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Research Paper

Screening of antioxidant property and inhibition of α-amylase activity from semi-purified flavonoids of selected pericarps of edible kernels in the Philippines

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ABSTRACT

Diabetes mellitus is a major health concern in the Philippines, projected to affect 7.5 million people by 2045 if left unmanaged. Flavonoid-rich foods with antioxidant and hypoglycemic properties may help slow disease progression, yet research has largely focused on fruits, vegetables, and grains. This study explored the potential of underutilized edible kernels—cashew, peanut, and pili nut—by assessing the antioxidant and hypoglycemic properties of their semi-purified pericarp extracts. Extracts were prepared via maceration with 70% ethanol, screened for phytochemicals, and semi-purified into flavonoids. Flavonoid concentration was measured using UV-Vis spectrophotometry against quercetin. Antioxidant activity was evaluated through the DPPH Radical Scavenging Assay, while hypoglycemic potential was tested via α-amylase inhibition. Results showed cashew exhibited antioxidant activity comparable to ascorbic acid ($p > 0.711$), while pili nut demonstrated the highest α-amylase inhibition (32.99%) with moderate flavonoid content. Cashew proved strongest in antioxidant capacity, whereas pili nut excelled in hypoglycemic activity. These findings highlight their promise as natural therapeutic agents, offering novel nutraceutical and pharmacological applications for diabetes management.

Key words: flavonoids, antioxidant, α-amylase, diabetes, cashew, peanut, pili nut, nutraceuticals

1. Introduction

Diabetes Milletus is a chronic metabolic disease that continuously poses a major health concern not only in the Philippines, but also globally. Its increase of adult cases within the country is projected to rise from 4.7 million in 2024 to approximately 8.6 million by 2050 if left unmanaged (International Diabetes Federation, 2024). The condition is identified through chronic hyperglycemia, which can develop further complications such as cardiovascular disease, neuropathy, and nephropathy. Thus, Diabetes management has become a serious public health concern. With this, plant-derived compounds have gained growing attention for their potential diabetes management, particularly through antioxidant and hypoglycemic properties. Among these plants, edible kernels such as *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) have shown promising bioactivities.

The *Anacardium occidentale* (Cashew) is a tropical tree native to Brazil, but widely cultivated in the Philippines. Cashew kernels are commonly consumed as a snack and are rich in essential nutrients such as healthy fats, proteins, and vitamins. Cashew nuts have historically been used to treat several ailments, including coughs, fevers, and rheumatism. In addition, its pericarp has long been used in folk medicine for its anti-inflammatory and antimicrobial properties. In recent studies, its phytochemical composition contains notable amounts of flavonoids, phenolic acids, and tannins, which are known for their antioxidant effects. Studies have also demonstrated that cashew extracts possess significant potential to manage blood glucose. Cashews contain compounds such as anacardic acid, which has exhibited α-amylase activity; this chemical compound is essential for controlling blood sugar levels. When included in a balanced diet, its low glycemic index and bioactive components also help stabilize blood sugar levels, which enhances glycemic control (Salehi et al., 2020).

Arachis hypogaea (Peanut) is another edible kernel that is an essential component of local dishes and snacks in the Philippines. Peanuts have long been used to support heart health and manage diabetes, in addition to being high in proteins, healthy fats, and fiber. The phytochemical profile of peanuts contains flavonoids, polyphenols, and other key biochemical compounds known for their antioxidant and anti-inflammatory properties (Nunes et al., 2023). Recent research suggests that peanuts can help to stabilize blood glucose levels, owing to their high fiber content and monounsaturated fatty acids, which slow sugar digestion and absorption (Zhao et al., 2020). The pericarps and other byproducts of peanuts have bioactive compounds that have also shown to inhibit α-amylase, potentially preventing postprandial hyperglycemia (Nunes et al., 2023).

Canarium ovatum (Pili Nut) is native to the Philippines and other Southeast Asian countries. It has long been utilized in Filipino cuisine and folk medicine, where it is thought to have characteristics that promote skin health and increase energy. Despite its prominence in culinary techniques, research on its phytochemical composition and therapeutic potential is still limited. With the recent available studies related to pili nuts, the kernel has high flavonoid, phenolic acid, and saponin levels, thus indicating strong antioxidant and anti-inflammatory properties (Dumandan et al., 2022). The pericarp of the pili nut is frequently discarded, yet studies have shown that it contains considerable antioxidants. This shows that pili nut pericarps have yet to be explored as a natural source for reducing oxidative stress and controlling hyperglycemia.

Although the kernels are being widely used, the pericarps – the outer covering that is mostly disposed as waste – are underutilized despite having potential bioactive properties. Repurposing these pericarps offers an opportunity to beneficially utilize their medicinal potentials, as well as ensuring sustainability by reducing agricultural waste and optimizing resource use. Integrating agricultural byproducts into scientific research and commercial applications enhances efficiency, reduces waste, and drives innovation in sustainable healthcare solutions, all while minimizing environmental impact. Pericarp-based therapeutic products can be developed to develop cost-efficient alternative solutions in diabetes treatment and have positive effects on the lives of local farmers and sustainable healthcare innovations. This concept builds the value chain of nut production, promotes eco-conscious practices, and follows the world sustainability initiatives.

This study aimed to investigate the antioxidant and hypoglycemic properties of semi-purified extracts of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) pericarps through DPPH Radical Scavenging Assay and α-Amylase Inhibition Assay. The objective was to identify which pericarp extract holds the highest potential to be developed as a natural therapeutic agent for diabetes management.

2. Materials and Methods

This is an experimental study of the determination of the phytochemical compounds, functional groups present (via Fourier Transform Infrared (FTIR) spectroscopy) total flavonoid content, antioxidant property, and hypoglycemic properties of the semi-purified flavonoids of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili nut) pericarps.

2.1. Plant Collection

The fresh pericarps of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) were collected in September 2024 under controlled conditions of 25–30°C to maintain their integrity and bioactivity. The *Anacardium occidentale* (Cashew) were harvested in Coron, Palawan with coordinates of 12° 1' 33.096" N, 120° 11' 13.902" E; *Arachis hypogaea* (Peanut) were from Lalog, Luna, Isabela Philippines, with coordinates of 16° 59' 36.5316" N, 121° 43' 51.4452" E; and *Canarium ovatum* (Pili Nut) was harvested in Sorsogon, Bicol, with coordinates of 13° 3' 34.9236" N, 124° 0' 50.2236" E. They were all harvested during clear and dry weather and were shelled immediately after harvest. They were all transported in jute bags to preserve their biochemical properties and prevent fungal growth. All samples were labeled with collection details and processed within 24 hours to prevent degradation, ensuring high-quality plant materials for phytochemical and pharmacological studies.

The *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) used in this study were authenticated by the Jose Vera Santos Memorial Herbarium of the Institute of Biology, College of Science located at Regidor Street, University of the Philippines Diliman, Quezon City. For privacy concerns, the identity of the botanist who verified the plants will not be disclosed. Nevertheless, the specimens were verified and submitted to the herbarium for confirmation. Despite the lack of specimen depositions or voucher numbers, the herbarium provided a certificate indicating the correct identification of the plant species used in the study.

2.2. Preparation of the Plant Crude Extract

The researchers thoroughly cleaned *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) pericarps using only water to eliminate extraneous material. The plant samples required air drying following light exposure at temperatures between 25°C and 27°C (Fazeli-Nasab, 2023). The dry samples underwent electric blender crushing followed by a 60-mesh sieve process for removing contaminants while achieving consistent size distribution.

2.3. Maceration Method

The coarsely ground kernels' pericarps were macerated with 70% ethanol at a ratio of 1:10, allowing them to soak for 24–74 hours in a container. The use of 70% ethanol was chosen due to its optimal polarity, which effectively extracts both polar and non-polar phytochemicals. The extraction process was optimized by sealing the container and keeping it at room temperature for three days with occasional stirring during this time. The mixture proceeded through maceration where the extract was filtered from solid residue through muslin cloth. After running the filtrate through a rotary evaporator at 80°C and 80 rpm, the researchers continued with extraction by using a water bath procedure. Following extraction, the extracts were measured and transferred into amber bottles. They were subsequently stored in a refrigerator at 4°C until further analysis could be conducted (Idrissi et al., 2022).

2.4. Phytochemical Screening

The preliminary separation, identification, and quantification of polyphenolic compounds in Anacardium occidentale (Cashew), Arachis hypogaea (Peanut), and Canarium ovatum (Pili Nut) ethanolic crude extracts—including alkaloids (Abubakar & Haque, 2020), flavonoids (Dahanayake et al., 2019), glycosides and phenolics (Farooq et al., 2023), and saponins, steroids, terpenoids, tannins, carbohydrates, proteins, fixed oils, and volatile oils (Thangjam et al., 2020)—were conducted using qualitative phytochemical testing.

2.5. Semi-Purification of Flavonoids

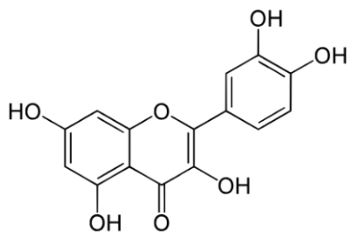
The researchers dissolved 20 grams of the extracted substance in 96% ethanol and added 40 mL distilled water. This mixture was placed into a separatory funnel and extracted with 100 mL of n-hexane. The funnel was shaken and then left to stand for 30 minutes until two layers appeared. The n-hexane layer, from this mixture, was separated and repeatedly fractionated until there was no reaction with the Liebermann-Burchard reagent. The n-hexane fraction was then concentrated through a rotary evaporator. The remaining residue was mixed with 100 mL of ethyl acetate and allowed to stand for 30 minutes to form two layers. The ethyl acetate layer was separated and fractionated repeatedly until there was no reaction with ferric chloride ($FeCl_3$) reagent. The ethyl acetate fraction was concentrated with the use of a rotary evaporator. The remaining layer which contains water was also concentrated through a rotary evaporator to obtain the water fraction (Nasution et al., 2022)

2.6. Flavonoid Confirmatory Tests

The semi-purified extract was subjected to another round of phytochemical screening specifically targeting flavonoids, serving as a qualitative confirmatory assessment to verify their continued presence following continuous extraction. The confirmatory tests included the Alkaline Test (Abubakar & Haque, 2020), Lead Acetate Test (Dahanayake et al., 2019), Shinoda Test (Kancherla et al., 2019), and Ethyl Acetate Test (Thangjam et al., 2020). All tests were performed in triplicate to ensure accuracy.

2.7. Total Flavonoid Content

Total Flavonoid Content analysis is a preliminary step used in evaluating a plant's overall antioxidant potential and quality, and is commonly conducted before a comprehensive phytochemical analysis. This assessment is typically performed through aluminum chloride colorimetric assay based on the creation of a complex between aluminum chloride and the keto group at the C-4 position, along with the hydroxyl groups at the C-3 or C-5 positions of flavones and flavonols. Through this reaction, it will yield a stable yellow compound (Nicolescu et al., 2025; Shraim et al., 2021).



Quercetin was used as a standard reference as it is abundant and a known flavonoid possessing powerful antioxidant activity with a known and consistent chemical structure. Specifically its flavonol structure of the keto group at the C-4 position and hydroxyl groups at C-3 or C-5, forming the same stable aluminum chloride complex. Quercetin's chemical structure is a 3,3,4,5,7-pentahydroxyflavone, consisting of 2 benzene rings connected by heterocyclic pyrone rings with 5 hydroxyl functional groups which are attributed to its biological activity. As for its chemical formula, it is $C_{15}H_{10}O_7$, while its IUPAC name is 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one (Batiha et al., 2020; Ghareeb et al., 2024; Vollmannová et al., 2024).

2.7.1. Preparation of Quercetin Standard Stock Solution

Ten milligrams (10 mg) of quercetin dissolved in methanol to reach a volume of 100 mL creating a solution with 100 ppm concentration of quercetin.

2.7.2. Determination of Quercetin Maximum Wavelength

Two (2) mL of the 100 ppm quercetin standard stock solution was pipetted. Then, 0.1 mL Aluminum Chloride ($AlCl_3$), 0.1 mL of Sodium Acetate (CH_3COONa), and 2.8 mL of distilled water were pipetted and incubated in a dark room for 40 minutes. The maximum wavelength was measured using a UV-Vis spectrophotometer in the range of 400–800 nm.

2.7.3. Creation of a Quercetin Calibration Curve

Quercetin standard solutions were prepared at concentrations of 6, 10, 14.5, 19.5, and 23 ppm. These were obtained by transferring 3.00, 5.00, 7.25, 9.75, and 11.50 mL, respectively, from a quercetin stock solution into separate 50 mL volumetric flasks, then diluting to the mark with methanol. From each solution, 2 mL was placed in a test tube and mixed with 0.1 mL aluminum chloride ($AlCl_3$), 0.1 mL sodium acetate (CH_3COONa), and 2.8 mL distilled water. The mixtures were incubated in the dark for 40 minutes. Absorbance was then measured at 438 nm using a UV-Vis spectrophotometer. The absorbance values were plotted against concentration to generate the quercetin calibration curve, from which the linear regression equation (y = ax + b) was derived.

2.7.4. Determination of Total Flavonoid Extract Level

The extract solution was prepared by dissolving 10 mg paste-like extract with 10 mL methanol solvent at 1000 ppm concentration. The 2 mL pipette section from this concentration received 0.1 mL of Aluminum Chloride ($AlCl_3$) and 0.1 mL of Sodium Acetate (CH_3COONa), which was mixed with 2.8 mL of distilled water. UV-Vis spectrophotometry analyzed solution absorbance after 40 minutes of dark incubation at the wavelength of 438 nm (Nasution et al., 2022).

2.8. Determination of Functional Group using Fourier Transform Infrared Spectroscopy (FTIR) for Ethanolic Crude Extracts and Semi-Purified Flavonoid Extracts

To determine the functional group present within the isolated and processed samples of the semi-purified flavonoid extract and the samples of the ethanolic crude extract, Fourier Transform Infrared Spectroscopy (FTIR) was conducted. The semi-purified and isolated extracts of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) pericarps were analyzed by an instrument model Agilent Cary 630 FTIR-ATR manufactured by Agilent Technologies (2019). This was performed at Centro Escolar University - Manila.

2.9. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a technique for assessing phytochemicals' and plant-derived extracts' antioxidant capacity. It is based on the reduction of the stable DPPH radical by antioxidants that donate electrons or hydrogen, which causes the color to change from deep violet to pale yellow at 515 nm, resulting in a detectable decrease in absorbance. This assay provides a quick, repeatable, and sensitive way to evaluate the ability to neutralize free radicals, which is mechanistically relevant to reducing oxidative stress linked to metabolic diseases like diabetes mellitus (Gulcin & Alwasel, 2023).

2.9.1. Preparation of DPPH Standard Stock Solution

A volumetric flask with a 50 mL mark received 10 mg of DPPH solution which was completed up to the line with methanol (200g/mL).

2.9.2. Preparation of Blank Solution

Ten (10) mL used the standard stock solution to fill a 50 mL volumetric flask. Methanol was used to fill up the volumetric flask up to the mark line (40 g/mL).

2.9.3. Determination of maximum wavelength

The DPPH solution at 40 g/mL concentration allowed researchers to determine the maximum wavelength which established the absorption wavelength between 400–800 nm.

2.9.4. Preparation of Sample Solution

The semi-purified plant sample of 100 mg was weighed, and then each was dissolved in a 100 mL volumetric flask with methanol until it reached the mark line (1000 μg/mL). The standard stock solution was pipetted with 5 mL, 10 mL, 15 mL, 20 mL, and 25 mL to obtain concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm into 50 mL volumetric flasks each. Then, into each volumetric flask, another 10 mL (200 g/mL) standard stock solution of DPPH was added along with methanol until it reached the mark line and homogenized. The solution was then left to stand for 30 minutes, and the absorption was measured using a UV-Visible spectrophotometer at the maximum absorption wavelength of 515 nm.

2.9.5. Determination of Operating Time

Two (2) mL of the DPPH solution from a 500 ppm sample solution was added. Its absorbance was measured over a period of 0–60 minutes at a wavelength of 516 nm.

2.9.6. Measurement of Antioxidant

In a 50 mL volumetric flask, the semi-purified plant sample solutions containing 100, 200, 300, 400, and 500 ppm were prepared. Ten (10) mL of the sample solution was pipetted into each solution, followed by 10 mL of DPPH solution. The solutions were then shaken until they were homogenous and incubated at room temperature for the operating time range. The absorbance was then measured at the maximum wavelength that was obtained (Nasution et al., 2022).

2.9.7. Data Analysis

The percent inhibition is calculated by the following formula:

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

2.10. α-Amylase Enzyme Inhibition

The α-Amylase inhibition assay assesses the ability of bioactive compounds to regulate carbohydrate metabolism by inhibiting α-Amylase, an enzyme that converts starch into glucose. The assay typically employs colorimetric detection methods, such as the 3,5-dinitrosalicylic acid (DNSA) assay, to assess the reduction in sugar production, which indicates an inhibitory action. This enzyme inhibition is a recognized pharmaceutical technique for controlling postprandial hyperglycemia, a major therapeutic target in type 2 diabetes management (Khadayat et al., 2020).

The National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Laguna, conducted the α-amylase enzyme inhibition assay based on the study journal's procedure, "*In Vitro* α-Amylase Enzyme Assay of Hydroalcoholic Polyherbal Extract: Proof of Concept for the Development of Polyherbal Teabag Formulation for the Treatment of Diabetes" (Quazi et al., 2022). The control used in this evaluation was acarbose. For ten minutes, 1 mL of the plant extract and 1 mL of α-amylase were incubated at 37°C in a test tube. Each tube was filled with 1 mL of 1% (v/v) starch solution following preincubation, and the tubes were then incubated for 15 minutes at 37°C. After stopping the reaction using 2 mL of 3,5-dinitrosalicylic acid (DNSA) reagent, the mixture was placed in a boiling water bath for 5 minutes, allowed to cool to room temperature, and then diluted. The absorbance was then measured at 546 nm using a Shimadzu spectrophotometer. The control procedure, which reflected 100% enzyme activity, did not include any plant extract. Acarbose and the extract were given at varying dosages (2–16 mg/mL). The semi-purified flavonoid plant extracts were utilized in the following concentrations: 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm.

The percentage inhibition formula was utilized to determine the α-amylase inhibitory activity:

$$\% \text{ Inhibition} = \frac{enzyme_{activity\ of\ control} - enzyme_{activity\ of\ extract}}{enzyme_{activity\ of\ control}} \times 100$$

The analysis calculated hypoglycemic activity based on percent inhibition results which led to a concentration-based plot of plant extract with IC50 value derivation for both the sample and positive control.

2.11. Statistical Analysis

All analyses were done in triplicate, and data were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) with a 4×5 factorial design and Tukey post-hoc test was done to assess the significant differences between means ($p < 0.05$) using SPSS. Comparisons between the experimental group and the control group were done using a pairwise comparison procedure.

3. Results

3.1. Identification Tests and Screenings

The pericarp of *Anacardium occidentale* (Cashew) ethanolic extract showed to have alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, triterpenoids, carbohydrates, proteins, and fixed oils. However, steroids and volatile oils were absent. *Arachis hypogaea* (Peanut) ethanolic extract demonstrated the presence of alkaloids, flavonoids, glycosides, phenolic compounds, tannins, triterpenoids, carbohydrates, proteins, and fixed oils, with steroids, saponins, and volatile oils yielding negative results. Lastly, *Canarium ovatum* (Pili Nut) exhibited alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, steroids, carbohydrates, and volatile oils, while tests for triterpenoids, proteins, and fixed oils yielded negative results (Table 1).

3.2. Characterization of the Functional Groups

The Fourier Transform Infrared analysis of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) fractions using ethanolic and

Table 1. Phytochemical Screening of Ethanolic Crude Extract of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut).

Test	*A. occidentale* (Cashew)	*A. hypogaea* (Peanut)	*C. ovatum* (Pili Nut)
Alkaloids			
Wagner's Test	+	+	+
Flavonoids			
Alkaline Test	+	+	+
Shinoda Test	+	+	+
Glycosides			
Keller-Killani Test	+	+	+
Phenolic Compounds and Tannins			
Ferric Chloride Test	+	+	+
Saponins			
Froth Flotation Test	+	–	+
Steroids and Triterpenoids			
Liebermann-Burchard Test	Steroids: – Triterpenoids: +	Steroids: – Triterpenoids: +	Steroids: + Triterpenoids: –
Carbohydrates			
Benedict's Test	+	+	+
Molisch Test	+	+	+
Proteins			
Biuret Test	+	+	–
Ninhydrin Test	+	+	–
Fixed Oils			
Spot Test	+	+	–
Volatile Oils			
Filter Paper Test	–	–	+
Sudan III Test	–	–	+

Note: (+) presence; (–) absence

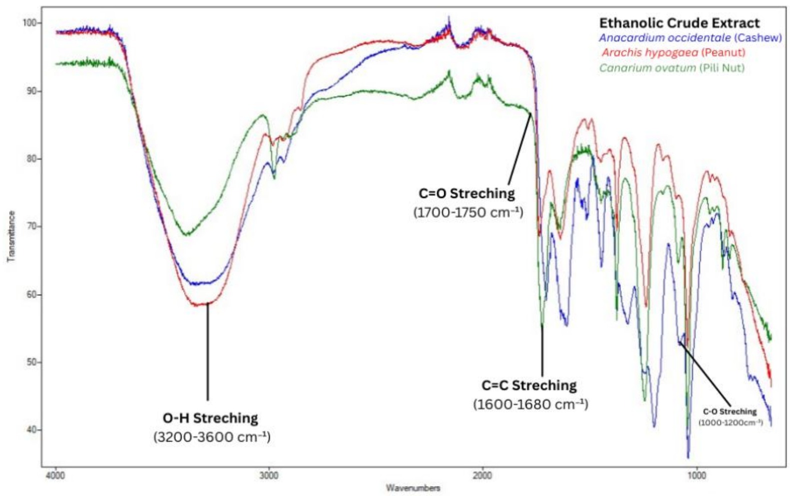


Figure 1. Analysis of Functional Group using Fourier Transform Infrared (FTIR) Spectroscopy of the Ethanolic Extract of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut). Note: Wavenumber unit is cm^{-1}.

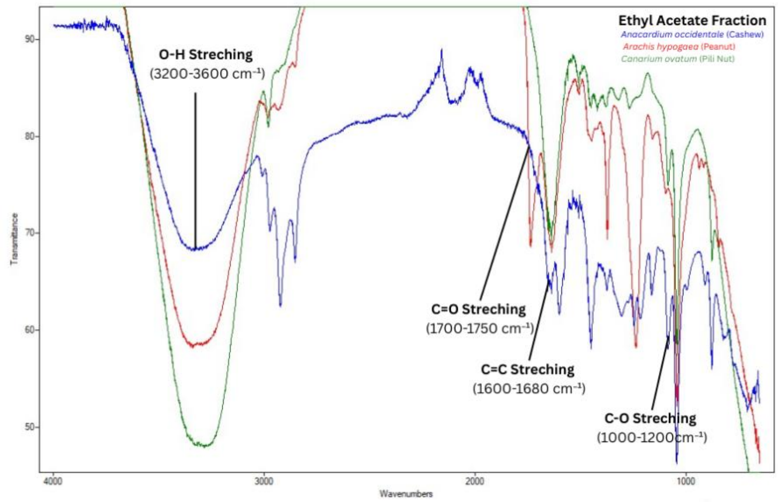


Figure 2. Analysis of Functional Group using Fourier Transform Infrared (FTIR) Spectroscopy of the Semi-Purified Flavonoid Extract of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), *and Canarium ovatum* (Pili Nut). Note: Wavenumber unit is cm^{-1}.

ethyl acetate allowed for functional group identification through detection of O-H and C=O stretching bands along with C=C stretching band. The obtained spectra showed distinct absorption bands between 3200–3600 cm^{-1} for O-H stretching and between 1700–1750 cm^{-1} for C=O stretching that indicate phenolic antioxidants and carbonyl components in tannins and other bioactive compounds (Figure 1). Analysis of C=C stretching (1600–1680 cm^{-1}) confirmed the existence of alkenes for antioxidant effects and C-O stretching (1000–1200 cm^{-1}) indicated carbohydrate structures responsible for inhibiting α-Amylase (Figure 2). These two spectra showed how different solvents and extraction methods influence the retention alongside the characterization of active biological compounds found in the sample.

3.3. Confirmatory Tests for Flavonoids

The confirmatory tests for flavonoids in *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) consistently demonstrated positive experimental results across all tests conducted: Alkaline Test, Lead Acetate Test, Shinoda Test, and Ethyl Acetate Test. Each test aligned theoretical expectations with observed experimental outcomes, confirming the successful detection of flavonoids in all three plant samples (Table 2).

3.4. Total Flavonoid Content (TFC)

Figure 3 shows the linearity of the graph with a regression equation of $y = 0.0367x + 0.0129$, where:

y is the dependent variable, representing the measured total predicted flavonoid concentration in a sample.

Table 2. Confirmatory Tests for the Semi-Purified Flavonoid Extract of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut).

Test	*A. occidentale* (Cashew)	*A. hypogaea* (Peanut)	*C. ovatum* (Pili Nut)
Alkaline Test	+	+	+
Lead Acetate Test	+	+	+
Shinoda Test	+	+	+
Ethyl Acetate Test	+	+	+

Note: (+) presence; (–) absence

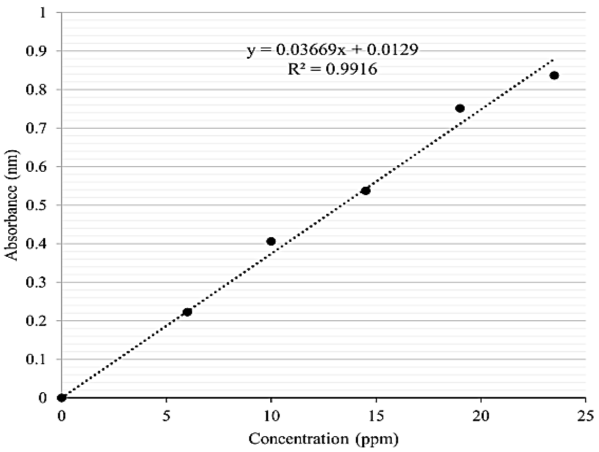


Figure 3. Quercetin Calibration Curve.

x is the concentration that influences the total predicted flavonoid concentration.
a is the slope of the line showing how responsive flavonoid yield is to changes in x.
b is the y-intercept representing the baseline flavonoid level in the absence of the influencing factor.

A R^2 of 0.9916 with an interpretation of a very strong model meant it predicted the dependent variable, and all the data points fell exactly on the regression line. This showed a highly significant relationship between quercetin content and absorbance, demonstrating the correctness and dependability of the calibration curve for analytical reasons.

The extraction process of dried *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) produced 20 g extract per specimen. The quantification of total flavonoid content happened through a UV-Vis spectrophotometric analysis that utilized $AlCl_3$ and sodium acetate because these reagents form flavonoid compound complexes. The reference standard Quercetin achieved the highest concentration of 37.985 µg/mL together with the highest flavonoid content of 36.878 mgGAE/g extract. The results indicate that *Anacardium occidentale* (Cashew) possessed the most abundant flavonoids with a total content of 20.682 mgGAE/g extract and concentration of 22.523 µg/mL which affirmed its effective antioxidant properties. The flavonoid analysis of *Canarium ovatum* (Pili Nut) showed an average consumption of 6.559 µg/mL and a total extracted amount of 4.523 mgGAE/g extract. The flavonoids in *Arachis hypogaea* (Peanut) were found in minimum amounts because they contained 2.417 mgGAE/g extract together with a concentration of 4.036 µg/mL. The study demonstrates *Anacardium occidentale* (Cashew) stands out as the highest flavonoid source compared to *Arachis hypogaea* (Peanut) and *Canarium ovatum* (Pili Nut) while demonstrating decreased flavonoid content and concentration (Table 3).

Table 3. Total Predicted Flavonoid Content of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), *and Canarium ovatum* (Pili Nut) and Quercetin (Reference standard).

Sample	± SD	Concentration (µg/mL)	Total Predicted Flavonoid Content (mgGAE/g extract)
A. occidentale (Cashew)	0.07	53.07	49.13
A. hypogaea (Peanut)	0.003	8.85	5.30
C. ovatum (Pili Nut)	0.009	14.31	9.87
Quercetin	0.12	82.31	79.91

Note: mgGAE/g extract: milligrams of gallic acid equivalents per gram of extract.

3.5. Antioxidant Property through DPPH Radical Scavenging Assay

Research data showed the antioxidant properties of semi-purified flavonoid extracts from *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) using percent inhibition values from 100 ppm to 500 ppm (Table 4).

Table 4. DPPH Assay of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), *and Canarium ovatum* (Pili Nut) *and* Ascorbic Acid (Positive Control), n=3.

Concentration (ppm)	*A. occidentale* (Cashew)		*A. hypogea* (Peanut)		*C. ovatum* (Pili Nut)		Ascorbic Acid	
	% Inhibition	± SD	% Inhibition	± SD	% Inhibition	± SD	% Inhibition	± SD
100	92.21	0.46	27.68	0.69	61.375	0.64	93.07	0.18
200	94.59	0.28	42.94	0.94	82.24	0.21	93.43	0.18
300	95.32	0.69	68.07	0.80	92.64	0.74	94.28	0.42
400	96.90	0.36	69.34	0.66	94.22	0.21	94.95	0.38
500	97.45	0.18	71.96	0.46	94.28	0.46	95.44	0.36

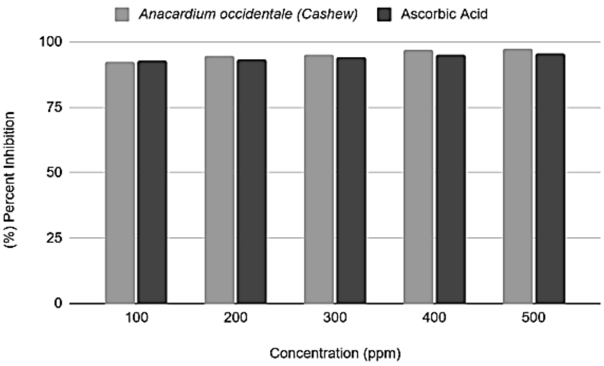


Figure 4. DPPH Assay of *Anacardium occidentale* (Cashew) compared to Ascorbic Acid (Positive Control).

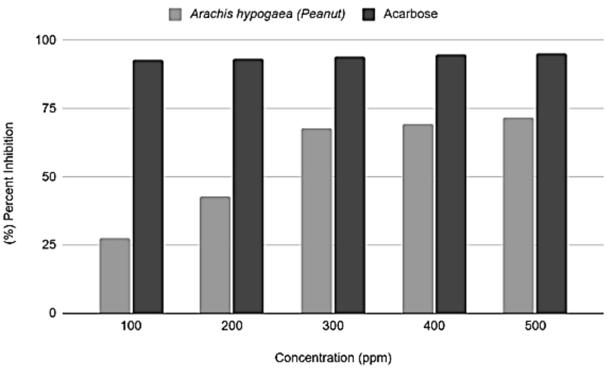


Figure 5. DPPH Assay of *Arachis hypogaea* (Peanut) compared to Ascorbic Acid (Positive Control).

The best-performing antioxidant source among the studied extracts was cashew, which showed a percent inhibition that was very similar to that of the positive control, ascorbic acid. The antioxidant performance of Cashew extract reached its peak at 97.45% inhibition following the initiation of 92.21% inhibition when tested from 100 to 500 ppm (Figure 4).

The antioxidant activity of *Arachis hypogaea* (Peanut) showed moderate effects when the inhibition percentage increased from 27.68% at 100 ppm to 71.96% at 500 ppm. Even though its activity levels were still far lower than those of cashew extract and ascorbic acid, the steady increase in inhibition across doses indicated that peanut extract may have an effect as an antioxidant, however, a weaker one (Figure 5).

Canarium ovatum (Pili Nut) presented antioxidant levels that varied from moderate to high at different quantity concentrations. Its antioxidant inhibition values increased gradually until they neared that of ascorbic acid at 300 ppm, which was a promising level. The results showed Pili Nut extract contained bioactive components that generated significant antioxidant benefits, although they had subtle activity at reduced concentration levels. The test results indicated potent antioxidant properties in Pili Nut extract at a concentration of 500 ppm, which suggests it could become an effective substance to minimize oxidative stress (Figure 6).

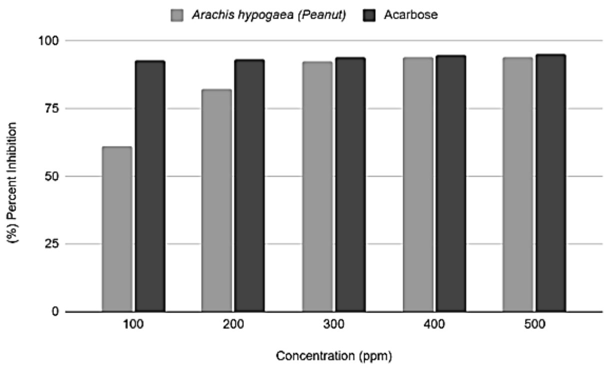


Figure 6. DPPH Assay of *Arachis hypogaea* (Peanut) and *Canarium ovatum* (Pili Nut) compared to Ascorbic Acid (Positive Control).

The ANOVA analysis confirmed significant differences in DPPH inhibition among the flavonoid extracts of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) compared with the positive control, Ascorbic Acid. A very high F-value (18910.59; $p < 0.001$) and Partial Eta Squared ($\eta^2 = 0.999$) indicated that treatment groups accounted for nearly all variability in antioxidant activity, underscoring the dominant role of bioactive composition over concentration effects (Table 5).

Further testing ($F = 979.44$; $p < 0.001$; $\eta^2 = 0.997$) showed that both treatment type and its interaction with dosage significantly influenced antioxidant potency. These findings highlight that the choice of treatment group and its dosage interplay are critical in optimizing antioxidant outcomes, providing a standardized basis for therapeutic applications (Table 5).

The antioxidant inhibition analysis compared *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) extracts with ascorbic acid

Table 5. Tests of Treatment Effects - Antioxidant Property Percentage.

Source	Type III Sum of Squares	df	Mean Square	F value	p-value	Partial Eta Squared
Treatment (IC50)	15144.65	3	5048.22	18910.59	<0.001	0.999
Concentration	3964.88	4	991.22	3713.10	<0.001	0.997
Treatment × Concentration	3137.55	12	261.46	979.44	<0.001	0.997
Error	10.68	40	0.27			

Note: <0.05 significant, while >0.05 not significant.

Table 6. Pairwise Comparison - Antioxidant Property Percentage.

Pairwise Comparisons	Mean Difference	Std. Error	p value
Ascorbic Acid vs Cashew	–1.058	2.841	0.711
Ascorbic Acid vs Peanut	38.236	2.841	<0.001
Ascorbic Acid vs Pili Nut	9.282	2.841	0.002

Note: <0.05 significant, while >0.05 not significant.

Table 7. α-Amylase Percent Inhibition of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) and Acarbose (Positive Control), n=3.

Concentration (ppm)	*A. occidentale* (Cashew)		*A. hypogea* (Peanut)		*C. ovatum* (Pili Nut)		Ascorbic Acid	
	% Inhibition	± SD	% Inhibition	± SD	% Inhibition	± SD	% Inhibition	± SD
20	10.61	0.18	7.311	0.05	12.55	0.22	30.99%	0.07
40	16.86	0.16	10.31	0.14	20.65	0.20	41.90%	2.33
60	18.48	0.05	15.32	0.16	23.13	0.02	63.30%	0.23
80	23.17	0.16	18.65	0.12	27.17	0.02	70.98%	0.39
100	26.19	0.29	21.08	0.01	32.99	0.13	86.00%	0.12

(positive control) using Tukey post hoc pairwise tests following One-Way ANOVA. Results showed that Peanut exhibited significantly lower antioxidant activity than all other treatments ($p < 0.001$), consistent with its reduced phenolic and flavonoid content. Cashew demonstrated significantly higher antioxidant potential than Pili Nut ($p < 0.001$), while ascorbic acid also outperformed Pili Nut ($p = 0.002$) (Table 6).

No significant difference was observed between Cashew and ascorbic acid ($p = 0.711$), with Cashew even showing a slightly higher mean inhibition value (mean difference = –1.058). These findings indicate that Cashew possesses antioxidant properties comparable to, and potentially exceeding, ascorbic acid, whereas Peanut and Pili Nut extracts remain inferior. Overall, Cashew emerges as a promising plant-based antioxidant source with potential therapeutic relevance comparable to the pharmaceutical standard (Table 6).

3.6. Inhibitory Effects on α-Amylase Activity

Different inhibitory properties were observed among the tested *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) semi-purified flavonoid extracts as well as Acarbose which served as the positive in α-amylase inhibition analysis. The extracts are varied with their inhibitory properties, which offer important information about their potential use as natural enzyme inhibitors to manage diabetes mellitus (Table 7).

The highest inhibitory action from the experimental group emerged from *Canarium ovatum* (Pili Nut) since percent inhibition increased from 12.55% at 20 μg/mL to 32.99% at 100 μg/mL. This potent enzyme inhibition occurs because of the bioactive components especially flavonoid chemicals, which can be seen through the observed dose-dependent reactions (Figure 7).

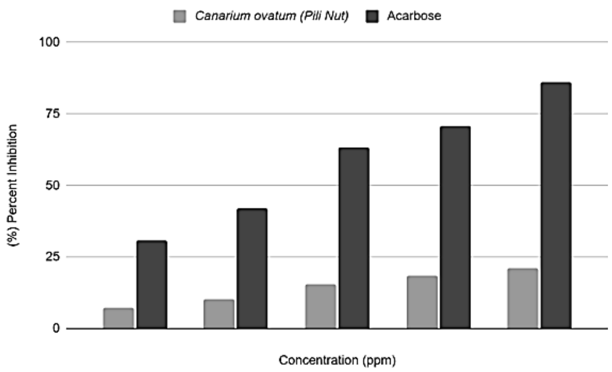


Figure 7. α-Amylase Percent Inhibition of *Canarium ovatum* (Pili Nut) compared to Acarbose (Positive Control).

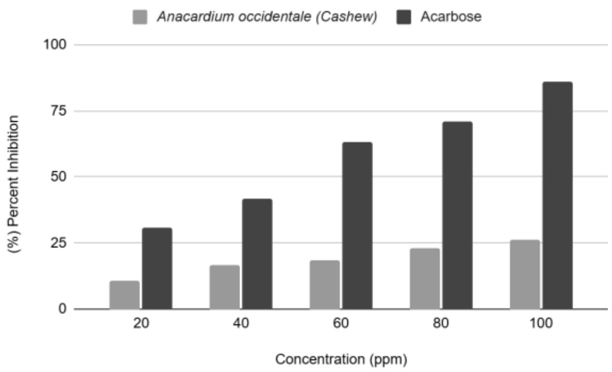


Figure 8. α-Amylase Percent Inhibition of *Anacardium occidentale* (Cashew) compared to Acarbose (Positive Control).

The inhibitory activity of *Anacardium occidentale* (Cashew) showed moderate effectiveness, as concentration directly influenced the inhibition values. Its flavonoid and phenolic content, which are known to impede the breakdown of carbohydrates, is probably responsible for its α-Amylase inhibitory capacity (Figure 8).

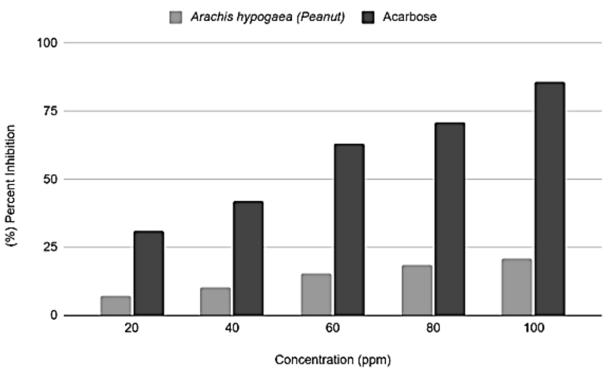


Figure 9. α-Amylase Percent Inhibition of *Arachis hypogaea* (Peanut) compared to Acarbose (Positive Control).

Among the examined samples, *Arachis hypogaea* (peanut) showed the weakest α-amylase inhibition; the percentage of inhibition increased steadily but moderately throughout doses. Although a study reported that the ethanolic extract of the peanut seed achieved 67.68% α-amylase inhibitory activity at 1.25 µg/mL, which is a close inhibition level to Acarbose with 72.34% at the same concentration, it's effectiveness of enzyme inhibition capacity is equaled by the limited presence of bioactive chemicals. Peanut extract has several nutritional advantages, but its ability to inhibit enzymes was not as strong as the Pili Nut or Cashew (Figure 9).

Among the tested substances, Acarbose functioned as the positive control by demonstrating maximal inhibitory power at all levels; thus confirming its position as the top α-amylase inhibitory agent. The drug's strength demonstrated its proven mechanism to regulate metabolic carbohydrates and manage blood glucose control after meals. The semi-purified flavonoid extracts demonstrated noteworthy potential, yet Pili Nut presented the most promising results among them, but fell short of Acarbose's inhibitory strength.

The ANOVA analysis revealed significant differences in α-Amylase inhibition among *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) compared with the positive control, Acarbose. A very high F-value (20739.52; $p < 0.001$) and Partial Eta Squared ($\eta^2 = 0.999$) indicated that treatment groups accounted for nearly all variability in inhibition, underscoring the dominant role of bioactive composition and dosage in determining enzymatic activity (Table 8).

Moreover, a significant interaction between treatment and concentration ($F = 660.69$; $p < 0.001$; $\eta^2 = 0.995$) demonstrated that inhibitory outcomes were dose-dependent. At higher concentrations, extracts rich in bioactive compounds produced stronger inhibition, highlighting the importance of both treatment selection and dosage optimization in maximizing α-Amylase inhibitory effectiveness (Table 8).

The inhibition assays revealed significant differences between Acarbose (positive control) and the semi-purified flavonoid extracts of *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut). Tukey post hoc comparisons confirmed Acarbose as the strongest inhibitor, with highly significant mean differences against Cashew (39.57, $p < 0.001$), Peanut (44.08, $p < 0.001$), and Pili Nut (35.35, $p < 0.001$) (Table 9).

Among the natural extracts, Pili Nut exhibited greater inhibitory activity than Peanut but remained weaker than Acarbose. Its superior performance relative to Cashew was attributed to higher phenolic and flavonoid content, which enhanced inhibition at elevated concentrations. Cashew demonstrated moderate inhibition, supporting its potential as a supplementary agent with dual antioxidant and enzyme-inhibitory properties.

Overall, the semi-purified flavonoid extracts displayed dose-dependent α-Amylase inhibition, though consistently lower than Acarbose. These findings highlight the therapeutic promise of natural inhibitors, particularly Pili Nut, as supportive agents in managing postprandial glucose levels. While Acarbose remains the most effective inhibitor, the plant-derived extracts provide complementary potential for functional food or adjunctive treatment applications.

Table 8. Tests of Treatment Effects α-Amylase Inhibition Percentage.

Source	Type III Sum of Squares	df	Mean Square	F-value	p-value	Partial Eta Squared
Treatment	18271.52	3	6090.51	20739.52	<0.001	0.999
Concentration	5078.10	4	1269.53	4323.02	<0.001	0.998
Treatment × Concentration	2328.28	12	194.02	660.69	<0.001	0.995
Error	11.75	40	0.29			

Note: <0.05 significant, while >0.05 not significant.

Table 9. Pairwise Comparison - α-Amylase Inhibition Percentage.

Pairwise Comparisons	Mean Difference	Std. Error	p-value
Acarbose vs Cashew	39.57*	2.45	<0.001
Acarbose vs Peanut	44.08*	2.45	<0.001
Acarbose vs Pili Nut	35.35*	2.45	<0.001

Note: <0.05 significant, while >0.05 not significant.

4. Discussion

Phytochemical screening confirmed that *Anacardium occidentale* (Cashew), *Arachis hypogaea* (Peanut), and *Canarium ovatum* (Pili Nut) contain diverse bioactive metabolites, particularly phenolics and flavonoids, which are consistently associated with antioxidant and antidiabetic activity. Cashew extracts exhibited the richest phytochemical profile, while Pili Nut contained moderate levels and Peanut the lowest. These compositional differences provided the biochemical basis for the variations observed in antioxidant and enzyme inhibition assays.

FTIR analysis supported these findings by identifying functional groups essential to bioactivity. O–H stretching bands (3200–3600 cm^{-1}) confirmed hydroxyl groups in phenolic antioxidants, while C=O stretching (1700–1750 cm^{-1}) indicated carbonyl groups in tannins and polyphenols. C=C stretching (1600–1680 cm^{-1}) reflected alkenes contributing to antioxidant reactivity, and C–O stretching (1000–1200 cm^{-1}) highlighted carbohydrate structures mechanistically linked to α-amylase inhibition. These spectral features reinforced the role of phenolics, flavonoids, and carbohydrates in both free radical scavenging and enzyme inhibition.

Flavonoid quantification further explained the observed biological activities. Cashew extracts contained the highest flavonoid levels (53.07 mg GAE/g extract; 49.13 μg/mL), consistent with their superior antioxidant activity, even comparable to ascorbic acid. Pili Nut demonstrated moderate levels (9.87 mg GAE/g extract; 14.31 μg/mL), while Peanut contained the lowest (5.30 mg GAE/g extract; 8.85 μg/mL), accounting for its weaker antioxidant response.

Antioxidant assays confirmed cashew as the most effective free radical scavenger, followed by Pili Nut and Peanut. In parallel, α-amylase inhibition assays revealed dose-dependent effects, with Pili Nut exhibiting the strongest inhibition (12.55% at 20 μg/mL to 32.99% at 100 μg/mL), attributed to secondary metabolites such as β-amyrin, epilupeol, and stigmasterol. Cashew showed moderate inhibition, consistent with its phenolic and flavonoid content, while Peanut demonstrated the weakest activity. ANOVA analysis confirmed significant differences among treatments, with Acarbose consistently outperforming all extracts but Pili Nut emerging as the most promising natural inhibitor.

Overall, the integration of phytochemical screening, FTIR characterization, flavonoid quantification, and bioassays demonstrates that cashew extracts are superior in antioxidant activity, while Pili Nut extracts are most effective in α-amylase inhibition. These complementary properties highlight the therapeutic potential of nut-derived bioactive compounds in managing oxidative stress and postprandial hyperglycemia, supporting their application in functional foods and adjunctive strategies for diabetes management.

5. Conclusion

This study demonstrated that Cashew exhibited the highest antioxidant property, while Pili Nut exhibited the highest α-amylase inhibition activity, highlighting their potential for nutraceutical and pharmacological applications in diabetes management. The antioxidant nature of *Anacardium occidentale* (Cashew) matched ascorbic acid levels and showed promise against oxidative stress, which assists diabetes development as well as insulin resistance. The antioxidant properties of Cashew make it a beneficial natural source for therapeutic applications. Pili Nut demonstrated potential benefits for glucose regulation because it exhibited remarkable α-Amylase inhibitory activity, which selectively suppresses important enzymes that break down carbohydrates. Pili Nut showed promise as a diabetes management agent because of its ability to block the said enzyme, leading to lower post-meal blood sugar elevation.

This research yielded enhanced biological properties of plant-derived extracts, demonstrating observable significant antioxidant and hypoglycemic activities. These activities that exhibit potent effects were derived from the preservation and concentration of vital functional groups, such as flavonoids.

In a broader context, this research highlighted the value of exploring underutilized plant parts, such as kernel pericarps, as sustainable and accessible resources for combating diabetes. For future researchers, they should consider to the use of higher concentrations of plant samples together with fully purified plant extracts in order to increase the efficiency and power of bioactive compounds. Increasing sample concentration may enhance α-amylase inhibitory effects, to make them more comparable to the positive control, acarbose at the same time purification serves as a way of isolating the most active compounds having maximum therapeutic potential. In order for these compounds to be proved to be safe and effective, testing for acute toxicity and animal studies are recommended. Additionally, additional investigation on the terms of bioactive characteristics of the identified compounds may be useful to delve into the potential of the compounds to be used in the drug development process. Ameliorating extracting techniques, in-depth biochemical testing, and testing pharmacological properties, future studies will help evolve plant-based therapies for diabetes treatment as well as integrating sustainable and innovative healthcare procedures.

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Conflict of Interest

The authors have no conflict of interest to declare.

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