fruit of *Cassia leiandra* is a woody type, cylindrical vegetable, with many seeds immersed in a juicy and bittersweet pulp [19]. The physical analysis of the fruits was carried out in order to characterize in dimensions and composition by parts of the constituents of the fruits. The mari-mari is a long and thin fruit, having 76.5±5.3 cm in length, 9.2 ± 1.0 cm in width and 9.2 ± 1.0 cm in thickness. The total weight of the fruits varied from 256.7 to 476.7 g, with an average of 342.6 ± 70.0 g, being represented by 27% of peel, 32.4% of pulp and 40.7% of seed. Among the constituents, the seed represented most of the fruit, different from other types of ingá, such as liana (23.0%), and açu (24.3%), where the majority is represented by 48% of peel.

The yield of the mari-mari pulp was 32.40%, compared to the pulp of other fruits native to the Amazon, found that the results were similar for ingá-açu (*Inga cinnamomea*), ingá-cipó (*Ingá edulis*), uxi (*Endopleura uchi*) and pitomba (*Talisia esculenta*) presenting 27.9%, 29.4%, 32.8% and 33.8%, respectively, being classified in the low income category (between 21% and 40%) according to Carvalho and Muller (2005) [7], which does not preclude economic exploitation to obtain pulps or other food products, mainly due to the exotic flavor they present.

3.2 Centesimal composition

The centesimal composition of the studied parts of the mari-mari fruit is shown in Table 1. The comparison of the composition of the different parts of the fruit showed that the moisture content of the pulp was higher than that of the seed and the peel, showing that there is 27.2%, 41.1% and 95.3% of dry residue (proteins, lipids, ashes, fibers and carbohydrates), respectively.

*Table 1: Physiochemical parameters of mari-mari (*Cassia leiandria *Benth).*

Sample	Moisture (%)	Ashes (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)
Pulp	72.76 ± 0.21^{a}	0.80 ± 0.09^{c}	0.17 ± 0.05^{b}	0.28 ± 0.08^{b}	25.99 ± 0.24^{c}
Seed	58.87 ± 2.70^{b}	1.16 ± 0.06^{b}	0.36 ± 0.04^{a}	0.68 ± 0.04^{a}	38.92 ± 2.81^{b}
Peel	4.68 ± 0.19^{c}	1.69 ± 0.12^{a}	0.32 ± 0.02^{a}	0.62 ± 0.17^{a}	92.68 ± 0.08^{a}

* Different letters show statistical difference by the Tukey test ($p < 0.05$).

Regarding the ash content, the peel had a high content, followed by the seed and the pulp. In general, all parts had a low content of lipids and proteins, with the seed having the highest value, which can be explained by being an organ that accumulates nutritional reserve for the development of the embryo [20]. In relation for total carbohydrates, the peel had a higher content, probably due to its high fiber content. Thus, the seed and peel have higher amounts of nutrients than the respective edible parts of the fruit, and can be used to obtain food products, such as flours for human consumption, which maintains the nutritional value, flavor and smell characteristic of the fruit and it allows practical storage and longer conservation time, as well as animal feed [21]. However, when incorporated into the human diet, they must be evaluated for toxicity, to ensure the safety of their use.

When compared to other fruits from the Amazon of high economical use, such as acerola, cupuaçu and soursop, the mari-mari pulp showed, respectively, lower values for moisture (90.5; 86.2; and 82.2%) and proteins (0.9; 1.2 and 0.8%), values close to ashes (0.4; 1.0 and 1.0), similar for lipids of acerola (0.2%) and soursop (0.2 %), and higher than the values of carbohydrates (8.0; 10.4 and 15.8) [22].

3.3 Phytochemical characterization

Fruit and vegetable by-products have a high potential for reuse, since most of them still contain many interesting compounds, such as phenolic, which according to Hassimotto et al. (2005) [23] have a positive influence on human health, reducing the risk of various diseases, such as cancer and cardiovascular diseases, in addition to providing consumers with pleasant sensory properties. To evaluate the phenolic compounds, present in the different parts of the fruit, different extraction methods were performed, as shown in Figure 2.

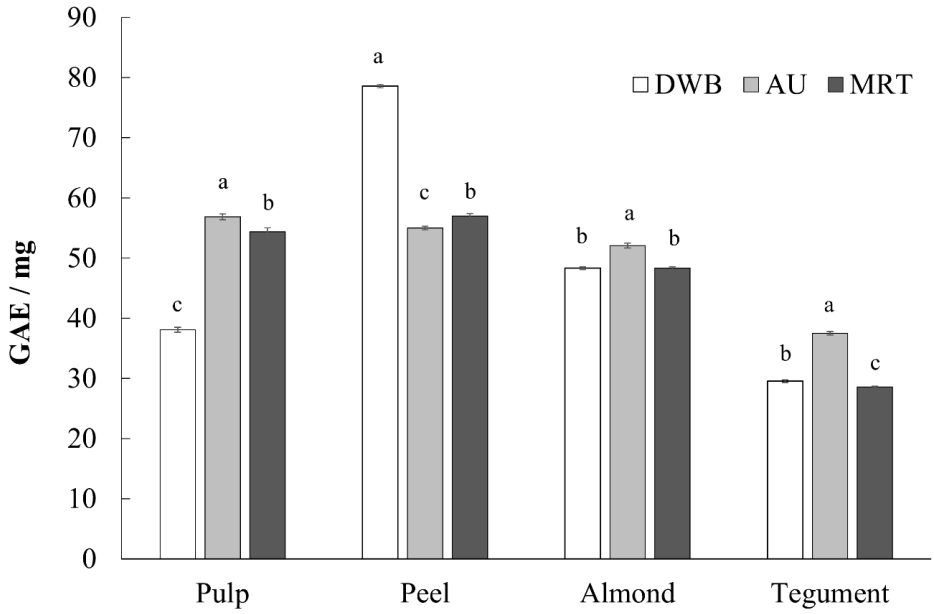

Figure 2: Evaluation of the content of total phenolic compounds extracted by different methods from different parts of the mari-mari fruit. AU: ultrasound bath at 37 °C; DWB: water bath at 60 °C; MRT: room temperature with shaking at 100 rpm.

For the extraction of phenolic compounds from pulp, almond and tegument, the assisted ultrasound extraction method was more efficient, followed by the decoction method and finally by the maceration method. As for peel, the decoction method at 60 °C extracted the phenolic compounds better, due to the temperature helping to soften the fibrous matrix, improving penetration and contact between the solvent and the sample particles. However, this method has the disadvantage of degradation and/or chemical transformation of these compounds, depending on the exposure time and the extraction temperature [24].

After evaluating the best method of extracting phenolic compounds for each part of the mari-mari fruit, the phytochemical characterization was performed according to Table 2. As the pulp is the only part of the fruit consumed *in natura*, the result was calculated both on a dry and wet basis.

Table 2: Phytochemical characterization of the different parts of the mari-mari fruit.

Sample	Total Phenolic (GAE/mg)	Total Flavonoids (RE/mg)	Condensed tannins (CE/mg)	Total Anthocyanins (mg/100g)
Pulp (WB)	15.49 ± 0.13	2.54 ± 0.02	13.27 ± 0.21	2.91 ± 0.32
Pulp (DB)	56.85 ± 0.48^{b}	9.31 ± 0.07^{b}	48.73 ± 0.76^{b}	10.69 ± 1.17^{d}
Peel (DB)	78.59 ± 0.29^{a}	8.18 ± 0.10^{c}	54.89 ± 3.70^{a}	30.99 ± 2.33^{c}
Nut (WB)	52.08 ± 0.42^{c}	19.60 ± 0.37^{a}	43.90 ± 2.33^{b}	53.63 ± 0.58^{a}
Tegument (WB)	37.50 ± 0.29^{d}	8.45 ± 0.33^{c}	35.49 ± 0.55^{c}	44.16 ± 3.67^{b}

* Different letters show statistical difference by the Tukey test ($p < 0.05$).
** WB – Wet Basis; DB – Dry Basis.

Among the different parts of the mari-mari fruit, the peel had a higher content of phenolic compounds, followed by pulp, almond and tegument. This result is in agreement with Ayala-Zavala, Vega-Vega (2011) [25] on the phenolic compounds are preferably located in the peel and seeds of the fruits, due to their role in defending the fruit against biotic and abiotic factors. Comparing the pulp of the mari-mari in fresh matter with other tropical fruits, the phenolic content

found was lower than the acerola pulps, but higher than those of soursop, tamarind and pitanga studied by Silva et al. (2014) [24], presenting values of 700.5, 547.0, 493.0 and 420.7 mg GAE/100 g, respectively.

Flavonoids represent one of the most important and diverse phenolic groups among products of natural origin. The analysis of flavonoids showed that these compounds were found to have a higher concentration in the almond, followed by the pulp and the lowest value in the peel and the tegument, showing no statistically significant difference. The highest concentration of condensed tannins was found in the peel, possibly presenting a greater astringent action in that part. Similar results were reported by Arruda et al. (2018) [26] who found that the Araticum contains different levels of specific phenolic for each part of the fruit, where the levels of total phenolic, flavonoids and condensed tannins of the fruits were higher in the peel, followed by the pulp and seed.

The content of anthocyanins showed higher values in the almond followed by the tegument, shell and pulp. Comparing with results obtained by Kuskoski et al. [27], mari-mari showed higher values for certain parts of tropical fruits, such as grapes, açaí and acerola (30.9, 22.8 and 2.7 mg.100g^{-1}, respectively).

3.4 Antioxidant capacity

The antioxidant capacity was evaluated against the DPPH, ABTS and FRAP methods, as shown in Figure 3.

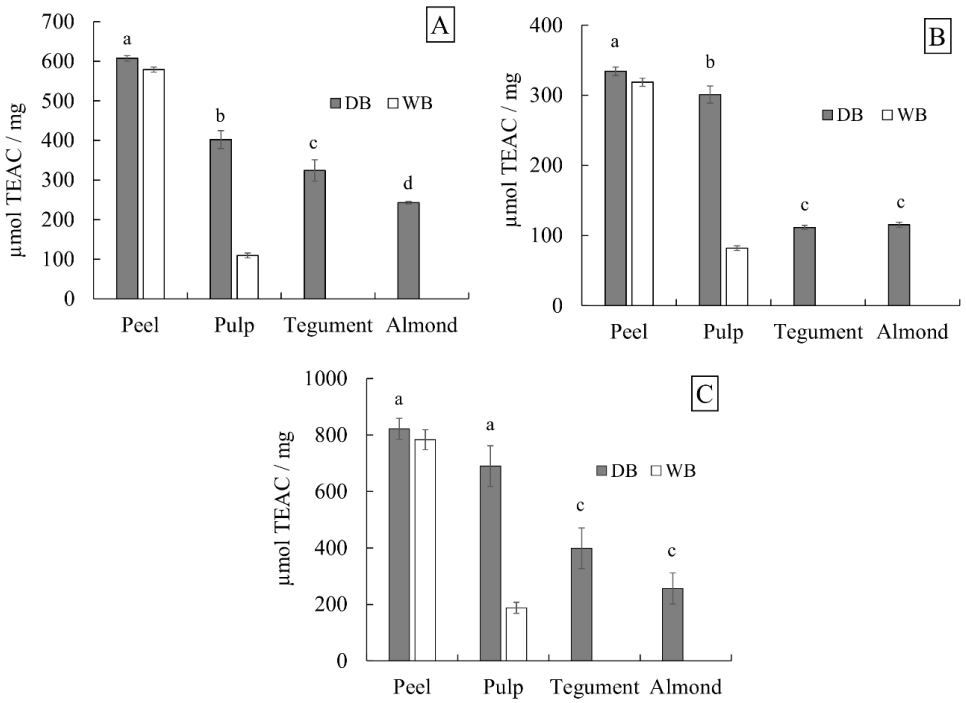

Figure 3: Evaluation of antioxidant capacity by the DPPH (A), FRAP (B) and ABTS (C) methods of the different parts of the mari-mari fruit in dry basis (DB) and wet basis (WB).

In general, the tests performed were based on the ability to sequester free radicals by samples that showed similar behavior, where the greatest capture was obtained by the peel, followed by the pulp, tegument and almond for all parts of the mari-mari fruit in dry basis. When processed to wet basis, the antioxidant capacity of the peel hardly changed, however there was a significant difference for the pulp, due to its high moisture content, but that did not cancel its antioxidant potential. Regarding the types of tests to assess antioxidant capacity, the samples showed greater activity by the ABTS method than by the DPPH and FRAP methods.

The analysis of the antioxidant capacity by the iron reducing power (FRAP), obtained the best result for the peel followed by the pulp and finally the almond and the tegument that did not present statistical difference (334.2 ± 6.1, 301.0 ± 12.2, 111.5 ± 3.0 and 115.3 ± 3.7 µmoL TEAC/mg DB, respectively). In the DPPH radical scavenging analysis, the peel also showed greater antioxidant capacity (607.23 µmol TEAC/mg DB). Comparing the results of the mari-mari pulp in fresh matter (109.5 ± 6.1 µmol TEAC/mg) with other commercial pulps, it was found that the values found were higher than the pulp of acerola, graviola and cupuaçu (68; 4.5 and 1.1 µmol/g, respectively) [27].

The peel (821.7 ± 37.1 µmol TEAC/mg DB) and the pulp (690.0 ± 72.1 µmol TEAC/mg DB; 188.0 ± 19.6 µmol TEAC/mg WB) of mari-mari showed greater capture of the radical ABTS in relation to the tegument and the fruit almond, in dry basis. Regarding the peel, higher values were found by Barros et al. (2017) [28] for tropical fruit peel as buriti, pomegranate and apple (16.3, 36,46 and 8.02 mM Trolox/g, respectively). Comparing the mari-mari pulp with the work of Rufino et al. (2010) [29] it was found that the values obtained were higher than the pulps of camu-camu, acerola, jaboticaba and açaí (153.0; 96.6; 37.5 and 15.1 µmol TEAC/g WB, respectively), by the ABTS radical method.

Evaluating the correlation of phenolic compounds with the methods of determining antioxidant capacity, there was a positive and significant correlation ($p < 0.05$) for all methods, being stronger for DPPH ($r = 0.837$) and FRAP (0.835), than for the ABTS method ($r = 0.755$). Results similar to those found by Rufino et al. (2010) [29] who also observed a positive and significant correlation between the levels of polyphenols and the antioxidant capacities determined by the ABTS ($r = 0.92$) and FRAP ($r = 0.89$) methods. Thus, we can see that all parts of the mari-mari fruit showed antioxidant potential, due to the considerable amounts of bioactive compounds, indicating promising prospects for the commercial exploitation of the fruit and its residues.

4. CONCLUSION

The mari-mari fruits can be described as a long thin fruit with low pulp yield. Chemical analyzes showed that the fruit peels have considerable amounts of nutrients, as well as their edible parts. The best method of extraction of phenolic compounds for pulp, almond fruits and tegument of Mari-Mari was ultrasonically assisted method and to the peel, the decoction. The peel of the mari-mari fruit showed the highest levels of all the groups of phenolic compounds evaluated in this study, as well as the highest antioxidant capacity. The inedible parts of the fruit showed potential as a promising raw material for the development of nutraceutical and food products.

5. ACKNOWLEDGMENTS

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