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

Background: Diabetes mellitus is one of the leading causes of death in the Philippines and is projected to affect 7.5 million people by 2045 if left unmanaged. Flavonoid-rich foods include fruits, vegetables, grains, legumes and nuts. Most of the studies focused on fruits, vegetables and grains, while other edible kernels were unexplored, but research is limited on the antioxidant and glucose-lowering properties of polyphenol-rich extracts from the *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut). Exploring this underutilized plant part from these nuts could provide innovative solutions for diabetes management.

Objective: This study aimed to assess the antioxidant and glucose-lowering properties of the semi-purified extracts of cashew, peanut and pili nut pericarps; identifying which had the highest potential as a natural therapeutic agent.

Methods: Kernel pericarps were extracted by the maceration method using 70% ethanol. The ethanolic extract was subjected to phytochemical screening, and then it was semi-purified into flavonoids through liquid-liquid extraction. The concentration of flavonoid was analyzed through a colorimetric method using a UV-Vis Spectrophotometer and compared to Quercetin (reference standard). Antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay compared to Ascorbic Acid (Positive Control), while glucoselowering properties were evaluated through the α-Amylase inhibition assay and compared to Acarbose (positive control).

Results: The result showed that there is no significant difference in the antioxidant property between Ascorbic Acid and A. occidentale (cashew) ($p>0.711$). Furthermore, there is a very significant difference between Acarbose and cashew in α-Amylase inhibition ($p<0.001$). *C. ovatum* (pili nut) displayed the highest α-Amylase inhibition (32.99%) and moderate flavonoid content.

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.

Key words: flavonoids, antioxidant, α-amylase

The ongoing escalation of cases of Diabetes Mellitus, a chronic metabolic disease that continuously poses a major health concern in the Philippines and around the world, necessitates a pressing need to investigate for an alternative and more cost-effective treatment. Research into plant-derived compounds offers valuable insight into potential therapeutic solutions, with edible kernels such as *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili) have shown promising results in managing diabetes due to their glucose-lowering and antioxidant properties. Cashews aid glycemic control with their low glycemic index and bioactive compounds, while peanuts provide stable blood sugar regulation through their high fiber content and healthy

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fats. Though less studied, pili nuts possess strong antioxidant and phytochemical profiles, suggesting potential benefits in glucose regulation.

However, 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 assess the antioxidant and hypoglycemic properties of semi-purified extracts of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili) 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.

MATERIALS AND METHODS

This is an experimental study of the determination of the phytochemical compounds, total flavonoid content, antioxidant property, and glucose- lowering properties of the semi-purified flavonoids of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut) pericarps.

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 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.

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.

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).

Phytochemical Screening

The preliminary separation, identification and quantification of *Anacardium occidentale* (cashew),

Arachis hypogaea (peanut) and *Canarium ovatum* (pili nut) ethanolic crude extracts' polyphenolic compounds, such as alkaloids, flavonoids, glycosides, phenolics, saponins, steroids and terpenoids, tannins, carbohydrates, proteins, fixed oils and volatile oils present in this plant, were conducted using qualitative phytochemical testing.

Semi-Purification of Flavonoids

The researchers placed 20 grams of extracted substance which had been dissolved in 96% ethanol together with 40 mL distilled water into a separatory funnel. The separation funnel required 100 mL of n-hexane which was shaken before allowing the mixture to stand for 30 minutes to form two separate layers. The n-hexane layer extraction was followed by fractionation steps until the Liebermann-Burchard reagent produced no reaction from the n-hexane layer. The evaporator. A mixture of 100 mL ethyl acetate with the residue leads to two distinct layers forming within 30 minutes. The researchers performed fractionation on the ethyl acetate layer until testing it with the ferric chloride ($FeCl_3$) reagent produced no reaction. The ethyl acetate fraction underwent concentration through usage of a rotary evaporator. The collection of the water fraction involves rotary evaporator concentration of the remaining water layer (Nasution, et al, 2022).

Flavonoid Confirmatory Tests

The semi-purified extract underwent another phytochemical screening using the tests for flavonoids, which served as the qualitative confirmatory test for the presence of flavonoids. The following confirmatory tests are the Alkaline Test, Lead Acetate Test, Shinoda Test and Ethyl Acetate Test. These were done in triplicate to ensure the accuracy of the results.

Total Flavonoid Content

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.

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.

Creation of a Quercetin Calibration Curve

A volumetric flask received 3 mL and 5 mL and 7.25 mL and 9.75 mL and 11.5 mL of pure quercetin solution followed by methanol addition up to the 50 mL mark to create solutions of 6 ppm, 10 ppm, 14.5 ppm, 19.5 ppm and 23 ppm. A combination of 2 mL solution per concentration was transferred to a test tube containing 0.1 mL of Aluminum Chloride ($AlCl_3$) together with 0.1 mL of Sodium Acetate (CH_3COONa) followed by 2.8 mL of distilled water. The mixed solution rested in complete darkness for a period of 40 minutes. UV-VIs spectrophotometry evaluated absorbance measurements from the solutions after their standing period at the 438 nm maximum wavelength. A quercetin calibration curve and its linear regression line equation $y=ax + b$ are established.

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.

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 in the samples in the ethanolic crude extract and the semi-purified flavonoid extract that were isolated and processed. 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.

2,2-Diphenyl-1-picrylhy –drazyl (DPPH) Radical Scavenging Assay

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).

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 (40g/mL).

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.

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 g/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 UVVisible spectrophotometer at the maximum absorption wavelength of 515 nm.

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.

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 was then measured at the maximum wavelength that was obtained.

Data Analysis

The percent inhibition is calculated by the following formula:

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

α-Amylase Enzyme Inhibition

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:

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

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.

Statistical Analysis

All analyses were done in triplicate, and data were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) with a 4x5 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.

RESULTS AND DISCUSSION

Identification Tests and Screenings

The ethanolic crude extracts of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut) showed distinct profiles in the phytochemical screening, which reflected the unique chemical composition of each sample.

The ethanolic extract of *Anacardium occidentale* (cashew) pericarp was found to have alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, triterpenoids, carbohydrates, proteins and fixed oils. However, steroids and volatile oils were absent. The results of this study align with the previous study (Archana, et al, 2021), which reported that cashew root extracts have potent antioxidant and antihyperglycemic qualities due to their high phenolic and flavonoid contents.

For *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. Similar to a study on peanut polyphenols (Aneklaphakij, et al, 2021), which highlights the presence of flavonoids and phenolic compounds as important active constituents in peanuts in contributing to antioxidant activity to reduce oxidative stress and offer potential health benefits.

Lastly, *Canarium ovatum* (pili nut) exhibited alkaloids, flavonoids, glycosides, phenolic compounds, tannins, saponins, steroids, carbohydrates and

Table 1. Phytochemical screening of ethanolic crude extract of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut)

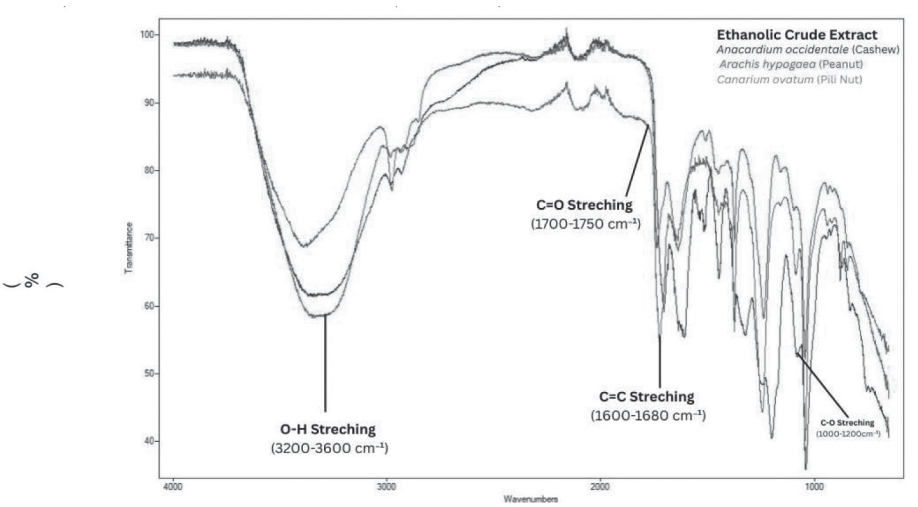
Test	*occidentale* (Cashew)	*hypogaea* (Peanut)	*ovatum* (Pili Nut)
Alkaloids			
Wagner's Test	+	+	+
Flavonoids			
Alkaline Test	+	+	+
Shinoda Test	+	+	+
Glycosides			
Keller-Killani Test	+	+	+
Phenolic Compounds and Tannins			
Ferric Chloride Test	+	+	+
Saponins			
Froth Flotation Test	+	-	+
Steroids 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

volatile oils, while tests for triterpenoids, proteins, and fixed oils yielded negative results. A study on native fruits in the Philippines (Recuenco, et al, 2020) found that *Canarium ovatum* (pili nut) is a source of cardiac glycosides and alkaloids. These results are in line with those findings. The aforementioned study also showed a substantial association between the levels of phenolic compounds and flavonoids and their antioxidant properties, which further emphasised the potential of *Canarium ovatum* (pili nut) as a significant source of bioactive compounds. In addition, the study found that *Canarium ovatum* (pili nut) contains terpenoids. This constituent supports its antioxidant qualities.

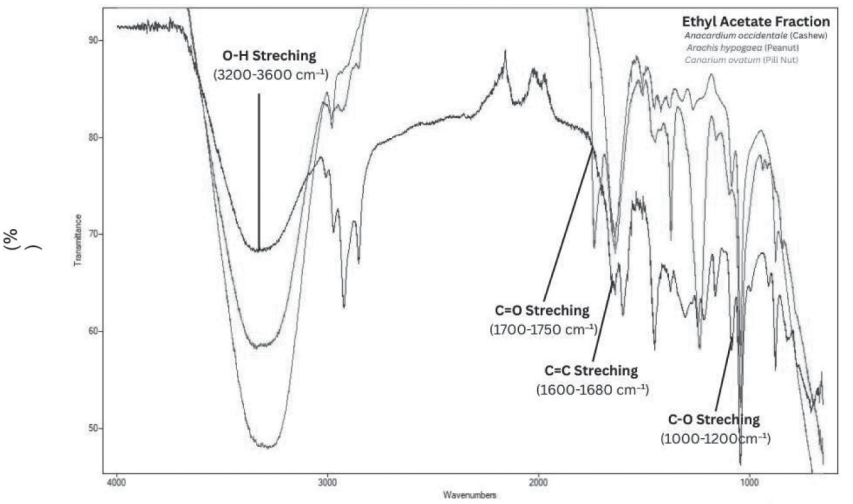
Characterization of the Functional Groups

The functional groups found in the fractions of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) that were extracted using ethanol and ethyl acetate solvents were revealed by the Fourier Transform Infrared (FTIR) analysis. The presence of bioactive substances essential to their biological activity is shown by the



Note: Wavenumber unit is cm^{-1}

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)

detection of distinctive absorption bands. Phenolic antioxidants contain several hydroxyl groups that produce O-H stretching bands within the 3200 and 3600 cm^{-1} region. The antioxidants protect cells by eliminating free radicals to reduce oxidative stress in diabetes and other degenerative disease treatments.

The existence of carbonyl groups, which are frequently present in tannins and other polyphenolic compounds, is suggested by the unique absorption bands for C=O which lie between 1700 and 1750 cm^{-1}. These carbonyl elements support the samples' antioxidant capacity and provide therapeutic advantages against illnesses linked to oxidative stress. Additionally, the presence of alkenes, which are recognized for their reactive qualities and role in antioxidant effects, was validated by the examination of C=C extending between 1600 and 1680 cm^{-1}. These functional groups enhance both stability and bioactivity in fighting oxidative damage.

Furthermore, the existence of C-O stretching bands between 1000 and 1200 cm^{-1} indicated the samples' carbohydrate structures, which are crucial in inhibiting the activity of α-Amylase. The extracts' capability to manage hyperglycemia was further highlighted by the direct correlation between this inhibition and the ability to regulate the digestive system's absorption of glucose.

Beyond these emphasized peaks, the fingerprint region (600–1500 cm^{-1}) provided important details regarding distinct absorption patterns that can be used to pinpoint particular bioactive substances in the samples. Strong hydrogen bonds may be indicated by the broadness or sharpness of peaks, such as the O-H stretching, which would increase the antioxidant activity. Other functional groups, like C-H stretching (2800–3000 cm^{-1}) and possible N-H stretching (3300–3500 cm^{-1}), could add layers to the characterization of bioactive components by offering more proof of aliphatic structures and amino groups connected to proteins or peptides. The evaluation between ethanol and ethyl acetate solvents increased the researchers' understanding regarding solvent-specific extraction effectiveness as well as active chemical preservation. The molecular makeup and possibility for treatment of the samples can be determined by these combined characteristics, which enhanced the analysis.

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. 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

Total Flavonoid Content (TFC)

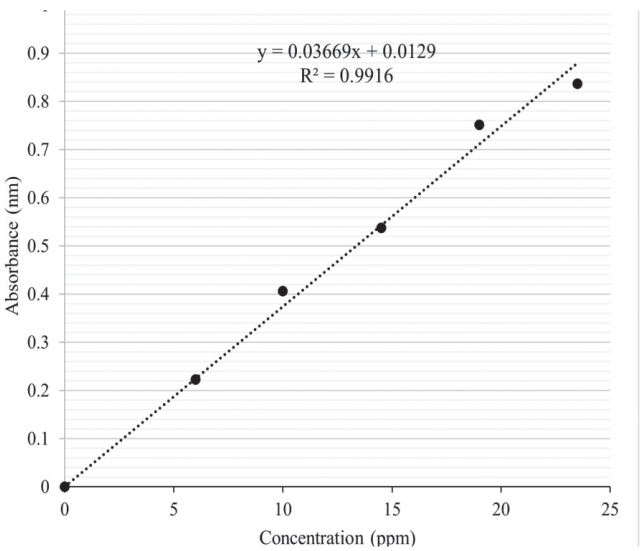


Figure 3. Quercetin calibration curve.

This 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

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.

AR^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.

Table 3. Total predicted flavonoid content of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut) and Quercetin (Reference standard)

Sample	Concentration (µg/mL)	Total Predicted Flavonoid Content (mgGAE/g extract)
Anacardium occidentale (Cashew)	53.07	49.13
Arachis hypogaea (Peanut)	8.85	5.30
Canarium ovatum (Pili Nut)	14.31	9.87
Quercetin	82.31	79.91

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

The reference standard Quercetin achieved the highest concentration of 82.31 µg/mL together with the highest flavonoid content of 79.91 mgGAE/g extract. The results indicate that *Anacardium occidentale* (cashew) possessed the most abundant flavonoids with a total content of 53.07 mgGAE/g extract and concentration of 49.13 µg/mL which affirmed its effective antioxidant properties. Cashew's high flavonoid concentration is consistent with prior research identifying cashew extracts as significant sources of polyphenols and flavonoids (Archana, et al, 2021). According to research, cashew leaves and bark contain high levels of quercetin, which contributes to their antioxidant and anti-inflammatory qualities. Cashew's high flavonoid content suggests that it could be used to regulate oxidative stress and prevent illness (Sassi A, et al, 2021).

The flavonoid analysis of *Canarium ovatum* (pili nut) showed an average consumption of 14.31 µg/mL and a total extracted amount of 9.87 mgGAE/g extract. The flavonoid content of pili nut extracts is consistent with prior research indicating the presence of polyphenols and flavonoids in pili fruit. Studies have found that pili nut pulp contains bioactive chemicals with antioxidant effects, albeit in lesser concentrations than cashew. The presence of flavonoids in pili nut enhances its potential medicinal applications, but at a lower degree than cashew (Recuenco, et al, 2020)

The flavonoids in *Arachis hypogaea* (peanut) were found in minimum amounts because they contained 5.30 mgGAE/g extract together with a concentration of 8.85 µg/mL. A 2020 study looked into flavonol synthase genes in *Arachis hypogaea* and their significance in flavonol production (Hou, et al, 2020).

The researchers discovered that flavonol content varied among peanut organs, with dried mature seeds having the highest quantities. Furthermore, a 2021 study investigated phytochemicals in peanut skin extract, discovering flavonoids among other beneficial substances. These findings confirm that, while peanuts contain flavonoids, their amounts may be lower than in pili nut, as observed in the current investigation (Kyei, et al, 2020).

The study demonstrated that *Anacardium occidentale* (cashew) stood out as the highest flavonoid source compared to *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) while demonstrating decreased flavonoid content and concentration. The comparison highlights the various degrees of efficacy within the experimental group and emphasizes quercetin's position as the gold standard in antioxidant investigations. Notwithstanding these variations, the edible kernels' flavonoid contents indicated promising antioxidant and medicinal potential, with *Anacardium occidentale* (cashew) being the most effective among the test samples.

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

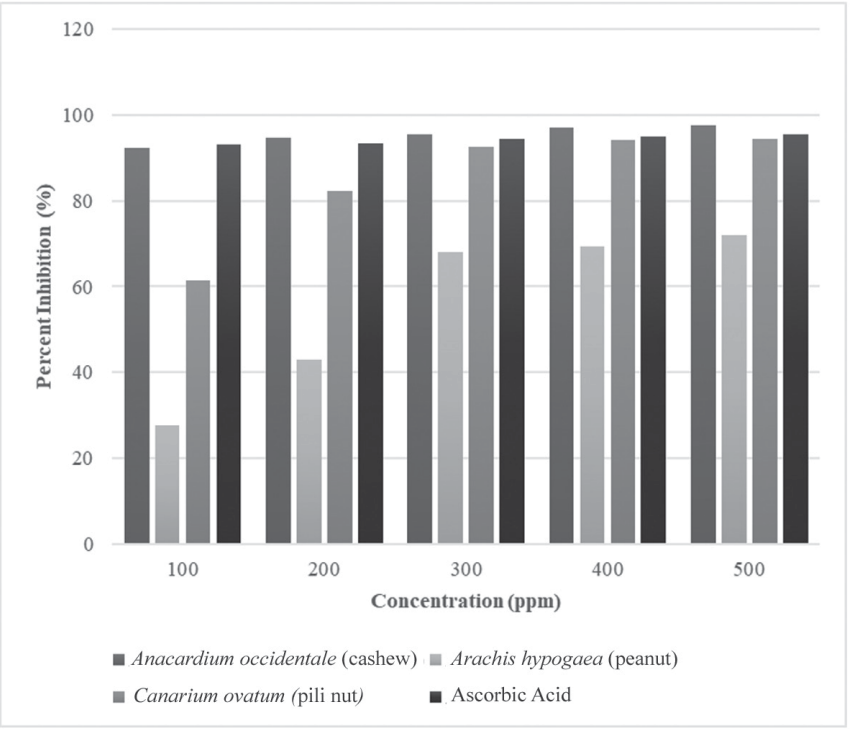


Figure 4. DPPH Percent Inhibition of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut) and Ascorbic Acid (positive control)

Table 4. DPPH percent inhibition of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) and Ascorbic Acid (positive control)

Concentration (ppm)	Percent Inhibition (%) (IC50)			
	***A. occidentale* (Cashew)**	***A. hypogea* (Peanut)**	***C. ovatum* (Pili Nut)**	**Ascorbic Acid**
100	92.21%	27.68%	61.375%	93.07%
200	94.59%	42.94%	82.24%	93.43%
300	95.32%	68.07%	92.64%	94.28%
400	96.90%	69.34%	94.22%	94.95%
500	97.45%	71.96%	94.28%	95.44%

Canarium ovatum (pili nut) using percent inhibition values from 100 ppm to 500 ppm.

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. The high antioxidant activity of cashew extract aligns with previous studies that have reported significant polyphenol and flavonoid content in cashew leaves and bark (Sassi, et al, 2022). Research has shown that cashew extracts exhibit strong free radical scavenging activity, contributing to their potential use in managing oxidative stress-related diseases (Archana, et al,

2021). The similarity in inhibition values between cashew extract and ascorbic acid further supports its effectiveness as a natural antioxidant.

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. Studies have indicated that peanut skins contain higher concentrations of polyphenols and flavonoids, suggesting that different plant parts may exhibit variations in antioxidant potential (Abigail & Frederick, 2021). Despite its lower inhibition values, peanut extract still demonstrates a steady increase in antioxidant activity across doses. Additionally, from the results of the previous tests, its reduced flavonoid content may be the cause of this mediocre competence, which restricts its capacity to efficiently neutralize free radicals.

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. Recent research has related the antioxidant properties of pili nut to its total flavonoid content and phytochemical profile. Pili nut has been recognized as a rich source of phenolic chemicals, which contribute to its ability to effectively scavenge free radicals. A previous study discovered that pili nut includes alkaloids, cardiac glycosides and terpenoids, all of which contribute to its antioxidant potential (Recuenco, et al, 2020). Another study investigated the pili nut ethanolic leaf extracts revealed significant antioxidant activity, confirming its promise as a natural antioxidant (Terrazola, et al, 2024).

The experimental data demonstrates that *Arachis hypogaea* (peanut) extracts exhibited a moderate stable antioxidant effect, but *Anacardium occidentale* (cashew) extracts possessed higher capabilities compared to ascorbic acid, and *Canarium ovatum* (pili nut) demonstrated effective results at elevated concentrations. These findings highlight the value of bioactive profiling in locating antioxidants found in plants and imply that the extracts, especially cashew, may be effective natural medicines.

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 x 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

The ANOVA results demonstrated that significant variations exist in the DPPH inhibition activities between the *Anacardium occidentale* (cashew) and *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) groups when compared to the positive control, ascorbic acid. A controlled analysis of independent DPPH inhibition effects was made possible by blocking variables associated with different concentrations and their treatment interactions.

An F-value of 18910.59 along with $p<0.001$ showed that statistical differences exist between DPPH percent inhibition values for *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), *Canarium ovatum* (pili nut) semi-purified flavonoid extracts and ascorbic acid (positive control). Each treatment is performed independently to affect antioxidant activity levels, according to this research, which showed selection of treatment groups is essential in maximizing antioxidant effectiveness.

The high partial $\eta^2=0.999$ demonstrated that the treatment group itself accounts for almost complete variability in percent inhibition, thus indicating its strong impact on the outcome. The treatment had a significant impact on antioxidant activity levels, which is supported by the high percentage of variability explained after considering all other factors affecting sample concentration. Study results showed that variations in the bioactive compounds provoke

the strongest impact on antioxidant performance because they override any single-factor effects linked to concentration differences.

The testing results demonstrated a significant change in the examined relationship because treatment conditions affected the antioxidant activity measurements at various concentrations ($F=979.44$; $p<0.001$; $\eta^2=0.997$). The data showed that both each treatment variation and its reaction when facing dosage variations impact antioxidant potency levels when combined. The systematic results showed that treatment composition interacts with dosage levels to produce optimal antioxidant outcomes, which sets a standardized approach for therapeutic applications.

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

This antioxidant inhibition analysis showed a detailed assessment between ascorbic acid (positive control) and the treatment groups *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut), and *Canarium ovatum* (pili nut). The Tukey post hoc test conducted pairwise comparisons to reveal the effectiveness levels of treatments which proved significant in the One-Way ANOVA analysis.

Research through pairwise comparison established *Arachis hypogaea* (peanut) presented lower antioxidant activity than every other treatment test condition ($p < 0.001$). The antioxidant capacity of *Arachis hypogaea* (Peanut) suffers from low bioactive compound contents which include phenolic compounds and flavonoids and thus results in poor inhibitory effect measurements.

Analysis between the *Anacardium occidentale* (cashew) and *Canarium ovatum* (pili nut) showed a statistically relevant distinction since cashew exhibited better antioxidant potential ($p < 0.001$). The pairwise comparison analysis revealed that ascorbic acid showed stronger antioxidant inhibition than *Canarium ovatum* (pili nut) because their difference was statistically significant ($p = 0.002$). The antioxidant capacity of pili nut remains lower than cashew and ascorbic acid based on these experimental results. The detected inhibition rates indicate that pili nut contains lower antioxidant properties and bioactive substances than cashew and ascorbic acid treatments.

The Tukey post hoc test revealed no statistically significant difference ($p= 0.711$) between Anacardium occidentale (cashew) antioxidant activity and ascorbic acid activity. The results show that cashew possesses better antioxidant properties than ascorbic acid, with a mean difference of -1.058. This proves that cashew possesses antioxidant properties higher than the positive control, which is commonly used to manage oxidative stress.

Moreover, the results revealed that ascorbic acid displayed higher antioxidant capacity than both *Arachis hypogaea* (peanut) along with *Canarium ovatum* (pili nut), thus indicating peanut and pili nut extracts provide inferior protection than ascorbic acid when used for DPPH percent inhibition. The antioxidant activity measurements between ascorbic acid and *Anacardium occidentale* (cashew) extracts yielded higher results, thus demonstrating cashew extract has superior antioxidant potential like ascorbic acid. This demonstrates that cashew shows promise as a plant-based antioxidant that matches the pharmaceutical industry standard.

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) semipurified 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.

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

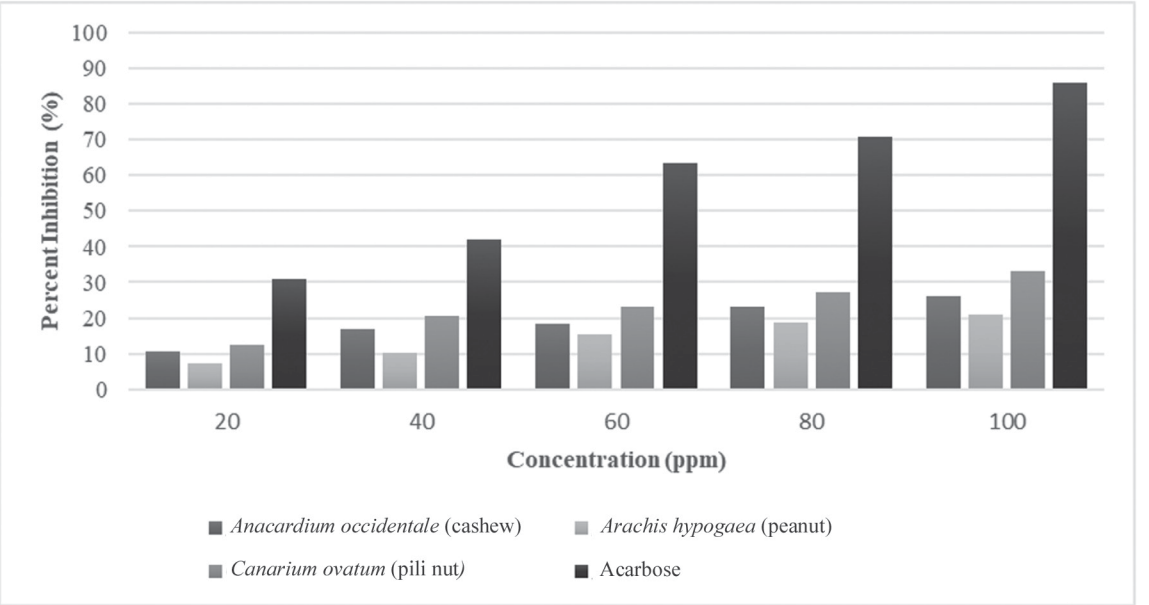


Figure 5. α-Amylase percent inhibition of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) and Acarbose (positive control)

Table 7. α-Amylase percent inhibition of *Anacardium occidentale* (cashew), *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) and Acarbose (positive control)

Concentration (ppm)	Percent Inhibition (%) (IC50)			
	A. occidentale **(Cashew)**	***A. hypogaea*** **(Peanut)**	***C. ovatum*** **(Pili Nut)**	**Acarbose**
20	10.61%	7.311%	12.55%	30.99%
40	16.86%	10.31%	20.65%	41.90%
60	18.48%	15.32%	23.13%	63.30%
80	23.17%	18.65%	27.17%	70.98%
100	26.19%	21.08%	32.99%	86.00%

reactions. These results were consistent with a prior study (Herrera, et al, 2022), which indicated that the secondary metabolites that were isolated from *Canarium ovatum* (pili nut), specifically β-amyrin, epilupeol and stigmasterol, demonstrated a greater binding affinity to α-amylase than the reference standard, Acarbose. Since these particular plant constituents are known to have α-amylase inhibitory effects, these results further support the potential of pili nut as an effective α-amylase inhibitor.

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. Similar to the results from a study (Haron, et al, 2020), the cashew leaf extracts contained bioactive polyphenols capable of inhibiting α-amylase and dipeptidyl peptidase IV (DPPIV) activities. In

addition to their findings, it was reported that soluble ester and insoluble-bound phenolic fractions from the mature leaves exhibited stronger α-amylase inhibition activity. Nevertheless, the semi-purified flavonoid extract's inhibitory levels were continuously lower than those of Acarbose and pili nut, suggesting that although cashew extract has potential as a supplemental inhibitor, pili nut is more effective.

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 (Sani, et al, 2020) reported that the ethanolic extract of the peanut seed achieved 67.68% α-amylase inhibitory activity at 1.25µg/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.

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 semipurified flavonoid extracts demonstrated noteworthy potential, yet pili nut presented the most promising results among them, but fell short of Acarbose's inhibitory strength.

Table 8. Tests of treatment effects α-amylase inhibition percentage.

Source	Type III Sum of Squares	df	Mean Square	F-value	p-value	Partial η^2
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 x 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

The ANOVA results demonstrated that significant variations exist in the α-Amylase inhibition activities between the *Anacardium occidentale* (cashew) and *Arachis hypogaea* (peanut) and *Canarium ovatum* (pili nut) groups when compared to the positive control, Acarbose. A controlled analysis of independent α-amylase inhibition effects was made possible by blocking variables associated with different concentrations and their treatment interactions.

The analysis produced a significant F-value, which reached 20739.52, indicating that the applied treatment groups successfully influenced α-Amylase inhibition. The unique enzymatic inhibitory capabilities of different treatment groups emphasized the importance of selecting proper treatment in determining α-Amylase inhibition effectiveness. The partial $\eta^2 = 0.999$ showed that treatment groups alone explain virtually all percent inhibition variability after considering concentration and other elements. The substantial fraction of data points demonstrated that treatment composition, which included bioactive compound types and dosages, played the biggest role in influencing enzyme inhibition results.

The ANOVA test revealed a significant interaction between treatment groups and concentration through its Fvalue of 660.69, which reached statistical significance ($p<0.001$) with $\eta^2 = 0.995$. The inhibitory results of each treatment group demonstrated specific outcomes that depend on the dosage adjustments through this interaction process. At elevated concentrations, the treatments containing potent bioactive contents showed better inhibition rates, leading to enhanced performance in blocking α-amylase activity.

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

The inhibition tests on the α- αmylase enzyme showed significant differences between Acarbose (positive control) and the experimental extracts *Anacardium occidentale* (cashew), *Arachis hypogaea*

(peanut) and *Canarium ovatum* (pili nut). The comparison results with the Tukey post hoc test established Acarbose as the strongest inhibitor compared to all experimental extracts with significant mean differences between them ($p < 0.001$).

The inhibitory effects found in *Canarium ovatum* (pili nut) were more potent than peanut yet weaker than Acarbose according to experimental results. The inhibition differences revealed by the study showed Acarbose outperforming cashew (39.57*, $p < 0.001$), peanut (44.08*, $p < 0.001$), and pili nut (35.35*, $p < 0.001$), while assessing inhibitory power between the drug Acarbose and natural inhibitors. The enzyme inhibition effectiveness depends heavily on phenolic compounds and flavonoid levels in plant materials.

The experimental semi-purified flavonoid extracts showed substantial dose-dependent α-Amylase inhibitory properties even though they exhibited lower inhibitory capabilities than Acarbose in terms of enzymatic function disruption. These natural extract findings show promise for therapeutic usage when used alongside Acarbose as supportive treatment. The bioactivity tests demonstrated that Canarium ovatum (pili nut) produced stronger α-Amylase inhibitory effects than *Anacardium occidentale* (cashew). Pili nut demonstrates superior bioactive compound potency including phenolics and flavonoids which leads to increased effectiveness at higher inhibitory concentrations. The moderate inhibition level of cashew preserves its potential value as a natural supplement that provides antioxidant effects and enzyme inhibition.

The research suggests that *Canarium ovatum* (pili nut) possesses significant dose-dependent inhibitory capability which establishes its potential as a natural treatment option but its enzyme-inhibiting effect remains weaker than Acarbose. The inhibition results from *Anacardium occidentale* (cashew) and *Arachis hypogaea* (peanut) revealed their potential to supplement with other agents that might help control post-meal glucose levels. The semi-purified flavonoid extracts show significant therapeutic potential even though they demonstrate reduced inhibitory capability compared to Acarbose. These findings stem from using semi-purified flavonoid extracts which demonstrates the therapeutic value of natural substances.

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 alpha-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 gave special attention to optimized extraction along with semi-purification methods that boost the biological properties of plant-derived extracts. These methods ensured the preservation and concentration of vital functional groups, such as flavonoids, which are responsible for the antioxidant and hypoglycemic activities observed.

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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