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Annona muricata leaf extracts obtained with subcritical water and conventional methods: evaluation of antioxidant activities, total polyphenol and rutin contents

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ABSTRACT. *Annona muricata* (soursop) is a native American plant and is well known for its leafy canopy and its fruits, widely consumed in South America and Southeast Asia. The objective of this work was to obtain *Annona muricata* leaf extracts and to evaluate their antioxidant activities, which were obtained by four different extraction methods: Soxhlet, maceration, ultrasound, and pressurized hot (subcritical) water. In order to assess and compare the extracts bioactivities, total polyphenol contents of the extracts were evaluated by the Folin-Ciocalteu method and the antioxidant activities were determined by DPPH and ABTS methods. High performance liquid chromatography was used to identify and quantify rutin, a special flavonoid, which was found to be the most representative component, for all extracts obtained. Rutin is known for its benefits has a potential natural antioxidant. Among all extraction methods investigated, extracts obtained by the Soxhlet method using ethanol as solvent showed a higher total polyphenol content, 2.83% of gallic acid equivalent/g plant material, as well as higher antioxidant activity. A correlation between rutin content and antioxidant activity was found. Soxhlet extractions revealed samples with the highest rutin content of 0.130 ± 0.003 g g^{-1}, what may explain the high antioxidant activity of the obtained *A. muricata* leaf extracts.

Keywords: soursop; phenolic compounds; bioactivity; pressurized hot water; extraction.

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Introduction

Annona muricata, soursop tree or gravioleira is a plant of the Annonaceae family, originally from the American continent, but also present in Southeast Asia. Its fruit can be used to produce juice, ice cream, jelly, or for fresh consumption (Orak, Bahrisefit, & Sabudak, 2019; Nugraha, Haritakun, Lambert, Dillon, & Keller, 2021). The fruit, peel, seeds and leaves are used in the treatment of respiratory, heart, gastrointestinal, kidney and liver diseases, being widely applied in patients with malaria, diabetes and cancer (Coria-Téllez, Montalvo-Gónzalez, Yahia, & Obledo-Vázquez, 2018; Chan, McLachlan, Hanrahan, & Harnett, 2020). These benefits can be attributed to phytochemicals such as acetogenins, alkaloids, phenolics, cyclopeptides and essential oils. Soursop leaves are a common residue in production areas and may be an important source of high-added value products, which may act as important natural antioxidants (Moghadamtousi et al., 2015).

The use of natural antioxidants as food and medicine additives or supplements increased in the last decades, due mainly to the restrictions imposed to synthetic ones. Thus, the recovery of specific chemical components from plant materials or agroindustrial residues, like phenolics, has been extensively investigated in order to characterize the bioactivities of these extracts and to identify the most representative components (Kintzios, Papageorgiou, Yiakoumettis, Baričevič, & Kušar, 2010).

Depending on the chemical characterization of each plant extract, results of antioxidant activities may vary due to the use of distinct analytical procedures. It is known that several methods for the evaluation of antioxidant activities are available and the use of one isolated method is not satisfactory to cover completely the mechanisms involved in the evaluation of antioxidant potentials (Michielin, Wiese, Ferreira, Pedrosa, &

Ferreira, 2011). Usually, phenolics, among other chemical components, are considered antioxidants due to their free radical scavenging potential, but other additional mechanisms may be responsible for overall antioxidant activities, such as metal chelating and singlet oxygen scavenger (Sharififar, Moshafi, Mansouri, Khodashenas, & Khoshnood, 2007; Frankel & Finley, 2008).

In order to recover high-added value components from agroindustrial residues, like stems, seeds, leaves and roots, the so-called biorefinery approach is considered one promising alternative in producing areas by using several separation techniques, like extraction. The use of conventional extraction methods, such as Soxhlet, hydrodistillation, maceration, ultrasound and others, may present some drawbacks, including the use of high temperatures, very long extraction times and large amounts of potentially toxic solvents. Given this, extraction using subcritical water, or pressurized hot water (PHW), is an interesting alternative, as it uses water, a safe solvent, at high temperatures and pressures, but below the critical point, which causes changes in the solvent mass transfer properties and solvent solubility (Guthrie et al., 2020; Asl & Niazmand, 2020). Thus, hydrophobic compounds can be easily dissolved and water can be completely removed from the extract (Plaza & Turner, 2015; Çam et al., 2019).

This work aims to evaluate the antioxidant activities and total polyphenol content of *A. muricata* leaf extracts obtained by PHW and compare to the extracts obtained by Soxhlet (SOX), maceration (MAC) and ultrasound-assisted extraction (ULT) methods. Additionally, rutin contents were also determined and the comparison between the mentioned extraction methods was performed.

Materials and methods

Herbal material

Annona muricata leaves were obtained from local producers in the city of Garanhuns, Pernambuco, Brazil. First, the leaves with the presence of fungi and visible imperfections were removed and the remaining material was subjected to drying in an oven with forced air circulation (Lucadema, Model 82/480, São José do Rio Preto, Brazil) at 318.15 K for three days, according to AOAC procedures (Association of Official Analytical Chemists [AOAC], 2005). Then, the leaves (8.12% moisture, w w^{-1}) were milled using a knife mill (Marconi, Model MA340, Piracicaba, Brazil) and particles with a mean diameter of 1 mm were chosen for the extraction experiments. The milled material was stored in closed flasks and the material was used in the experiments within 120 h to avoid long storage times and possible deterioration, especially by fungi. The material was processed only once for all experiments.

Reagents

The reagents used were all from Sigma Aldrich, including: ethanol (99.5%, absolute), DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate; HPLC, 1 g) and ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]; HPLC, 1 g) reagents, rutin (96% HPLC), Folin & Ciocalteau's phenol, potassium persulfate (99.0%), sodium carbonate (99.5%), gallic acid (99.5%), and Trolox (6-hydroxy-2,5,7,8- tetramethylchromane-2-carboxylic acid, 97%).

Conventional extraction methods

To obtain extracts using the three conventional methods proposed (Soxhlet, maceration and ultrasound), the solvent used was ethanol and the solid/solvent mass ratio used was 1:10. All procedures described below for the mentioned conventional methods used 10 g of milled leaves and were performed in duplicate.

Soxhlet extraction (SOX)

The Soxhlet extraction process was performed according to the procedure proposed by Michielin et al. (2011), with modifications. The extracts were obtained in a conventional Soxhlet apparatus in cycles of 5 h and were then stored in amber glass flasks for further analysis.

Maceration extraction (MAC)

The extractions were performed according to Sousa et al. (2007), with modifications. The milled plant material (leaves) and solvent were placed in Erlenmeyer flasks, which were covered with plastic film to prevent evaporation of the solvent. The process was carried out over 96 hours, without stirring, at room temperature. Finally, the system was filtered, and the extracts stored in amber glass flasks.

Ultrasound-assisted extraction (ULT)

The procedure was adapted from Mazzutti, Riehl, Ibáñez, and Ferreira (2017). 10 g of plant material (milled leaves) were placed in Erlenmeyer flasks with the solvent (ethanol). The ultrasound probe (Ultronique, Model QR 500, Eco-Sonics, Indaiatuba, Brazil) was used with frequency and power of 18 kHz and 297 W, respectively. The extraction time was 15 min.

Pressurized hot water (PHW) extraction: experiments, factorial design and data analysis

The extracts were obtained using a semi-continuous flow reactor made entirely of 316 L stainless steel, with an internal volume of 50 mL. Figure 1 shows the flowchart of the PHW extraction equipment (CITUA, Model HPE, Campinas, Brazil).

To supply pressurized deionized water to the reactor, a high-pressure double piston pump (CITUA, Model DPP, Campinas, Brazil) was used, with a water preheating system at its outlet. Deionized water was obtained using a water purification system (Milli-Q A10, Millipore, USA). The pressure in the electrically heated reactor was monitored by a conventional manometer and the process temperature was controlled by type K thermocouples, one positioned at the inlet of the preheater and the other at the outlet of the reactor. The decompression of the system was performed by a manual micrometric valve placed at the outlet of the reactor. The extracts were collected in bottles and stored for further analysis. All extractions were performed in duplicate.

Factorial design

In this work, a factorial design was applied only to PHW extraction. Such design allows investigating the effect of factors on the desired response, being able to determine the best-operating conditions among those tested. To study the effect of the amount of plant material, temperature and pressure on the content of polyphenols in the extract, a 2^3 full factorial design was carried out to obtain the largest possible total polyphenols content from the herbal material in 30 min., based on preliminary tests. The levels used are presented in Table 1 and were chose according to equipment limitations. High temperatures (398.15 and 423.15 K) were associated with the pressures evaluated (20 and 22.5 MPa) in order to optimize the depressurization of the system downstream the extractor.

In this procedure, the water flow in the system was kept constant at 5.67 mL $min.^{-1}$ due to pump limitations. When using 2 and 4 g of raw material during 30 min. of extraction, the solvent/solid ratio for the upper and lower levels were 42.5 and 85.0 g solvent g^{-1} solid, respectively.

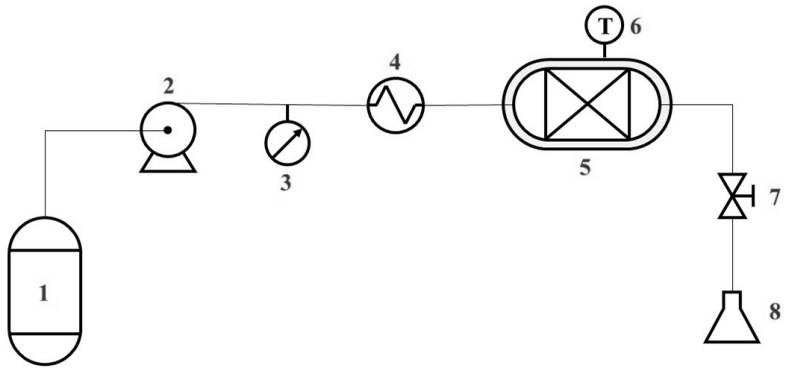

Figure 1. Layout of the equipment used for PHW extractions: 1 - Water tank; 2 - Water pump; 3 - Pressure gauge; 4 - Water preheater; 5 - Extractor vessel and heater; 6 - Temperature sensor; 7 - Needle valve; 8 - Collector flask.

Table 1. Levels and variables of the 2^3 full factorial design.

Variables	Levels	
Mass (g)	2[a]	4[b]
Temperature (K)	398.15	423.15
Pressure (MPa)	20	22.5

[a]solvent/solid ratio of 42.5 g solvent g^{-1} solid; [b]solvent/solid ratio of 85.0 g solvent g^{-1} solid.

Data analysis

The effects of variables on the PHW process were assessed with the aid of Statistica 8.0 software (StatSoft, USA). The results obtained for the phenolic compounds content (TPC) and antioxidant activities were

expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA), while the means were subjected to Tukey test with a significance level of 5%. In addition, a correlation was made between TPC and antioxidant activity by DPPH using the Pearson method.

Quantification of total polyphenol content (TPC)

To quantify the total polyphenols present in the extracts, the Folin-Ciocalteu methodology was used with modifications (Mazzutti et al., 2017). In a 25 mL flask, 1 mL of the Folin-Ciocalteu reagent and 10 mL of distilled water were added. Afterwards, 225 µL of this sample was pipetted, with the volume completed with aqueous sodium carbonate solution (Na_2CO_3), at 10.75% (w v^{-1}). The solutions were kept at rest for 30 min. in the absence of light at room temperature and, soon after, their absorbances were measured in a spectrophotometer at 760 nm, with the distilled water used as a blank solution in the measurements. The standard solution used was gallic acid (1 mg mL^{-1}). The analysis of the standard solution was carried out following the same procedure but using only 100 µL of gallic acid instead of the sample. All measurements were performed in triplicate and the values reported represent the average value. The total polyphenol content was reported as a percentage of gallic acid equivalent (GAE) per gram of extract.

Total antioxidant activity (TAA) by the DPPH method

The analysis of TAA by the DPPH method was carried out according to the methodology described by Dias et al. (2019) with modifications. In a dark environment, solutions of *A. muricata* leaf extracts in concentrations of 500, 250, 125, 50, 25, 10 and 5 µg mL^{-1} were prepared in triplicate. 1000 µL of DPPH reagent (0.3 mM) was added to each solution, which was then homogenized on a shaker. The resulting solutions were then maintained at room temperature for 30 min. in the absence of light and then analysed on a spectrophotometer at 517 nm. For each concentration, only one blank was analysed (the extract of the respective concentration with the extraction solvent). In addition, a negative control containing only the dilution solvent and DPPH was used. The percentage of inhibition of the sample tested on the DPPH radical was calculated by

$$AA(\%) = 100 - \frac{\left(Abs_{sample} - Abs_{blank}\right) \cdot 100}{Abs_{control}} \qquad (1)$$

where:
AA (%) denotes the antioxidant activity in percentage; Abs_{sample} is the absorbance of the sample; Abs_{blank} is the absorbance of the blank solution and $Abs_{control}$ represents the absorbance of the control solution. From these results, a graph of concentration *versus* antioxidant activity was plotted and the respective line equation was obtained by linear regression.

Finally, antioxidant activity was reported as the minimum effective concentration required to decrease the initial amount of DPPH by 50%, known as IC_{50}. To calculate IC_{50}, the line equation for each concentration was used.

Total antioxidant activity (TAA) by the ABTS method

Antioxidant activity by $ABTS^{\bullet+}$ method was performed according to the methodology described by Nenadis, Wang, Tsimidou, and Zhang (2004), with modifications. The $ABTS^{\bullet+}$ radical was formed by reacting 5 mL of ABTS solution (7 mM) with 88 µL of potassium persulfate solution (140 mM), incubated at room temperature, and in the absence of light for 16 hours. Once formed, the ABTS radical was diluted with ethanol until the absorbance value of 0.700 ± 0.020 was obtained at 734 nm. Then, a standard solution of Trolox (2 mM), a synthetic antioxidant analogous to vitamin E, was prepared. From this one, solutions were prepared in volumetric flasks of 10 mL in concentrations of 0, 20, 50, 100, 500, 1000, 1500 and 2000 µM. In a dark environment, 30 µL of each Trolox solution was transferred to test tubes, containing 3 mL of the ABTS radical solution. The measure was performed after 6 min. of mixing. Ethanol was used as blank in the calibration. Therefore, Trolox concentrations (µM) were plotted in the abscissa and the respective absorbances in the ordinate, determining the equation of the Trolox analytical curve.

Subsequently, from the extracts obtained by different extraction methods (SOX, MAC, ULT, and PHW), solutions with four different concentrations (1000, 500, 250 and 100 µg mL^{-1}) were prepared for each extract. In a dark environment, a 30 µL aliquot of each dilution of the extracts was transferred to test tubes with 3 mL of the ABTS radical, in triplicate. The measure was made at 734 nm after 6 min. of reaction, and ethanol was used as blank. From the absorbances obtained from the different dilutions of the extracts, a curve was

constructed by plotting the absorbance on the y-axis and the concentration of the solution (in mg L^{-1}) on the x-axis, to then determine the equation for this curve. The TAA was calculated by

$$AA = \frac{a \cdot 10^6}{b - Abs_{1000}} \quad (2)$$

where:
AA denotes the antioxidant activity expressed in μM Trolox/g leaf, *a* is the slope of the standard curve of each extract in L mg^{-1}, b is the linear coefficient of that curve and Abs_{1000} is the absorbance equivalent to 1000 μM of the standard Trolox, determined from the Trolox analytical curve. The factor 10^6 corresponds to unit conversions.

High performance liquid chromatography (HPLC) analysis

Following the methodology of Xie et al. (2011), the extracts were diluted in water, obtaining solutions with a concentration equal to 1 mg mL^{-1}. These solutions were filtered through 0.45 μm pore size membranes. HPLC analysis was performed on an Ultimate 3000 HPLC system (Thermo Fisher Scientific, USA), coupled to a photodiode array detector (DAD; Thermo Fisher Scientific, USA) and equipped with a binary pump (HPG-3x00RS, Thermo Fisher Scientific, USA), degasser and automatic sampler equipped with a 20 μL loop (ACC-3000, Thermo Fisher Scientific, USA). The wavelengths were fixed at 210, 254, 280 and 350 nm for the detection of hydrolysable tannins and flavonoids. Chromatographic separations were obtained with a C_{18} column (250 × 4.6 mm i.d., 5 μm, Dionex®, USA) equipped with a pre-column (C_{18}; 4 mm × 3.9 m; Phenomenex®), at 299.15 K. The mobile phase consisted of ultrapure water (A) and methanol (B), both acidified with 0.05% trifluoroacetic acid, and the flow rate was adjusted to 0.7 mL $min.^{-1}$. A concentration gradient for the mobile phase was applied as follows: initially, the percentage of B in A was 10% and increased linearly to 25% in 10 min., and then to 40% up to 15 min. of analysis and 75% until 30 min., where the concentration remained constant until the end of the experiment. For data analysis and processing, the Chromeleon 6.8 software (Dionex/Thermo Fisher Scientific, USA) was used.

Results and discussion

Quantification of TPC using PHW

The results obtained for the total polyphenol content using PHW at different conditions (Table 1) are presented in Table 2. By analyzing these results, it can be observed that the best extraction condition with PHW among those studied was found to be when 4 g of soursop leaves, i.e. 85 g solvent g^{-1} solid (solvent-to-feed ratio), were used at 423.15 K and 22.5 MPa, with a value of 2.37% gallic acid equivalent (GAE) per gram of plant material (leaves). This value was below the one found by Ibrahim and Abdullahi (2015) for the ethanolic extract of soursop leaves obtained by Soxhlet, which was 27.83% GAE g^{-1} leaves. However, this can be attributed to the different extraction method and solvent and the TPC determination methodology employed. Additionally, Gyesi, Opoku, and Borquaye (2019) obtained TPC in essential oils extracted from *A. muricata* leaves by hydrodistillation, with a result of (4.38 ± 0.42)% GAE g^{-1} leaves.

Table 2. Parameter levels and average TPC of the extracts obtained by PHW.

Levels			TPC
Mass (g)	Temperature (K)	Pressure (MPa)	(% GAE g^{-1} leaves)
2[a]	398.15	20	0.75 ± 0.05
4[b]	398.15	20	0.74 ± 0.00
2[a]	423.15	20	1.49 ± 0.02
4[b]	423.15	20	0.94 ± 0.23
2[a]	398.15	22.5	0.97 ± 0.20
4[b]	398.15	22.5	0.64 ± 0.04
2[a]	423.15	22.5	1.51 ± 0.37
4[b]	423.15	22.5	2.37 ± 0.35

[a]solvent/solid ratio of 42.5 g solvent g^{-1} solid; [b]solvent/solid ratio of 85.0 g solvent g^{-1} solid.

The factorial design also allowed the evaluation of the effect of each variable related to the increase the total polyphenol content in the extract. The Pareto chart in Figure 2a shows, on average, that the main effects of temperature and pressure were highly significant at a 95% confidence level. Among all interaction effects evaluated, only the interaction between mass and temperature was not significant at the same confidence level.

In Figure 2b, which presents the response surface corresponding to the factorial design, the slope of the surface at the highest point was very steep, indicating that the point of maximum extraction of polyphenols has not been reached. However, higher operational conditions (temperature and pressure) could not be investigated in this work.

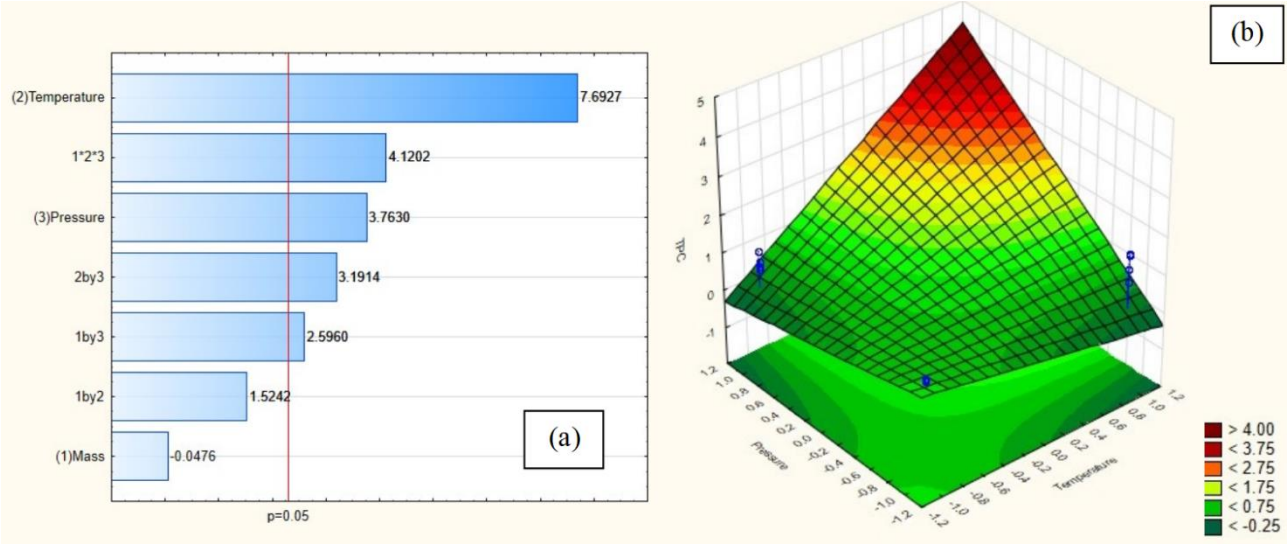

Figure 2. Pareto chart (a) and response surface (b) for the 2^3 factorial design of PHW extraction of *A. muricata* leaves on TPC of the obtained extracts.

Results of total antioxidant activity (TAA) analyses

For comparison with the extracts obtained by the conventional methods used in this work (SOX, MAC, and ULT), the result of pressurized hot water extraction was used under the conditions that showed the highest extraction of polyphenols among the studied ones (85 g solvent g^{-1} solid, 423.15 K and 22.5 MPa). Table 3 shows the amount of total polyphenols and the antioxidant activities obtained by DPPH and ABTS for each extraction method. To verify which values were statistically different from the others, the Tukey test was used at a 95% significance level.

To evaluate the antioxidant activity by ABTS, the extract with the highest concentration of Trolox per gram of plant material (leaf) was the one considered with the highest activity. On the other hand, the IC_{50} has to be evaluated in an inverse way, where lower values represent higher activities. Both methods (DPPH and ABTS) are able to scavenge free radicals by single electron transference, but a direct comparison between the obtained values can not be made and they must be evaluated separately. From the data in Table 3, the following conclusions can be drawn: the most suitable method among the extracts analyzed for obtaining polyphenols (TPC) was the Soxhlet extraction (2.83% GAE g^{-1} leaf). Also, extracts obtained by Soxhlet extraction showed the highest antioxidant activity (both by DPPH and ABTS methods). The pressurized hot water extraction obtained the second highest values for TPC and antioxidant activity by ABTS, only below the Soxhlet extraction, but presented the lowest TAA by the DPPH method.

It is known that polyphenols are polar (hydrophilic) components and are soluble in ethanol and water. Among the conventional extraction methods investigated (SOX, MAC and ULT), all using ethanol as solvent, the SOX method was the only one that used a temperature different from room temperature (around 351.15 K, the boiling point of ethanol), what suggests the influence of increasing temperature when using a polar solvent for the recovery of polyphenols from *A. muricata* leaves. PHW experiments used higher temperatures and pressures at subcritical region (Table 2), what may decrease the water solvent power for polar solutes.

Table 3. Total polyphenol content and total antioxidant activity of *A. muricata* leaf extracts obtained by different methods.

Extraction Method	TPC[1] (% GAE g^{-1} leaves)*	DPPH[1] (IC_{50} µg mL^{-1})	ABTS[1] (mM Trolox g^{-1} leaves)
SOX	$2.83^{d} \pm 0.00$	$228.86^{a} \pm 2.07$	$12.24^{b} \pm 0.80$
MAC	$1.41^{a} \pm 0.14$	$257.20^{b} \pm 17.46$	$10.79^{a,b} \pm 3.30$
ULT	$1.66^{b} \pm 0.06$	$266.39^{b} \pm 5.70$	$8.61^{a} \pm 1.76$
PHW	$2.37^{c} \pm 0.35$	$394.32^{c} \pm 5.70$	$11.06^{a,b} \pm 1.12$

[1]In the same column, means followed by the same letter do not differ statistically. Tukey test at 95% significance level; *% gallic acid equivalent per gram of plant material (leaves).

Regarding to the TAA result using the DPPH method, Kalidindi et al. (2015) reported similar trend when assessing the antioxidant activity of *Annona squamosa* Linn. leaf extract using different solvents, where extraction with water showed lower activity (IC_{50} 439.6 μg mL^{-1}) compared to extracts obtained with chloroform and methanol (IC_{50} 308.3 and 342.5 μg mL^{-1}, respectively). For the essential oil of soursop leaves obtained by Gyesi et al. (2019), the IC_{50} value was 244.8 ± 3.2 μg mL^{-1}, showing activity slightly lower than that of the extract obtained by Soxhlet in the present work.

Moraes et al. (2016) obtained a higher TPC result compared to that obtained in our study when evaluating the cooking of soursop leaves with water (3.3% GAE g^{-1} leaves) at 363.15 K for 10 min. Choi et al. (2020) found considerably higher values, around 7% GAE g^{-1} leaves, obtained also by cooking, but at 394.15 K, and minimum time of 1 hours. In turn, using araticum pulp, the extracts obtained by Arruda, Pereira, Morais, Eberlin, and Pastore (2018) presented TPC in the range of 0.12 to 1.82% GAE g^{-1} plant material.

This wide variation in values is the result of the effect of several factors, such as method and conditions of extraction, type of solvent and the part of the plant used. In addition, the concentration and variety of bioactive compounds in plants are also influenced by genetic, physiological and evolutionary factors, environmental conditions, geographical variations and harvest time (Sodeifian, Sajadian, & Ardestani, 2016).

On the other hand, Magalhães, Segundo, Reis, and Lima (2008) reported the possibility of interference of certain chemical compounds in the evaluation of antioxidant activity by ABTS, if they have a reducing potential lower than that of $ABTS^{\bullet+}$, which can react with this radical and overestimate the values obtained.

Results of HPLC analyses

In this study, distinct chemical compounds, not only phenolics, were observed in the chromatogram of the samples resulting from pressurized hot water extraction, whereas for the conventional extraction processes, in which the solvent was ethanol, only four peaks were found. The results of PHW extractions suggest that this technique may be a good alternative for the extraction of phenolic compounds among the evaluated methods, but are beyond the objectives of this work. PHW may become attractive because organic compounds are readily dissolved by water in the almost critical and supercritical region until the total miscibility. On the other hand, the solvent power of water decreases for inorganic compounds near the critical region and beyond it (Brunner, 2009).

Among the compounds obtained by all extraction methods, the flavonoid rutin was identified as the most representative compound. Rutin contents were calculated based on the areas related to the corresponding peak, as shown in Table 4. Other compounds found showed UV spectra as cinnamic derivatives, flavonoids and ellagic acid, but were not evaluated in this work. This is in agreement with the fact that several cinnamic derivatives and flavonoids, as well as ellagic acid, have previously been reported in species of the genus *Annona* (Sarker, Latif, & Gray, 2005).

Table 4. Values obtained for rutin contents by HPLC and Pearson correlation for DPPH considering the different extraction methods investigated.

Extraction method	Average rutin content (g g^{-1})	Pearson correlation
SOX	0.130 ± 0.0003	-0.805
MAC	0.070 ± 0.0002	0.092
ULT	0.062 ± 0.0001	-0.835
PHW	0.072 ± 0.0003	0.441

Therefore, it is possible to correlate rutin contents with the total antioxidant activities (DPPH and ABTS) by the Pearson method, since rutin was the most abundant polyphenol in the extracts. However, the antioxidant activity is not only dependent on rutin since other phenolic compounds may be present in the extracts in lower concentrations.

In the studies by Jiménez, Gruschwitz, Schweiggert, Carle, and Esquivel (2014), sixteen phenolic compounds were extracted in two fractions of dry *A. muricata* pulp samples and partially characterized by HPLC with diode matrix and detection by mass spectrometry. Among the compounds identified, the derivatives of cinnamic acid, hexoses of coumaric acid, derivatives of caffeic acid, caffeoylquinic acid, feruloylglycosides, and methyl esters of coumaric acid, among others, stand out. The study of Nugraha et al. (2021) isolated five alkaloids from the root of *Annona muricata* by HPLC (coclaurine, reticuline, argentinine, atherosperminine, and xylopine), while Lage et al. (2014) isolated five flavonoids from an ethanolic extract of *Annona classifora* Mart leaves (four pyranosides and one catechin). There is a lack of information related to

the presence of rutin in the aerial parts of *Annona* species in the literature, especially leaves. Pharmacological studies show that flavonoids (mostly flavones and flavonols, such as rutin) have been associated with UV-B protection. This may be the explanation for their high concentrations in leaves, acting in the cellular protection of the organelles responsible for photosynthesis. In addition, synergetic effects between distinct phenolic components may potentialize this protection, and flavonoids also act against insects and other extern pathogens (Santos & Salatino, 2000).

A correlation coefficient analysis by the DPPH method was carried out to determine the influence of rutin on the antioxidant activity of the soursop leaf extracts. For the extracts obtained by SOX and ULT, the rutin showed a strong negative correlation with IC_{50}, that is, the increase in the rutin concentration leads to a lower IC_{50} value, indicating a high antioxidant activity of the extract. For the extracts obtained with PHW, the correlation was weaker. Similar results were observed by Arruda et al. (2018) in their studies with parts of *Annona crassiflora* Mart., demonstrating a strong correlation of rutin with antioxidant activities by the DPPH and ABTS methods. In addition, they reported the influence of other phenolic compounds as important as rutin, such as catechin and protocatechuic acid, in determining the antioxidant activity of the extracts.

Conclusion

Through the proposed methodologies it was possible to identify and quantify rutin in the soursop leaf extracts using different extraction methods. TPC and TAA presented a dependence on the extraction method: SOX using ethanol achieved the highest results for total phenolic compounds and antioxidant activities. The highest rutin content was achieved when using SOX, followed by PHW and MAC. A correlation was found between rutin content and antioxidant activities. This corroborates the idea that TAA of extracts can be determined by its phenolic contents. The results obtained suggested that leaf extracts of *A. muricata* present potential for use as antioxidants.

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