Lawal, J Biomed & App Sci FUD (2025) 4:1
www.jobas.fud.edu.ng
ISSN: 2814-3221 (print), ISSN: 2955-0874 (online)

JOBASFUD
Journal of Biomedical and Applied Sciences

RESEACH ARTICLE

SCREENING OF METHANOL EXTRACT OF *PICRALIMA NITIDA* SEEDS FOR A-AMYLASE AND A-GLUCOSIDASE INHIBITORS THROUGH IN-VITRO AND MOLECULAR DOCKING ANALYSES

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Abstract

Background: The activities of α-amylase and α-glucosidase have been linked to postprandial hyperglycaemia, which, if not properly controlled, can lead to diabetes mellitus. Diabetes mellitus is a metabolic disorder characterised by persistent high blood sugar levels due to the body's inability to produce or effectively use insulin. If this condition is not properly managed, it can lead to various complications such as cardiovascular disease, nephropathy, neuropathy, retinopathy, and an increased risk of infections. Synthetic drugs used to treat this condition have been found to have harmful effects on the kidneys, heart, and liver functions in some patients. Consequently, there is a need to find natural products with minimal side effects to manage this disorder. This study examined various fractions of methanol extract from *Picralima nitida* seeds for their ability to inhibit α-amylase and α-glucosidase. **Results:** The most potent fraction was identified as PF6 from column chromatography, with the least IC_{50} of 48.17±1.61 and 25.32±1.81 µg/mL against α-amylase and α-glucosidase, respectively. Sub-fraction 4 (SF4) obtained from PF6 showed the highest potency with an IC_{50} of 52.48±6.54 and 30.20±2.02 against α-amylase and α-glucosidase, respectively. The standard drug acarbose exhibited IC_{50} values of 25.34±1.22 and 22.28±2.03, making it more potent than the fractions and Sub-fractions against the two enzymes. FTIR analysis revealed the presence of C=O, O-H, C-O, C=C, C-N, Ar-CH, C=S, Ar-O-C, CH_2, and CH_3 functional groups. LCMS analysis tentatively identified genipin, genistein, cirantin, bezafibrate, and usnic acid in SF4. Molecular docking analysis revealed that cirantin and genistein are potent inhibitors for α-amylase and α-glucosidase, with docking scores of -7.9928 and -8.8359 kcal/mol, respectively. At the same time, acarbose exhibited scores of -9.0021 and -8.3366 kcal/mol against the two enzymes. The identified compounds have exhibited strong molecular docking interactions with α-amylase and α-glucosidase, indicating their potential to inhibit these two enzymes effectively. **Conclusion**, further research should be conducted on these compounds, particularly cirantin and genistein, as they are potentially used as antidiabetic agents due to their inhibitory effects on carbohydrate digestive enzymes.

Keywords: *Picralima nitida*; alpha amylase; alpha glucosidase; molecular docking; diabetes mellitus

INTRODUCTION

Enzymes responsible for breaking down carbohydrates, such as α-amylase and α-glucosidase, have been linked to high blood sugar levels after meals, which could potentially lead to diabetes if not properly managed (Li *et al.*, 2022). Diabetes mellitus is a metabolic condition characterized by unusually high levels of glucose in the bloodstream (hyperglycaemia). Diabetes is associated with various complications including retinopathy, heart disease, nephropathy, neuropathy, stroke, and lower limb amputation (WHO, 2023). Current antidiabetic medications have a limited effect on post-meal blood sugar spikes and, therefore, do not reduce blood glucose levels to the baseline (Lawal *et al*., 2021). The enzymes alpha-glucosidase and alpha-amylase, which are responsible for breaking down carbohydrates during digestion, lead to an increase in blood glucose levels after meals (Akmal *et al*. 2024, Lawal, 2022). Managing the breakdown of carbohydrates in individuals with diabetes requires inhibiting these enzymes (Li *et al.*, 2022). Medications like acarbose and miglitol work by blocking

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alpha-glucosidase, thereby preventing the absorption of carbohydrates in the small intestine. Despite potential side effects, these inhibitors are employed for treating type 2 diabetes (Akmal *et al*. 2024). The current research focused on examining the hypoglycaemic properties of the aqueous extract of *Picralima nitida* seeds. The study looked into the inhibitory effects of the fractions and Sub-fractions of the extract on the activities of α-amylase and α-glucosidase.

Picralima nitida, a member of the Apocynaceae family, is a medicinal plant commonly found in the tropical rainforests of Africa (Igwe & Mgbemena, 2014; Alhassan *et al*., 2017). In Nigeria, *Picralima nitida* is known as 'Osi-igwe' among the Igbo-speaking people and as 'Akuama' or 'Abere' among the Yoruba-speaking people (Nkere & Iroegbu, 2005; Kazeem *et al*, 2013). This plant has been traditionally used for medicinal purposes and has gained significant attention due to its pharmacological properties. Studies have indicated that the extracts derived from the seeds of *Picralima nitida* exhibit the potential to lower blood sugar levels (Alhassan *et al*., 2017). The coconut water extract obtained from the seeds of *Picralima nitida* has shown promising potential in lowering blood glucose levels. This effect has been observed in studies involving alloxan-induced diabetic albino rats (Salihu *et al*., 2009) and alloxan-induced diabetic rabbits (Adegoke & Oloyede, 2013). These findings suggest that the coconut water extract from *Picralima nitida* seeds may hold value as a potential natural solution for managing blood glucose levels (Alhassan *et al*., 2017).

The computational method known as molecular docking is widely used in drug discovery (Agu *et al.*, 2023). It helps identify new therapeutic compounds, predict interactions between ligands and targets at a molecular level, and explore structure-activity relationships (Pinzi & Giulio, 2019). Importantly, this method does not require prior knowledge of the chemical structure of other target modulators (Saikia and Bordoloi 2019). Molecular docking plays a crucial role in structural molecular biology and the computer-aided design of drugs. Specifically, ligand-protein docking aims to predict how a ligand binds to a protein with a known three-dimensional structure. Effective docking methods can navigate complex spaces and use a scoring function to rank potential docking accurately (Meng *et al.*, 2011). Additionally, docking can be used to conduct virtual screening on large compound libraries, prioritize the results, and propose structural hypotheses regarding how the ligands interact with the target (Stanzione *et al.*, 2021).

The current study investigated the antidiabetic potential of *Picralima nitida* seeds by assessing their ability to inhibit α-amylase and α-glucosidase and by conducting molecular docking of the most active components to interpret the protein-ligand interactions.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh *Picralima nitida* seeds were obtained locally and identified at the herbarium units of the Biological Sciences Department, Bayero University, Kano. They were then assigned reference numbers BUKHAN622 after identification. The seeds were thoroughly washed and dried in the shade until they reached a constant weight. The dried seeds were ground into a coarse powder using a mortar and pestle. Fifty grams (50g) of the powder was soaked in 500 mL of methanol. The mixture was allowed to stand for 72 hours with intermittent shaking and filtered through a mesh cloth. The liquid mixture was further filtered using Whatman filter paper, grade 1. The resulting crude extract (filtrate) was then dried in a water bath at 40°C to produce a solid residue. The solid residue was stored in a plastic container labelled "methanol extract of *Picralima nitida* seed (MEPNS)".

Fractionation

Column Chromatography

Silica gel (mesh size 120G) was used as the stationary phase, with various solvent combinations as the eluent. A slurry of 200 g silica gel and 500 mL hexane was poured into the column, leaving the tap open to let the solvent flow into a beaker below. After packing, the tap was closed, and the setup was left undisturbed for 24 hours. The clear solvent on top was drained down to the silica gel meniscus, and the column flow rate was recorded.

Five grams (5 g) of the dried extracts were mixed thoroughly with 10 g of silica gel and then gently layered on the top of the column. Elution of the column was done with various solvent combinations of varying polarity. The following solvent systems were used in the elution process; hexane: ethyl acetate 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100. For each solvent combination, the elution was done until each solvent ratio became clear. The eluted fractions were collected in an aliquot volume of 50 mL.

Thin Layer Chromatograph, TLC (Analytical)

The column chromatography fractions were pooled using analytical thin-layer chromatography (TLC). Each fraction was applied to a pre-coated aluminium silica gel plate (Macherey-Nagel, Germany, 2023) and developed in a glass chromatographic tank using methanol as the solvent. A sample spot was placed 1.0 cm from the base, and the plate was dried before being immersed in a solvent-saturated tank. The solvent ascended until it reached about ¾ of the plate length, after which it was dried again. The plate was then sprayed with a mixture of p-anisaldehyde, glacial acetic acid, and sulfuric acid and heated at 105°C for 10 seconds to visualize the bands. The colour reaction was observed, and the relative retention factor (Rf) was calculated. Fractions with the same number of spots and Rf values were combined. Each pooled fraction was tested for its inhibitory activity against α-amylase and α-glucosidase.

Preparative Thin Layer Chromatograph, PTLC

The most active pooled fraction was subjected to PTLC, where it was further separated into various Sub-fractions. The separated compounds were viewed under UV light, and the fluorescent areas were scrapped, eluted with a suitable solvent (methanol), air-dried, and weighed. The PTLC process was designed as follows using the method of Davies & Johnson, 2007 with slight modification.

The glass plates (20 x 20cm) were cleaned and dried, then coated uniformly (thickness, 0.25 mm) with a slurry of silica gel in water (1:2, w/v). They were left to dry at room temperature for 30 minutes, then heated in an oven at 100°C for 1 hour to activate the adsorbent. Finally, the dried plates were stored in a desiccator over silica gel to prevent moisture adsorption. For sample application, a distance of about 2.5 cm from one end of the glass plate was left. The sample and standards were applied as streaks using a syringe, ensuring the adsorbent did not flake off at the application point. The sample was allowed to dry for repeated spotting.

A developing solvent (chloroform-methanol, 1:1) was poured into the tank to a depth of 1.5 cm to develop the chromatogram. The tank was then covered and allowed to stand for at least an hour to ensure the atmosphere within the tank became saturated with solvent vapour (i.e. equilibration. The cover plate was removed, and the thin layer plate with the sample applied was placed vertically in the tank. The cover plate was then replaced, and compounds were separated as the solvent moved upward. Once the solvent reached the top of the plate, it was removed from the tank, dried, and proceeded for the identification of the separated compounds.

To identify the compounds, the separated compounds were examined under UV light and appeared as blue, green, and black spots. The plates were inspected to locate compounds that absorb UV light or fluoresce. The fluoresced areas were extracted using methanol as the solvent, dried, and weighed.

Screening for Alpha Glucosidase Inhibition

The inhibitory effect of each fraction and Sub-fraction on α-glucosidase activity was determined following the method described by Dej-Adisai *et al.,* (2021) with slight modifications. Various concentrations (10, 30, 60, and 80 µg/mL) of the fractions were prepared in a 20 mM sodium phosphate buffer solution with a pH of 6.9. 100 µL of each fraction concentration was incubated with 100 µL of α-glucosidase solution (1.0 U) at 37 °C for 10 minutes. The mixture was then incubated with 100 µL of 10 mM p-NPG solution at 37 °C for another 10 minutes. The reaction was then stopped by adding 5 mL of 20 mM Na_2CO_3 and the absorbance was measured at 405 nm. The procedure was repeated without the fraction (using buffer instead) as a negative control. A positive control was also carried out where the fraction was replaced with the standard drug, acarbose. The absorbance was converted to the amount of *p*-nitrophenol released using a *p*-nitrophenol standard curve. The enzyme activity was determined using Equation 1.

$$\text{A unit of α-glucosidase activity} = \frac{pNP(mg) \times DF}{T \times E(mg)} \quad ...Equation\ 1$$

Where pNP is the amount of p-nitrophenol released, DF is the dilution factor, T is the reaction time, and E is the amount of the enzyme (α-glucosidase) in the reaction mixture.

The percentage of α-glucosidase activities was determined from the Equation 2.

$$\text{α-glucosidase activity}(\%) = \frac{A_{in}}{A_{uin}} \times 100 \quad ....Equation\ 2$$

Where A_{in} is the activity of the inhibited enzyme and A_{uin} is the activity of the uninhibited enzyme.

A plot of percentage enzyme activity was made against the concentrations of the fractions and the concentration

of the fraction that inhibits 50% enzyme activities (IC_{50}) was determined graphically.

SCREENING FOR α-AMYLASE INHIBITION

This assay was carried out using a method of Alp *et al.*, (2023) with slight modification. The fractions ' different concentrations (0.08, 0.16, 0.24, 0.32, and 0.40 mg/mL) were prepared in 20 mM sodium phosphate buffer solution, pH 6.9. Exactly 250 µL of each extract concentration was incubated with 250 µL of α-amylase solution (0.5 mg/mL) at 25°C for 10 minutes. The mixture was thereafter incubated with 250 µL of 1% starch solution at 25°C for another 10 minutes. The reaction was then quenched by adding 500 µL of DNSA, boiled for 5 minutes, cooled to room temperature, made up to 10 mL with distilled water and its absorbance was taken at 540 nm. The procedure was repeated without the extract (i.e., by replacing the fraction with buffer-negative control). Positive control was carried out as described above, and a positive control was carried out, but the extract was replaced with acarbose. A blank was also prepared in the same manner but with the enzyme replaced by the buffer. The absorbance was converted to the amount of maltose released using a maltose standard curve, and enzyme activities were determined using Equation 3.

$$\text{A unit of α-amylase activity} = \frac{M\left(mg\right) \times DF}{T \times E\left(mg\right)} \quad \textit{...Equation 3}$$

The percentage of enzyme activities was determined using Equation 4.

$$\text{α-amylase activity} = \frac{A_{in}}{A_{uin}} \times 100 \quad \textit{....Equation 4}$$

Where A_{in} is the activity of the inhibited enzyme and A_{uin} is the activity of the uninhibited enzyme.

A plot of percentage enzyme activity was made against the concentrations of the fractions and the concentration of the fractions that corresponded to 50% enzyme activities (Inhibitory concentration of 50% enzyme activities; IC_{50}) was determined graphically.

LCMS Analysis Protocol and Identification of Compounds

A standard method using the LC Waters e2695 separation module with W2998 PDA and coupled to ACQ-QDA MS is employed for LCMS analysis. The samples were analysed using a liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by Piovesana *et al.,* (2018) with some modifications. The fraction sample was reconstituted in methanol and filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter. After filtration, 10.0 µL of the filtrate was injected into the LC system and allowed to separate on a Sunfire C18 5.0µm 4.6mm x 150 mm column. The run was conducted at a 1.0 mL/min flow rate, with sample and column temperature at 25°C. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient as explained thus:

The chromatography maintained a ratio of A to B at 95:5 for 1 minute, then at 5:95 for 13 minutes, and back to 95:5 until 20 minutes (Table 1). The PDA detector was set to scan from 210nm to 400nm with a resolution of 1.2nm and a sampling rate of 10 points per second. Mass spectra were obtained with a scan range from m/z 100 to 1250, using the ESI source in positive and negative ion modes, a capillary voltage of 0.8kV for both positive and negative modes, a probe temperature of 600°C, a flow rate of 10mL/min, and a nebuliser gas pressure of 45 psi. The MS was set to automatic mode with a fragmentation voltage of 125V. The data was processed using Empower 3. Compounds were identified based on base peak m/z generated using the MassBank Europe Mass Spectral Database library (https://massbank.eu/MassBank/Search). The identified compounds from MS were docked against α-amylase and α-glucosidase using MOE software.

Molecular Docking

The structures of ligands were prepared by ChemSketch software and imported into the MOE database, where structures were converted into 3D format by adding hydrogen atoms, partial charge calculation with MMFF94x forcefield, and by minimizing the structures until the RMS gradient was reached to 0.1 kcal/mol/Å. The refined protein model was taken for docking, which was performed with AMBER14: EHT force field and Triangle Matcher docking algorithm, and each conformation of ligand was scored with the London dG scoring function.

The scoring method was GBVI/WSAdG, and the top 10 poses for each tested substance were chosen from a pool of 30 poses using rigid receptor docking as the refining process. The MDB file for the examined ligands was then supplied to the application, and the calculations for the

ongoing docking were carried out automatically. After everything was done, the resulting poses were analysed, and the best ones with the largest scores, reasonable RMSD values, and better ligand−protein target interactions were picked and kept for subsequent studies.

RESULTS AND DISCUSSION

Results

The results for the fractions (F1 to F35) obtained through column chromatography, along with the pooled fractions (PF1 to PF7) and the corresponding number of spots, are summarized in Table 1. Fractions that exhibited the same number of spots and Rf values, as determined by analytical TLC analysis, were combined into pooled fractions PF1 to PF7. These pooled fractions (PF1 to PF7) were then evaluated for their inhibitory effects against α-amylase and α-glucosidase.

Table 1: Pooled Fractions Obtained from Column Chromatography

MEPNS Fraction	Pooled Fraction	No of Spot with Similar Rf
F1 – F5	PF1	0
F6 – F11	PF2	5
F12 – F18	PF3	6
F19 – F25	PF4	2
F26 – F29	PF5	3
F30 – F32	PF6	4
F33 – F35	PF7	3

The outcomes of inhibitory activities of the pooled fractions against α-glucosidase and α-amylase, as indicated by their respective IC_{50} values, are presented in Table 2. Among these fractions, PF6 is the most potent, with IC_{50} values of 25.32±0.84 for α-glucosidase and 48.17±1.61 for α-amylase. The PF6 was further subjected to PTLC analysis.

Table 2: IC_{50} of Pooled Fractions

Pooled Fractions	IC_{50} (µg/mL)	
	α-Glucosidase	α-Amylase
PF1	-	-
PF2	321.12±9.70	480.00±13.05
PF3	463.21±12.44	541.75±16.06
PF4	263.43±5.77	335.18±11.17
PF5	160.93±4.33	132.32±3,14
PF6	25.32±1.84	48.17±1.61
PF7	102.42±3.42	179.05±57.97

Table 3 shows the Sub-fractions (SF1 – SF4) obtained from PTLC analysis. The retardation factors (R_f) and percentage yield of the respective Sub-fractions were depicted. Sub-fraction 4 (SF4) has the lowest R_f (0.78) but the highest percentage yields (38.08%). The Sub-fractions were subjected to inhibitory activities against α-glucosidase and α-amylase.

Table 3: Fractions Obtained From PTLC

Sub-fractions of PF6	Retardation Factor (Rf)	% Yield
SF1	0.94	15.07
SF2	0.92	18.09
SF3	0.86	25.76
SF4	0.78	38.08

Table 4 depicts the inhibitory activities (IC_{50}) of Subfractions (SF1 – SF4) against α-glucosidase and α-amylase. Acarbose, a standard drug used as a positive control, is more potent than the sub-fractions against the two enzymes. The most potent sub-fraction (SF4) was subjected to FTIR analysis and LCMS analysis.

Table 4: IC_{50} of Sub-fractions of PF6

Sub-fractions of PF6	IC_{50}(µg/mL)	
	α-Glucosidase	α-Amylase
SF1	76.28±8.20	272.60±32.21
SF2	117.82±20.10	250.17±23.21
SF3	117.47±25.50	254.45±31.76
SF4	30.20±2.02	52.48±6.54
Acarbose	22.28±2.03	25.34±1.22

The outcomes of the FTIR analysis of the most potent sub-fractions are depicted in Table 5.

Table 5: Functional Groups of Compounds Present in SF4 using FTIR

Functional Group	Peak (cm^{-1})
C=O	1693
O–H	3249
C–O	1078
C=C	1618
C–N	1587
C-Cl	785
Ar–CH	3191
Ar–O–C	1089
CH_2, CH_3	29259, 2880

Figure 1 shows the chromatogram of SF4 analysed using LCMS. The x-axis represents retention time, and the y-axis indicates the absorbance unit, which corresponds to the detector response.

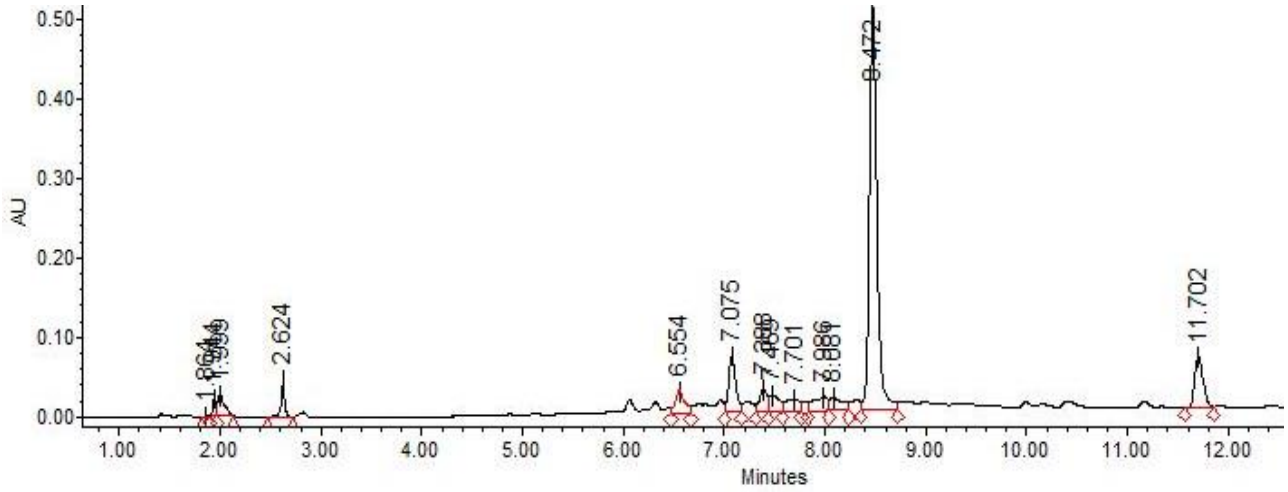

Figure 1: LCMS Chromatogram of SF4

Table 6 displays the retention time and base peak (m/z) from the analysis of SF4 using LCMS (negative mode). It also includes the potential actual mass and potential compounds based on a database library.

Table 6: Possible Components of SF4 from LC-MS Analysis (negative mode) and Database Library

Retention time	Base peak (m/z)	Actual mass	Tentative compound
1.864	225.085	226.085	Genipin
6.554	431.342	432.342	Genistin
7.075	609.352	610.352	Cirantin
7.469	360.246	361.246	Bezafibrate
11.702	343.134	344.134	Usnic acid

The chemical structures of the identified compounds are depicted in Figure 2.

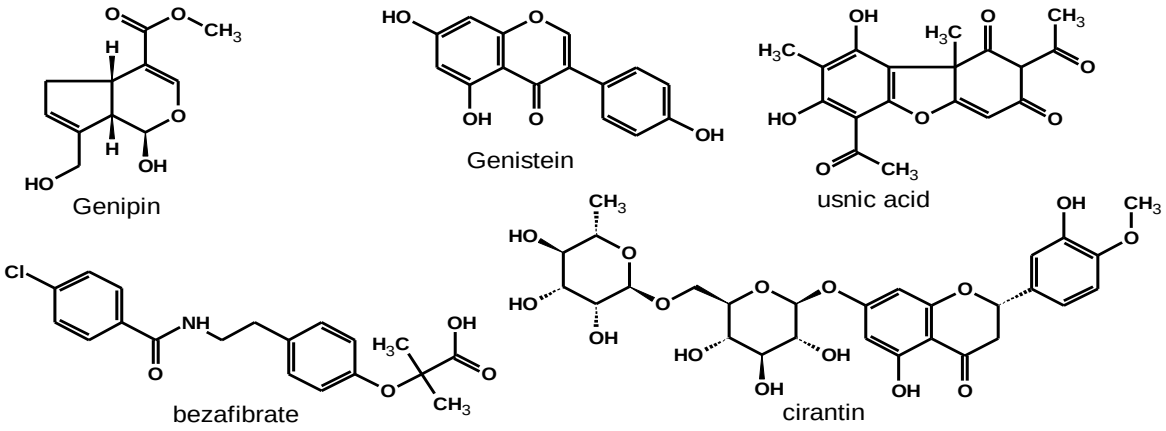

Figure 2: Chemical structures of the identified compounds

The docking scores of the potential compounds with the respective α-amylase and α-glucosidase are presented in Table 7. The CID refers to the compound identification number in the PubChem database. Acarbose is used as the positive control standard. Cirantin achieved the highest docking score (−7.9928) against α-amylase, although not as high as acarbose (−9.0021). On the other hand, Genistin has the highest docking score (−8.8359) against α-glucosidase, slightly greater than the standard drug, acarbose (−8.3366).

Table 7: Docking scores of the identified compounds with α-amylase and α-glucosidase

CID	Tentative compound	Docking Score (kcal/mol)	
		α-Amylase	α-Glucosidase
442424	Genipin	−53895	−54528
5281377	Genistin	−68605	−8.8359
10621	Cirantin	−7.9928	−7.9056
39042	Bezafibrate	−6.9217	−68659
5646	Usnic acid	−5.9203	−6.1302
41774	Acarbose	−9.0021	−8.3366

Figures 3 to 13 depict the interactions between the components (ligands) obtained and α-amylase and α-glucosidase. Interactions between acarbose and α-amylase and α-glucosidase are included for comparison.

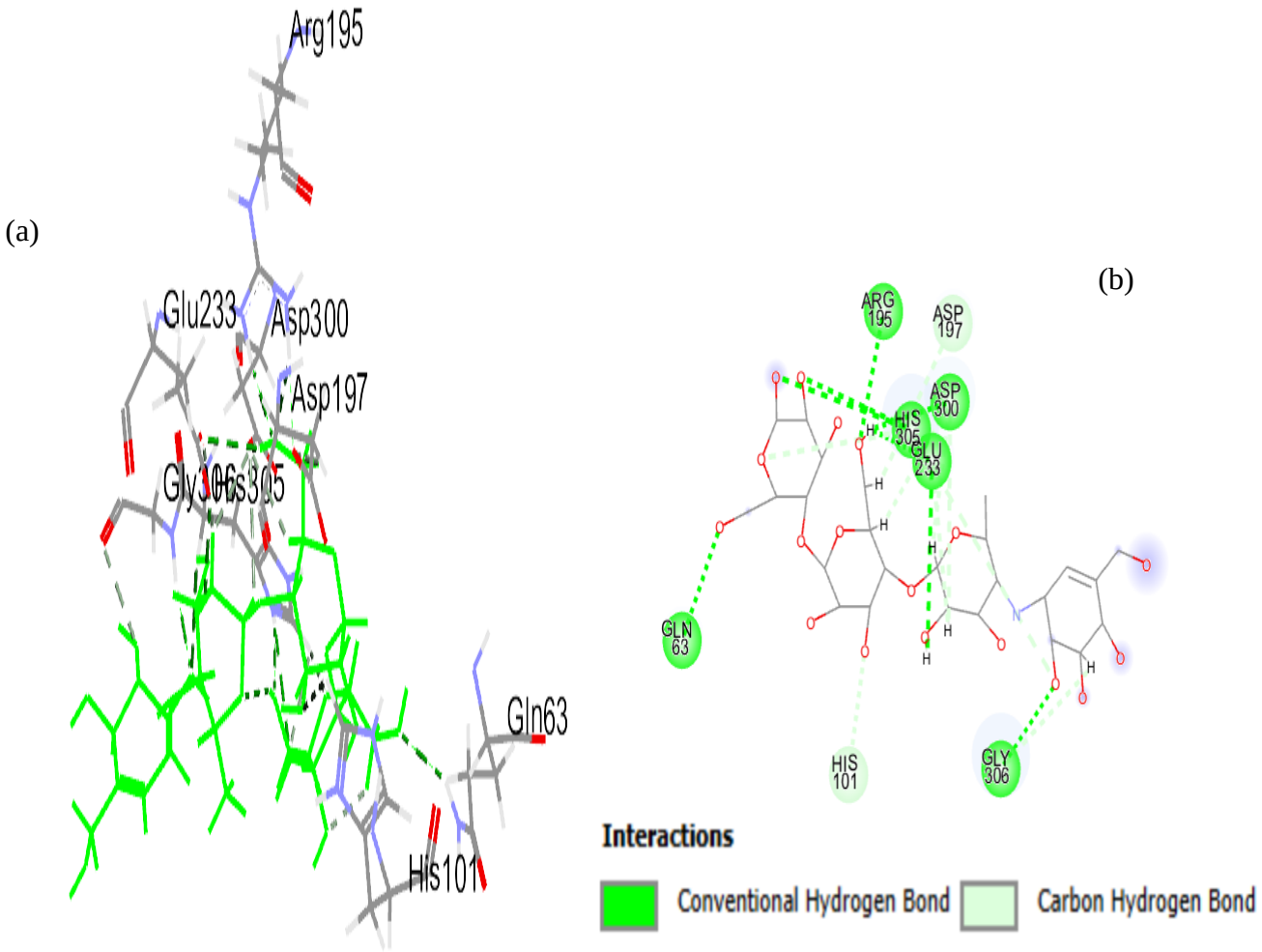

Figure 3: Interaction between Acarbose (CID:41774), the standard drug, and α-amylase in 3D (a) and 2D (b) conformations

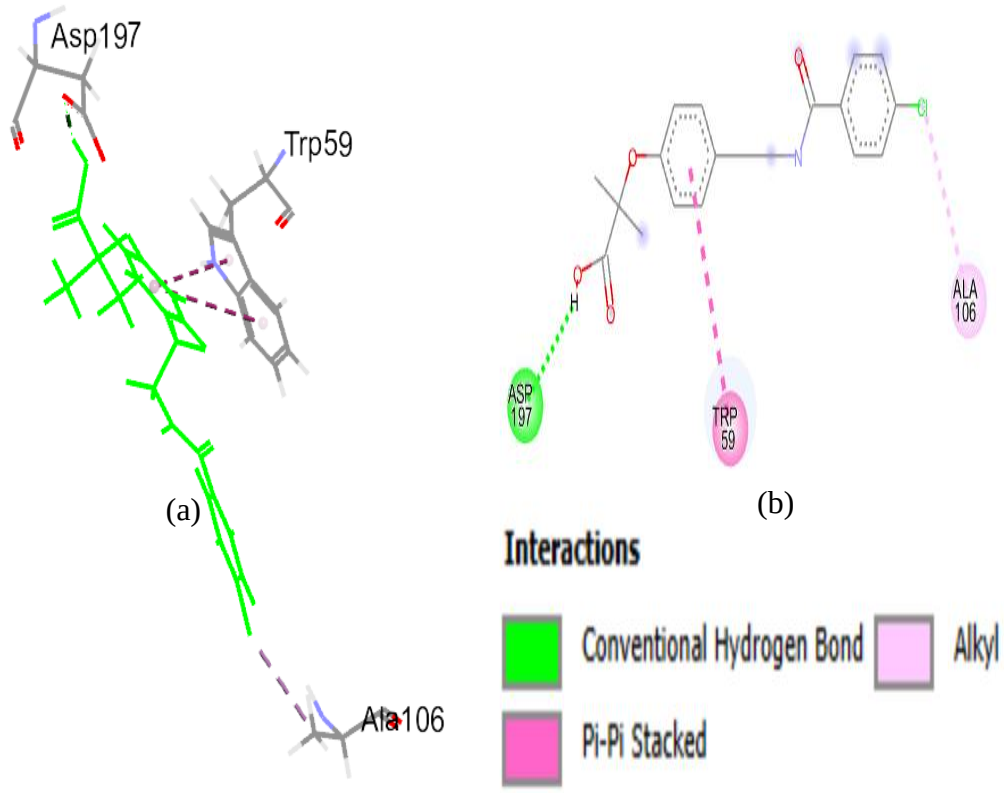

Figure 4: Interaction between Bezafibrate (CID: 39042) and α-amylase in 3D (a) and 2D (b) conformations

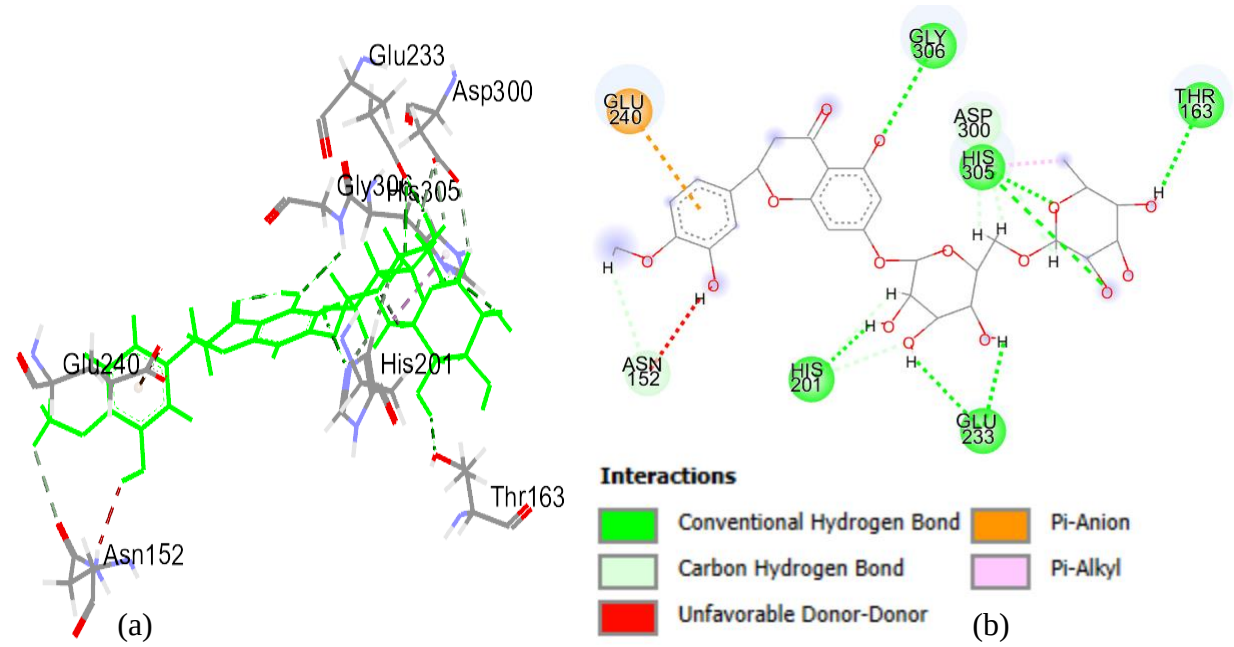

Figure 5: Interaction between Cirantin (CID: 10621) and α-amylase in 3D (a) and 2D (b) conformations

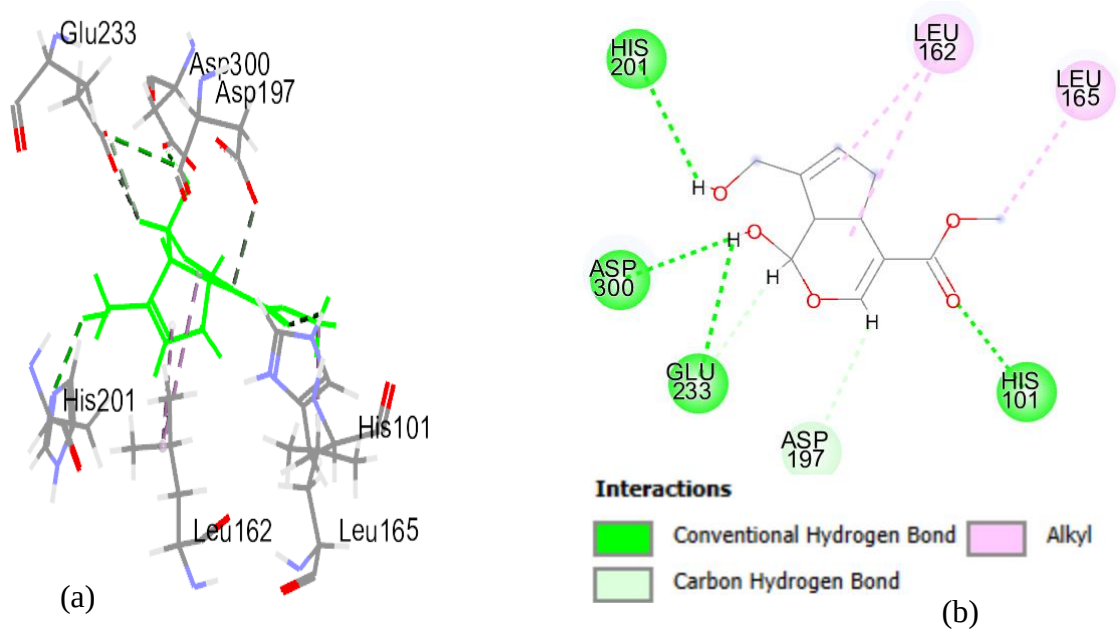

Figure 6: Interaction between Genipin (CID: 442424) and α-amylase in 3D (a) and 2D (b) conformations

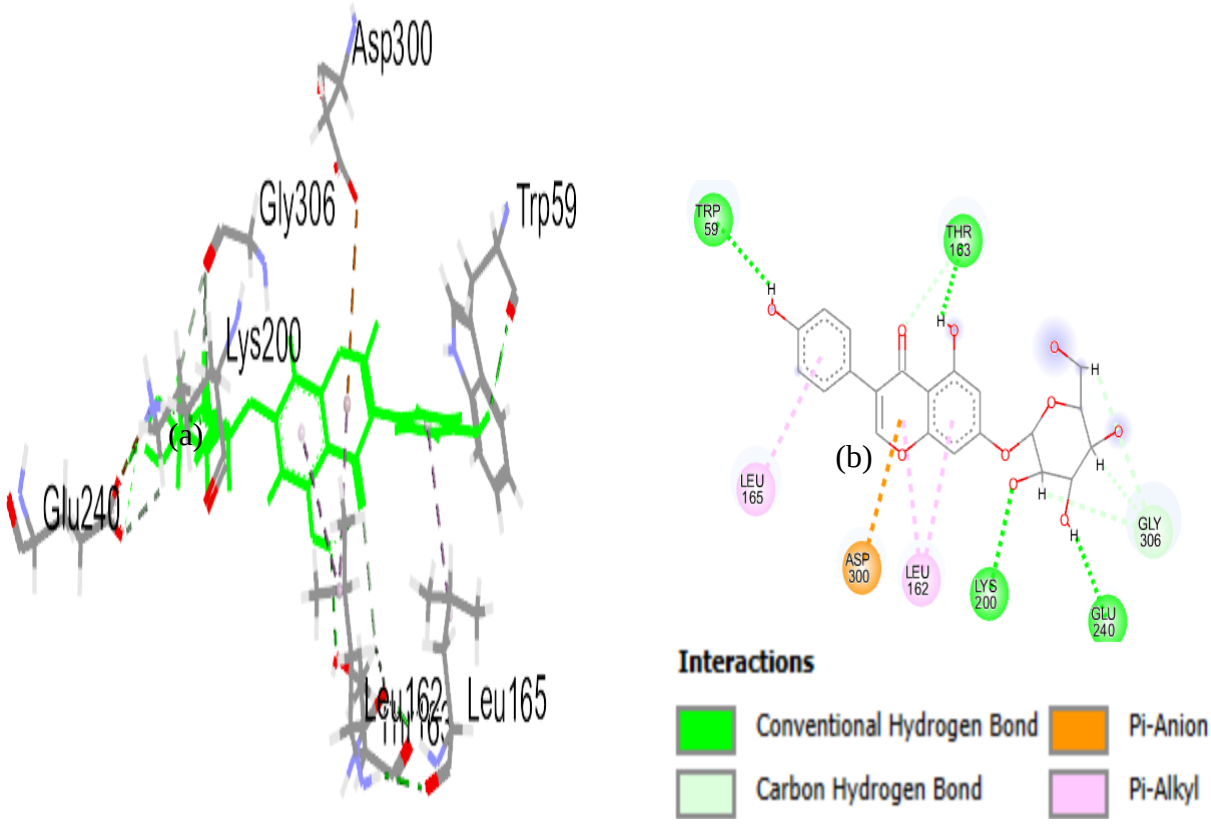

Figure 7: Interaction between Genistin (CID: 5281377) and α-amylase in 3D (a) and 2D (b) conformations

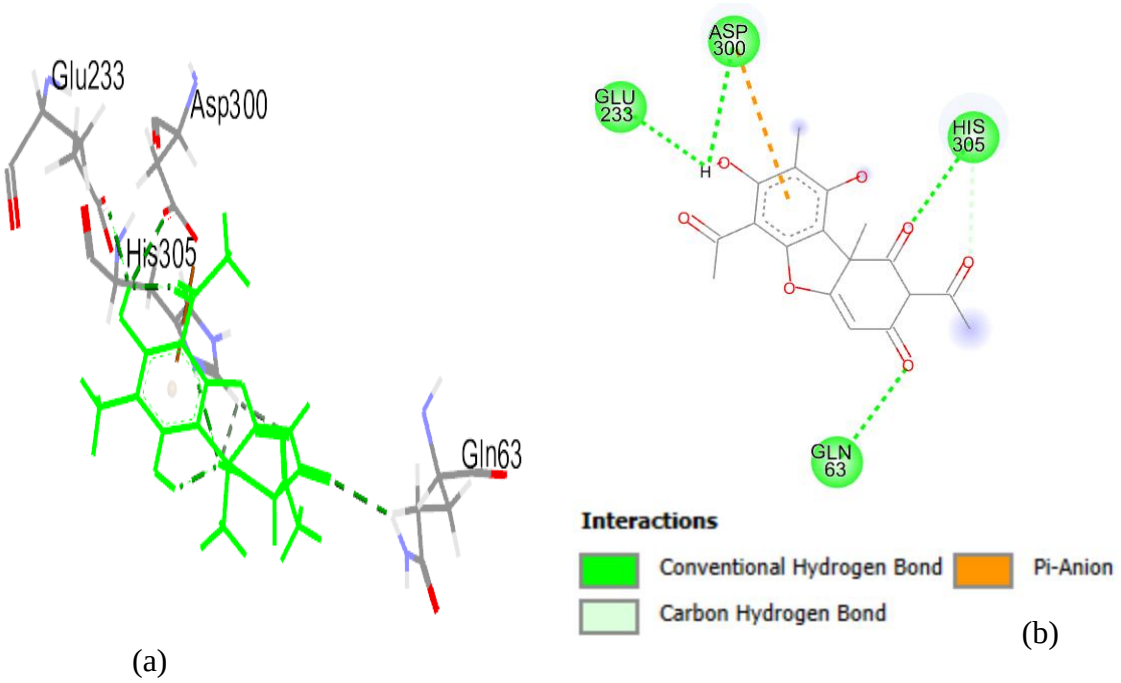

Figure 8: Interaction between Usnic acid (CID: 5646) and α-amylase in 3D (a) and 2D (b) conformations

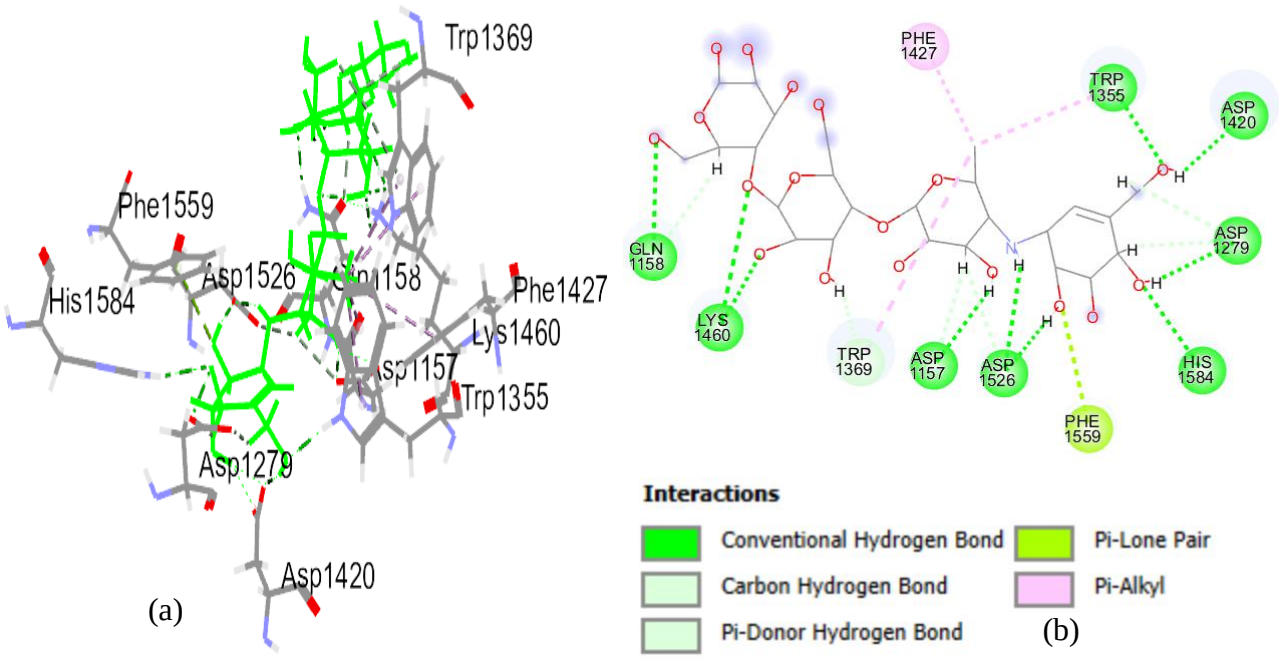

Figure 9: Interaction between Acarbose (CID: 41774) and α-glucosidase in 3D (a) and 2D (b) conformations

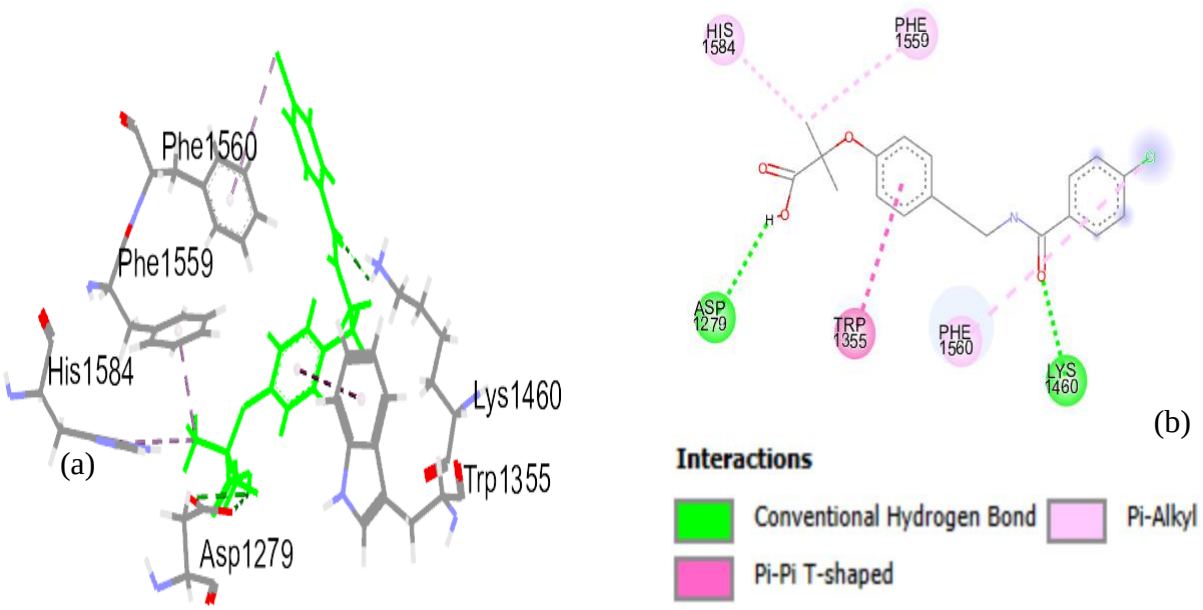

Figure 10: Interaction between Bezafibrate (CID:39042) and α-glucosidase in 3D (a) and 2D (b) conformations

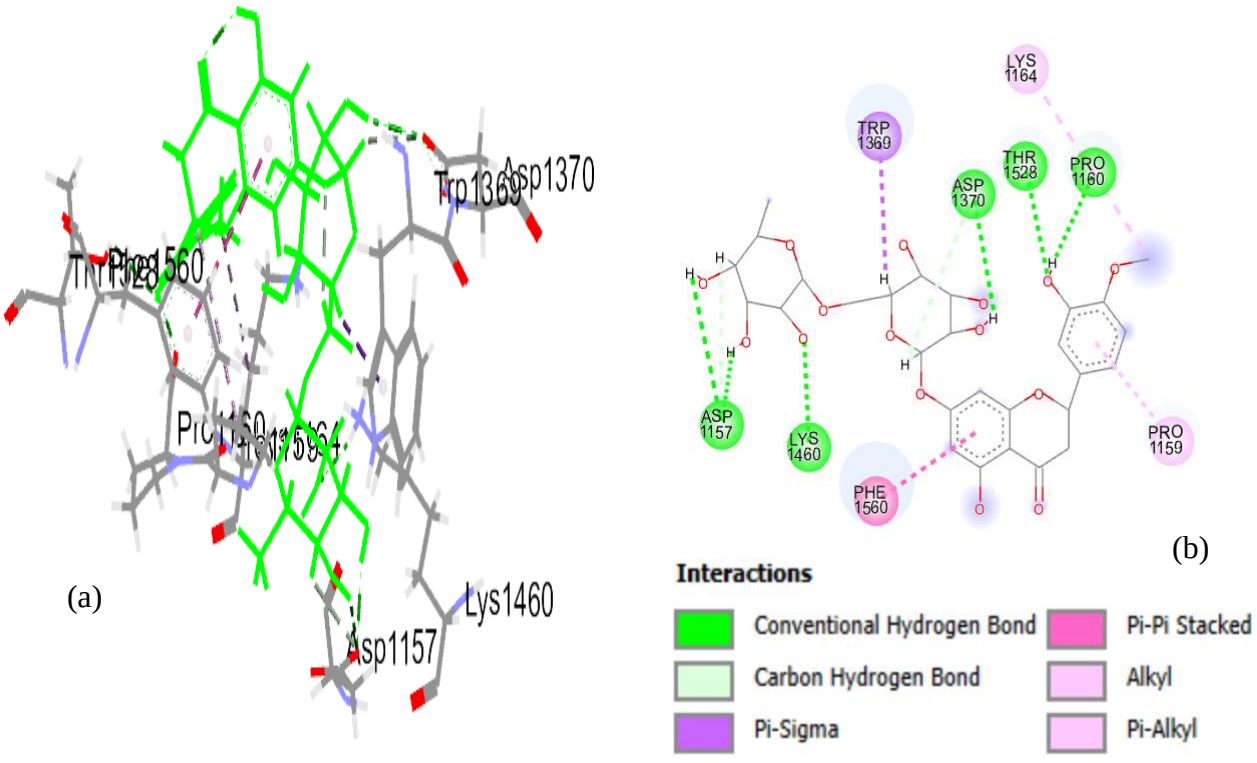

Figure 11: Interaction between Cirantin (CID: 10621) and α-glucosidase in 3D (a) and 2D (b) conformations

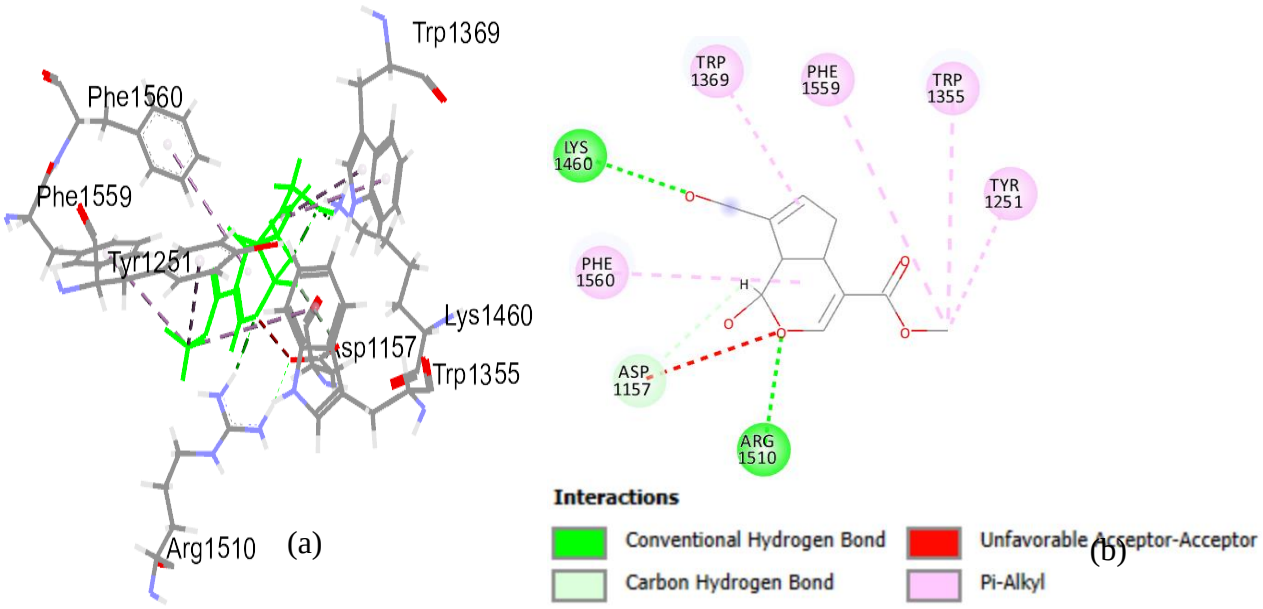

Figure 12: Interaction between Genipin (CID: 442424) and α-glucosidase in 3D (a) and 2D (b) conformations

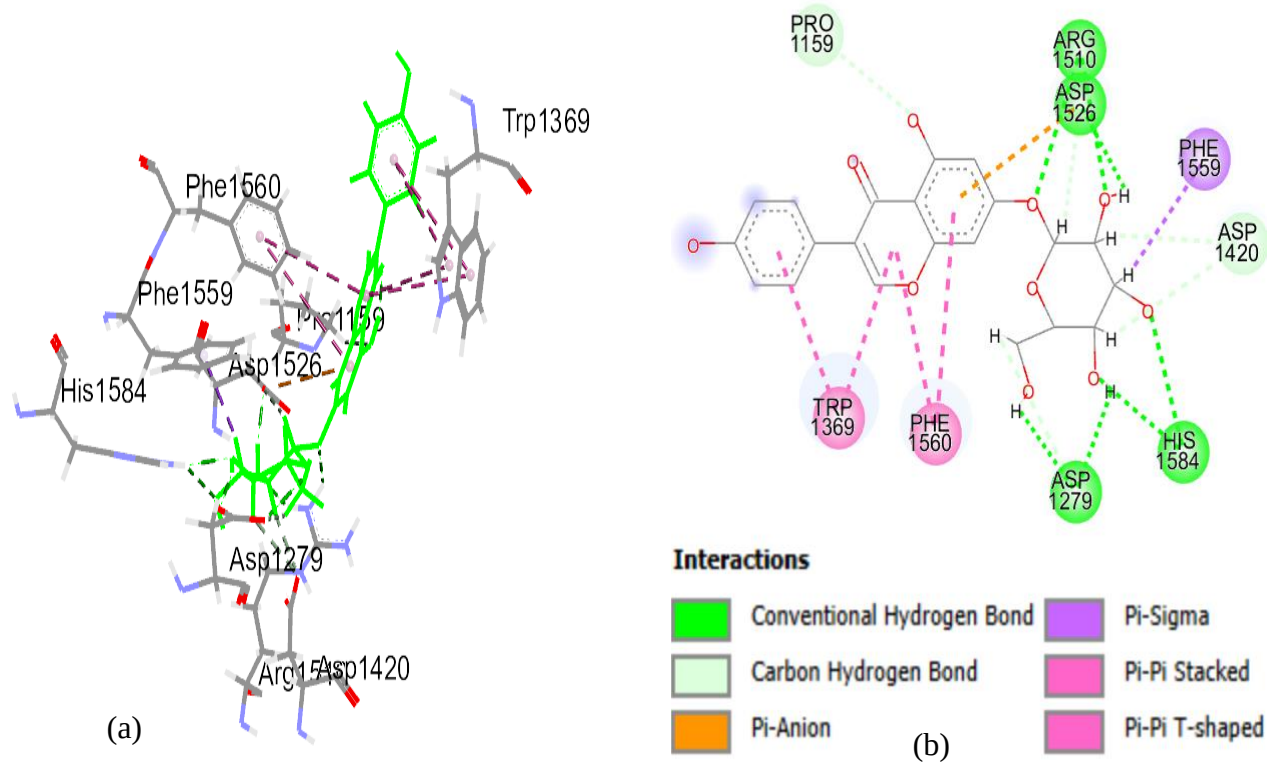

Figure 13: Interaction between Genistin (CID: 5281377) and α-glucosidase in 3D (a) and 2D (b) conformations

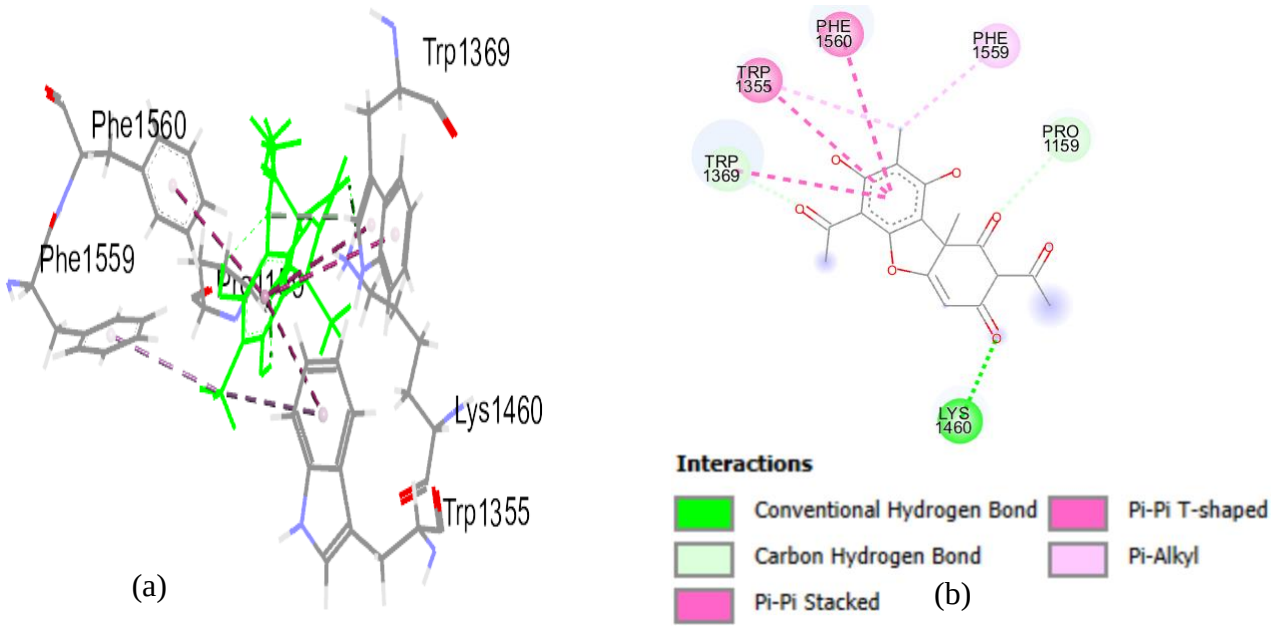

Figure 14: Interaction between Usnic acid (CID: 5646) and α-glucosidase in 3D (a) and 2D (b) conformations

The summary of the various ligand-protein interactions is illustrated in Table 8. Cirantin has the highest number of hydrogen bonds for both α-amylase and α-glucosidase.

Table 8: Summary of the types of interactions between components of MEPNS and α-amylase and α-glucosidase.

S/N	Components	Interactions and Types	
		α-amylase	**α-glucosidase**
1.	Cirantin	**H-Bond:** Gly306, His305, Thr163, Glu233, His201 **C-H Bond:** Asp300 **pi-Anion:** Glu240 **pi-Alkyl:** His305	**H-bond:** Asp1370, Thr1528, Pro1160, Asp1157, Lys1460 **C-H Bond:** Asp1157, Asp1370 **pi-Sigma:** Trp1369 **pi-pi stacked:** Phe1560 **Alkyl/pi-Alkyl:** Pro1159, Lys1164
2.	Genipin	**H-Bond:** His201, Asp300, Glu233, His101 **C-H Bond:** Asp197 **Alkyl:** Leu162, Leu165	**H-Bond:** Lys1460, Arg1510 **C-H Bond:** Asp1157 **pi-Alkyl:** Trp1369, Phe1559, Trp1355, Tyr1251, Phe1560
3.	Genistin	**H-Bond:** Trp59, Thr163, Lys200, Glu240 **C-H Bond:** Gly306 **pi-Anion:** Asp300 **pi-Alkyl:** Leu165, Leu162	**H-Bond:** Asp1279, His1584, Asp1526 **C-H Bond:** Pro1159, Asp1420 **pi-Anion:** Asp1526 **pi-Sigma:** Phe1559 **pi-pi Stacked/pi-pi T-shaped:** Trp1369, Phe1560
4.	Usnic acid	**H-Bond:** Glu233, Asp300, His305, Gln63 **C-H Bond:** His305 **pi-Anion:** Asp300	**H-Bond:** Lys1460 **C-H Bond:** Pro1159, Trp1369 **pi-pi Stacked/T-shaped:** Trp1355, Phe1560 **pi-Alkyl:** Phe1559
5.	Bezafibrate	**H-Bond:** Asp197 **pi-pi stacked:** Trp59 **Alkyl:** Ala106	**H-Bond:** Asp1279, Lys1460, **pi-Alkyl:** His1584, Phe1559, Phe1560 **pi-pi T-shaped:** Trp1355
6.	Acarbose	**H-Bond:** Arg195, Asp300, His305, Glu233, Gln63, Gly306 **C-H Bond:** Asp197, His101	**H-Bond:** Gln1158, Lys1460, Asp1157, Asp1526, His1584, Asp1279, Asp1420, Trp1355 **C-H/pi Donor H-Bond:** Trp1369, Gln1158, Asp1157, Asp1526, Asp1279 **pi-Alkyl:** Phe1427, Trp1355, Trp1369 **pi-lone pair:** Phe1559

DISCUSSION

The activities of α-amylase and α-glucosidase are associated with high blood sugar levels after meals, which, if uncontrolled, can lead to diabetes mellitus (Lawal, 2022). Poorly managed diabetes can lead to complications such as heart disease, kidney damage, nerve issues, eye damage, and increased vulnerability to infections (Gong *et al.*, 2020). This investigation involved fractionating the methanol extract of *Picralima nitida* into different partitions, and each partition was screened for its α-amylase and α-glucosidase inhibitory activities. The partition that exhibited the most potent inhibitory activities (Table 4) was further analysed using LC-MS (Liquid Chromatography-Mass Spectrometry). The base peak generated by LCMS (Figure 1 & Table 6) was subjected to compound identification through a database library to determine the specific compounds in the partition showing the highest inhibitory activities. FTIR analysis result (Table 5) confirms the presence of functional groups of the compounds identified.

The compounds identified in the study (Table 2) — genistein, genipin, usnic acid, cirantin (also called hesperidin), and bezafibrate — are attracting attention for their significant potential in managing hyperglycaemia. These compounds may exert their effects primarily through the inhibition of key carbohydrate digestive enzymes, specifically α-amylase and α-glucosidase. Genipin, a compound derived from the fruit of the gardenia plant, has emerged as a particularly powerful agent in the treatment of type II diabetes, demonstrating promising results in studies conducted on murine models (Wu et al., 2023). Its mechanism of action may involve the modulation of glucose metabolism, which is crucial for controlling blood sugar levels. Genistein, an isoflavone predominantly found in soy products, has been extensively reviewed and is recognized for its robust antidiabetic properties. Recent clinical studies indicated by Jain et al. (2022) suggest that genistein not only delays the onset of type 2 diabetes but also alleviates various symptoms associated with the disease, such as insulin resistance and inflammation. Usnic acid, a lichen-derived compound, has also shown substantial inhibitory effects on the α-glucosidase enzyme, suggesting its potential as a future therapeutic agent in the realm of diabetes treatment (Maulidiyah et al., 2022). This inhibition is critical as it may lead to reduced absorption of carbohydrates, consequently lowering postprandial blood glucose levels. Cirantin, or hesperidin, is a type of bioflavonoid found in citrus fruits, which has demonstrated capabilities not just in treating diabetes but also in preventing various complications linked to the condition (Dhanya & Jayamurthy, 2020; Mirzaei et al., 2023). Its antioxidant properties might play a vital role in combatting oxidative stress, a common issue in diabetic patients. Lastly, bezafibrate has emerged in clinical settings as a significant agent with antidiabetic effects. Clinical evidence indicates that bezafibrate can effectively lower blood glucose levels in patients diagnosed with type 2 diabetes, thus presenting itself as an important option in diabetes management (Flory et al., 2009). Its multifaceted approach, potentially improving lipid profiles alongside glycaemic control, underscores its clinical relevance and efficacy. Overall, these compounds offer exciting possibilities as therapeutic interventions for hyperglycaemia and related metabolic disorders, warranting further investigation to unravel their full potential in diabetes care.

The molecular docking analysis results, presented in Table 7 and Figures 3 to 14, indicate that cirantin and genistein show the highest potential as inhibitors of the enzymes α-amylase and α-glucosidase, respectively. This conclusion is supported by their superior docking scores, which reflect a strong binding affinity and effective interaction with these enzymes. In comparison, acarbose demonstrated competitive performance. It achieved higher docking scores than cirantin when interacting with α-amylase; however, it fell slightly short of genistein's scores against α-glucosidase. This suggests that while acarbose is a potent inhibitor of α-amylase, genistein may be more effective against α-glucosidase, an enzyme critical for carbohydrate metabolism. Additionally, all five compounds studied showed significant molecular docking interactions with both α-amylase and α-glucosidase. This consistent interaction among the various compounds highlights their potential as effective inhibitors of these enzymes, which play a vital role in managing blood glucose levels in individuals with diabetes mellitus. Given these findings, further research is essential to explore the pharmacological properties of these compounds, particularly cirantin and genistein. Their demonstrated inhibitory effects on carbohydrate-digesting enzymes position them as promising candidates for development as antidiabetic agents, offering hope for new therapeutic strategies to control hyperglycaemia.

Acknowledgements

The author of this article appreciates the contribution of Mallam Haruna Tsoho Bala from the Department of Biochemistry at Bayero University Kano (BUK) to this investigation.

Ethics Approval

Ethical guidelines for in-vitro and in-silico studies were strictly followed, and the appropriate authorities approved the procedures.

Conflict of Interest

The author declares that he has no conflict of interest.

Funding

This research work did not receive grants from public, commercial, or not-for-profit funding agencies.

Author's Contributions

LTA was responsible for developing the investigation methods, overseeing the research project, and preparing the published work. Specifically, he wrote the first draft and made significant contributions to the revision, planning, and execution of the work. He provided the resources for the study, conducted statistical analysis, and was involved in typesetting, editing, formatting, and investigation. Additionally, he performed the in silico investigation process, collected data for further analysis, and contributed to the revision of the manuscript. LTA also participated in LC-MS analysis, interpreted the results, and contributed to the writing of the manuscript. Furthermore, he played a role in the manuscript's typesetting, editing, and formatting.

Article History:

Received: 08th November 2024.

Accepted: 26th January 2025.

Published online: 16th May 2025.

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