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Antidiabetic potential and antioxidant effect of aqueous and ethanolic extracts obtained from soursop (*Annona muricata* L.) seeds and peels

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Abstract

Currently, large quantities of fruit residues like soursop peels and seeds, which could be used to obtain bioactive compounds with applications in traditional medicine due to their potential benefits, are discarded. Therefore, the aim of the present work was to evaluate the inhibitory effect on carbohydrate metabolism enzymes, and the antioxidant activity of aqueous extracts from soursop (*Annona muricata* L.) peels and seeds, for their potential application as nutraceuticals or functional food ingredients. The peels and seeds were obtained as wastes from pulp extraction, and processed to obtain aqueous and ethanolic extracts. The inhibitory effects of extracts on α-amylase and α-glucosidase were evaluated. The total phenolic content was determined by the Folin-Ciocalteu method, and the antioxidant capacity was evaluated with DPPH (2, 2'-diphenyl-1-picrylhydrazyl) and $ABTS^+$ (2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). Phenolic compounds were also identified by HPLC. The inhibitory effect of the aqueous extract of peels was 92.80% for α-amylase, and 91.22% for α-glucosidase, while the ethanolic extract of peels presented a total phenolic content of 0.0576 mg GAE/mg. The sample with the highest antioxidant capacity for DPPH and $ABTS^+$ was the aqueous extract of peels, which showed the presence of 26 phenolic compounds, including gallic acid, vanillic acid, ferulic acid, and catechin. Overall, soursop peels could have antioxidant and glycosidic metabolism benefits, offering an alternative use to the inedible parts of the fruit for potential use in the formulation of functional foods.

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Introduction

Among the health problems currently prevalent in the world, we can find diabetes mellitus 2 (DM2), metabolic syndromes, obesity, oxidative stress, and cancers. The current data on the prevalence of these diseases is alarming since, for example, in the last decades, an increase in diabetes has been observed with approximately 422 million people suffering from this disease, of which 62 million sufferers are in the American continent, and approximately 1.5 million deaths per year have been reported (Alcocer-Díaz *et al.*, 2023). Furthermore, the World Health Organization (WHO, 2018) has established cancer as one of the main causes of mortality, and affects the life expectancy of many countries. In 2022, it was estimated that there were 20 million cases of cancer worldwide, and it was projected that by 2050, this figure will increase by 77%, reaching up to 35 million people affected by this disease (WHO, 2024). Oxidative stress is another condition that can result in the development of various diseases, including obesity, diabetes, and cancers (Oguntibeju, 2019). Due to these problems, treatments have been developed to control the effects caused by these diseases. However, some therapeutic processes could cause collateral damage to patients, even being invasive, such as the constant administration of insulin or chemotherapies. In addition to patent medicines, alternative treatments based on various natural bioactive compounds focused on the needs of people with these and other chronic degenerative diseases have been used (Vaou *et al.*, 2021).

In the last decade, several studies have been conducted using fruit plant wastes. Wresdiyati *et al.* (2015) concluded that ethanolic and aqueous extracts

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of mahogany (*Swietenia mahagoni* (L.) Jacq.) fruit seeds applied in rats had an inhibitory effect on the enzyme α-glucosidase, suggesting that this plant species may act as an antidiabetic agent. Similarly, mulberry (*Morus alba* L.) green leaves have been used for tea brewing, demonstrating a decrease in postprandial glucose in diabetic patients 90 min after ingestion (Banu *et al.*, 2015). Muñóz-Bernal *et al.* (2017) found that avocado (*Persea americana* Mill.) leaf extracts showed a higher total phenolic content compared to other species analysed, like habanero chili (*Capsicum chinense* Jacq.) and anise (*Pimpinella anisum* L.). Menezes *et al.* (2019) established that soursop (*Annona muricata* L.) seeds had unsaturated chain fatty acids such as oleic, linoleic, and palmitic acids, as well as phenolic compounds such as rutin and coumaric acid, which have shown effects on weight loss in people with obesity. Nurul *et al.* (2016) indicated the presence of six types of annonaceous acetogenins, which are considered to be potential anticarcinogenic compounds from ethanolic extracts of sousop leaves.

Some recent research has focused on the use of certain inedible by-products derived from the processing of fruits such as soursop, which generates up to tonnes of waste per year, including peels, seeds, leaves, and stems of the plant (Menezes *et al.*, 2019). The results of some *in vivo* and *in vitro* studies on the biological properties found in the leaves compared to other residues have been highlighted in numerous investigations, with emphasis on their antidiarrheal effects (Afroz *et al.*, 2020), reproductive performance (Tchoffo *et al*., 2023), antidiabetic, antihypertensive (Adefegha *et al.*, 2015), anticancer, antimicrobial, and antioxidant properties (Raybaudi-Massilia *et al.*, 2015). Soursop leaves possess a broad spectrum of phytochemicals, such as phenolic, antiangiogenic, and antioxidant compounds that give them potential for multiple pharmacological applications (Santos *et al*., 2023). In this sense, certain wastes such as peels and seeds, which have not been sufficiently researched, could offer potential effects based on the bioactive properties of their phenolic compounds, as well as enzyme inhibitory activity, thus broadening the sources of raw materials for these purposes. Therefore, the objective of the present work was to determine the inhibitory percentage of enzymes of glycaemic metabolism, and the antioxidant effect *in vitro* of extracts from seeds and peels of soursop.

Materials and methods

Sample collection

Whole soursop fruits from the March - April 2022 harvest were obtained at random from different markets in Merida, Yucatan, Mexico. The soursop pulp used for the production of juices and ice cream was selected at physiological maturity (free of cuts and physical damage), firm to the touch, and with the stalk still attached. The fruits were washed with a sponge and drinking water at room temperature, being careful to eliminate any residue or foreign agent adhered to the peel; a 1% hypochlorite solution was used to disinfect the sample. The peels and seeds were manually removed from the cleaned and dried fruits to obtain the inedible by-products. These fruit wastes were subjected to a second wash with purified water to remove any pulp that may have adhered to the sample. The yield of each by-product was expressed as a percentage of the total weight of the fruit.

For the drying of the samples, a modification of the methodology described by Iyanda-Joel *et al.* (2019) was used, which consisted of leaving both the soursop peels and seeds to dry at room temperature on absorbent paper for 1 h. Subsequently, the samples were transferred to a Mabe® model FRD05W4MPS FRIGIDAIRE refrigerator at 7°C for 4 w to complete the drying process. Once dried, the peels were crushed and pulverised (150 µm) using a Moulinex® food processor model AR9868. The dried seeds were pulverised using a PULVEX® model Mini 100 mill (150 µm), and defatted by a Soxhlet system using hexane as solvent at 68 - 70°C. Four refluxes with an approximate duration of 70 min/reflux were performed on each batch. The pulverised and defatted seeds were placed in a Fisher Scientific air convection oven at 50°C for 1 h to completely evaporate the solvent.

The moisture content of both by-products was determined following the AOAC 925.09 method (oven drying) (AOAC, 1997); the plant material was placed in a Fisher Scientific® air convection equipment at 100°C until constant weight.

Ethanolic and aqueous extracts of soursop seeds and peels

The experiment was conducted based on a 2 × 2 factorial design with three replicates per treatment. The factors and levels evaluated were the type of

extract (aqueous or ethanolic) and the inedible part of the fruit (peel or seed). To obtain the ethanolic extracts of soursop seeds and peels, a modification of the methodology described by Moncada *et al.* (2012) was used. First, 100 g of defatted soursop seed flour was weighed and mixed with 400 mL of 95% ethanol to obtain a 1:4 (w/v) ratio. The mixture was subjected to a constant magnetic stirring process for 24 h at room temperature. The extract was filtered using Whatman No. 3 filter paper. The sample was concentrated to 10% of the amount placed using a BUCHI® R-215 rotary evaporator at reduced pressure and a temperature of 40°C for 50 min. The same procedure was done to prepare the ethanolic extracts of peels.

For aqueous extracts, in different assays, 10 g of ground seeds or peels were weighed, and 90 mL of distilled water (1:9 (w/v) ratio) was added. The mixtures were shaken, filtered, and concentrated under the same conditions used for the ethanolic extraction. Each extract was stored for preservation in amber glass vials at 7°C for subsequent analyses. The response variables were inhibition of α-amylase, α-glucosidase, total phenolic content, and antioxidant activity.

In vitro inhibitory activity of soursop extracts on α-amylase enzyme

The methodology reported by Dineshkumar *et al.* (2010) was used. Tubes with the following nomenclature were used: AC^+ as positive control and AC^- as negative control; As^+ sample with enzyme, Ab^- sample without enzyme (2% extract); and AAs^+ inhibitor with enzyme and AAb^- inhibitor without enzyme (acarbose, 2 mg/mL). The methodology consisted of incubating 200 µL of a 10% commercial starch (Maizena®) suspension prepared with the addition of a buffer (1.336 g Tris-Neutral and 222 mg $CaCl_2$ adjusted 6. 9 pH) in a VWR® Heating Circulator water bath model 1130-2S, at 100°C for 5 min. Subsequently, they were left to cool to 25°C, and incubated again at 37°C for 5 min. To the control tubes, 200 µL of 50% dimethyl sulfoxide (DMS, CH_3SOCH_3) was added. To the remaining tubes, 200 µL of the aqueous or ethanolic extract to be evaluated was added, considering that each sample was diluted to 1% in distilled water. To all tubes labelled positive (AC^+, As^+, and AAs^+), 200 µL of α-amylase Sigma Aldrich, CAS-No. 9000-90-2 was added, and to those labelled negative (AC^-, Ab^-, and AAb^-), 100 µL of buffer. All samples were incubated at 37°C for 10 min, and added with 500 µL of 0.1% 3,5-dinitrosalicylic acid (DNS) (200 mg DNS, 320 mg NaOH, and 6 g of sodium potassium tartrate in deionised water); they were incubated for 10 min at 100°C, and then cooled to room temperature for addition of 1,000 µL of buffer. The samples were read at 540 nm absorbance using a Thermo Scientific® Evolution 300 UV-Vis spectrophotometer. The equipment was bleached with the buffer used. The calculation of the percentage of α-amylase inhibition was performed using Eq. 1. The IC_{50} value of the extract with the highest inhibitory effect on the enzyme was calculated by means of a linear regression equation, considering the fit of the data in relation to the percentage of inhibition *vs*. the extracts used. The following formula was used to obtain the calculation: IC_{50} = (50 - b)/m, where 50 = 50% of inhibition; b = ordinate of origin; and m = slope of the line.

$$\%\,\alpha - amylase\ inhibition = \frac{[(AC+)-(AC-)]-[As-Ab]}{[(AC+)-(AC-)]}\ x\ 100 \qquad \text{(Eq. 1)}$$

In vitro inhibitory activity of soursop extracts on α-glucosidase enzyme

The *in vitro* determination of the inhibition of the enzyme α-glucosidase was performed by a modification of the method described by Dineshkumar *et al.* (2010). The same groups used for the determination of α-amylase were used for this assay. The concentration of the aqueous and ethanolic extracts of peels and seeds using distilled water for dilution was 2%; the concentration of the positive control (acarbose) was 2 mg/mL. The experiment consisted of pouring into tubes labelled positive (AC^+, As^+, and AAs^+), 100 µL of the enzyme α-glucosidase Sigma Aldrich, CAS-No. 9001-42-7 (2 U/mL) in solution. Then, 100 µL of the sample was added only to the tubes corresponding to the groups containing the sample and control. All tubes were then incubated at 37°C for 5 min in a Cole-Parmer® model 5 L water bath, series 106400716. Subsequently, 100 µL of *p*-nitrophenyl-β-D-glucopyranoside Sigma N7006® (3.01 mg/10 mL buffer KH_2PO_4 and K_2HPO_4) was added to all test tubes, and incubated for 1 h at 37°C; the samples were left to cool at room temperature, and 250 µL of 1.0 M Na_2CO_3 (52.99 g/500 mL water) were added. The absorbance of the sample was measured at 405 nm in a Thermo Scientific® Evolution 300 UV-Vis

spectrophotometer; phosphate buffer was used as blank. To obtain the results of the inhibitory percentage of α-glucosidase as well as its IC_{50}, the same equations used in the determination of α-amylase were used.

Total phenolic content

For the determination of total phenolic content, the methodology reported by Hui *et al.* (2021) was adapted. Standard curves with gallic acid for aqueous and ethanolic extracts of both soursop seeds and peels were obtained. For the determination of the samples, 500 µL of each extract was taken and poured into 10 mL test tubes. Next, 2.5 mL of 0.2 M Folin-Ciocalteu's reagent was added to each tube, as well as 2 mL of 7.5% sodium carbonate solution. Each tube was shaken for 30 s to homogenise the contents. Subsequently, the samples were incubated for 30 min in a water bath at 40°C; they were cooled to room temperature, and the absorbance was measured in a Bibby Scientific® JENWAY model 7305 spectrophotometer at 760 nm wavelength. Results were calculated based on the standard curve; values were expressed as mg gallic acid equivalent (GAE)/g of sample.

Trolox equivalent antioxidant capacity (TEAC)

A modification of the methodology described by Floegel *et al.* (2011) was used to determine *in vitro* the antioxidant effect of soursop seed and peel extracts. A standard curve was performed in triplicate, considering the concentration of the Trolox reagent against absorbance at nine different concentrations; the equation of the line was obtained by linear regression. In 5 mL test tubes, 0.1 mL of the sample was poured, and 3.9 mL of DPPH (2, 2'-diphenyl-1-picrylhydrazyl) reagent solution was added. The tubes with the samples and reagents were shaken using a vortex for 30 s to homogenise the mixture, and incubated in the dark for 30 min at 38°C. Then, the samples were measured using a Bibby Scientific® JENWAY model 7305 spectrophotometer at a wavelength of 515 nm. The DPPH results were obtained by extrapolation to the standard curve, and expressed as µmol Trolox equivalent/g sample.

Antioxidant activity by ABTS assay

For the determination of the *in vitro* activity with $ABTS^+$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), the methodology described by Floegel *et al.* (2011) was followed. A nine-point standard curve was performed in triplicate to obtain the equation of the line; a solution was prepared from the $ABTS^+$ cation by reacting 9.5 mL of a standard solution of the cation at 7 mM concentration. Next, 245 µL of 100 mM potassium persulphate ($K_2S_2O_8$) was added; the solution was incubated in a dark room at room temperature for 16 min before the use of the reagent. Then, $ABTS^+$ cation was diluted to pH 7.4. Soursop peel and seed samples were evaluated using different concentrations until curve fitting; the same preparation conditions as in the curve were used. The absorbance was determined using a Bibby Scientific® JENWAY model 7305 spectrophotometer at a wavelength of 734 nm. Prior to reading, the samples were incubated in a dark room at room temperature for 6 min. The Trolox reagent was used as a standard. The results were expressed as µmol Trolox equivalent/g sample.

Identification of total phenolics by HPLC

The qualitative analysis of phenolics from the aqueous extract of peels, which showed the highest *in vitro* effect was performed using the methodology described by Can-Cauich *et al.* (2017). The sample was vacuum dried for 48 h at -50°C at a pressure of 5 mtorr (9.67×10^{-5} psi) in a freeze dryer (Labconco® FreeZone 4.5, USA). The equipment used was an HPLC-1220 infinity system (Agilent Technologies, Palo Alto, CA, USA), which was equipped with a manual injector (20 µL coil (LOOP)) and a UV-Vis detector; the equipment was controlled by an OpenLAB CDS-ChemStation edition software (Agilent Technologies). The freeze-dried extract was reconstituted with 5 mL of water and 2.5 mL of methanol, both HPLC grade. The sample was centrifuged and filtered using a 0.45 µm Whatman filter. The following chromatographic conditions were considered: temperature column at 25°C; nucleosil C18 column (250 × 4. 6 mm, 5 µm particle size); detection of l = 280 nm; flow rate = 0.5 mL/min; injection volume = 20 µL; solvent A = mobile phase water/formic acid (99:1, v/v); and solvent B = HPLC grade acetonitrile. To filter the mobile phase, a 0.45 µm membrane filter was used with the application of vacuum. Before column conditioning, the sample was degassed using an ultrasonic bath for 15 min; the linear gradient profile was 2 - 100% in a time from 0 to 70 min. The nucleosil column was equilibrated for 10 min, considering the aforementioned conditions. In a 100 mL volumetric flask, a methanol:water mixture

(90:10, v/v) was made, adding exact amounts (100 mg) of the standard components for phenolic identification: gallic Sigma-G7384, caffeic Sigma-C0625, ellagic Sigma-E2250, trans-cinnamic Sigma-133760, quercetin Sigma-Q4971, catechin Sigma-43412, epicatechin Sigma-E4018, ferulic Sigma-128708, myricetin Sigma-M6760, sinapic Sigma-D7927, r-hydroxy benzoyl Sigma-821814, chrysin Sigma-C80105, and chlorogenic-Sigma C3878. A calibration curve was made using different concentrations of each standard; the values were calculated by linear regression analysis using the R^2 value ($R^2 > 0.98$ for each sample). To determine the retention time, the solutions were filtered and injected into the HPLC system. The elution order of each sample was compared with the retention time of the standard compounds to identify phenolics from soursop extracts.

Statistical analysis

Extracts and experimental determinations were performed in triplicate. The analysis of the results of inhibition and antioxidant effects were done using descriptive statistics with measures of central tendency and dispersion. The independent variables were the type of extract (aqueous or ethanolic) and the inedible part of the fruit (peel or seed); the response variables were the percentage of inhibition of the enzymes used, the total phenolic content, and antioxidant activity. A two-way analysis of variance (ANOVA) and Duncan's multiple range test were used to identify significant differences between means at the 95% confidence level.

Results and discussion

Soursop peel and seed yields

The moisture content of soursop peel samples was 7.77 ± 0.002%, and of the seeds was 4.28 ± 0.106%. The higher water content in peels was likely due to two factors: the nature of the plant structure and the way the sample was cold-dried (7°C). In this sense, the results were within the values reported by Vergara *et al.* (2018), who obtained 7.07% in leaves. Ortiz *et al.* (2019) have shown that drying temperatures higher than 40°C can influence the extraction process of thermolabile secondary metabolites, causing the loss of bioactive compounds present in different parts of the plant, and eliminating part of the potential benefits provided by the plant.

A soursop seed yield of 2.59% was obtained; this value was comparable to that reported by Lima *et al.* (2023), who presented a waste projection for a fruit production chain with a seed generation between 3 and 5%, and peels between 7 and 9.7%, being higher than in the present work (3.57%).

Likewise, when comparing the yields of both plant structures in this research, it was observed that both non-edible wastes could serve as a source of compounds with biological activity to be used in the food industry in various applications. For example, the peels and seeds could be used to obtain flour, and generate various products with the bioactive benefits of these parts of the plant. This is relevant because the post-harvest losses of fresh fruit, and therefore the by-products derived from it, can be as high as 75.8%, which would indicate sufficient available material and practically zero cost, as established by Lima *et al.* (2023). These same authors indicated that extracts of leaves, peels, and seeds have been a used strategy by various industries, such as pharmaceuticals and agronomy, for the production of different components. Gul *et al.* (2016) reported its possible application as a nutraceutical, presenting benefits to those who consume them. The use of non-edible soursop material can help reduce the excessive amount of waste generated annually, helping to reduce environmental pollution from solid waste.

Inhibition of α-amylase enzyme by extracts

The percentage inhibition of peel and seed extracts on α-amylase enzyme is shown in Figure 1. Statistical analysis indicated that there was a significant difference ($p < 0.05$) between all the treatments evaluated, showing that the inhibitory effect was due to both the type of extract and the part of the fruit. The aqueous extract of peels presented the highest inhibitory effect (92.80%) compared to the ethanolic extracts of both plant parts, as well as the aqueous extract of seeds. This higher inhibition was comparable to that presented by acarbose (positive control) of 96.8%; therefore, the effect caused by certain waste products, such as soursop extracts, could be used as a food supplement or serve as an adjuvant (Gul *et al.*, 2016) to traditional medicine treatment for diseases such as diabetes or cardiovascular diseases.

Adefegha *et al.* (2015) evaluated the inhibition of α-amylase in aqueous extracts (1:5, w/v) of freeze-dried soursop fruits, pericarp, and seeds, showing

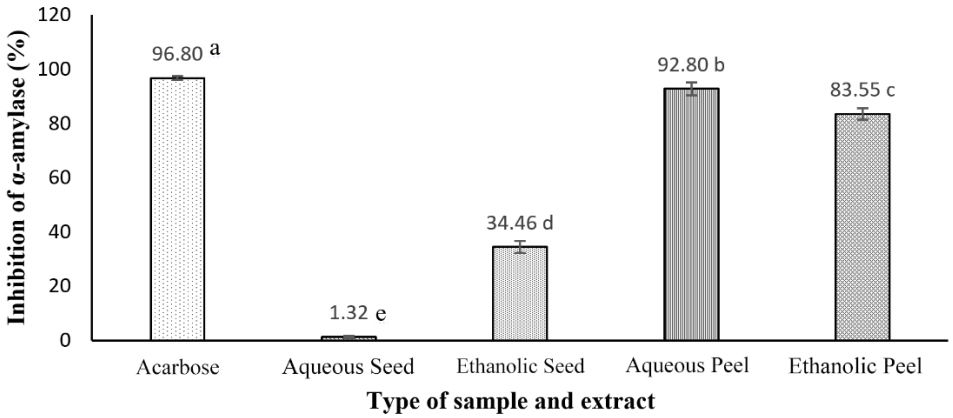

Figure 1. *In vitro* inhibitory effect of seed and peel extracts of soursop on α-amylase enzyme. Error bars indicate standard deviation. Lowercase letters indicate significant difference between means ($p < 0.05$).

positive results, especially in the pericarp extract. In their study, the authors showed that the highest inhibition was with the peel extract (63.5%), and the lowest with seeds (50%), agreeing with the present work's results. The higher inhibition in the aqueous extract of the present work (92.80%) could be attributed to a better preservation of the bioactive compounds due to the cold-drying (7°C). The low inhibition of the aqueous seed extract (1.32%) could be related to the loss of bioactive compounds due to leaching during defatting, as well as to the heat applied and the evaporation of the solvent during this process.

The aqueous extract of soursop peels had an IC_{50} value of 0.0220 mg/mL, which was numerically lower than that found by Agu *et al.* (2019), with an IC_{50} of 1.846 mg/mL in methanolic extracts of leaves of this species. This indicated that the inhibitory effect presented by the peel extracts was considerably higher. The IC_{50} values of the present work were comparable to those of the methanolic extract of soursop pulp, which was 0.0216 mg/mL (Agu *et al.*, 2019). Bae *et al.* (2012) indicated that different organic solvents, such as methanol or ethanol, could have a significant impact on the amount of potentially extractable plant compounds. Meanwhile, inorganic solvents such as water can produce extracts with bioactive effects, which makes this method a safer option due to its non-toxicity and ease of production, as indicated by Pohanka (2016).

Inhibition of α-glucosidase enzyme by extracts

The results indicated that there was a significant influence ($p < 0.05$) of the type of extract and plant part on the inhibition of α-glucosidase enzyme. A significant difference ($p < 0.05$) was observed between all treatments and the control (Figure 2). All the extracts of both plant parts inhibited the enzyme to different degrees, highlighting the aqueous extract of peels for its greater inhibitory effect (91.22%) in contrast with the ethanolic extract, and those obtained from seed.

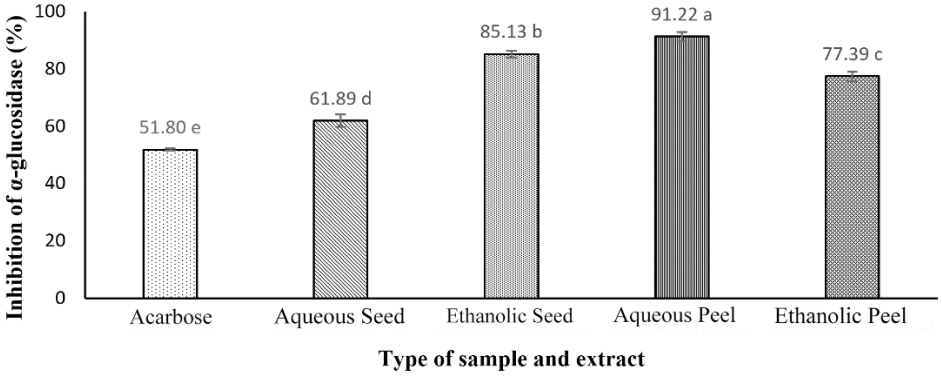

Figure 2. *In vitro* inhibitory effect of seed and peel extracts of soursop on α-glucosidase enzyme. Error bars indicate standard deviation. Lowercase letters indicate significant difference between means ($p < 0.05$).

Hedrington and Davis (2019) demonstrated that the enzyme α-glucosidase can cleave bonds in polysaccharides and oligosaccharides to produce glucose, which could trigger insulin resistance, a precursor to DM2, if not properly controlled. Therefore, inhibitors of this enzyme could slow the breakdown of carbohydrate chains, and regulate postprandial glucose levels. The extract with the highest percentage of inhibition showed that the bioactive components were inhibited even more than the positive control (51.80%). This behaviour has been indicated in a study by Agu *et al.* (2019), in which methanolic extracts of soursop leaves (69%) and stem bark (75%) outperformed acarbose (43%). Seed extracts showed the lowest inhibition percentages, possibly due to the loss of metabolites by the effect of heat during the defatting process.

The highest inhibition percentages for both α-glucosidase and α-amylase were obtained with aqueous extracts of soursop peels, probably due to the nature of the solvent used. In this sense, Babbar *et al*. (2014) stated that the solubility of the chemical substances and the polarity of the solvents used in the extraction are determinants for obtaining bioactive compounds.

According to Agu *et al*. (2019), the delay in the absorption of glucose after the consumption of bioactive compounds from soursop extracts could be because enzymes, such as α-amylase and α-glucosidase, catalyse various hydrolytic reactions of starch, glycogen, and other carbohydrates, letting the inhibitors of these to decrease the cleavage of α 1-4 bonds. This allows disaccharides, such as sucrose, to pass through the small intestine, and continue their journey to be eliminated by the body, reducing glucose consumption, and preventing people with insulin resistance or with DM2 from increasing their glycaemic levels.

Comparing the inhibition percentages among the enzymes studied, it could be observed that the highest values were obtained with the enzyme α-glucosidase; this agreed with the findings of Adefegha *et al*. (2015) in their *in vitro* study on antioxidant properties, antidiabetic potential, and phenolic content in different parts of soursop. In said research, the highest inhibitory percentage was also with α-glucosidase; this was likely due to the bioactive compounds present in the soursop extracts, which indicated a higher affinity for the receptors of said enzyme compared to those of α-amylase.

The aqueous extract of soursop peels presented an IC_{50} of 0.0334 mg/mL, a value that was twice higher than the methanolic extract of soursop leaves (IC_{50} = 0.0162 mg/mL) reported by Agu *et al.* (2019). This indicated that the methanolic extract would cause a greater effect on α-glucosidase compared to the aqueous extract; however, caution should be exercised when consuming methanolic extracts due to the likely toxicity of the extraction solvent (Pohanka, 2016). Agu *et al.* (2019) reported an IC_{50} of 0.0634 mg/mL for soursop pulp, indicating that α-glucosidase inhibitors are most likely found in higher concentrations in leaves and peel than in the fruit pulp itself, providing an alternative use for these plant parts that are generally considered as waste.

Total phenolic content

The statistical interaction between the type of extract and the plant part showed that there was a significant influence ($p < 0.05$) on the amount of extracted polyphenols. The highest content (0.0576 mg GAE/mg) was obtained in the ethanol-extracted peel compared to the other extracts (Figure 3). There was no significant difference ($p > 0.05$) between the type of extract for soursop seeds; however, despite the similarity between the values of the extracts of this plant part, the highest concentration of polyphenols was found in the aqueous extracts, which coincided with their greater inhibitory effect on α-amylase and α-glucosidase enzymes. This suggested a higher affinity of seed components for water compared to ethanol, probably due to the higher polarity of the former. Seeds, despite being considered waste material during pulp extraction, have a significant content of polyphenols, secondary metabolites present in different parts of plants that may have beneficial effects on human health (Bae *et al.*, 2012). In this regard, it is important to note that the type and concentration of polyphenols in plant matter can vary based on factors such as extraction method and climatic conditions (Jeszka-Skowron *et al*., 2014), which probably had an impact on the results obtained from soursop extracts.

The ethanolic extracts of soursop peels that showed the highest content of total phenolics had similar values to those presented by aqueous extracts of the same plant part, offering a potential antioxidant effect. Can-Cauich *et al.* (2017) established that phenolic compounds could act as metal ion chelators and free radical scavengers, allowing greater stability

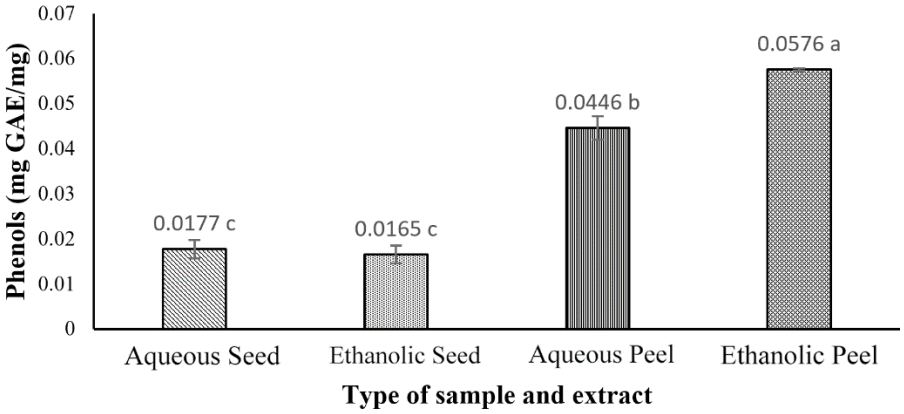

Figure 3. Total phenolic content of peel and seed extracts of soursop. Error bars indicate standard deviation. Lowercase letters indicate significant difference between means ($p < 0.05$).

to the molecule. Consequently, regular consumption of polyphenols as a nutraceutical by-product from non-edible parts of soursop could present antioxidant activity, helping to protect cellular components against the natural oxidation effect.

Antioxidant capacity of DPPH and ABTS$^+$ radicals

The antioxidant activity for DPPH and ABTS$^+$ from peel and seed extracts of soursop are shown in Table 1. Statistical analyses for both trials indicated that there was a significant influence ($p < 0.05$) of extract type and plant part on antioxidant capacity. The highest value for DPPH determination was for the aqueous extract of peels (2493.09 µmol Trolox eq/100 g). These results were higher than those obtained by Can-Cauich *et al.* (2017) in methanolic extracts of *Annona squamosa* peels (39.81 µmol Trolox eq/100 g) and of *Anacardium occidentale* (1593 µmol Trolox eq/100 g) (Moo-Huchin *et al.* 2015). Despite this, the ethanolic extract of peels (753.53 µmol Trolox eq/100 g) of soursop, as well as both seed extracts, were lower than reported by Moo-Huchin *et al*. (2015). This variation between extracts and different species could be likely due to the nature of the plant structure of the botanical species, the solvent used, and the type of extraction (Shah *et al.*, 2014).

Table 1. Antioxidant activity and TEAC (Trolox equivalent antioxidant capacity) obtained by DPPH and ABTS$^+$ methods from peel and seed extracts of soursop (*Annona muricata* L.), compared to other peel studies.

Type of extract	Plant species	Plant structure	TEAC (µmol/100 g DB)	
			DPPH	ABTS$^+$
Aqueous	*Annona muricata* L.	Peels	2493.09 ± 179.81^a	2.128 ± 0.50^a
Aqueous	*Annona muricata* L.	Seeds	202.73 ± 22.22^b	0.9121± 0.09^b
Ethanolic	*Annona muricata* L.	Peels	753.53 ± 54.40^c	0.5146 ± 0.03^c
Ethanolic	*Annona muricata* L.	Seeds	108.53 ± 1.04^d	0.850 ± 0.05^d
Methanolic*	*Annona diversifolia* Saff.	Peels	4.63 ± 0.66	11.70 ± 1.47
Methanolic*	*Annona reticulata* L.	Peels	0.33 ± 0.02	2.12 ± 0.12
Methanolic*	*Annona squamosa* L.	Peels	39.81 ± 0.91	39.10 ± 1.87
Methanolic*	*Chrysophyllum cainito* L.	Peels	1680 ± 75.8	3310 ± 32.4
Methanolic**	*Anacardium occidentale* L. (yellow)	Peels	1579 ± 121.5	3322 ± 49.2
Methanolic**	*Anacardium occidentale* L. (red)	Peels	1593 ± 67.87	3050 ± 188.9

(*) Can-Cauich *et al.* (2017). (**) Moo-Huchin *et al.* (2015). Lowercase superscripts in same column indicate significant difference between means ($p < 0.05$).

The ability of the antioxidant compounds of soursop to neutralise $ABTS^+$ radicals was superior in the peels of the aqueous extract compared to the others. In particular, the $ABTS^+$ value (2.128 µmol Trolox equivalent/100 g) was close to that reported in a methanolic extract of *Annona reticulata* peels (2.12 µmol Trolox equivalent/100 g), but lower than that of *Annona squamosa* (39.10 µmol Trolox equivalent/100 g) indicated by Can-Cauich (2017). This suggested that even within the same genus, antioxidant effects may differ between species. According to Dulf *et al.* (2017), the antioxidant effect could vary according to the part of the plant or fruit analysed due to the presence of polyphenols, flavonoids, lipids, proteins, or carbohydrates in the plant structure. Likewise, when compared with the methanolic extracts studied by Moo-Huchin *et al.* (2015), it was found that the $ABTS^+$ values for soursop extracts were lower, probably because methanol extracts a higher amount of compounds such as anthocyanins, terpenoids, saponins, tannins, flavones, and polyphenols compared to water, which has only shown extraction of anthocyanins, tannins, saponins, and terpenoids (Goyal *et al.*, 2019). However, aqueous and ethanolic extracts are the most widely used in the food industry (Oroian and Escriche, 2015), offering the opportunity to obtain natural exogenous antioxidants from non-edible materials that are discarded as waste.

The antioxidant activity of aqueous extracts from soursop peels indicated a capacity for the elimination of free radicals or hydrogen donation, which were probably related to the higher deprotonation of the hydroxyl groups present in the aromatic rings of the compounds in the peel of soursop. This would lead to a transfer of H^+, and a decrease in the ionisation potential, so there would be an increase in the electron-donating capacity as indicated by Hui *et al*. (2021).

Aqueous peel extracts with high antioxidant effect also showed the highest inhibition of α-amylase and α-glucosidase enzymes; this suggested a relationship between glucose metabolism and the antioxidant effect since high postprandial glucose is directly related to oxidative stress and inflammation, as well as to chronic diabetic complications (Banu *et al.*, 2015). Due to this, soursop peels with a high content of phenolic compounds showed antioxidant activity, being able to act on the active site of these enzymes, and demonstrate a potential anti-diabetic effect due to the reduction of oxidative stress (Oguntibeju, 2019). This was likely because certain phenolic compounds contain glycosides that protect against this oxidative process in cells (Mazewski *et al*., 2019), reducing the risk of chronic diseases such as obesity and DM2 (Abountiolas and Do Nascimiento, 2018).

Identification of phenolic compounds

Aqueous peel extracts showed a better inhibitory effect on α-amylase and α-glucosidase enzymes, as well as higher antioxidant activity compared to ethanolic and seed extracts of soursop. Due to this, phenolic compounds were identified in this extract. Chromatographic analysis revealed 26 phenolic compounds; six were confirmed with standards, and the remaining by comparison with literature based on their retention times and observed peaks (Figure 4). The confirmed compounds were caffeic acid, ferulic acid, and catechins, also reported in aqueous extracts of soursop leaves and seeds (Mesquita *et al.*, 2023). Gallic acid was identified in the aqueous peel extract, similar to that found in the pulp and seeds of the fruit using ultrasound-assisted extraction (Aguilar-Hernández *et al.*, 2019; Mesquita *et al.*, 2023).

Compounds were identified as 12-15 *cis*-escuamostatin-A at 6.25 min, escuamostatin-A at 16.06 min, escuamocin at 29.41 min, isodesacetyluvaricin at 32.11 min, and deacetyluvaricin at 57.46 min. These findings were similar to those reported by Nurul *et al.* (2016) on ethanolic extracts of soursop leaves, and suggested that despite differences in the composition of inedible parts of the fruit and extraction solvents, significant components can be found at similar retention times. The compounds detected in soursop peel extracts corresponded to annonaceous acetogenins, bioactives with anticancer properties composed of fatty acid chains of 32 to 34 carbons, tetrahydrofuran rings, and methylated γ-lactone. The coincidence in retention times with this reference study confirmed that certain plant components can be present in different structures, such as leaves, seeds, and peels, and contain aromatic rings, alkanes, alkenes, and hydroxyl groups in their composition.

The chromatogram identified vanillic acid, as well as gallic, caffeic, and ferulic acids, which are considered simple polyphenolic compounds. However, polyphenols encompass a wide variety of structures, including more complex compounds such as flavonoids (flavonols, flavones, and anthocyanins)

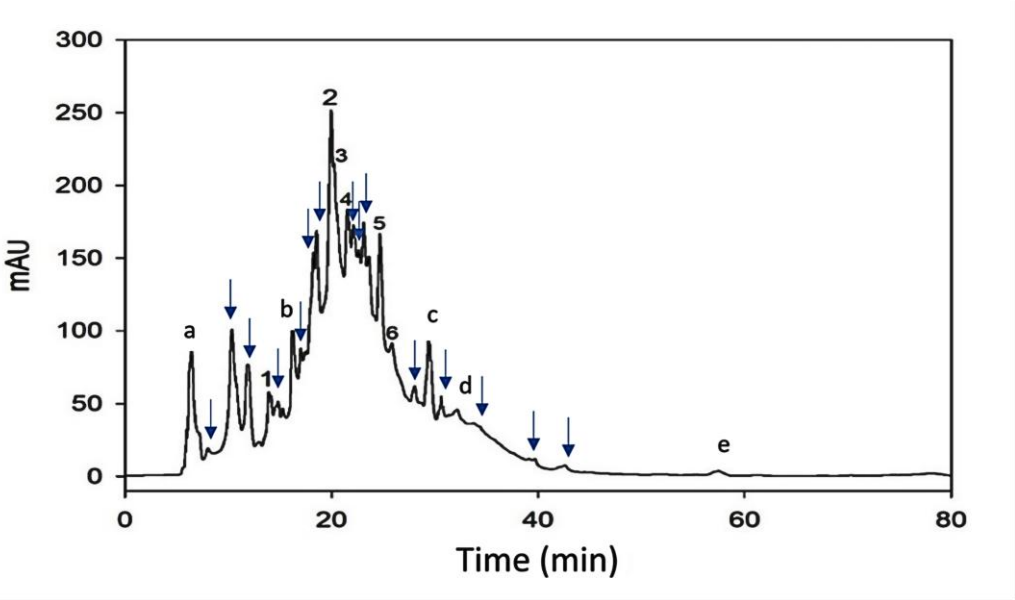

Figure 4. HPLC chromatogram. Numbers correspond to compounds of aqueous extracts of soursop peels, confirmed with standard: 1 = gallic acid; 2 = catechin; 3 = vanillic acid; 4 = caffeic acid; 5 = epicatechin gallate; and 6 = ferulic acid. Letters correspond to annonaceous acetogenins, confirmed with literature: (a) 12-15-*cis* escuamostatin-A; (b) escuamostatin-A; (c) escuamocin; (d) isodesacetyluvaricin; and (e) deacetyluvaricin. Arrows indicate other compounds found with the methodology used.

and tannins (Abbas *et al.,* 2017). The present work revealed the presence of flavonols such as quercetin at 32.11 min, and catechin at 19.94 min retention time; the latter was also found in methanolic extracts of *Annona schematosa* peels according to Can-Cauich *et al.* (2017), coinciding with those present in soursop. In addition, *Annona diversifolia* and *Annona reticulata* species in the referenced study showed the presence of hydrobenzoic acid. Likewise, caffeic acid and ferulic acid were detected in methanolic extracts of *Annona schematosa* and *Annona diversifolia*, in agreement with the present work with soursop. These findings suggested that species of the Annonacea family, including some of their non-edible parts, share key phenolic compounds that could have bioactive properties useful in the food industry, and probably in the pharmaceutical industry.

Conclusion

The aqueous extract of soursop peels showed significant inhibitory activity on α-amylase and α-glucosidase enzymes, indicating a potential effect on the glycosidic metabolism due to a possible delay in the digestion and absorption of glucose. The ethanolic extracts of soursop seeds showed a higher inhibitory percentage than the aqueous extracts; however, it was lower than the effect shown by the peel extracts. The differences in phenolic content were attributed to the solvent used for the extraction of the inedible parts of the fruit. Experimental assays with DPPH and $ABTS^{+}$ indicated an antioxidant effect in aqueous peel extracts. Of the 26 compounds evaluated by HPLC, six were confirmed with the standard, highlighting their potential importance as bioactive molecules. Soursop peels could be used to obtain bioactive compounds for use as an ingredient in functional foods, offering a potential application as a nutraceutical adjuvant. This adds value to this by-product by diversifying the use of all fruit parts to harness their full advantage. In addition, non-toxic or low-toxicity solvent extracts obtained from natural products, or their non-edible parts may be beneficial for patients with DM2 due to their bioactive effects, offering applications in public health. Further studies are needed, however, to verify the effects in *in vivo* experimental models.

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References

Abbas, M., Saeed, F., Anjum, F., Afzaal, M., Tufail, T., Bashir, M., ... and Suleria, H. 2017. Natural polyphenols: An overview. International Journal of Food Properties 20: 1689-1699.

Abountiolas, M. and Do Nascimiento, N. C. 2018. Polyphenols, ascorbic acid and antioxidant capacity of commercial nutritional drinks, fruit juices, smoothies and teas. International Journal of Food Science and Technology 53: 188-198.

Adefegha, S., Oyeleye, S. and Oboh, G. 2015. Distribution of phenolic contents, antidiabetic potential, antihipertensive properties, and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro*. Biochemistry Research Corporation 2015: 347673.

Afroz, N., Ahsanul Hoq, M., Jahan, S., Mainul Islam, M., Ahmed, F., Shahid-Ud-Daula, A. F. M. and Hasanuzzaman, M. 2020. Methanol soluble fraction of fruits of *Annona muricata* possesses significant antidiarrheal activities. Heliyon 6(1): e03112.

Agu, K., Eluehike, N., Ofeimun, R., Abile, D., Ideho, G., Ogedengbe, M., ... and Elekofehinti, O. 2019. Possible anti-diabetic potentials of *Annona muricata* (soursop): Inhibition of α-amylase and α-glucosidase activities. Clinical Phytoscience 5: 21.

Aguilar-Hernández, G., García-Magaña, M., Vivar-Vera, M., Sáyago-Ayerdi, S., Sánchez-Burgos, J., Morales-Castro, J., ... and González, M. 2019. Optimization of ultrasound assisted extraction of phenolic compounds from *Annona muricata* by products and pulp. Molecules 24: 904.

Alcocer-Díaz, S., Marcillo-Castro, A., Macías-Miranda, L. and Medranda-Pin, A. 2023. Prevalence of type 2 diabetes mellitus, overweight and obesity in adults at global level. Revista Arbitrada Multidisciplinaria Pentaciencias 5(5): 353-363.

Association of Official Analytical Chemists (AOAC). 1997. Official methods of analysis of AOAC International. United States: AOAC.

Babbar, N., Oberoi, H., Sandhu, S. and Bhargav, V. 2014. Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. Journal of Food Science and Technology 51(10): 2568-2575.

Bae, H., Jayaprakasha, G., Jifon, J. and Patil, B. S. 2012. Variation of antioxidant activity and the leaves of bioactive compounds in lipophilic and hydrophilic extracts from hot pepper (*Capsicum* spp.) cultivars. Food Chemistry 134: 1912-1918.

Banu, N., Jabir, N., Manjunath, N., Khan, M., Asharf, G., Kamal, M. and Tabrez, S. 2015. Reduction of post-prandial hyperglycemia by mulberry tea in type-2 diabetes patients. Saudi Journal of Biological Sciences 22(1): 32-36.

Can-Cauich, C., Sauri-Duch, E., Betancur-Ancona, D., Chel-Guerrero, L., González Aguilar, G. A., Cuevas-Glory, L., ... and Moo-Huchin, V. 2017. Tropical fruit peel powders as functional ingredients: Evaluation of their bioactive compounds and antioxidant activity. Journal of Functional Foods 37: 501-506.

Dineshkumar, B., Mitra, A. and Manjunatha, M. 2010. Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (xanthone glucoside) in streptozotocin-induced type 1 and type 2 diabetic model rats. International Journal of Advances in Pharmaceutical Sciences 1: 75-85.

Dulf, F., Vodnar, D., Dulf, E. and Pintea, A. 2017. Phenolic compounds, flavonoids, lipids and antioxidant potential of apricot (*Prunus americana* L.) pomace fermented by two filamentous fungal strains in solid state system. Chemistry Central Journal 11: 92.

Floegel, A., Kim, D., Chung, S., Koo, S. and Chun, O. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Journal of Food Composition and Analysis 24(7): 1043-1048.

Goyal, M., Hafiz, S. and Joy, P. 2019. Plant secondary metabolites for human health - Extraction of bioactive compounds. 1st ed. United States: Apple Academic Press.

Gul, K., Singh, A. K. and Jabeen, R. 2016. Nutraceuticals and functional foods: The foods for the future world. Critical Reviews in Food Science and Nutrition 56(16): 2617-2627.

Hedrington, M. and Davis, S. 2019. Considerations when using alpha-glucosidase inhibitors in the treatment of type 2 diabetes. Expert Opinion on Pharmacotherapy 20(18): 2229-2235.

Hui, X., Wu, G., Han, D., Gong, X., Wu, X., Tang, S., … and Brennan, C. 2021. The effects of bioactive compounds from blueberry and blackcurrant powder on oat bran pastes: Enhancing *in vitro* antioxidant activity and reducing reactive oxygen species in lipopolysaccharide-stimulated RAW264.7 macrophages. Antioxidants 10: 388.

Iyanda-Joel, W., Ajetunmobi, O., Chinedu, S., Iweala, E. and Adegbite, O. 2019. Phytochemical, antioxidant and mitochondrial permeability transition studies on fruit-skin ethanol extract of *Annona muricata*. Journal of Toxicology 2019: 7607031.

Jeszka-Skowron, M., Flaczyk, E., Jeszka, J., Krejpcio, Z., Król, E. and Buchowski, M. 2014. Mulberry leaf extract intake reduces hyperglycaemia in streptozotocin (STZ)-induced diabetic rats fed high-fat diet. Journal of Functional Foods 8(1): 9-17.

Lima, I., Da Cruz, A., Amante, E. and Meller, L. 2023. Soursop (*Annona muricata*) properties and perspective for integral valorization. Foods 12(7): 1448.

Mazewski, C., Kim, M. and González de Mejia, E. 2019. Anthocyanins, delphinidin-3-O-glucoside, and cyanidin-3-O-glucoside inhibit immune checkpoints in human colorectal cancer cell *in vitro* and *in silico*. Scientific Reports 9(1): 11560.

Menezes, E., Olivera, E., Carvalho, G., Guimares, I. and Queiroz, F. 2019. Assessment of chemical, nutritional and bioactive properties of *Annona crassiflora* and *Annona muricata* waste. Food Science and Technology 39(2): 662-672.

Mesquita, P., Rodrigues, L., Mazzutti, S., Silva, M., Vitali, L. and Lanza, M. 2023. Intensified green-based extraction process as a circular economy approach to recover bioactive compounds from soursop seeds (*Annona muricata* L.). Food Chemistry 12: 100164.

Moncada, M., Giraldo, A. and Landazuri, P. 2012. Antioxidant activity of ethanolic and aqueous extracts of the peel and seed of *Annona muricata* and of the leaves of *Brownea ariza*. Revista de Asociación Colombiana de Ciencias 24: 143-151.

Moo-Huchin, V., Moo-Huchin, M., Estrada-León, R., Cuevas-Glory, L., Estrada-Mota, I., Ortiz-Vázquez, E., ... and Sauri-Duch, E. 2015. Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chemistry 166: 17-22.

Muñóz-Bernal, O., Torres-Aguirre, G., Núñez-Gastélum, J., De la Rosa, L., Rodrigo-García, J., Ayala-Zaval, F. and Álvarez-Parrilla, E. 2017. New approach to the interaction of the Folin-Ciocalteu reagent with sugars during the quantification of total polyphenols. Revista Especializada en Ciencias Químico-Biológicas 20(2): 23-28.

Nurul, N., Harisun, Y. and Mohamad, N. 2016. Acetogenins of *Annona muricata* leaves: Characterization and potential anticancer study. Integrative Cancer Science and Therapeutics 3(4): 543-551.

Oguntibeju, O. 2019. Type 2 diabetes mellitus, oxidative stress and inflammation: Examining the links. International Journal of Physiology 11: 45-63.

Oroian, M. and Escriche, I. 2015. Antioxidants: Characterization, natural sources, extraction and analysis. Food Research International 74: 10-36.

Ortiz, L. M., Delgado Alvarado, A., Herrera Cabrera, E., Árevalo Galarza, M. and Barrera Rodríguez, A. 2019. Effect of two drying methods on total phenolic compounds, L-DOPA and antioxidant activity of *Vicia faba* L. Nova Scientia 11(23): 00010.

Pohanka, M. 2016. Toxicology and the biological role of methanol and ethanol: Current review. Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc, Czech Republic 160(1): 54-63.

Raybaudi-Massilia, R., Suárez, A., Arvelo, F., Sojo, F., Mosqueda-Melgar, J., Zambrano, A. and Calderón-Gabaldón, M. 2015. An analysis *in-vitro* of the cytotoxic, antioxidant and antimicrobial activity of aqueous and alcoholic extracts of *Annona muricata* L. seed and pulp. Current Journal of Applied Science and Technology 5(4): 333-341.

Santos, I. L., Rodrigues, A. M. C., Amante, E. R. and Silva, L. H. M. 2023. Soursop (*Annona muricata)* properties and perspectives for integral valorization. Foods 12: 1448.

Shah, M., Bosco, S. and Mir, S. A. 2014. Plant extracts as natural antioxidants in meat and meat products. Meat Science 98(1): 21-33.

Tchoffo, H., Momo, C. M. M., Ayemle, P. F., Manfouo, R., Nyembo, C. K., Adamou, M., ... and Ngoula, F. 2023. Effect of Incorporating soursop (*Annona muricata*) leaves powder on reproductive performance of Japanese quail (*Coturnix coturnix* japonica). Journal of World's Poultry Science 3(3): 42-52.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C. and Bezirtzoglou, E. 2021. Towards advances in medicinal plant anitimicrobial activity: A review study on challenges and future perspectives. Microorganism 9: 2041.

Vergara, A., Paúcar, K., Morales, C., Castro, O., Pizarro, P. and Díaz, J. 2018. Obtaining and phytochemical study of extracts of *Annona muricata* (guanábana) induced by its corrosion inhibiting effect. Revista de la Sociedad Química del Perú 84(1): 119-132.

World Health Organization (WHO). 2018. Global health observatory. Geneva: WHO.

World Health Organization (WHO). 2024. Global cancer burden growing, amidst mounting need for services. Geneva: WHO.

Wresdiyati, T., Sa´diah, S., Winarto, A. and Febriyani, V. 2015. Alpha-glucosidase inhibition and hypoglycemic activities of *Sweitenia mahagoni* seeds extract. HAYATI Journal of Biosciences 22: 73-78.