



Nutritional composition and antioxidant and cancer chemopreventive activities of fruits of *Psidium myrtilloides* (O. Berg)

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ABSTRACT: *Psidium myrtilloides* fruits food industries produce by-products such as liqueurs, ice cream, jellies, and juices. However, there needs to be more information about the nutritional composition of fruits, as well as the biological potential, mainly of seed flour. Therefore, the present study aimed to evaluate the nutritional composition and antioxidant and cancer chemopreventive activities of pulp and seed of fruits of *Psidium myrtilloides*. Moisture content, total minerals and mineral profile, total lipids and fatty acids profile, total proteins, dietary fiber (soluble and insoluble) and total carbohydrates were analyzed. Vitamins C and complex B, organic acids and the profile of phenolic compounds were also determined. DPPH and ABTS methods evaluated the antioxidant activity, and cancer chemopreventive activity was evaluated by quinone reductase induction and NF- κ B inhibitory activity. The fruits are a good source of thiamine, iron, phosphorus, potassium, fiber, and protein. Linoleic acid was the major fatty acid in both pulp and seed. Quercetin (1600 μ g/100g) and pyrogallol (819 μ g/100g) were the major phenolic compounds pulp and seed, respectively. The fruits showed strong antioxidant capacity, mainly the seeds, and the ability to induce quinone reductase activity, highlighting a cancer chemopreventive activity. Seed flour has a high potential to enrich food, in addition to valuing agro-industrial by-products, where we can highlight 25%, 13% and 37% of the recommended daily intake for iron (1.6 mg/100g), phosphorus (58 mg/100g) and vitamin B1 (450 μ g/100g), respectively, in addition to antioxidant and cancer chemopreventive activities.

Key words: NF- κ B, quercetin, vitamins, quinone reductase, arça-úna.

Composição nutricional e atividades antioxidante e quimiopreventiva de câncer de frutos de *Psidium myrtilloides* (O. Berg)

RESUMO: Frutos de *Psidium myrtilloides* são utilizados na produção de subprodutos como licores, sorvetes, geleias e sucos, porém, existem poucas informações sobre a composição nutricional das frutas, bem como o potencial biológico, principalmente da farinha de sementes. Dessa forma, o objetivo do presente estudo foi avaliar a composição nutricional e as atividades antioxidante e quimiopreventiva de câncer da polpa e sementes de frutos *Psidium myrtilloides*. Foram analisados o teor de umidade, minerais totais e perfil de minerais, lipídios totais e perfil de ácidos graxos, proteínas totais, fibra alimentar (solúvel e insolúvel) e carboidratos totais. Também foram determinadas as vitaminas C e do complexo B, ácidos orgânicos e perfil de compostos fenólicos. A atividade antioxidante foi avaliada pelos métodos DPPH e ABTS, e a atividade quimiopreventiva de câncer foi avaliada pela indução da enzima quinona redutase e pela atividade inibitória do NF- κ B. Os resultados mostraram que os frutos são boas fontes de vitamina B1, ferro, fósforo e potássio, além de fibras (polpa: 7%; semente: 25%) e proteínas. O ácido linoléico foi o principal ácido graxo tanto na polpa quanto na semente. Quercetina (1600 μ g/100g) e pirogalol (819 μ g/100g) foram os principais compostos fenólicos encontrados na polpa e na semente, respectivamente. A fruta apresentou forte capacidade antioxidante, principalmente as sementes, que também apresentam capacidade de induzir a atividade da enzima quinona redutase, ressaltando atividade quimiopreventiva de câncer. A farinha de semente tem grande potencial para enriquecer alimentos, além de valorizar subprodutos agroindustriais, em que podemos destacar 25%, 13% e 37% da ingestão diária recomendada para ferro (1,6 mg/100g), fósforo (58 mg/100g) e vitamina B1 (450 μ g/100g), respectivamente, além de atividades antioxidantes e quimiopreventivas do câncer.

Palavras-chave: NF- κ B, quercetina, vitaminas, quinona redutase, arça-úna.

INTRODUCTION

A diet rich in fruits and vegetables positively impacts health, as they are sources of essential nutrients such as vitamins, minerals and fibers that perform various functions in the body, bringing benefits to human beings. Recent studies have shown that fruit consumption can prevent chronic noncommunicable diseases due to the

presence of compounds with pharmacological properties such as antioxidant, anti-inflammatory and cancer chemoprevention (AROLA-ARNAL et al., 2019; MOLEHIN et al., 2021).

Brazil is known for presenting different climates, which favors the diversity of fruit species distributed around the six significant biomes (Amazon, Caatinga, Cerrado, Atlantic Forest, Pantanal and Pampa). Despite the abundance of fruits

in Brazil, there are still a large number of poorly studied species that may be a possible source of active compounds, such as *Psidium myrtooides* (O. Berg). It belongs to the Myrtaceae family, considered one of the most relevant angiosperm families in the country, and it is widely found in almost all Brazilian biomes. Previous works performed with *P. Myrtooides* focused on the composition and antimicrobial activity of the essential oil of the leaves, such as antifungal (MACÊDO et al., 2020) and antibacterial (DIAS et al., 2018). Earlier studies analyzed physical characteristics such as fruit size, total phenolics and tannins, besides the antioxidant activity of the leaves. The authors conclude that *P. Myrtooides* is a promising target for further phytochemical studies and other biological tests (DIAS et al., 2022). However, the nutritional composition of the fruits is still unknown. Therefore, the present study aimed to evaluate the nutritional composition, and the antioxidant and cancer chemopreventive activities of pulp and seeds of *Psidium myrtooides* fruits.

MATERIALS AND METHODS

Samples

We collected seven kilograms of fruit, randomly chosen from three specimens considering the degree of ripeness, in the city of Guarapari – ES, (20°32'32.3"S 40°36'09.1"W) (-20.542296, -40.602540), selecting those with dark purple color. The samples were immediately transported in thermal boxes to the laboratory, where they were washed with water and manually separated into peels with pulp (5.0 Kg) and seeds (2.0 kg) since the peel is edible. Half of the samples were frozen at -80 °C and freeze-dried for 48h (Enterprise I Lyophilizer, Terroni), and the other part, still fresh, was homogenized in a food processor to analyze proximate composition. The botanical identification was held at Vila Velha University (ES, Brazil), by Dr. Solange Schneider, and the voucher of the species n 2620 was deposited at the Vila Velha University herbarium. Fruits were measured for weight and length (n = 100).

Proximate composition

Moisture was determined in the oven at 75 °C until constant weight; ash was quantified by carbonization at 550 °C until constant weight; total proteins were obtained by the Kjeldahl method, and total fat was quantified by the Goldfish method (AOAC, 2016). Total, soluble and insoluble dietary fiber were determined by enzymatic-gravimetric method (AOAC 991.43) using the total dietary fiber

analysis kit (Sigma®). Total carbohydrate content was obtained by subtraction (moisture + ash + lipid + protein + total dietary fiber). All analyzes were performed in triplicate.

Elemental composition

The sample digestion was performed in a Speedwave four microwave oven (Berghoff Instruments, Eningen, Germany) with a 12-tube rotor, 60 mL capacity, maximum power of 2000 W, pressure, and temperature limit of 100 bar and 230 °C, respectively, was used for sample digestion. The samples were weighed (0.5 g) in tube barges with 2 mL of HNO₃ (70%) and 0.5 mL of H₂O₂ (30%). After, the solution was transferred to a polypropylene tube and the volume was filled with ultrapure water to 10 mL. For analysis, iCAP 6000 (Thermo Fisher Scientific) with axial and radial vision was used. The conditions for analysis were: 1200 W of RF power, 12 L/min plasma gas flow, 0.5 L/min auxiliary gas flow, 0.65 L nebulizer gas flow/min, 0.6 mL/min sample flow with a concentric nebulizer and cyclonic nebulization chamber. All samples were analyzed in triplicate. Quantification was done by an external curve (5 points) for each metal using individual standard solutions (Certified Reference Material).

Organic acids

The extraction was performed by weighing 1.0 g of lyophilized sample in a 50 mL volumetric flask and adjusted with phosphate buffer (KH₂PO₄ - 0.01M, pH 2.6). After 15 minutes in an ultrasonic bath (80 Hz), the solution was filtered on filter paper, filtered through a 0.45 µm membrane, and injected into the chromatograph. Tartaric, malic, ascorbic, and citric acids were determined on a liquid chromatograph (Breeze, Waters) (SCHERER et al., 2012). An RP-C18 column (150 x 4.6 mm, 5.0 µm) was used for separation. The mobile phase consisted of 0.01 mol/L KH₂PO₄ buffer solution (pH = 2.60 adjusted with o-phosphoric acid), using an isocratic elution procedure with a flow rate of 0.5 mL/min. Detection was done in a UV detector at 210 nm for tartaric, malic and citric acids, and 250 nm for ascorbic acid. Quantitation was performed by a calibration curve (7 points). The detection (LD) and quantification (LQ) limits were obtained by the signal-to-noise ratio, where LD was defined as concentration producing a signal three times higher than the noise amplitude and six times for the LQ.

B-group vitamins

An amount of 0.5 g of lyophilized samples was extracted with 10 mL of ethanol/H₂O (1:1) solution

with 200 μM HCl in an ultrasonic bath (10 minutes). We centrifuged the samples for 1 min (12,000 rpm), and 1.0 mL of solution was diluted with 1.0 mL H_2O , filtered (0.45 μm), and injected into the LC/MS/MS. We used a liquid chromatograph Acquity UPLC (Waters) coupled to a triple quadrupole mass detector (Xevo TQ-S micro; Waters) with electrospray ionization (LC-ESI-MS/MS) in positive mode $[\text{M} + \text{H}]^+$. An Acquity UPLC BEH (C18; 100 mm x 2.1 mm; 1.7 μm column) was used at 45 $^\circ\text{C}$. The mobile phase consisted of an aqueous solution of formic acid (0.1%) (A) and acetonitrile (B) using a gradient elution at 0.4 mL/min, starting with 1% B from 0 to 2 min, 1-55% B in 2-3 min, 55-99% B in 3.0-3.1 min, 99% B in 3.1-4.0 min, 99-1% B in 4.0-4.1 and 1% B from 4.0 to 5.0 min for column conditioning for the next injection. The ESI ion source parameters were capillary voltage of 3.0 kV, cone voltage as optimized for each compound, source temperature of 130 $^\circ\text{C}$, desolvation temperature and flow were 650 $^\circ\text{C}$ and 1200 L/h, respectively. The analyses were performed in Selected Reaction Monitoring mode. Quantitation was performed by calibration curve (7 points). The detection (LD) and quantification (LQ) limits were obtained by the signal-to-noise ratio, where LD was defined as concentration producing a signal three times higher than the noise amplitude and six times for the LQ.

Fatty acids

Bligh and Dyer method was used to extract lipids from seeds and pulps and then derivatized using a 14% BF₃ methanolic solution, according to Joseph and Ackman (JOSEPH & ACKMAN, 1992). We analyzed the esters in a gas chromatograph (Shimadzu GC-2014) coupled to a flame ionization detector. A capillary column (HP-INNOWAX; 50 m x 0.20 mm; 0.20 μm) (Agilent) was used under the following conditions: injector 250 $^\circ\text{C}$, split mode 1:10 for 1.0 min; nitrogen flow at 1.2 mL/min; detector temperature: 260 $^\circ\text{C}$; temperature ramp: 150 $^\circ\text{C}$, increasing 10 $^\circ\text{C}/\text{min}$ to 260 $^\circ\text{C}$ and kept for 9 min. The alkane standard solution (C7-C30, Sigma-Aldrich) was injected under the same conditions for compound identification. Quantification was performed by the internal standard method using methyl tricosanoate. All analyses were performed in triplicate.

Phenolic compounds profile by LC-HRMS

The phenolic compounds were analyzed by liquid chromatography coupled with high-resolution mass spectrometry (Orbitrap) (BARBOSA et al., 2018). Freeze-dried samples (0.1 g) were

extracted in ultrasound using 10 mL of extraction solvent (acetone/water/HCl; 70/29.9/0.1). After centrifugation (3500 rpm, 15 min), the supernatant was filtered (0.45 μm) and kept at -4 $^\circ\text{C}$ until analysis. We analyzed the extracts on a UHPLC/Q-Exactive Orbitrap mass detector (Thermo Fisher Scientific). We utilized a C18 reversed-phase column (150 x 2.1 mm, 2.7 μm ; Supelco) for separation in a gradient elution with water (A) and acetonitrile (B), both acidified with 0.1% formic acid. The gradient was as follows (0.3 mL/min): 0-1 min 10% B; 1-20 min linear to 95% B; 20-23 min 95% B; 23-24 min 10% B; and 24 to 30 min for column conditioning. We used the electrospray ionization source (HESI-II) operated in negative and positive ionization mode. HESI-II heater temperature at 350 $^\circ\text{C}$ and capillary voltage at -2.5 kV was applied. Retention time, mass error, isotopic patterns, and product ion were used for identification and confirmation purposes.

Extracts

The extraction was performed by ultrasound-assisted maceration method using 20 g of freeze-dried samples with 200 mL of ethanol. After two cycles of 30 min, the extracts were filtered, and the process was repeated twice. The organic fractions were mixed, and ethanol was removed on a rotary evaporator (Fisatom[®] 802 - 1200W), with subsequent freeze-drying for 24 h.

Antioxidant activity

The antioxidant activity of the samples was evaluated by the DPPH method (SCHERER & GODOY, 2009). The following equation calculated the activity: $I (\%) = [(Abs_0 - Abs_1) / Abs_0] \times 100$, where Abs₀ is the absorbance of the blank and Abs₁ is the absorbance of the samples at different concentrations. According to RE et al. (1999), the antioxidant activity was also determined, adapted to 96-well microplates. The ABTS[•] radical was formed by the reaction among 7.0 mM ABTS (50% ethanol) and 2.45 mM potassium persulfate. The reagent was diluted with ethanol (50%) until the absorbance reached 0.98 - 1.02 at 734 nm. ABTS[•] (270 μL) and the samples (30 μL) were added into 96-well microplates. The blank was 30 μL of ethanol. After 10 min in the dark, the reading was performed at 734 nm using a microplate reader. The following equation calculated the activity: $I (\%) = [(Abs_0 - Abs_1) / Abs_0] \times 100$, where Abs₀ is the absorbance of the blank and Abs₁ is the absorbance of the samples at different concentrations. The results were expressed as IR₅₀ (concentration capable of reducing 50% of

the free radicals) calculated by calibration curve by plotting the final concentration of the extract versus the corresponding I (%).

Cancer chemopreventive activity

Quinone reductase assay

Quinone reductase induction assay was performed following the method described by GERHÄUSER et al. (1997). For evaluation of quinone reductase induction by the extracts, rat hepatoma cell culture was used (Hepa1c1c7). The extracts were tested at 20 µg/mL. Results were expressed as percentage inhibition (IR%).

NF-κB inhibitory activity assay

For the NF-κB inhibition, the 293-NF-κB cell line (NF-κB-luciferase transfected renal embryonic cells 293.12 PTA – 5554) was used induced by TNF-α (5 ng/mL). The extracts were tested at a concentration of 20 µg/mL and Promega® Luciferase Assay. Na-tosyl-L-phenylalanine-chloromethyl ketone was used as a positive control (IC₅₀ = 3.8 nM). Results were expressed as percentage inhibition (HOMHUAL et al., 2006).

Statistical analysis

The statistical analysis was performed by ANOVA using PRISMA software. Tukey's test was used to significant differences (P < 0.05).

RESULTS AND DISCUSSION

Proximate composition

The size of fruits ranged from 1.4 to 3.0 cm in diameter, with an average weight of 3.40 ± 0.97 g. The yield of pulp with peel (thin and edible) obtained was 72.1%, and 27.9% of seeds, values that indicate a potential use as fresh fruit or for industrial use, for example, in the production of juice pulps. The results of the proximate composition of the pulp and the seeds of fruits are shown in table 1. The pulp presented a high amount of moisture, carbohydrates and fiber, mainly insoluble fiber, and the seed flour presented a high amount of fiber and carbohydrate. Recent studies have demonstrated the impact of dietary fiber intake on the intestinal microbiota, helping the intestinal microbiota to exert its beneficial effects on human metabolism, preventing cardiovascular diseases, diabetes, obesity, inflammatory processes, and others (KUMAR et al., 2020).

Elemental composition

Both pulp and seed presented considerable potassium amounts (Table 1). This mineral is significant to cardiovascular health, exerting a vasoprotective function, particularly in the context of hypertension and prehypertension (OKAYAMA et al., 2016). The seed can be considered a source of iron and phosphorus, with 25 and 13% of DRI

Table 1 - Proximate (g/100g) and elementary (mg/100g) composition of pulp and seed flour.

	-----Pulp-----	-----Seed flour-----
Kcal (kcal/100g)	50.0	145
Moisture	79.2 ± 0.1	-
Ash	0.8 ± 0.03b	1.3 ± 0.03a
Total carbohydrates	11.5	26.9
Protein	0.7 ± 0.01b	1.6 ± 0.1a
Fat	0.2 ± 0.01b	3.5 ± 0.03a
Total dietary fiber	7.7 ± 0.55b	66.8 ± 0.6a
Insoluble fiber	6.7 ± 0.27b	61.2 ± 0.9a
Soluble fiber	1.0 ± 0.61b	5.6 ± 1.06a
Calcium	< 0.5	63.3 ± 0.7
Iron	0.2 ± 0.0b	1.6 ± 0.0a
Phosphorus	5.4 ± 0.1b	57.7 ± 0.5a
Magnesium	5.0 ± 0.1b	34.6 ± 0.4a
Potassium	114 ± 1.5b	215 ± 0.9a
Sodium	14.8 ± 0.2b	20.0 ± 0.2a
Zinc	0.1 ± 0.00b	0.5 ± 0.0a
Silicon	0.7 ± 0.01b	1.06 ± 0.0a

Results are expressed as mean ± standard deviation (n = 3). Different letters on the same line correspond to significant differences (P < 0.05).

values, respectively (INSTITUTE OF MEDICINE, 2006). Other minerals were analyzed, such as selenium, manganese, chromium, lithium, copper, cobalt and aluminum. However, they did not present relevant values.

Organic acids and B-group vitamins

Tartaric and citric acids were the main organic acids present in the fruit (Table 2). Despite having vitamin C, araçáúna cannot be considered a good source, since the fruit consumption should be around 1.7 kg to meet the recommendation of daily vitamin C consumption for an adult (INSTITUTE OF MEDICINE, 2006).

B-group vitamins are known to act as coenzymes of macronutrient catabolism reactions, generating energy production for the body (KHALIL et al., 2021). Seven B-complex vitamins were analyzed, and the results are in table 2. The results showed that pulp reaches 6% of the daily recommendation of Thiamine (B1), while seed flour reaches 37%, according to the values used as reference by the Dietary Reference Intakes (RDI) (INSTITUTE OF MEDICINE, 2006). The presence of this vitamin is required in carbohydrate and energy metabolism, participating as a coenzyme for enzyme complexes. The deficiency of such compounds in the body causes some symptoms, such as weakness, nervous disorders, and protein-caloric malnutrition (COMBS JUNIOR, 2017).

Fatty acids profile

Table 3 presents the profile of fatty acids present in the pulp and seed of araçáúna. Unsaturated

fatty acids were predominant concerning saturated fatty acids. While the pulp presented higher omega 3 and palmitic acid levels, the seeds presented omega 6 and omega 9. It is noteworthy that pulp presented a 2:1 ratio of omega 6 and omega 3, which is within the recommended for promoting a higher conversion of alpha-linolenic acid to docosahexaenoic acid (DHA) (MARTIN et al., 2006). There is a relationship between an unbalanced proportion of n-6/n-3 and the occurrence of inflammatory diseases, atherosclerosis, or tumor proliferation. One of the reasons for this is the growth of the food industry that has marketed foods with high amounts of vegetable oils coupled with a decrease in fruit and vegetable consumption, resulting in a poor diet (LIU et al., 2015).

Phenolic compounds profile

In the quantitative analysis of phenolic compounds in extracts, 10 compounds were identified (Table 4). Quercetin was the major phenolic compound in the pulp, a flavonoid recognized as an antioxidant, which plays a relevant role in the metabolic pathways involved in the prevention of nephropathy, carcinogenesis, inflammation, and cardiovascular diseases, besides contributions to the regulation of bone homeostasis (GUSS et al., 2017; XU et al., 2019). Furthermore, quercetin beneficially affects the composition of the intestinal microbiota, improving metabolic disorders (SHABBIR et al., 2021). The main phenolic in the seed was pyrogallol, which exerts antioxidant, antibacterial and antiseptic activity (WANG et al., 2018). A previous study reported that pyrogallol is an effective anticancer with low toxicity for colon cancer. In addition, it has

Table 2 - Content of B-group vitamins and organic acids of pulp and seed flour.

Compound	--Linearity (r ² --	--LD (µg/mL)--	--LQ (µg/mL)--	-----Pulp-----	-----Seed flour-----
Ascorbic acid (C)	0.9983	0.2	0.5	3.50 ± 0.07	< LQ
Thiamine (B1)	0.9903	1.4	4.3	71.5 ± 2.9	452 ± 9.1
Riboflavin (B2)	0.9960	1.3	3.8	17.2 ± 0.8	43.4 ± 1.5
Nicotinic acid (B3)	0.9927	0.4	1.1	< LQ	< LQ
Pantothenic acid (B5)	0.9840	0.3	0.8	18.0 ± 1.3	46.8 ± 1.2
Pyridoxine (B6)	0.9940	0.1	0.4	1.2 ± 0.05	6.38 ± 0.1
Biotin (B7)	0.9955	1.5	4.7	0.11 ± 0.0	0.8 ± 0.0
Cyanocobalamin (B12)	0.9995	0.3	0.9	< LQ	< LQ
Tartaric acid	0.9995	1.3	2.7	3.10 ± 0.2	< LQ
Malic acid	0.9997	2.7	5.4	< LQ	< LQ
Citric acid	0.9997	0.5	1.1	2.10 ± 0.1	< LQ

Results are expressed as mean ± standard deviation (n = 3). LD: Limit of detection; LQ: Limit of quantification; B-group vitamins: µg/100g; Vitamin C: mg/100g; Citric, malic and tartaric acids: g/100g.

Table 3 - Fatty acids profile of pulp and seed flour.

Fatty acid	-----Pulp-----		-----Seed-----	
	%	mg/g	%	mg/g
C10:0	1.9	4.0	-	-
C12:0	1.5	3.1	-	-
C14:0	3.8	7.8	0.1	0.7
C15:0	1.4	2.8	0.0	0.3
C16:0	24.0	48.3	9.9	72.3
C16:1 ω -7	0.7	1.3	0.2	1.2
C17:0	0.3	0.5	0.1	0.7
C18:0	7.2	14.2	6.8	48.3
C18:1 ω -9	9.4	18.4	16.8	119
C18:2 ω -6	32.0	62.6	64.9	458
C18:3 ω -3	16.2	31.4	0.4	2.5
C20:0	0.8	1.6	0.4	3.1
C20:1 ω -9	0.3	0.6	0.2	1.2
C22:0	0.4	0.7	0.1	1.0

antitumor properties in hepatocellular carcinoma cells (AHN et al., 2019; REVATHI et al., 2019).

Antioxidant and cancer chemopreventive activities

According to the results obtained (Table 5), the seeds presented a significantly ($P < 0.05$) more substantial antioxidant activity than the pulp in both evaluated methods. In a previous work executed with araçauína fruits, high antioxidant activity was also reported for pulp extracts, similar to the activity of quercetin (DIAS et al., 2022). However, the seeds were not evaluated. As previously reported, phenolic compounds such as quercetin and pyrogallol were found in the extracts, possibly contributing to the antioxidant activity.

In the quinone reductase assay, the result was expressed as induction ratio (IR), where $IR \geq 2.0$ means an inhibitory capacity of quinone reductase. The *P. myrtiloides* seed showed significant activity, presenting $IR = 2.0 \pm 0.0$. On the other hand, the pulp was not able to induce quinone reductase, expressing an IR value of 1.0 ± 0.0 . This result demonstrates that the seed can be a potential chemopreventive agent to be explored in future studies. Both samples were not considered toxic for cells, as they showed cell survival values greater than 80%. Percentages of inhibition and survival evaluated results for NF- κ B inhibition (I%) and cell survival. For NF- κ B inhibition, those with values above 50% are considered inhibitory, and for

Table 4 - Phenolic compounds in pulp and seed flour obtained by LC-MS/MS (μ g/100g).

Compound	---[M-H] ⁻ ---	--Exact mass m/z--	-Experimental m/z-	Mass error* (ppm)	----Pulp----	--Seed flour--
(-)- Epicatechin	C15H13O6	289.0706	289.0719	0.583	31 \pm 0.4	10.9 \pm 0.01
4-Hydroxybenzoic acid	C7H5O3	137.0233	137.0242	-1.221	116 \pm 4.1	-
4-O-Caffeoylquinic acid	C16H17O9	353.0867	353.0879	0.438	110 \pm 2.0	-
Chlorogenic acid	C16H17O9	353.0867	353.0879	0.438	25.5 \pm 1.0	-
Ethyl gallate	C9H9O5	197.0444	197.0458	1.387	10.4 \pm 0.6	-
Gallic acid	C7H5O5	169.0131	169.0140	-1.281	166 \pm 0.6	774 \pm 2.7
Polydatin	C20H21O8	389.1230	389.1244	1.306	1.1 \pm 0.00	5.8 \pm 0.00
Pyrogallol	C6H5O3	125.0233	125.0244	-0.139	170 \pm 12	819 \pm 3.2
Quercetin	C15H9O7	301.0342	301.0354	1.201	1601 \pm 41	34.8 \pm 1.3
Vanillin	C8H7O3	151.0389	151.0396	-2.962	10.1 \pm 0.10	-

Results are expressed as mean \pm standard deviation (n = 3). - : Not detected. * The highest value was considered.

Table 5 - Antioxidant activity of pulp and seed flour.

Method	Pulp	Seed flour
DPPH	173 ± 5.5 a	117 ± 1.0 b
ABTS	116 ± 6.4 a	90.4 ± 1.4 b

Results are expressed as mean ± standard deviation (n = 3). Result expressed in IR₅₀ (µg/mL).

survival, values below 80% are considered toxic. The pulp (I = 9.6 ± 1%) and seed (I = 22.5 ± 2%) samples were not able to inhibit NF-κB, as they did not reach results above 50%. However, they were not considered toxic for cell survival, as they presented results above 80%, 113 ± 0.1% for the pulp, and 108 ± 1% for the seed.

Quinone reductase, one of the phase II enzymes, is a significant detoxification pathway, being a biomarker for the research of chemopreventive agents against the initial phase of cancer. Several flavonoids and phenolic compounds present in fruits and vegetables are among the most commonly identified as inducers of enzymatic phase II (ZHANG et al., 2018). An earlier study suggests an antitumor potential for flavonoids such as quercetin via possible modulation of quinone reductase gene expression (ZHANG et al., 2021). Besides, flavonoids inhibit cell proliferation by suppressing the NF-κB pathway (HAZAFI et al., 2020). A previous study showed that blueberry (*Vaccinium myrtillus*) has high chemopreventive and antioxidant activity by inhibiting the proliferation, growth and migration of the human lung carcinoma cell line (A549). The authors suggest that blueberries' chemopreventive and antiproliferative action was not limited to a single content of bioactive compounds but rather several of them acting synergistically (BABY et al., 2018). As mentioned above, the phenolic pyrogallol was identified in the seed, which the literature already mentions as a compound with anticancer activity (AHN et al., 2019; REVATHI et al., 2019). In the same way, the results suggest that pyrogallol and the other compounds of the seed extract may be acting in synergy in the chemopreventive activity of cancer.

CONCLUSION

P. myrtiloides fruits have great nutritional and technological potential that are still little explored. The pulp and the seeds are sources of minerals, vitamins and substances of pharmacological interest. The results show that the fruit can be produced for

consumption and industrialization, as it has a good pulp yield with moderate acidity indicated for juices, pulps, jellies, and fermented beverages. Also, in fruit processing, the seeds are usually discarded. However, using these by-products, such as seed flour, has aroused the interest of consumers. In this way, *P. myrtiloides* seed flour becomes an excellent source of dietary fiber, iron, calcium, potassium, phosphorus, magnesium, and thiamine, as well as 1.6% of proteins, 3.5% of lipids, and 27% of carbohydrates, in addition to antioxidant and cancer chemopreventive activities, and thus, can be used to fortify foods in populations with nutritional deficiency.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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