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Evaluation of antioxidant and antidiabetic properties of ethyl acetate fraction from *Anacardium occidentale* leaves, *in-vitro and in-silico*

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ABSTRACT

Enzymes that break down carbohydrates, specifically α-amylase and α-glucosidase, play a significant role in post-meal high blood sugar levels. Inadequate control of these enzyme functions may lead to the onset of diabetes mellitus. Furthermore, the buildup of free radicals within biological systems has been identified as a contributing factor to the advancement of this condition. The current research examined the antioxidant and antidiabetic effects of different solvent fractions obtained from the methanolic extract of Anacardium occidentale. This included assessing their ability to neutralize DPPH radicals and chelate metal ions, as well as their potential to inhibit the activities of α-amylase and α-glucosidase. The most active fraction was analysed using LCMS to identify its components through a database library. The three-dimensional configurations of the identified compounds were studied for possible interactions with the α-amylase and α-glucosidase enzymes using MOE software. The results revealed that the ethyl acetate fraction exhibited the highest levels of antioxidant and antidiabetic activity, with an IC_{50} of 73.16±3.03 for DPPH scavenging and 99.68±5.10 µg/mL for metal chelation. In comparison, the reference substances ascorbic acid and EDTA showed IC_{50} values of 68.97±2.08 and 65.01±3.01 µg/mL, respectively. Furthermore, the IC_{50} values for the ethyl acetate fraction's inhibitory effects on α-amylase and α-glucosidase were found to be 10.20±1.02 and 12.23±3.13 µg/mL, respectively. In contrast, the standard drug acarbose displayed IC_{50} values of 9.48±1.06 and 6.82±0.86 µg/mL against these enzymes. The LC-MS analysis identified the following base peaks (m/z): 433.56, 447.50, 469.67, and 561.43, corresponding to 4-di-(2-ethylhexyl) trimellitate, maritimetin-6-O-glucose, glycyrrhetinic acid, and isoflavone base, respectively. Molecular docking analyses indicate that the isoflavone base achieves the most favourable score of -9.1736 and -75239, while the standard acarbose recorded scores of -8.6791 and -8.9104 kcal/mol against α-amylase and α-glucosidase, respectively. This study highlights that the constituents within the ethyl acetate fraction exhibit promising antioxidant and antidiabetic properties, potentially aiding in diabetes management. These components have displayed capabilities in DPPH radical scavenging and metal chelation, as well as the inhibition of α-amylase and α-glucosidase activities. In conclusion, the findings from this investigation could significantly contribute to the development of effective therapies for oxidative stress and diabetes mellitus.

Keywords: *Anacardium Occidentale; molecular docking; antioxidant; alpha amylase; alpha glucosidase; diabetes mellitus*

INTRODUCTION

Diabetes mellitus refers to a group of metabolic disorders that are characterized by elevated blood sugar levels. This condition can arise from either excessive glucose production or insufficient insulin secretion [1].

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Diabetes is a chronic condition that currently has no known cure; however, it can be effectively managed through medication, a balanced diet, and regular exercise [2]. One approach to managing diabetes involves enzyme inhibition [3]. Diabetes is a long-lasting condition marked by elevated blood sugar levels, which can greatly affect overall health and result in serious long-term complications. These complications can affect various organ systems, resulting in problems such as hardened arteries, heart disease, and an increased risk of stroke. Individuals with diabetes may also experience infections, gangrene, foot ulcers, kidney disease, and nerve damage. This long-term damage is primarily caused by consistently elevated blood sugar levels, emphasizing the importance of effective diabetes management to prevent these negative outcomes [4], [5].

Enzymes responsible for digestion, such as alpha-glucosidase and alpha-amylase, cause a rise in blood glucose levels after meals [6]. Regulating the breakdown of carbohydrates in people with diabetes involves blocking these enzymes. Alpha-amylase and alpha-amylase break down complex carbohydrates into glucose and maltose. Drugs like acarbose and miglitol inhibit alpha glucosidase, preventing carbohydrate absorption in the small intestine. Despite potential side effects, these inhibitors are used to treat type 2 diabetes [7].

Antioxidants function as free radical scavengers, neutralising harmful free radicals to prevent or minimize cell and organ damage. As reducing agents, they remove free radical intermediates and prevent further oxidation [8]. The DPPH assay serves as a technique for evaluating the antioxidant potential of extracts obtained from medicinal plants. Chelation involves the binding of molecules to metal ions. Chelating medications remove harmful metal ions from intracellular or extracellular spaces by forming structures that can be easily eliminated from the body [9].

The cashew tree, *Anacardium occidentale*, is found in tropical regions of Africa, America, and Asia. Research has shown that specific parts of the tree have anti-diabetic properties. For example, studies have demonstrated that extracts from the stem bark have a hypoglycaemic effect in diabetic rats [10]. In addition, diabetic mice with impaired kidney function and abnormal protein metabolism have been treated with stem-bark extract [11]. The leaves and stem bark of the tree can also be used for the treatment of bacterial infections and inflammation.

Molecular docking is an essential computational method in the field of drug discovery, allowing scientists to find novel therapeutic agents and anticipate the interactions between ligands and their target proteins on a molecular scale [12]. It provides insights into structure-activity relationships without needing prior knowledge of the chemical structures of other target modulators [13]. This technique is crucial in structural molecular biology and the computational design of pharmaceuticals. The main goal of ligand-protein docking is to predict how a ligand will interact with a protein that has a known three-dimensional structure. Effective docking methods can explore complex molecular environments and use scoring functions to accurately evaluate potential docking results [12]. Furthermore, docking enables the virtual screening of extensive compound libraries, allowing researchers to prioritize their discoveries and develop structural theories regarding ligand interactions, which are essential for optimizing lead compounds [13].

The present research explored the antioxidant and antidiabetic properties of Anacardium occidentale leaf extract by evaluating its capacity to scavenge DPPH radicals, chelate metallic ions, and inhibit α-amylase and α-glucosidase. Furthermore, the study performed molecular docking of the most potent components and thoroughly analysed the interactions between the proteins and ligands.

METHODOLOGY

Sample Collection

Cashew leaves from the *Anacardium occidentale* species were obtained from the Botanical Garden of Biological Sciences at Bayero University (BUK) in Kano State, Nigeria. The collection was authenticated by Dr. Yusuf Nuhu, a plant taxonomist in the herbarium section, who assigned it the accession number BUKHAN0413. After being collected, the leaves were carefully rinsed under running tap water to remove any dirt, dust, or contaminants. Following this wash, they were placed in a shaded area with good air circulation to facilitate drying. This drying process lasted for two weeks, during which the leaves were periodically checked to ensure they were not exposed to direct sunlight, as this could affect their properties. The drying continued until the leaves achieved a constant weight, indicating that all moisture had been effectively removed.

Sample Preparation

The dried leaves were processed by pulverizing them with an electric blender, specifically the Silvercrest Multifunction Blender (Model: SC-1589). After blending, the resulting powder was stored in an airtight container, ensuring its preservation until it was needed for future work.

Sample Extraction

The methanol extract of *A. occidentale* leaves was obtained through a cold maceration method. Initially, 250 grams of powdered leaves were combined with 700 millilitres of methanol in a stoppered container and left to sit at room temperature for 72 hours, with regular agitation to enhance extraction. After this period, the mixture underwent filtration to separate the solid material, known as the marc, from the liquid. The marc was then pressed to extract any residual liquid. The liquids collected from the filtration process were clarified by passing through the Whatman filter paper to eliminate impurities and solid particles, resulting in a clear extract. This methanol extract was subsequently concentrated to one-quarter of its original volume using a rotary evaporator at 100 °C and stored in a refrigerator for future use.

Fractionation Procedure

A crude methanol extract of *Anacardium occidentale*, weighing 5 g, was combined with 20 g of silica gel (60–120 mesh), which is suitable for column chromatography. To this mixture, 5 mL of n-hexane was added, and the components were stirred briefly to ensure proper mixing. The mixture was subsequently air-dried before being placed into a column chromatography system that measured 300 mm in diameter and 15 cm in height. After the column was properly packed and settled, various solvents were introduced in ascending order of polarity: n-hexane, chloroform, ethyl acetate, acetone, ethanol, and distilled water. In the procedure, 50 mL of each solvent was introduced into the column, and the resulting fractions were collected in distinct beakers. Once all fractions had been eluted, the solvent was evaporated to obtain dried fractions, specifically from ethyl acetate. These dried fractions were then analysed for their antioxidant properties and evaluated for enzyme inhibition.

Antioxidant Evaluation

(a) DPPH Scavenging Properties

Various fractions' radical scavenging activity (RSA) was assessed using a modified DPPH method of [14]. Each fraction at 100 to 600 µg/mL concentrations was mixed with a 0.1 mM DPPH solution, which is dark purple before adding the samples. After incubating in the dark at room temperature for 30 minutes, the absorbance was measured at 517 nm to determine the decline in absorbance, indicating the neutralization of DPPH radicals. Controls included a DPPH solution with methanol and methanol alone as a blank. The percentage of RSA was calculated by using Equation 1.

$$\%\ RSA\ of\ DPPH = \frac{A_0 - A_1}{A_0} \times 100 \qquad \ldots equation\ 1$$

The analysis measures absorbance values (A_0 for control and A_1 for test fractions) to evaluate radical scavenging activity (RSA) against varying concentrations of test fractions. The IC_{50} value, indicating the concentration needed to inhibit 50% of radical activity, was determined from a graphical plot. The most potent fraction, with the lowest IC_{50}, was further examined for its inhibitory effects on α-amylase and α-glucosidase, aiming to explore its potential in glucose regulation and diabetes management.

(a) Metal Chelating Properties

The study evaluated the chelation of Fe^{2+} ions using various fractions, following methods of [15] and [16] with slight modifications. Fractions were prepared at concentrations of 100 to 600 µg/mL and incubated with 2 mM ferrous chloride ($FeCl_2$) and 5 mM ferrozine, forming a coloured complex measurable at 562 nm using a spectrophotometer. A positive control with EDTA was included for comparison. The efficiency (percentage) of metal chelation was quantified using Equation 2.

$$\%\ Metal\ Chelation = \frac{A_0 - A_1}{A_0} \times 100 \qquad \ldots Equation\ 2$$

The absorbance values of a standard sample (A_0) and various test samples (A_1) were measured to evaluate metal chelation. A graph was created to plot the percentage of metal chelation against fraction concentration, enabling the determination of the IC_{50} value. The fraction with the lowest IC_{50}, indicating the highest potency, was further studied for its effects on α-amylase and α-glucosidase activities.

Inhibitory Potentials

(a) Alpha-Amylase Inhibitory Assay

The assay utilized a modified protocol of [17] to evaluate the inhibition of α-amylase. Fractions were prepared in a 20 mM sodium phosphate buffer (pH 6.9) at concentrations between 0.08 and 0.40 mg/mL. Each fraction (250 µL) was mixed with α-amylase solution (0.5 mg/mL) and incubated at 25°C for 10 minutes, followed by the addition of 1% starch solution for another 10 minutes. The reaction was terminated with dinitrosalicylic acid (DNSA), boiled, cooled, and diluted to 10 mL for absorbance measurement at 540 nm. Control experiments included a negative control with buffer, a positive control using acarbose, and a blank without the enzyme. Each test was performed in triplicate, with the spectrophotometer zeroed using the blank solution. The percentage inhibition was calculated using a Equation 3.

$$\%\ \alpha\text{-amylase Inhibition} = \frac{A_0 - A_f}{A_0} \times 100 \quad \ldots equation\ 3$$

The absorbance in the absence of fractions or acarbose was labelled as A_0, and A_f was the absorbance with tested fractions. A graph was constructed to show the percentage inhibition of α-amylase at various concentrations of the fractions and acarbose. This led to determining the IC_{50}, the concentration needed for 50% enzyme inhibition. The fraction with the highest effectiveness was identified by the lowest IC_{50} value and further analysed using LC-MS.

(a) Alpha-Glucosidase Inhibitory Assay

The study evaluated the effects of various plant extracts on α-glucosidase activity using a modified method of [18]. Plant extracts at concentrations of 10, 30, 60, and 80 µg/mL were dissolved in a 20 mM sodium phosphate buffer (pH 6.9) and mixed with an α-glucosidase solution, followed by incubation at 37°C. After adding p-NPG solution and further incubation, the reaction was terminated with Na_2CO_3, and absorbance was measured at 405 nm. A negative control without extracts and a positive control with acarbose were included. The experiment was conducted in triplicate, and the percentage inhibition of α-glucosidase was calculated using Equation 4.

$$\%\ \alpha\text{-glucosidade Inhibition} = \frac{A_0 - A_f}{A_0} \times 100 \quad \ldots equation\ 4$$

In the equation, absorbance without the tested fraction (A_0) and with the tested fraction or acarbose (A_f) were determined to assess α-glucosidase inhibition. A graph was constructed to show the relationship between inhibition percentage and varying concentrations, from which the concentration that resulted in 50% inhibition (IC_{50}) was determined. The fraction with the lowest IC_{50}, indicating the highest efficacy, was chosen for LCMS analysis.

Protocol for LCMS Analysis

The LCMS analysis was performed using an LC Waters e2695 separation module, a PDA, and an ACQ-QDA mass spectrometer. Samples were prepared following modified protocols of [19], involving reconstitution in methanol and filtration through a 0.45 µm PTFE membrane filter. A 10.0 µL aliquot was injected into the LC system, utilizing a Sunfire C18 column at a flow rate of 1.0 mL/min and a temperature of 25°C. The mobile phase comprised 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The chromatography used a solvent ratio of 95:5 for the first 13 minutes, then switched to 5:95 for 13 minutes, and returned to 95:5 for 20 minutes. The PDA detector scanned wavelengths from 210 nm to 400 nm with a resolution of 1.2 nm and a sampling rate of 10 points/second. Mass spectrometry was performed with a scan range of m/z 100 to 1250, using an ESI source in both positive and negative ion modes. Key settings included a capillary voltage of 0.8 kV, a probe temperature of 600°C, a flow rate of 10 mL/min, and a nebulizer gas pressure at 45 psi. The mass spectrometer operated in automatic mode with a fixed fragmentation voltage of 125 V. Data processing was done with Empower 3, identifying compounds based on fragmentation patterns, which were then docked against α-amylase and α-glucosidase using MOE software.

Molecular Docking

The ligand structures were sourced from PubChem in 3D format and prepared in ChemSketch before being imported into the MOE database. In the database, hydrogen atoms were added, and partial charges were calculated using the MMFF94x force field. The structures were minimized until the RMS gradient reached 0.1 kcal/mol/Å. A refined protein model was used for docking with the AMBER14: EHT force field and Triangle Matcher docking algorithm, and each ligand conformation was evaluated using the London dG scoring function. The scoring approach used was GBVI/WSAdG. Starting with 30 poses for each tested substance, the top 10 were selected through rigid receptor docking for further refinement. An MDB file with the ligands facilitated automatic calculations during docking. After docking, the resulting poses were evaluated for top candidates based on scores, acceptable RMSD values, and favourable ligand-protein interactions, which were visualized with BIOVIA Discovery Studio 2024.

RESULTS AND DISCUSSIONS

RESULTS

Antioxidants

Table 1 displays the inhibitory concentrations of different fractions concerning DPPH radical scavenging and metal chelating activities at 50% (IC_{50}). The findings show that the ethyl acetate fraction has the greatest effectiveness, revealing the lowest IC_{50} values for both antioxidant indicators. However, its effectiveness remains lower compared to the standard substances, ascorbic acid and EDTA, respectively.

Table 1: Inhibitory concentration of 50% (IC_{50}) of various fractions against DPPH radical scavenging and metal chelating activities

Fractions	DPPH scavenging IC_{50} (µg/mL)	Metal chelating IC_{50} (µg/mL)
Ethanol	152.76±17.00	250.23±21.11
Aqueous	206.87±23.12	269.78±22.15
Acetone	336.13±26.98	279.17±22.50
Ethyl acetate	73.16±3.03	99.68±6.10
Chloroform	108.36±28.00	221.87±20.00
n-Hexane	366.12±28.93	282.45±22.23
Ascorbic Acid	68.97±2.08	-
EDTA	-	65.01±3.01

Inhibitory Activities of Digestive Enzymes

Table 2 displays the IC_{50} values for various fractions affecting α-amylase and α-glucosidase activities. The ethyl acetate fraction shows the lowest IC_{50} value, making it the most effective against these enzymes. However, acarbose, the standard drug, exhibits greater potency than any of the tested fractions.

Table 2: IC_{50} of fractions of against α-amylase and α-glucosidase activities

Fractions	α-amylase IC_{50} (µg/mL)	α-glucosidase IC_{50} (µg/mL)
Ethanol	76.28±8.20	77.88±08.11
Aqueous	78.82±12.20	82.90±06.32
Acetone	117.47±25.50	119.48±11.06
Ethyl acetate	10.20 ± 1.02	12.23±03.13
Chloroform	76.28±8.20	77.88±10.11
n-Hexane	117.82±20.10	128.20±15.32
Acarbose	9.48±1.06	6.82 ± 00.86

LCMS Analysis Result

Table 3 presented below shows the base peaks, retention durations, precise masses of the compounds, and the names of the identified compounds from the ethyl acetate fraction.

Table 3: Ethyl acetate fraction LC-MS negative mode (M-H) base peak and the corresponding exact mass of the components.

Retention Time (min)	Base Peak (m/z)	Exact compound Mass	Identified Compounds
5.584	433.56	434.6	2,4-Di-(2-ethylhexyl) trimellitate
7.571	447.50	448.4	maritimetin-6-O-glucoside
8.847	469.67	470.7	Glycyrrhetinic acid
11.319	561.43	562.5	Isoflavone base + 1O, 1MeO, O-Pen-Hex

The total ion chromatogram (TIC) in Figure 1 displays the components of the ethyl acetate fraction, showcasing the intensity of detected ions over time. Each peak represents different compounds, indicating their relative concentrations and elution times from the chromatographic column.

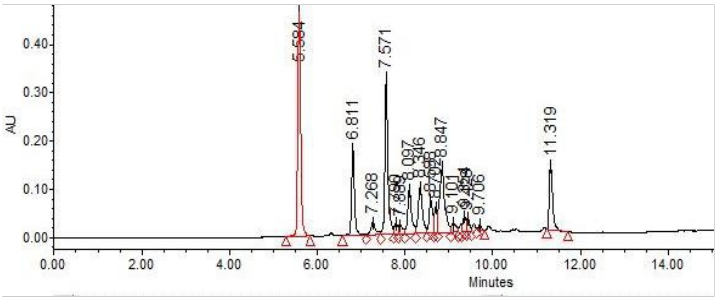

Figure 1: Total ion chromatogram (TIC) of ethyl acetate fraction components

The various structures of the identified components are illustrated in detail in Figure 2.

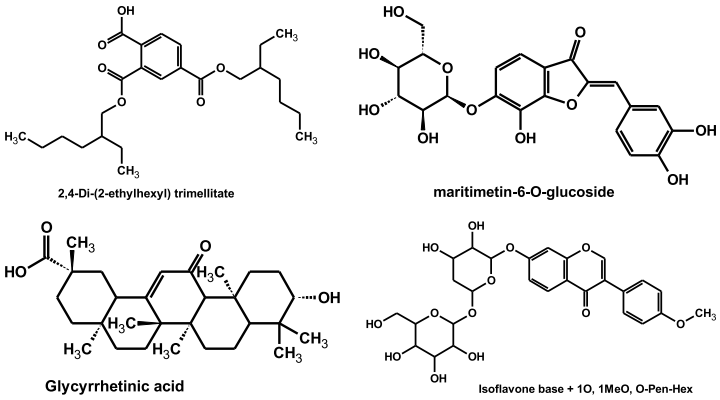

Figure 2: Structures of the identified compounds

Identification of Compounds from Database Library

The compounds derived from the LC-MS analysis (in negative ionization mode) base peak (m/z) of the ethyl acetate fraction of Anacardium occidentale, together with their docking scores for α-amylase and α-glucosidase, are shown in Table 4 below. The 3D molecular structures of the compounds utilized for docking were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Table 4: Docking scores of identified compounds and the standard drug, acarbose

Identified Compounds	PubChem CID	Docking Scores (S), (kcal/mol)	
		α-amylase	α-glucosidase
2,4-Di-(2-ethylhexyl) trimellitate	14048412	−7.7130	−8.2295
maritimetin-6-O-glucoside	45934452	−7.7600	−7.3314
Glycyrrhetinic acid	10114	−6.1403	−5.4752
Isoflavone base + 1O, 1MeO, O-Pen-Hex	139291975	−9.1736	−7.5239
Acarbose	41774	−8.6971	−8.9104

The diagrams presented in Figures 2 through 12 provide a comprehensive visualization of both the two-dimensional (2D) and three-dimensional (3D) interactions between the identified compounds (ligands) and the target proteins, α-amylase and α-glucosidase. These figures detail the docking results, highlighting how the ligands engage with the active sites of the enzymes. Additionally, the standard antidiabetic drug, acarbose, is included in this analysis for comparative purposes. The illustrations depict the spatial orientation, bonding interactions, and structural conformations that underscore the potential efficacy of these compounds in modulating enzyme activity.

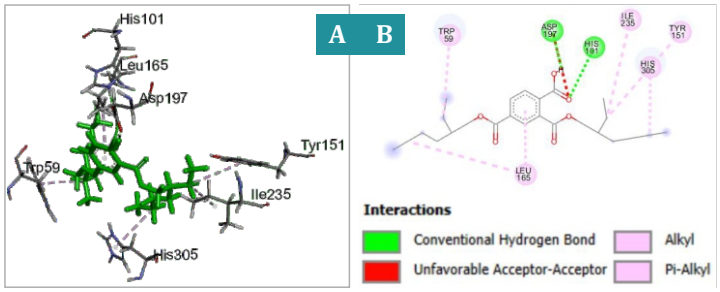

Figure 3: Interaction of 2,4-di-(2-ethylhexyl) trimellitate (CID: 14048412) in 3D (a) and 2D (b) structures with α-amylase (1OSE)

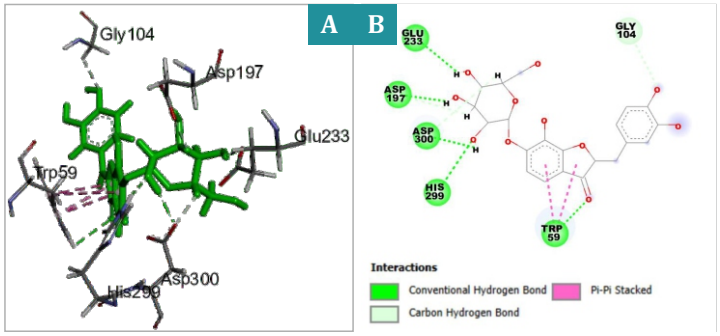

Figure 4: Interaction of Maritimetin-6-O-glucoside (CID: 45934452) in 3D (a) and 2D (b) structures with α-amylase (1OSE)

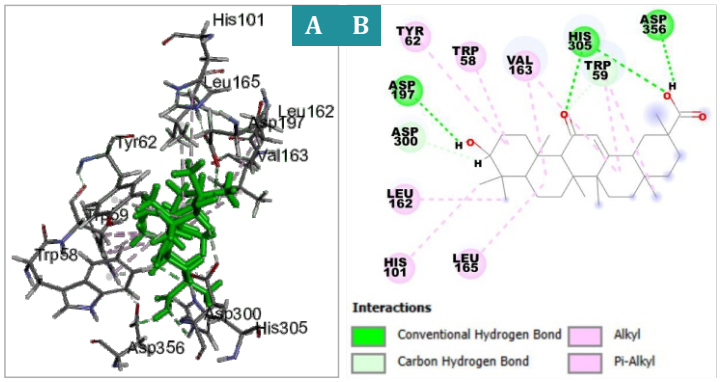

Figure 5: Interaction of Glycyrrhetinic acid (CID: 10114) in 3D (a) and 2D (b) structures with α-amylase (1OSE)

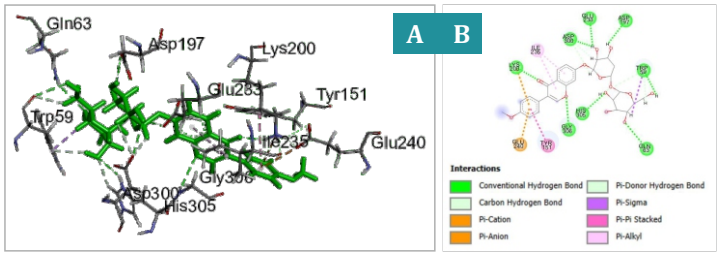

Figure 6: Interaction of Isoflavone Base + 1O, 1MeO, O-Pen-Hex (CID: 139291975) in 3D (a) and 2D (b) structures with α-amylase (1OSE)

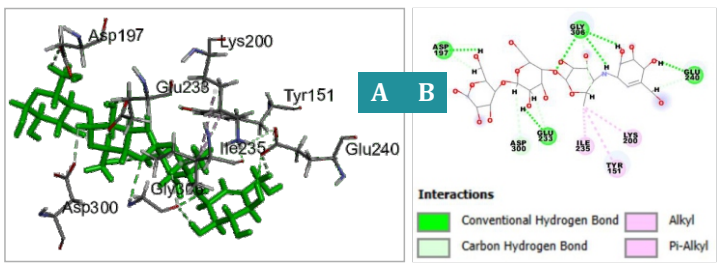

Figure 7: Interaction of Acarbose (CID: 41774) in 3D (a) and 2D (b) structures with α-amylase (1OSE)

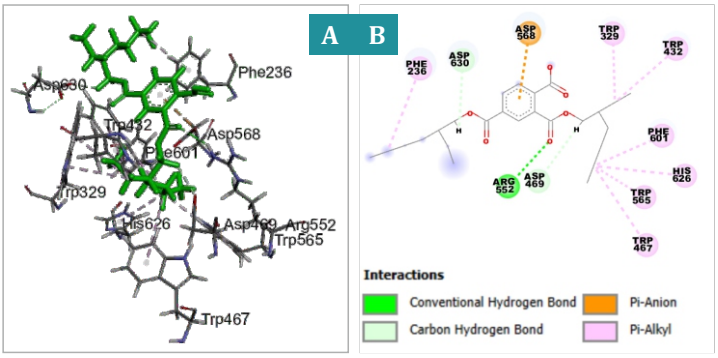

Figure 8: Interaction of 2,4-di-(2-ethylhexyl) trimellitate (CID: 14048412) in 3D (a) and 2D (b) structures with α-glucosidase (3W37)

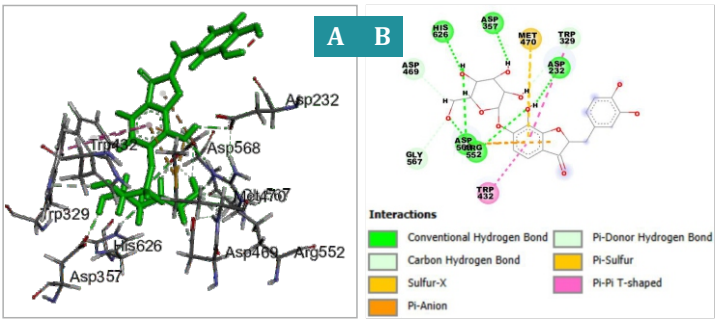

Figure 9: Interaction of Maritimetin-6-O-glucoside (CID: 45934452) in 3D (a) and 2D (b) structures with α-glucosidase (3W37)

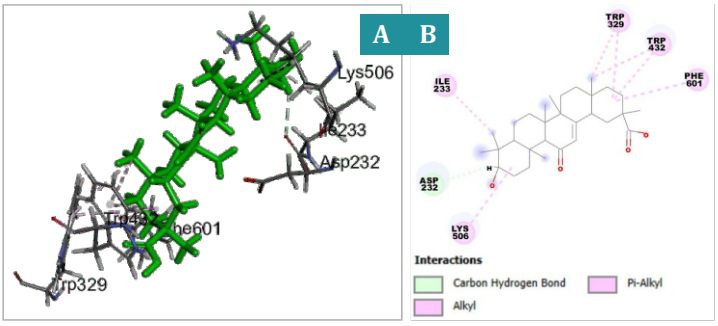

Figure 10: Interaction of Glycyrrhetinic acid (CID: 10114) in 3D (a) and 2D (b) structures with α-glucosidase (3W37)

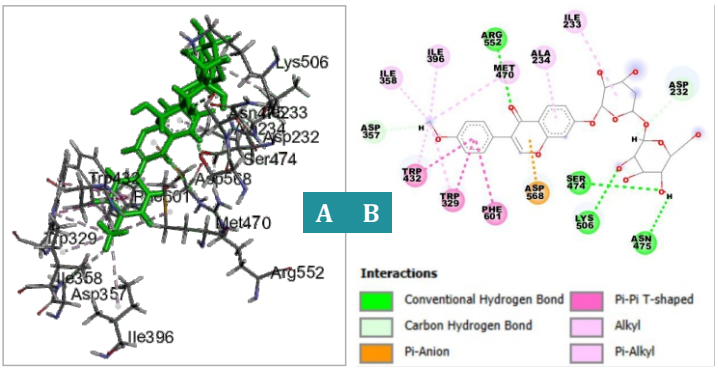

Figure 11: Interaction of Isoflavone Base + 1O, 1MeO, O-Pen-Hex (CID: 139291975) in 3D (a) and 2D (b) structures with α-glucosidase (3W37)

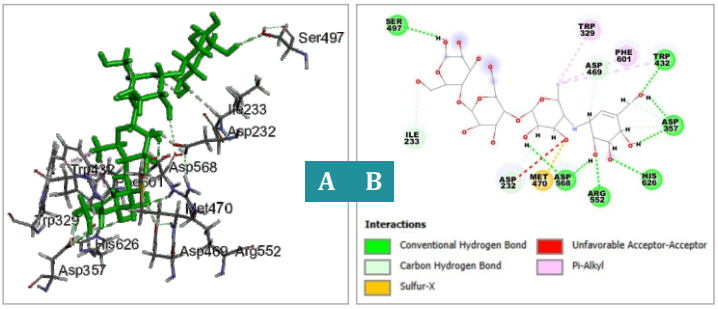

Figure 12: Interaction of Acarbose (CID: 41774) in 3D (a) and 2D (b) structures with α-glucosidase (3W37)

Table 5: Summary of Ligand-protein interactions of components of ethyl acetate fraction of Anacardium occidentale methanolic extract

S/N	Compound Name	Name/Type of Interaction with α-amylase	Name/Type of Interaction with α-glucosidase
1.	2,4-Di-(2-ethylhexyl) trimellitate	H-Bond: Asp197, His101 Alkyl & pi-Alkyl: Try59, Ile235, Tyr151, His305, Leu165	H-Bond: Arg552 C-H Bond: Asp630, Asp469 pi-Anion: Asp568 pi-Alkyl: Phe236, Trp329, Trp432, Phe601, His626, Trp565, Trp467
2.	maritimetin-6-O-glucoside	H-bond: Asp233, Asp197, Asp300, His299, Trp59 C-H Bond: Gly104 pi-pi stacked: Trp 59	H-Bond: His626, Asp357, Asp232, Asp568, Arg552 C-H Bond: Gly567, Asp357, Asp232, Asp568, Arg552 Pi-Donor-H Bond: Trp329 Sulphur-X/pi-Sulphur: Met470 pi-Anion: Asp568 pi-pi T-shaped: Trp432, Trp329
3.	Glycyrrhetinic acid	H-Bond: Asp179, His305, Asp356 C-H Bond: Trp59, Asp300 Alkyl/pi-alkyl: Leu165, His101, Leu162, Tyr62, Trp58, val163, Trp59	H-Bond: Asp232 Alkyl/pi-Alkyl: Lys506, Ile233, Trp329, Trp432, Phe601
4.	Isoflavone base + 1O, 1MeO, O-Pen-Hex	H-Bond: Lys200, Asp300, Glu233, Asp197, Trp59, Gln63, His305, Gly306 C-H Bond/pi-donor: Trp59, Asp300 pi-cation/pi-anion: Gly240, Lys200 pi-sigma: Trp59 pi-pi stacked: Tyr151 pi-alkyl: Ile235	H-Bond: Arg552, Ser474, Lys506, Asn475 C-H Bond: Asp357, Asp232 pi-Anion: Asp568 pi-pi T-shaped: Trp432, Trp329, Phe601 Alkyl/pi-Alkyl: Ile358, Ile396, Met470, Ala234, Ile233, Trp432, Trp329
5.	Acarbose	H-Bond: Asp197, Gly306, Glu240, Glu233 C-H Bond: Asp300, Gly306, Glu233, Glu240, Asp197 Alkyl/pi-alkyl: Ile235, Tyr151, Lys200	H-Bond: Ser497, Trp432, Asp357, His626, Arg552, Asp568 C-H Bond: Ile233, Asp469, Asp232 Sulphur-X: Met470 pi-Alkyl: Trp329, Phe601

The interactions between the proteins, α-amylase and α-glucosidase, and the ligands found in the ethyl acetate fraction of Anacardium occidentale are summarized in Table 5. Among the ligands, the isoflavone base shows the highest number of interactions with both α-amylase and α-glucosidase, exceeding the interaction count of the standard drug, acarbose.

Discussion

Enzymes such as α-amylase and α-glucosidase perform essential functions in the metabolic process of carbohydrates, specifically in their hydrolysis, which can lead to elevated levels of glucose in the bloodstream after meals [20]. Dysregulation of these crucial enzymes may significantly contribute to the pathogenesis of diabetes mellitus and the associated complications [6]. Moreover, the clinical manifestation of diabetes is increasingly recognised to be exacerbated by the accumulation of free radicals, alongside reactive oxygen species (ROS) and reactive nitrogen species (RNS) within the organism. These reactive species are notorious for inducing oxidative stress, leading to cellular damage and further complicating the disease's progression [21].

Our research highlights that various extracts from the cashew tree (*Anacardium occidentale*) exhibit robust antioxidant capabilities, indicating their potential to alleviate the complications linked with diabetes mellitus. This observation aligns with findings by [22], which emphasize that incorporating antioxidant-rich foods into one's diet may reduce the risk of developing type 2 diabetes. Among the examined extracts, the ethyl acetate fraction emerged as the most promising, demonstrating the highest levels of antioxidant activity [23], suggesting that it may harbour the most effective bioactive components for countering oxidative stress.

The methanolic extract derived from the cashew tree, scientifically known as Anacardium occidentale, underwent a series of fractionation processes to isolate its components. Among the various fractions tested for their ability to inhibit the activities of the enzymes α-amylase and α-glucosidase, both critical targets in managing carbohydrate metabolism, the ethyl acetate fraction stood out as the most potent inhibitor.

This promising result suggests that the ethyl acetate fraction likely contains the most effective bioactive compounds that specifically target these enzymes, which play vital roles in carbohydrate digestion. In comparison, acarbose, a well-known pharmaceutical agent used to treat type 2 diabetes, showed a higher level of inhibitory potency than any individual fractions from the Anacardium occidentale extract. Nevertheless, the significant inhibitory activity displayed by the ethyl acetate fraction underscores its potential as a natural alternative for managing postprandial hyperglycaemia. Additionally, this observation aligns with the findings of [24] & [25], which highlight how extracts from the roots of *Anacardium occidentale* possess the capability to mitigate hyperglycaemia and oxidative stress in an in vitro setting. These insights collectively point to the essential role that this plant extract could play in the development of complementary strategies for glycaemic control and the promotion of overall metabolic health.

The compounds identified through the LC-MS analysis, including 2,4-di(2-ethylhexyl)trimellitate, maritimetin-6-O-glucose, glycyrrhetinic acid, and various isoflavone derivatives, are primarily classified as flavonoids. This classification is reinforced by the findings of [26], which revealed a significant correlation between the structural characteristics, specifically the quantity and arrangement of hydroxyl groups, and the biological activities of flavonoids. These structural features play a crucial role in enhancing their antioxidant capabilities and antidiabetic effects. Notably, flavonoids are particularly effective at scavenging DPPH radicals, a common method for assessing antioxidant potency. Additionally, further research by [27] highlighted the role of polyphenolic compounds as potent antioxidants. According to their study, these compounds can effectively neutralize DPPH radicals, thereby mitigating oxidative stress. Furthermore, they have been shown to exhibit significant antidiabetic properties by inhibiting key digestive enzymes, specifically α-amylase and α-glucosidase, which are critical in carbohydrate metabolism. This dual action of flavonoids not only aids in reducing oxidative damage but also plays a vital role in managing blood sugar levels, showcasing their potential health benefits in preventing diabetes and related metabolic disorders.

The molecular docking analysis identified four novel compounds that inhibit the digestive enzymes α-amylase and α-glucosidase, with the isoflavone base showing the strongest inhibitory effects due to its hydroxyl group arrangement. While these compounds demonstrate significant potential, acarbose remains more potent. The study enhances the understanding of how molecular structure relates to biological function, providing insights for developing new diabetes treatments. These results align with the work of [28], who emphasized the considerable potential of two flavonoids that interact with α-glucosidase via in-silico analysis. Additionally, certain components from the extract exhibit antioxidant properties and effective inhibitory activities against the targeted enzymes, highlighting their potential in managing diabetes mellitus and oxidative stress.

LIST OF ABBREVIATIONS

2D: Two-Dimensional
3D: Three-Dimensional
CID: Compound identification number
DNSA: 3,5-dinitrosalicylic acid
DPPH: 2,2-diphenyl-1-picrylhydrazyl
EDTA: Ethylenediaminetetraacetic acid
IC_{50}: Inhibitory concentration of 50% reduction in activity
LC: Liquid Chromatography
LCMS: Liquid Chromatography Mass Spectrometry
MOE: Molecular Operating Environment
MS: Mass Spectrometry
PDB: Protein Data Base
pNPG: p-nitrophenyl-β-D-glucopyranoside
PTFE: polytetrafluoroethylene
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
RSA: Radical scavenging activity
TIC: Total ion Chromatogram

DECLARATIONS

Ethics Approval

The ethical guidelines for in-vitro and in-silico research were carefully followed, and the methods employed were approved by the relevant authorities.

Conflict of Interest

The authors state that they hold no conflicting interests.

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Authors' Contributions

Lawal TA performed the investigative techniques, managed the research initiative, and prepared the work for publication. Specifically, he authored the initial draft. He made significant contributions to the revision of this work, including its planning and execution. Sholadoye QO supplied the study resources, conducted statistical analysis, and was involved in the typesetting, editing, formatting, and investigation processes. Ononamadu CJ engaged in the in-silico investigation gathered data for additional analysis and assisted in revising the manuscript. Musa B performed the LC-MS analysis, interpreted the findings, and helped with manuscript writing. Akinjoko VO was involved in the investigation of the in-vitro α-amylase and α-glucosidase inhibitory assays. She also contributed to the typesetting, editing, and formatting of this manuscript. Segba DE participated in the evaluation of in-vitro DPPH radical scavenging and metal chelating assays. He also assisted in the typesetting, editing, and formatting of this manuscript.

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