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# Effects of age and food processing of sapodilla leaves for botanical beverage application

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**Abstract**

Botanical beverages are the latest trend in the health drink industry. Natural plant extracts bring many health benefits. The objective of this research was to investigate changes in the biological activity of sapodilla (*Manilkara zapota*) leaf powder production and extraction for functional food applications. The first investigation determined the effect of different leaf ages. The leaves were dried using a hot air oven. After leaf powder extraction, phytochemical content, antioxidant capacity and antihyperglycemic activities were monitored. Optimal condition for sapodilla leaf powder production was drying mature leaves at 55 °C, giving total phenolic content (TPC) at 36.10 mg GAE/g extract and $IC_{50}$ values of α-amylase and α-glucosidase 0.054 and 0.001 mg/mL, respectively. Extraction of sapodilla leaf powder using 50% acetone solvent gave TPC 260.54 mg GAE/g extract and $IC_{50}$ of α-amylase and α-glucosidase 0.004 and 0.00 mg/mL, respectively with glucose uptake into HepG2 cells increased from 290% to 710%. Using sapodilla leaves to make botanical beverages provided antioxidant benefits and inhibited digestion enzymes.

**Keywords:** *Manilkara zapota*; antihyperglycemic activity; antioxidant capacity; botanical beverage; leaf age.

**Practical Application:** Sapodilla leaves were used to make a botanical beverage (plant tea) that provided antioxidant benefits and inhibited digestion enzymes.

## 1 Introduction

The coronavirus epidemic has infected almost 530 million people worldwide (Worldometer, 2022). Guo et al. (2020) suggested that diabetes should be considered a risk factor for the rapid progression and poor prognosis of COVID-19 because higher than normal sugar levels impact the optimal functioning of the body's immune system. Natural botanical functional foods and beverages are gaining popularity as people become more health-conscious. Functional products, such as plant teas containing phenolic compounds had a global market capitalization of more than US$ 260 million in 2021, and this is likely to grow further (Kunal, 2022). Plants contain important phytochemicals such as phenolic compounds (GutiErrez-Grijalva et al., 2016) and their biological activity depends on many factors including the age of the plant and drying and thermal extraction processes (GutiErrez-Grijalva et al., 2016).

*Manilkara zapota*, commonly known as sapodilla or sapota, belongs to the family Sapotaceae. This popular crop grows well in tropical countries (Bano & Ahmed, 2017). In folk medicine, its leaves are used to treat coughs, colds and diarrhea due to phytochemicals that have antioxidant, anti-inflammatory, antipyretic, analgesic and gas-protective effects and antihyperglycemic activity (Hernández-Bolio et al., 2019; Islam et al., 2020; Maslikah et al., 2021). This research extracted sapodilla leaf powder and investigated the antioxidant, total phytochemical and antihyperglycemic activities of the botanical beverage.

## 2 Materials and methods

### *2.1 Chemicals and reagents*

HPLC grade acetonitrile and acetic acid were purchased from Merck (Darmstadt, Germany). Standard reagents as gallic acid, protocatechuic acid, vanillic acid, catechin, Trolox, tannic acid, caffeic acid, α-amylase (2 U/mg protein), α-glucosidase (16 U/mg protein) acarbose, MTT and 2-NBDG were purchased from Sigma-Aldrich (St. Louis, MO, USA). Syringic acid, p-coumaric acid, ferulic acid and sinapic acid were obtained from Acros (Geel, Belgium). DMEM, fetal bovine serum, non-essential amino acid cell culture supplement and Penicillin-Streptomycin solution were bought from Thermo Fisher Scientific (Bangkok, Thailand). HepG2 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Other chemicals were bought from MT Instrument Co., Ltd. (Bangkok, Thailand) and A&A Reagent Ltd (Songkhla, Thailand).

### *2.2 Plant material*

Sapodilla leaves were collected in Nakhon Ratchasima, Thailand, stored at 4 °C and transported to the Laboratory of the Biotechnology Department, Kasetsart University.

#### *Sample preparation and extraction to determine the effect of leaf age*

The leaf samples were divided into three groups. The first group comprised leaves collected at the tip of the branch, called the young leaves group. The second group comprised leaves

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collected from the middle of the branch, called mature leaves, while the third group comprised fallen leaves under the tree as dried mature leaves. All the leaves were washed under a running tap to remove dirt, drained using a plastic sieve, freeze-dried and stored at -60 °C. The dried samples were then pulverized and kept in airtight containers at -20 °C before analysis, following the method of Ademiluyi et al. (2018).

The aqueous extraction method was modified from Ademiluyi et al. (2018). One gram of sapodilla leaf powder was mixed with 100 mL of water and then placed in an orbital shaker for 24 h before centrifuging at 1,000 x g for 10 min and concentrating in a hot air oven at 45 °C for 30 min.

*Sample preparation and extraction to determine the effect of drying temperature*

Sapodilla leaves were washed thoroughly with water and allowed to dry. The leaves were subjected to different drying temperatures in a hot air oven at 55, 65 and 75 °C using the same aqueous extraction method.

*Determination of the effect of extraction method using dried leaf powder*

First group: A tea bag containing 1 g of dried leaf powder was placed in 120 mL of water at 60 °C for 3, 4 and 6 min.

Second group: Samples were extracted using acetone following the modified method of Nagani et al. (2012). Ten grams of sapodilla leaf powder were added to 100 mL of acetone. Three acetone concentrations diluted with water at 50%, 75% and 100% (v/v) were placed in a conical flask with a cotton swab stopper and subjected to rotary shaking at 120 rpm for 24 h. The extracts were then filtered and centrifuged at 8,600 x g for 10 min. The supernatants were collected and evaporated using a rotary vacuum evaporator.

### *2.3 Total phenolic content determination*

Total phenolic content (TPC) was determined according to the method of Ademiluyi et al. (2018). Appropriate dilutions of the extracts (200 µL) were oxidized with 62.5 µL of 10% (v/v) Folin-Ciocalteu reagent and neutralized by 50 µL of 7.5% (w/v) sodium carbonate solution. The reaction mixture was incubated for 90 min at 25 °C and the absorbance was measured at 765 nm by a microplate reader (Bioteksynergy HTX multi-mode reader). Total phenolic content was calculated as gallic acid (0.01-0.05 mg/mL) equivalent.

### *2.4 Total flavonoid content determination*

Analysis of total flavonoid content (TFC) was modified from Dewanto et al. (2002). The sample was diluted to an appropriate concentration of 30 µL, mixed with 120 µL of distilled water and 10 µL of 5% (w/v) sodium nitrite and shaken for 6 min. Then, 10 µL of concentrated aluminum chloride 10% (w/v) volume was added and the mixture was shaken for another 5 min before adding 1 M sodium chloride 60 µL and distilled water 70 µL. The mixture was then incubated at 25 °C for 10 min. The absorption was measured at a wavelength of 510 nm. Total flavonoid content was calculated using catechin (0.2-1 mg/mL) as the standard.

### *2.5 High performance liquid chromatography analysis*

The method for extraction of free phenolic acid followed Butsat & Siriamornpun (2010). One gram of sample was extracted with 10 mL of 80% (v/v) methanol for 1 h by shaking in an incubator at 25 °C, then centrifuging at 4,300 x g for 20 min. The supernatant was removed and the extraction was repeated. Supernatants were evaporated at 40 °C until the volume was less than 5 mL. Methanol was then added to a final volume of 10 mL. The extract was subjected to HPLC using a Spectra System P-4000 pump (Thermo Separation Products-TSP, Riviera Beach, CA, USA), with a Phenomenex Luna C18 column (5 µm, 4.6 x 150 mm), and a guard column, Spectra System UV-2000 detector, wavelength 280 nm and analyzed by the Chrome Quest program. The mobile phase was Solution A 1% acetic acid and solution B acetonitrile. Gradient elution was performed as 0-5 min, linear gradient from 5% to 9% solvent B 5-15 min held at 9% solvent B; 15-22 min, linear gradient from 9% to11% solvent B; 22-38 min, linear gradient from 11% to 18% solvent B, 38-43 min from 18% to 23% solvent B, 43-44 min and 23% to 90% solvent B. A washing period of 6 min with 80% solvent B and a re-equilibration period of 15 min with 5% solvent B were performed between individual runs. Total flow rate was 1 mL/min.

### *2.6 Antioxidant assays*

Antioxidant assays were adapted from Ajiboye et al. (2018) using Trolox (0.36-1.8 mg/mL) as the standard, with a microplate reader as the detector.

The DPPH method was performed by mixing 100 µL of the sample with 0.4 mM of DPPH substance in 100 µL of methanol before curing in a dark place at room temperature for 30 min. Absorbance measurements were conducted at 516 nm.

ABTS was generated by reacting 7 mM of ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, concentration) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.70 ± 0.02 with ethanol. Thereafter, 50 µL of an appropriate dilution of the extract was added to 100 µL of ABTS solution and the absorbance was measured at 734 nm after 15 min.

The FRAP was measured by preparing a solution of FRAP solution, mixing 5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 5 mL of 10% (w/v) trichloroacetic acid was added. A 15 mL aliquot of the solution was mixed with an equal volume of distilled water and 2 mL 0.1% (w/v) ferric chloride. Then, 50 µL of an appropriate dilution of the extract was added to 150 µL of FRAP solution and the absorbance was measured at 700 nm.

### *2.7 Antihyperglycemic activities*

*α-amylase inhibitory activity*

The α-amylase inhibitory activity assays were adapted from Irondi et al. (2017). The α-amylase inhibitory potential was

investigated by reacting different concentrations of the extracts with α-amylase enzyme and starch solution. A mixture of 250 µL of sample and 250 µL of 0.02 M sodium phosphate buffer (pH = 6.9) containing α-amylase (0.5 mg/mL) was incubated at 37 °C for 10 min. Then, 250 µL of 1% (w/v) starch solution was added to the reaction mixture and incubated at 37 °C for 15 min. Thereafter, 500 µL of DNS was added and the mixture was incubated in a boiling water bath for 15 min before the absorbance was measured at 540 nm. Acarbose (2-10 µg/mL) was used as a positive control (Equation 1).

$$\alpha\text{-Amylase inhibition\%} = \left(\frac{\text{A control-A test}}{\text{A control}}\right) \times 100\% \quad (1)$$

*α-glucosidase inhibitory activity*

The α-glucosidase inhibitory activity assays were adapted from Telagari & Hullatti (2015) using pNPG as the substrate. Briefly, a mixture of 80 µL sample, 40 µL of 0.1 M sodium phosphate buffer (pH = 6.8) containing α-glucosidase (2 U/mL) and 200 µL of buffer was incubated at 37 °C for 10 min. After preincubation, 80 µL of 5 mM pNPG solution in 0.1 M sodium phosphate buffer (pH = 6.8) was added. The reaction mixture was incubated at 37 °C for 20 min. After incubation, 200 µL of 0.1M sodium carbonate was added and the absorbance was determined at 405 nm. The α-glucosidase inhibitory activity was expressed as percentage inhibition and the 50% inhibitory concentration ($IC_{50}$) was determined. Acarbose (1.2-6 mg/mL) was used as a positive control (Equation 2).

$$\alpha\text{-Glucosidase inhibition\%} = \left[1 - \left(\frac{\text{A test}}{\text{A control}}\right)\right] \times 100\% \quad (2)$$

### *2.8 Cell culture*

*Cell viability by MTT assay*

Cell viability was determined by MTT assay (Tong et al., 2018). HepG2 cells were cultured in 96-well plates at $4 \times 10^4$ cells/well for 24 h. All cell culture medium was removed from each well. The samples were then added to the desired concentration at 100 µL/well and incubated for another 24 h before all samples were removed from each well. Subsequently, 100 µL of MTT (0.5 mg/mL) was added to each well and incubated at 37 °C for 1 h. The supernatant was then removed and 100 µL of DMSO was added to dissolve the formaldehyde crystals before centrifugation at 2,500 x g for 15 min. The absorbance was measured at 570 nm with a microplate reader (Equation 3).

$$\text{Cell viability \%} = \left(\frac{\text{A test}}{\text{A control}}\right) \times 100\% \quad (3)$$

*Glucose uptake by 2-NGDG in HepG2 cells*

Glucose uptake in HepG2 cells was assessed using 2-NBDG (Zou et al., 2005) cultured in 96-well plates of $4 \times 10^4$ cells/well and tested after 24 h of cell incubation. The medium was then removed from each well and replaced with 100 µL of cell culture medium containing 10 µM insulin. Incubation was continued for 24 h. All cell culture medium was removed from each well, replaced with 100 µL/well of sample and incubated for another 24 h. All samples were removed from each well and replaced with 0.1 µM of insulin, containing cell culture medium, and then incubated for another 30 min. Then, 100 µL of 2-NBDG 40 µM cell culture medium was added to the cell culture medium and incubated for another 30 min. The cell culture medium was then removed and the cells were cleaned with phosphate-buffered saline at 4 °C for 3 cycles before fluorescence analysis at wavelengths of 485 and 528 nm (Equation 4).

$$\text{Glucose uptake \%} = \left(\frac{\text{A test}}{\text{A control}}\right) \times 100\% \quad (4)$$

### *2.9 Statistical analysis*

The results of three replicate experiments were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance was used to analyze the mean, while post hoc treatment was performed using SPSS with significance accepted at $p \leq 0.05$ (Version 12, SPSS Inc., Chicago, USA).

## 3 Results and discussion

### *3.1 Total phytochemicals*

Total phenolic content in the samples was determined because the main factor affecting plant antioxidant capacity was secondary metabolites. Phenolic compounds are the most common group in plants (GutiErrez-Grijalva et al., 2016). A previous study found that sapodilla leaves had the highest total phenolic content, followed by the flowers (Chunhakant & Chaicharoenpong, 2021). Sapodilla leaves were freeze-dried to determine the effect of leaf maturity on phytochemical content. Young leaves had the highest total phenolic content (50.91 ± 2.77) followed by mature leaves (47.65 ± 2.33) and dry leaves (41.28 ± 0.86), while flavonoid contents were 1.30 ± 0.20, 1.71 ± 0.21 and 0.86 ± 0.21 CE/g extract, respectively (Figure 1). Antidiabetic activity quantitation of seven free phenolic acids (Table 1) was studied including gallic acid, protocatechuic acid, sinapic acid, ferulic acid, vanillic acid and caffeic acid (Adefegha et al., 2015; Malunga et al., 2018; Nithya & Subramanian, 2017). Sreelatha & Padma (2009) found that older leaves had higher DPPH antioxidant capacity with higher phenolic and flavonoid content than young leaves, while Zimmermann & Zentgraf (2005) identified an association between enzyme and non-enzyme-based antioxidant plant mechanisms with oxidative stress during plant aging and development. Thus, mature leaves were chosen for further study. Differences in drying temperature were studied by choosing a temperature range of not more than 75 °C because temperatures above 70 °C affected the content of phenolic compounds and their antioxidant capacity (Katsube et al., 2009). Drying leaves at 75 °C gave the lowest total phenolic and flavonoid contents (19.59 ± 0.67 mg GAE/g extract and 0.66 ± 0.01 mg CE/g extract) (Figure 1). Miranda et al. (2010) observed that an increase in drying temperature had a significant effect on total phenolic content, especially at high temperatures such as 60, 70 and 80 °C. The sapodilla leaf powder was divided into two groups.

The first group was extracted with water to simulate consumption as a tea drink from plants. Maslikah et al. (2021) used software to predict the absorption of phenolic compounds studied in sapodilla and found that they promoted intestinal absorption.

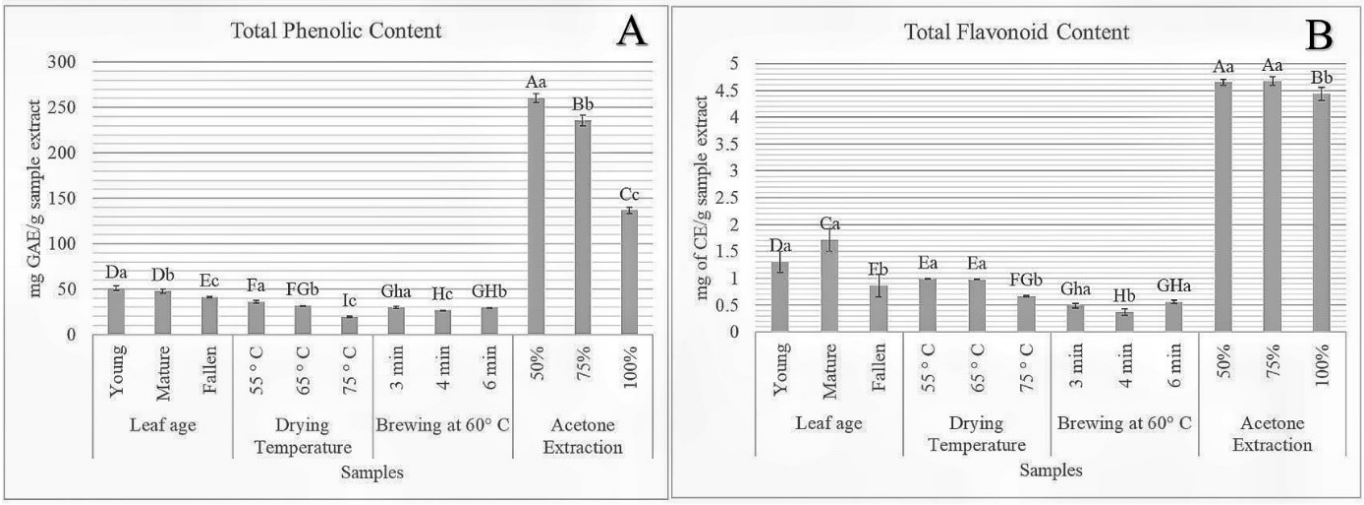


**Figure 1**. Total phytochemicals of sapodilla and effect of leaf age and food processing. Different uppercase letters indicate significant difference between a process. Different lowercase letters indicate significant difference within a group ($p < 0.05$).

**Table 1**. Quantitation of free phenolic acid in each age of sapodilla leaves by HPLC method.

| Free phenolic compounds | Young leaves (μg/g) | Mature leaves (μg/g) | Fallen leaves (μg/g) |
|---|---|---|---|
| Gallic acid | 242.6 ± 23.2c | 196.3 ± 12.4b | 330.6 ± 19.2a |
| Protocatechuic acid | 20.65 ± 1.47b | 65.34 ± 5.57a | 9.90 ± 1.20c |
| Vanillic acid | 7.60 ± 1.20b | 26.58 ± 8.22a | 12.58 ± 3.09b |
| Caffeic acid | 11.10 ± 1.73 | 12.58 ± 4.21 | 11.47 ± 3.58 |
| Coumaric acid | 0.84 ± 1.58c | 23.90 ± 2.02a | 9.82 ± 6.41b |
| Ferulic acid | 4.89 ± 0.60b | 16.86 ± 2.72a | 8.84 ± 3.03b |
| Sinapic acid | 21.82 ± 4.87b | 34.97 ± 3.55a | 14.91 ± 6.40b |

Data are expressed as mean ± standard deviation (n = 3). Means followed by the same letter within a row indicate significant differences among samples ($p < 0.05$).

The second group was extracted with acetone. Nagani et al. (2012) investigated the antioxidant activity of sapodilla leaves extracted with four polar solvents. Results showed that the $IC_{50}$ value of the acetone extract was very close to the value of the control sample of vitamin C. The sapodilla leaf powder was boiled at 60 °C and differences in the brewing time of the leaf powder were studied. At 3 and 6 min, total phenolic content was not different for sapodilla leaves dried at 65 °C, while total flavonoid content was not significantly different from sapodilla leaves dried at 75 °C. The phenolic and total flavonoid contents of acetone-extracted sapodilla leaves were significantly highest (Figure 1). Different concentrations of acetone influenced the total phenolic and flavonoid contents. Sapodilla leaf powder extracted with 50% acetone had the highest total phenolic content (260.54 ± 4.98), followed by 75% acetone (235.89 ± 5.93) and 100% (136.66 ± 3.19), with total flavonoid contents of 4.65 ± 0.06, 4.67 ± 0.08 and 4.44 ± 0.12 mg CE/g extract, respectively. Studies on other leaves found that using ethanol as the solvent gave significantly higher total phenolic and flavonoid contents than the aqueous extract (Iwansyah et al., 2021), while Ngo et al. (2017) found that the solvent played an important role in the extraction of solid and phytochemical elements.

### *3.2 Antioxidant activity*

Phenolics and polyphenols are very important plant secondary metabolites that possess antioxidant activities (Esmaeili et al., 2015). The $IC_{50}$ antioxidant activities of Trolox in DPPH and ABTS assays were 2.10 ± 0.15 and 1.78 ± 0.17 mg/mL, respectively (Figure 2), and not statistically different for leaf age groups, while FRAP of the young leaves gave the highest antioxidant efficiency of 61.31 ± 3.54 mg TEAC/g extract. Antioxidant capacity decreased with increasing temperature. Drying leaves at 55 °C gave the highest antioxidant capacity (35.00 ± 0.73 mg TEAC/g extract), while 75 °C gave the lowest antioxidant capacity for DPPH, ABTS and FRAP assays (0.035 ± 0.00, 0.035 ± 0.00 mg/mL and 37.86 ± 0.43 mg TEAC/g extract). Dehydration results in the binding of polyphenols to other compounds or changes in the chemical structure of polyphenols (Miranda et al., 2010).

Hernández-Bolio et al. (2019) found that drying methanolic extract from sapodilla leaves at 100 °C gave the highest antioxidant activity tested by DPPH assay, while the brewing leaf group gave the lowest value of FRAP assay compared with the other groups (10.33-10.59 mg TEAC/g extract). Hot extracts had less antioxidant activity than ambient extracts (Sasidharan & Menon, 2011). Different $IC_{50}$ values were recorded between brewing times. Excessive temperatures and boiling times destroyed the phenolics in the sample (Kowalska et al., 2021). The acetone extract group showed the highest antioxidant activities. There was a positive correlation between phenol content and antioxidant capacity (Nagani et al., 2012). Acetone at 50% had significantly highest ability in DPPH, ABTS and FRAP assays (0.003 ± 0.00, 0.003 ± 0.00 mg/mL and 494.77 ± 54.68 mg TEAC/g extract). Ngo et al. (2017) confirmed 50% acetone as an ideal solvent for the extraction of phenolic compounds as secondary metabolites for further separation and use.

### *3.3 Antihyperglycemic activity*

Inhibition of digestive enzymes is a mechanism commonly used to test the hyperglycemic effect. The ability to inhibit enzymes

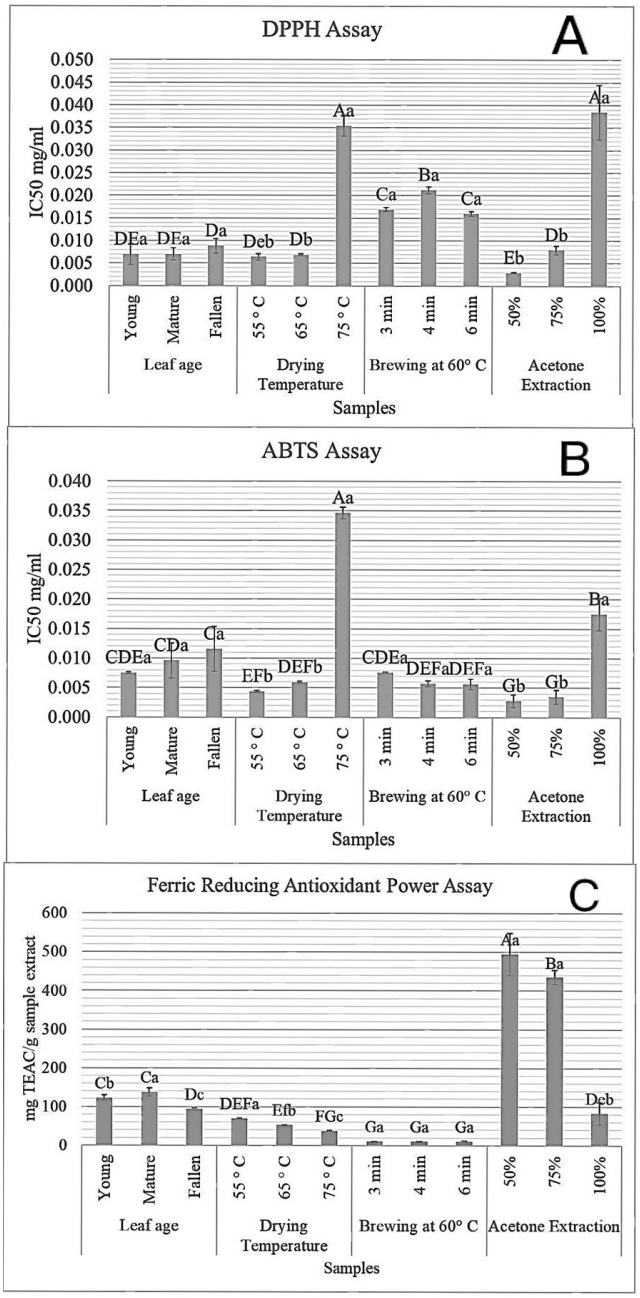


**Figure 2**. Antioxidant activities of sapodilla and effect of leaf age and food processing. Different uppercase letters indicate significant difference between a process. Different lowercase letters indicate significant difference within a group ($p < 0.05$).

was related to total phenolic content (Alam et al., 2017). $IC_{50}$ activities of acarbose in α-amylase and α-glucosidase inhibition were 346.6 ± 21.30 and 0.598 ± 0.04 mg/mL, respectively (Figure 3). Young and mature leaves showed significantly different abilities to inhibit α-amylase and α-glucosidase compared with fallen leaves. Mature leaves had the lowest $IC_{50}$ for α-glucosidase inhibition for the leaf age group (0.002 ± 0.00 mg/mL). Quantitation of free phenolic acid is shown in Table 1.

Hydroxycinnamic acids (caffeic, ferulic and sinapic acid) are characterized by a C = C double bonds conjugated with a carbonyl group in their structure that stabilizes the binding force with the active site of α-amylase, while ferulic, sinapic and vanillic acids have methoxy groups in their aromatic rings that may be responsible for α-glucosidase inhibition (Aleixandre et al., 2022). The $IC_{50}$ value of dried leaves at 55 °C showed the lowest values of α-amylase and α-glucosidase inhibition (0.054 ± 0.01, 0.001 ± 0.00 mg/mL) and significantly different from 75 °C. (0.104 ± 0.01 and 0.003 ± 0.00 mg/mL). In the brewing leaf group, optimal inhibitory effect on α-glucosidase was recorded for leaves brewed for 3 min ($IC_{50}$ 0.002 mg/mL), while leaves brewed at 60 °C in water for 4 min had optimal inhibitory effect on α-amylase ($IC_{50}$ 0.054 mg/mL). For the acetone extract group, 50% extraction gave the lowest $IC_{50}$ for α-amylase (0.004 ± 0.00 mg/mL), while inhibiting α-glucosidase using acetone 50% and 75% gave 0.001 ± 0.00 and 0.001 ± 0.00 mg/mL. Islam et al. (2020) found that methanol leaf extract significantly inhibited α-glucosidase activity, even at 1 µg/mL ($IC_{50}$ 2.51 ± 0.15 µg/mL). The ability to inhibit the enzymes of each sample was not the same. Our results concurred with Li et al. (2022) who found that inhibitors of these two enzymes were specific and that enzyme inhibition acted in different ways. α-Glucosidase and α-amylase are important enzymes involved in the digestion of carbohydrates. α-Amylase is involved in the breakdown of long-chain carbohydrates, while α-glucosidase breaks down starch and disaccharides into glucose. Among phenolic acid properties, the best known is inhibition. α-Glucosidase and α-amylase are the two main enzymes responsible for converting dietary carbohydrates into glucose (Kumar & Goel, 2019).

### *3.4 Cell culture*

Several mechanisms inhibit the absorption of sugar into the bloodstream, impacting enzyme digestion and glucose absorption into liver cells. Sapodilla leaf powder extractions at 60 °C water for 3 min and 50% acetone concentration were selected for testing. Maximum concentration of brewing leaf, giving cell survival more than 70% compared to the control was 1 mg/mL (Figure 4A), while maximum concentration of acetone extract was 1.5 mg/mL (Figure 4B). Higher concentrations inhibited the growth of liver cancer cells. Tan et al. (2018) showed that the percentage of viable cells was significantly inhibited in samples at higher concentrations (25-200 µg/mL) compared to the control group. The samples were tested for glucose uptake into HepG2 cells. The control represented normal people, while IR samples represented diabetic people. The acetone extract showed a significantly higher ability to draw sugars into liver cancer cells than the control at 290 to 710% (Figure 4C) because the combination of organic solvents and water facilitated extraction, whereas the aqueous extract was not different from the control (78-93%). Do et al. (2014) showed that all extracts obtained using organic solvents gave stronger radical scavenging capacity than water extracts. Aqueous extracts contain more non-phenolic compounds, with less active groups of phenolic compounds than acetone (Do et al., 2014). Kumar & Goel (2019) explained that phenolic acid influenced the activity of glucose and insulin receptors by increasing the expression of GLUT2, glucose transporter in pancreatic β cells (insulin-producing), and promoted the translocation of GLUT4 through PI3K/Akt and AMP activated protein kinase pathways.

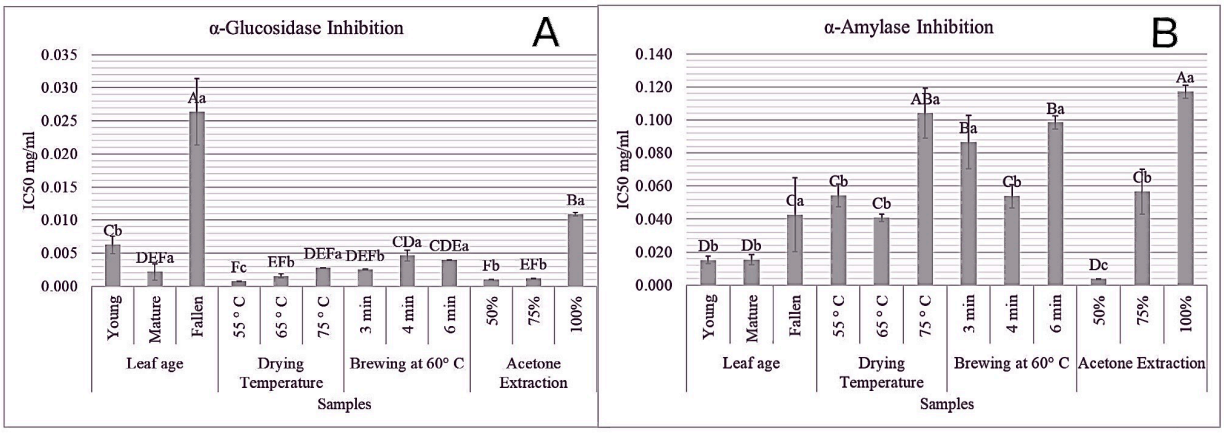


**Figure 3**. Enzyme inhibition of sapodilla and effect of leaf age and food processing. Different uppercase letters indicate significant difference between a process. Different lowercase letters indicate significant difference within a group ($p < 0.05$).

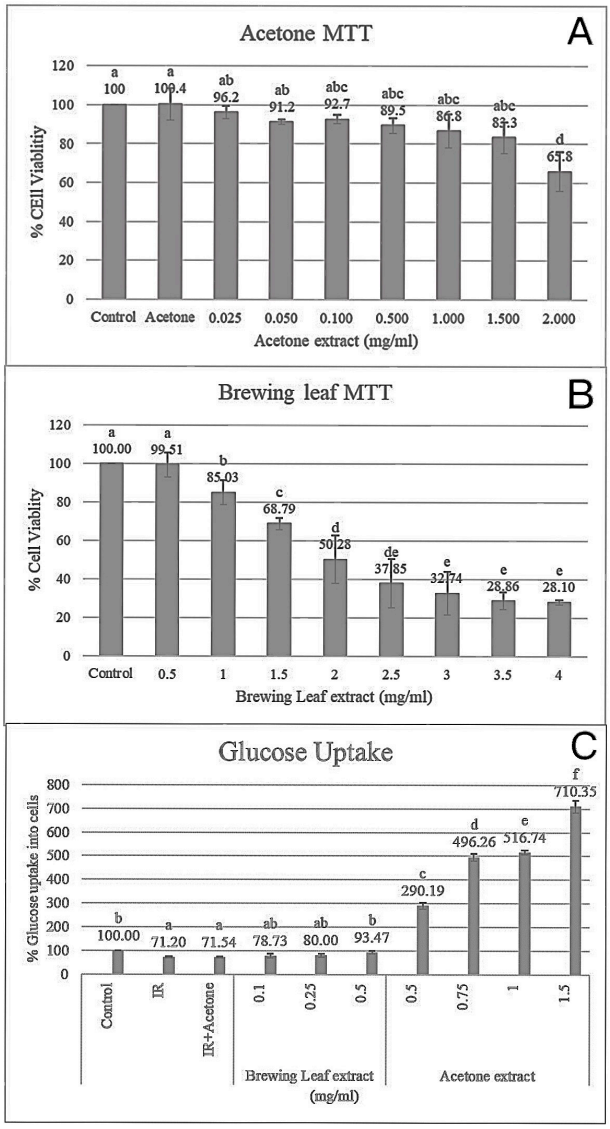


**Figure 4**. A and B present cell viability in HepG2 cells. C presents glucose uptake in HepG2 cells. Results are mean ± standard deviation of three analyses ($p < 0.05$).

Results indicated that young sapodilla leaves, old leaves or even fallen leaves can be used in the health food industry as plant tea. All leaf types showed potential for antioxidant and antihyperglycemic activities. Freeze-drying was used during the treatment of dry leaf powder. However, this would drive up the cost of production. A hot air oven with temperature limitation of no more than 65 °C was also applied. Optimal phenolic compound extraction from sapodilla leaves was achieved at 50% acetone. The extract will require purification before consumption.

Various plant leaves have been studied as functional ingredients in the food industry including olive leaves, bilimbi leaves, feijoa leaves and moringa leaves (Ademiluyi et al., 2018; Aminudin et al., 2022; Cebi & Sagdic, 2022; Iwansyah et al., 2021; Saibandith et al., 2017). Using plant leaves as health foods has the potential to reach many people around the world.

## 4 Conclusion

Sapodilla leaves contain phenolic compounds with antioxidant and antihyperglycemic health beneficial activities. Leaf age and food processing techniques including drying temperature and extraction solvent impacted extract concentrations. Sapodilla leaves were used to make botanical beverages with antioxidant benefits that inhibited digestion enzymes. Leaf extracts containing high amounts of phenolic compounds can be used to reduce blood sugar levels.

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