Avaliação de compostos bioativos da casca e sementes de Sapodilla (*Manilkara zapota*) obtidos pela técnica de ultrassom

Evaluation of bioactive compounds from Sapodilla (*Manilkara zapota*) peel and seeds obtained by ultrasound-assisted technique

Evaluación de compuestos bioactivos de cáschara de zapote (*Manilkara zapota*) y semillas obtenidas por técnica asistida por ultrasonido

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**Resumo**

Sapoti é uma fruta exótica consumida em vários países, que gera uma quantidade significativa de resíduos que pode ser usada como fonte de compostos bioativos. Nesse contexto, este trabalho teve como objetivo extrair compostos bioativos de cascas e sementes de sapoti através da técnica assistida por ultrassom. Este trabalho trata-se de uma pesquisa explicativa quantitativa baseada em experimentos laboratoriais. A extração foi realizada com água destilada, metanol e etanol em concentrações entre 40% e 80%, submetidas ao ultrassom por 1 h. Os maiores teores de compostos fenólicos foram obtidos no extrato de casca de metanol a
40% (126,0 mg de EAG/ 100 g de resíduo) e no extrato de semente de metanol a 80% (65,3 mg de EAG/100 g de resíduo). Em relação aos flavonoides totais, os níveis mais altos foram encontrados no extrato de casca de etanol a 80% (90,0 mg QCE/100 g) e no extrato de semente de etanol a 80% (33,3 mg QCE/100g). A maior atividade antioxidante desses extratos foi obtida pelo método ABTS, em torno de 700,0 μM Trolox /g de resíduo. Sete compostos polifenólicos foram identificados e quantificados por HPLC, sendo o ácido gálico o principal composto, seguido pela epigallocatequina e catequina. A técnica de ultrassom foi eficiente para obtenção de extratos bioativos de resíduos de sapoti com potencial para aplicação futura como fonte natural de compostos bioativos.

**Keywords:** Phenolic; Flavonoid; Residue; Fruit.

**Abstract**

Sapodilla is an exotic fruit consumed in several countries, which generates a significant amount of waste which can be used as a source of bioactive compounds. In this context, this work aimed to extract bioactive compounds from sapodilla peel and seeds through an ultrasound-assisted technique. This work is an explanatory quantitative research based on laboratory experiments. Extraction was carried out with distilled water, methanol and ethanol at concentrations between 40% and 80%, subjected to ultrasound for 1 h. The highest levels of phenolic compounds were obtained in 40% methanol peel extract (126.0 mg GAE/100 g of residue) and in 80% methanol seed extract (65.3 mg GAE/100 g of residue). In relation to total flavonoids, the highest levels were found in 80% ethanol peel extract (90.0 mg QCE/100 g) and in 80% ethanol seed extract (33.3 mg QCE/100g). The highest antioxidant activity for these extracts was obtained by the ABTS method, around 700.0 μM Trolox/g of residue. Seven polyphenolic compounds were identified and quantified by HPLC, with gallic acid being the major compound, followed by epigallocatechin and catechin. The ultrasound technique was efficient for obtaining bioactive extracts of sapodilla residues with potential for future application as a natural source of bioactive compounds.

**Keywords:** Phenolic; Flavonoid; Residue; Fruit.

**Resumen**

El zapote es una fruta exótica consumida en varios países, que genera una cantidad significativa de desechos que pueden utilizarse como fuente de compuestos bioactivos. En este contexto, este trabajo tuvo como objetivo extraer compuestos bioactivos de la cáscara de zapote y semillas a través de una técnica asistida por ultrasonido. Este trabajo es una
investigación cuantitativa explicativa basada en experimentos de laboratorio. La extracción se realizó con agua destilada, metanol y etanol a concentraciones entre 40% y 80%, sometidos a ultrasonido durante 1 h. Los niveles más altos de compuestos fenólicos se obtuvieron en extracto de cáscara de metanol al 40% (126.0 mg de EAG/100 g de residuo) y en extracto de semilla de metanol al 80% (65.3 mg de EAG/100 g de residuo). En relación con los flavonoides totales, los niveles más altos se encontraron en extracto de cáscara de etanol al 80% (90.0 mg QCE/100 g) y en extracto de semilla de etanol al 80% (33.3 mg QCE/100g). La mayor actividad antioxidante para estos extractos se obtuvo mediante el método ABTS, alrededor de 700.0 μM Trolox/g de residuo. Se identificaron y cuantificaron siete compuestos polifenólicos por HPLC, siendo el ácido gálico el compuesto principal, seguido de epigalocatequina y catequina. La técnica de ultrasonido fue eficiente para obtener extractos bioactivos de residuos de zapote con potencial para su futura aplicación como fuente natural de compuestos bioactivos.

Palabras clave: Fenólico; Flavonoide; Residuo; Fruto.

1. Introducción

Sapodilla (Manilkara zapota/Achras zapota) o sapota es el most well-known fruit species in the Sapotaceae family (Junior et al., 2014); native to Central America, it originated in southern Mexico or Central America (Oliveira et al., 2011). In Brazil, Pernambuco is the largest national producer, followed by states such as Bahía, Ceará, Pará, Paraíba, Rio Grande do Norte and Sergipe, mainly in the centre-south region, in municipalities such as Lagarto and Boquim (Junior et al., 2014). In general, sapodilla is consumed in its fresh form, juices, ice cream and jams, while in international industry it is used for the manufacture of sweets, soft drinks, preserves, jams and syrups (Costa et al., 2017).

Fruit processing commonly causes the generation of tons of waste, often not used. As a consequence, over the years, several studies have evaluated the composition of agro-industrial waste in the search for bioactive compounds of industrial interest. Over the years, researchers have determined bioactive compounds such as total phenolics, total flavonoids, anthocyanins and ascorbic acid in sapodilla peel, pulp leftovers and seeds (Silva et al., 2014; Sancho et al., 2015; Singh et al., 2016; Can-Cauich et al., 2017) through the conventional method of extraction with organic solvents and orbital shaking. However, this method is not efficient for the extraction of phenolic compounds present in the residue matrix in bound form. In addition, traditional extraction methods possess drawbacks such as long extraction
periods, the necessity of using solvents with high purity, low extraction selectivity, solvent consumption in huge quantities and degradation of heat-labile components (Koçak and Pazır, 2018).

As an alternative, novel techniques such as ultrasound-assisted extraction have been employed. Ultrasound waves are certain types of electromagnetic radiation which propagate through a medium with a frequency range between 20 and 100 MHz by generating compression and expansion (Chemat et al., 2011). Ultrasound extraction is a very simple procedure, where plant material or plant cells are disrupted by the application of ultrasound waves that promote the release of bioactive compounds into the surrounding solvent. The ultrasound extraction technique has advantages such as: shorter operating time, easier handling, reduced temperature, less solvent use and energy savings; and has the potential to increase extraction yields due to cavitation and improved mass transfer phenomena (Sharayeia et al., 2019). In this context, the aim of this work was to extract bioactive compounds from sapodilla peel and seeds using organic solvents associated with an ultrasound-assisted technique.

2. Methodology

This article is a quantitative explanatory research (Pereira et al., 2018) developed by the first author in the master's thesis under supervision of the third author. In this study, extracts of sapodilla seeds and peel were obtained using the assisted ultrasound technique associated with extraction with organic solvents. Next, the experimental methodologies used for the development of the work will be presented.

2.1 Materials

Mature sapodilla fruits were supplied from a commercial market in Aracaju city (Sergipe, Brazil). Ethanol and methanol were acquired from Neon Comercial (São Paulo, Brazil). Aluminium chloride, sodium carbonate, acetic acid, ferric chloride, glucose monohydrate and potassium persulphate were acquired from Dinâmica (Indaiatuba, Brazil). Hydrochloric acid was acquired from Proquimios (Rio de Janeiro, Brazil). Acetate, Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid (Trolox), 2,2-diphenyl-1-radical picryl-hydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS +), ferric reducing antioxidant power (FRAP) reagent and acetonitrile (98%
purity) were purchased from Sigma-Aldrich Brazil Ltda (São Paulo, Brazil). Formic acid (98% purity) was purchased from Fluka Analytical (Munich, Germany). The chromatographic standards gallic acid (97.5% purity), catechin (≥ 96% purity), epicatechin (≥ 90% purity), epigallocatechin (98% purity), epigallocatechin gallate (≥ 97.0% purity), ethyl gallate (≥ 96% purity), protocatechuic acid (≥ 97% purity) and vanillic acid (≥ 97% purity) were acquired from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Treatment of fruits

The fruits were immersed for 10 min in a chlorinated solution at 200 ppm and washed with water. Subsequently, the peel and seeds were separated manually from pulp. The peel and seeds were dried at 50 °C in a drying oven (Pardal, Brazil) for 24 h and crushed in a blender (Safdar et al., 2017 with modifications). The flours of sapodilla peel and seeds were named FPS and FSS, respectively.

2.3 Acquisition of extracts

The peel and seed extracts were obtained using 2 g of solid material, 10 mL of distilled water and hydroalcoholic solutions of ethanol at concentrations of 40%, 50%, 60%, 70% and 80% (Valvi et al., 2011). The solutions were placed in a USC-1400A ultrasonic bath (Unique, São Paulo, Brazil) for 60 min at a temperature of 30 °C, 50 kHz frequency and 250 VA of force (Rezende et al., 2017 with modifications). After that, the samples were filtered on filter paper and the supernatants (extracts) were analysed for total phenolic and total flavonoid content.

2.4 Determination of total phenolics in the extracts

The total phenolic content (TPC) was determined by a modified Folin–Ciocalteu method using gallic acid as standard (Shetty et al., 1995). In test tubes, 1 mL of extracts was added to 1 mL of 95% ethanol solution, 5 mL of distilled water and 0.5 mL of 1 N Folin–Ciocalteu reagent, followed by homogenisation. Then, 1 mL of 5% (w/v) sodium carbonate solution was added and homogenised, and kept in the dark for 60 min. The absorbances of the samples were determined at 725 nm using each solvent as a blank sample. A calibration curve was constructed from different concentrations of gallic acid (0–150 mg/L) and results
expressed in milligrams of gallic acid equivalent (GAE) per 100 g of FPS or FSS on a dry basis (d.b.).

2.5 Determination of total flavonoids in the extracts

To determine the total flavonoid content (TFC), extracts (2 mL) were added to 2% (w/v) aluminium chloride (2 mL) in test tubes, vortexed and maintained in the dark for 30 min. The absorbances of samples were measured at 415 nm (Meda et al., 2005 with some modifications). A calibration curve was constructed using different concentrations of quercetin (0–50 mg/L) and the results expressed in milligrams of quercetin (QCE) per 100 g of FPS or FSS (d.b.).

2.6 In vitro antioxidant activity of the extracts

The extracts with higher TPC and TFC were analysed for antioxidant activity (AA) by ABTS, DPPH and FRAP methods. The ABTS method was performed according to Nenadis et al. (2004) with modifications, using 30 μL of extract and 3.0 mL of ABTS reagent. The solution was vortexed for 6 min and the absorbance was measured at 734 nm. The calibration curve was obtained with different concentrations of Trolox (100–1600 μmol Trolox/L) and the results expressed as μmol Trolox/g of flour (wet basis). For the DPPH method (Kwon et al., 2006 with modifications), 250 μL of extract was mixed with 1.25 mL of DPPH reagent for 5 min and the absorbance was measured at 517 nm. The calibration curve was constructed using 50–250 μmol/L of Trolox. The blank was 95% ethanol. The results were expressed as μmol Trolox/g of flour (wet basis). For the FRAP method (Thaipong et al., 2006), 90 μL of the extract, 270 μL of distilled water and 2.7 mL of FRAP reagent were mixed, vortexed and maintained at 37 °C in a water bath. After 30 min, the absorbance was measured at 595 nm. To construct the calibration curve, 100–1200 μmol Trolox/L of Trolox was used and the results expressed as μmol Trolox/g of flour (wet basis).
2.7 Identification and quantification of bioactive compounds by high performance liquid chromatography

The extracts with higher TPC and TFC were analysed by HPLC in an ultra-fast liquid chromatograph (Shimadzu, Columbia, Maryland, USA) equipped with two LC-20AD pumps, one SIL-20A auto-injector, a CTO-20A column oven, a CBM-20A system controller and an SPD-M20A diode array detector. A Kinetex C18 column (particle size of 5.0 μm and 150 × 4.6 mm i.d.; Phenomenex, California, USA) and injection volume of 10 μL were used. Aqueous solution of 0.1% formic acid (v/v) (A) and acetonitrile acidified with 0.1% formic acid (B) were used as mobile phases. The flow rate was 1.0 mL/min, under an elution gradient at 40 °C as follows: 1–6 min, 15% B; 7–12 min, 25% B; 13–16 min, 30% B; 17–20 min, 35% B; 21–24 min, 40% B; 25–27 min, 45% B; 28–29 min, 50% B; 30–32 min, 15% B (Gomes et al., 2018 with modifications). Comparison of retention times and absorption spectra with analytical standards was used to identify compounds. The calibration curves were obtained from the standards identified in the samples. For quantification of compounds, the peak areas of individual compounds in relation to the peak areas of the corresponding analytical standard were used. All analyses were performed in triplicate.

2.8 Statistical analysis

The results were expressed as mean ± standard deviation and compared by Tukey’s test at a 5% level of significance (p < 0.05), using Sisvar 5.6 software.

3. Results and Discussion ......

3.1 Total phenolic and total flavonoid content in the extracts

The TPC was determined for the extracts obtained in different solvents from FPS and FSS (Figure 1A and 1B).
Figure 1. Total phenolics on (A) sapodilla peel flour and (B) sapodilla seeds flour. Results are expressed as mean ± standard deviation (n = 3). a–f different lowercase letters indicate significant differences (p < 0.05) between the mean values according to Tukey’s test.

In this Figure, the values of total phenolic compounds obtained in distilled water, ethanol and methanol extracts from sapodilla peel and seeds are showed. The same letters in the graphs mean that the values of these compounds not differed statistically among them and the different letters mean that the values differed in the analyzed extracts.

The TPC varied between 34.0 and 126.0 mg GAE/100 g of FPS (d.b.) and 25.0 and 65.3 mg GAE/100 g of FSS (d.b.) for peel and seeds, respectively. The greatest extraction of these compounds from peel and seeds was obtained in 40% and 80% methanol, respectively, values differing statistically from those obtained for the other extracts (p > 0.05).

The solvents used in this study have different polarities; as molecular weight of solvent increase the polarity decrease, the lower the polarity. The order of magnitude in relation to polarity index (PI) is (Snyder, 1974): distilled water (PI = 9.0) > methanol (PI = 6.6) > ethanol (PI = 5.2). Based on the results obtained, the phenolic compounds extracted from sapodilla peel and seeds were of high polarity with affinity for methanol, the compounds
from seeds being more polar since they were extracted with 80% methanol. In general, methanol, being highly polar, increases the solubility of bioactive compounds, facilitating extraction (Horincar et al., 2019). Horincar et al. (2019) and Safdar et al. (2017) also obtained greater extraction of bioactive compounds in aubergine and citrus peel with methanol as solvent. Despite of this, the phenolic content in 60% ethanol extract not differed statistically from obtained by 40% methanol extract in PSF. In this case, the use of ethanol would be more interesting, since that this solvent is low cost and the most preferred among alcohols due to its low boiling point, quick recovery and ‘generally regarded as safe’ status (EFSA, 2011).

Can-Cauich et al. (2017) obtained a phenolic content in sapodilla peel (210 mg GAE/100 g d.b.) close to that obtained in the present work, after two extractions with methanol in a shaking incubator (160 rpm) at 25 °C for 24 h. On the other hand, Singh et al. (2016) obtained the highest TPC of 1151.4 mg GAE/100 g (d.b.) in sapodilla peel, after two extractions with 80% methanol in an orbital agitator for 2 h. Sancho et al. (2015) obtained only 4.35 mg GAE/100 g of residue (d.b.) in sapota residue extract (peel and seeds) obtained with ethanol in Soxhlet apparatus operating at 60 °C for 6 h. Silva et al. (2014) obtained a TPC of 1053.43 mg GAE/100 g (d.b.) for sapodilla by-products (peel, pulp leftovers and seeds) through extraction with 50% ethanol at room temperature for 1 h, followed by extraction with 70% (v/v) acetone for 60 min at room temperature.

Regarding the TFC, values varied between 6.6 and 90.0 mg QCE/100 g of FPS (d.b.) and 6.3 and 33.3 mg QCE/100 g of FSS (d.b.), in FPS and FSS, respectively (Figures 2A and 2B).
**Figure 2.** Total flavonoids on (A) sapodilla peel flour and (B) sapodilla seeds flour Results are expressed as mean ± standard deviation (n = 3). Different lowercase letters indicate significant differences (p < 0.05) between the mean values according to Tukey’s test.

Source : This work

In this Figure, the values of total flavonoid compounds obtained in distilled water, ethanol and methanol extracts from sapodilla peel and seeds are showed. The same letters in the graphs mean that the values of these compounds not differed statistically among them and the different letters mean that the values differed in the analyzed extracts.

Eighty percent ethanol (90.0 mg QCE/100 g of FPS d.b.) and 80% methanol (33.3 mg QCE/100 g of FSS d.b.) were the most effective solvents for the extraction of total flavonoids in peel and seeds, respectively; the values differed statistically (p > 0.05) from those obtained in the other extracts. This result shows the presence of more polar flavonoid compounds in sapodilla seeds and less polar ones in the peel. Ethanol would be a more advantageous solvent as it is considered GRAS (generally recognised as safe), being preferred for application in food systems. Some researchers have obtained a higher TFC than that obtained in this work: Singh et al. (2016), after two extractions with 80% methanol in an orbital agitator for 2 h,
obtained 564.5 mg QCE/100 g (d.b.) in sapodilla peel; Can-Cauich et al. (2017) obtained 430 mg QCE/100 g of dry weight residue in sapodilla peel after two extractions with methanol in a shaking incubator (160 rpm) at 25 °C for 24 h. These differences in TFC may be due to the origin, planting and chemical composition of the fruit as well as the extraction method used.

3.2 Antioxidant activity of sapodilla residue extracts

The extracts with the highest TPC and TFC (sapodilla peel in 40% methanol and 80% ethanol and seeds in 80% methanol) were analysed for in vitro AA using the ABTS, FRAP and DPPH methods (Table 1).

<table>
<thead>
<tr>
<th>Residue of sapodilla</th>
<th>Solvent</th>
<th>ABTS (μM de Trolox/g of residue)</th>
<th>FRAP (μM de Trolox/g of residue)</th>
<th>DPPH (μM de Trolox/g of residue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peel</td>
<td>40% methanol</td>
<td>695.7 ± 0.6b</td>
<td>70.6 ± 0.8b</td>
<td>92.1 ± 1.4a</td>
</tr>
<tr>
<td>Seeds</td>
<td>80% methanol</td>
<td>702.3 ± 3.3a</td>
<td>69.7 ± 1.7b</td>
<td>85.8 ± 3.0b</td>
</tr>
<tr>
<td>Peel</td>
<td>80% ethanol</td>
<td>700.7 ± 1.6ab</td>
<td>74.9 ± 0.8a</td>
<td>86.3 ± 1.2b</td>
</tr>
</tbody>
</table>

Results expressed as mean ± standard deviation (n = 3). a,bDifferent lowercase letters in the same column indicate significant differences (p <0.05) between the mean values in the same column. Source : this work

In this Table, it is important to note which extract showed the highest antioxidant activity. Also compare the values obtained in each method (same column) noting that the same letters mean that the values not differed statistically among them and different letters mean that they differed.

The highest AA values were obtained by the ABTS method; those for 80% methanol seed (702.3 μM of Trolox/g of residue) and 80% ethanol peel (700.7 μM of Trolox/g of residue) extracts did not differ statistically (p < 0.05). This result may be due these extracts having hydrophilic and lipophilic compounds, which are more reactive with the ABTS radical than the other radicals (Gulçin, 2012). The 80% ethanol and 40% methanol peel extracts showed higher AA by FRAP and DPPH methods, respectively, differing from the values obtained for other extracts (p > 0.05).

The AA of the extracts was higher than the values obtained by Almeida et al. (2011) for 60% methanol sapodilla pulp extract (0.99 and 0.17 μM of Trolox/g of fresh pulp, for ABTS and DPPH, respectively) and by Can-Cauich et al. (2017) for methanol FPS extract (1.16 μM Trolox/g dry of residue for ABTS and 0.60 μM Trolox/g dry of residue for DPPH).
3.3 Identification and quantification of bioactive compounds in sapodilla residue extracts

Seven polyphenolic compounds were identified in the extracts, three phenolic acids from the subclass of hydroxybenzoic acids (gallic acid, protocatechuic acid and vanillic acid) and four from the flavonoid class (catechin, epigallocatechin, ethyl gallate and epigallocatechin gallate) (Table 2).

**Table 2. Polyphenolic compounds in sapodilla residue extracts.**

<table>
<thead>
<tr>
<th>Polyphenolic compounds (mg/g dry)</th>
<th>Extracts of sapodilla residues</th>
<th>Peel</th>
<th>Seeds</th>
<th>Peel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40% methanol</td>
<td>80% methanol</td>
<td>80% ethanol</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.045±0.000</td>
<td>0.065±0.000</td>
<td>0.047±0.000</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>0.038±0.000</td>
<td>0.034±0.001</td>
<td>0.040±0.000</td>
<td></td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>&lt;LQ</td>
<td>&lt;LQ</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>0.015±0.001</td>
<td>0.016±0.000</td>
<td>0.017±0.000</td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0.000±0.000</td>
<td>0.001±0.000</td>
<td>0.001±0.000</td>
<td></td>
</tr>
<tr>
<td>Ethyl gallate</td>
<td>0.003±0.000</td>
<td>0.003±0.000</td>
<td>0.003±0.000</td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>0.003±0.000</td>
<td>0.004±0.000</td>
<td>0.003±0.000</td>
<td></td>
</tr>
</tbody>
</table>

LQ: limit of quantification; ND: not detected. Source: This work

This Table shows the concentrations of individual polyphenolic compounds obtained in the selected sapodilla peel and seed extracts, which were determined by chromatographic method. The detailed discussion is presented below.

In all extracts, gallic acid was the major compound, with the highest concentration (0.065 mg/g dry) in 80% methanol seed extract. Gallic acid (3,4,5-trihydroxybenzoic acid) is considered one of the main phenolic acids, and has attracted the interest of researchers for its antioxidant capacity, and actions such as antimicrobial, antifungal, anticancer and anti-inflammatory, among others (Fernandes & Salgado, 2016). Epigallocatechin and catechin were found in the extracts at concentrations of between 0.034 and 0.040 mg/g dry matter and 0.015 and 0.017 mg/g dry matter, respectively. The catechins possesses antimicrobial, antioxidant, neuroprotective, cardioprotective, antitumour and anti-inflammatory effects and vasodilator activity and offer macular protection (Velayutham et al., 2008; Pedro et al., 2019). Protocatechuic acid was identified only in peel extracted in 40% methanol and seeds in 80% methanol. This compound has demonstrated anti-apoptotic and pro-survival effects in hypertensive hearts (Deng et al., 2014). Vanillic acid, ethyl gallate and epigallocatechin gallate were found at lower concentrations. Vanillic acid has shown an anti-osteoporotic...
effect (Tanaka et al., 2019) and epigallocatechin gallate has shown antioxidant and anti-inflammatory effects (Roubalova et al., 2015).

4. Final Considerations and Suggestions

In this work, the bioactive compounds of sapodilla peel and seeds were obtained using different solvents and an ultrasound-assisted technique. Sapodilla peel showed higher TPC and TFC than seeds. Methanol was the more effective solvent for extracting polyphenolic compounds. The extracts with higher TPC and TFC showed higher AA by the ABTS method. Gallic acid was the major compound found in the extracts, followed by epigallocatechin and catechin. The compounds were efficiently extracted from sapodilla residues by the ultrasound-assisted technique, showing the potential of these extracts to be explored in future studies as sources of bioactive compounds of industrial interest.

In future works, antimicrobial activity and in vivo antioxidant activity of these extracts can be evaluated. In addition, studies on the application of extracts as a source of pharmacological compounds, as well as source of natural additives for food preservation, are suggested.

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**Porcentagem de contribuição de cada autor no manuscrito**

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Narendra Narain- 15%
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