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Multiple Targets of Polyphenols from *Picralima nitida* on Blood Glucose Regulatory Enzymes

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ABSTRACT

Background and Purpose: *Picralima nitida* (Apocynaceae) is traditionally used in many African communities to manage diabetes mellitus. This therapeutic effect may be attributed to the presence of different phytochemicals that act on multiple targets. This study aimed to profile polyphenols in the stem of *P. nitida*, evaluate its antioxidant potential, and investigate the interactions of the identified polyphenols against selected antidiabetic protein targets through molecular docking.

Methods: High Pressure Liquid Chromatography was used to profile the methanol-dichloromethane extract of *P. nitida* (MDPN), while antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and Ferric Reducing Antioxidant Power (FRAP) assays. Molecular interaction of the identified polyphenols with α-glucosidase (AGL) and Dipeptidyl peptidase-4 (DPP-4) was evaluated through an *in silico* study.

Results: Seven polyphenols were identified, with kaempferol (136.01 ppm) and vanillic acid (134.54 ppm) occurring as the most abundant. A concentration-dependent increase in percentage inhibition was observed in DPPH and FRAP assays. The IC_{50} values for DPPH and FRAP were 89.37 µg/mL (R^2=0.962) and 10.271 µg/mL (R^2=0.973), respectively. Polyphenols intermingle with the AGL complex by hydrophobic interactions involving amino acid residues: Trp432, Trp329, Phe601, hydrogen bonds: Asp232, Asp568, His626, Asp357, Arg552, and van der Waals: Trp467, Ile358, Trp565, Phe476, Ser474, Ile396. Also, the DPP-4 complex engaged in hydrophobic interactions with the amino acid residues Phe357, Tyr666, Tyr662, hydrogen bonds with Asn710, and van der Waals interactions with His740, Glu206, Ser209, Tyr631, Val656, Val711, and Trp659. Epigallocatechin and myricetin exhibited the highest binding affinities and inhibitory potentials against AGL, whereas myricetin, quercetin, and kaempferol demonstrated superior binding affinities towards DPP-4. Epigallocatechin, epicatechin, vanillic acid, and gallic acid showed selective preference for DPP-4, while myricetin, quercetin, and kaempferol displayed greater selectivity for AGL.

Conclusion: These findings suggest that MDPN contains polyphenols that exhibit antioxidant activity via hydrogen-atom and electron-donation mechanisms and selectively target AGL and DPP-4.

INTRODUCTION

Polyphenols are naturally occurring micronutrients found in plants and are classified based on their chemical structures into flavonoids, phenolic acids, stilbenes, and lignins. Flavonoids constitute a large and diverse group of naturally occurring polyphenolic compounds predominantly seen in vascular plants. They are responsible for the vivid color in fruits and vegetables and are well known for their antioxidant property (Hu *et al*., 2025; Lemanowicz *et al*., 2026). This antioxidant property is largely attributed to their ability to neutralize unstable radicals generated during metabolic processes, thereby protecting cellular components such as DNA, proteins, and lipids from oxidative damage (Zahra *et al*., 2024).

The antioxidant activity of flavonoids is dependent on their chemical structure, specifically the arrangement and number of hydroxyl groups attached to the aromatic rings (Kumar and Pandey, 2013). Some of the well documented ways by which flavonoids exhibit antioxidant effect include the scavenging of free radicals and terminating chain reactions that cause oxidative stress (Jomova *et al*., 2025), inhibition of certain enzymes that initiate or trigger the production of reactive oxygen species (Stachelska *et al*., 2025), stimulation of the inert antioxidant defense system that regulate Nuclear factor erythroid 2-related factor 2 (Nrf-2) to promote the production of glutathione which is considered as a natural antioxidant (Suraweera *et al*., 2020).

Phenolic acids account for roughly one-third of dietary polyphenols and are abundant in fruits and vegetables. They are classified into hydroxybenzoic acids and hydroxycinnamic acids (López-Herrador *et al*., 2025). Stilbenes contain two phenyl rings connected by a central double-ethylene bridge and primarily function in plant defense mechanisms (Waffo-Teguo et al., 2008). The most widely studied stilbene is resveratrol, commonly found in grapefruit and red wine (Tsai *et al*., 2017). Lignans are biphenolic compounds formed through the oxidative dimerization of two phenylpropanoid residues and are mostly seen in seeds, whole grains, and cereals (Gnabre *et al*., 2015). Collectively, phenolic acids, stilbenes, and lignans are potent antioxidants (Mihaylova *et al*., 2024) and act as multi-target agents against Type 2 diabetes by inhibiting blood glucose regulatory enzymes through targeted structural binding, potentially mimicking and outperforming synthetic pharmaceuticals (Sarkar *et al*., 2022). Two such regulatory enzymes are Dipeptidyl peptidase-4 and Alpha-glucosidase.

Dipeptidyl peptidase-4 (DPP-4) is a serine exopeptidase enzyme responsible for catabolizing and inactivating incretin hormones in the human body. The incretin hormones glucagon-like peptide-1 (GLP1) and glucose-dependent insulinotropic polypeptide (GIP) are gut-derived peptide hormones released after a meal that reduce blood glucose by stimulating glucose-dependent insulin release. These hormones bind to receptors on pancreatic beta cells, increasing intracellular cyclic Adenosine monophosphate (cAMP), and promoting exocytosis of stored insulin into the bloodstream. Consequently, DPP-4 inhibition prolongs incretin activity and improves glycemic control.

Another enzyme of interest involved in complex carbohydrate metabolism is Alpha-glucosidase (AGL). This enzyme resides in the brush border of the small intestine and breaks down complex carbohydrates into absorbable glucose, thereby elevating blood glucose. Inhibition of AGL delays carbohydrate digestion and glucose absorption, thereby reducing postprandial hyperglycemia.

Plants from the Apocynaceae family show strong postprandial glycemic potential by blunting sugar cravings and inhibiting enzymes that digest carbohydrates. They regulate blood sugar spikes by delaying intestinal glucose uptake and preventing the rapid conversion of starches into glucose (Lima *et al*., 2025). *Picralima nitida* (Stapf) T. Durand & H. Durand. (Apocynaceae), The only species in the genus Picralima, commonly called Osu Igwe by the Igbo-speaking people in Nigeria, is indigenous to Nigeria, Cameroon, Togo, Ghana, Benin, and Côte d'Ivoire, and is used in traditional medicine to manage diabetes (Akabassi *et al*., 2024). In Southern Nigeria, especially among the people of Agbor in Delta State, the stem is used to lower elevated blood glucose levels. Previous studies have reported the hypoglycemic effects of aqueous and methanol seed extracts in rabbits and rats (Aguwa *et al*., 2001; Yao *et al.,* 2023). The stem and leaves have also been reported to possess antioxidant potential (Ogbeide *et al*., 2024). Furthermore, *in silico* studies have demonstrated interactions between stem bark phytochemicals and Nrf2-related antioxidant pathways (Erharuyi and Azubuike, 2025).

Several alkaloids have been isolated from the seeds of *P. nitida*, including akuammina, pseudo-akuammigine, akuammicine, akuammiline, pacraline, akuammidine akuammiline N-oxide rhazimol , and alstonine (Creed *et al*., 2021; Laryea *et al*., 2024; Gour *et al*., 2025). However, limited information is available on the polyphenol composition of the stem and its contribution to antioxidant and antidiabetic activities. Therefore, this study aimed to profile the polyphenols in the stem of *P. nitida*, evaluate its antioxidant potential, and investigate the interaction of the identified phytochemicals with selected antidiabetic targets using molecular docking.

MATERIALS AND METHODS

Collection, Identification, and Preparation of Plant Material

Fresh stems were carefully harvested from mature plants using a clean cutting tool by Mr. Friday Aliemeke in

January 2025 from Agbor-Nta (Latitude 6.2245° N, Longitude 6.1434° E), Ika South Local Government Area, Delta State, Nigeria. Botanical identification and authentication were performed on the fruit, leaves, and stem of the plant by Prof. Henry Adewale Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. A voucher specimen was deposited at the departmental herbarium for future reference following the issuance of voucher number UBH-P424.

Stems of *Picralima nitida* were thoroughly washed with running clean water to remove dirt and other extraneous materials. The stems were cut into smaller pieces and air-dried in the shade at room temperature for 21 days until a constant weight was reached. This drying method was adopted to prevent degradation of heat-sensitive phytoconstituents. Dried stem material was pulverized using an electric grinder and stored in an airtight container at room temperature until use.

Extraction

Fine-powdered stem material was macerated in methanol: dichloromethane (1:1 v/v) at a ratio of 10 g of powdered material to 100 mL of solvent for 72 h. The mixture was intermittently shaken every 30 min for 2 h to ensure proper dispersion of the powder's fine particles in the solvent mixture. After 3 days of soaking, the resulting mixture was first filtered through muslin cloth and through Whatman No. 1 filter paper to obtain the filtrate. This was then concentrated using a rotary evaporator at 47±5ºC until a semi-solid crude extract (MDPN) was obtained. and stored in a refrigerator at 4°C until further analysis.

High Pressure Liquid Chromatography Analysis of Polyphenols in MDPN

Quantitative analysis of seven polyphenols in MDPN was performed using the previously described protocol of Odion *et al*. (2025), with some modifications. Five microliter (5.0 µL) of diluted (1 mg/mL) of MDPN was injected into a reverse phase HPLC system, utilizing Agilent LiChrospher 100-5RP8 (250 x 4.6 mm) (C18) as the stationary phase and 0.1 % formic acid and methanol/acetonitrile in gradient elution mode as mobile phase, with a column temperature of 35ºC and Diode Array Detector for UV-visible detection of polyphenolic compounds. A standard mix of the seven flavonoids at 5, 10, and 20 ppm was used for the analysis.

Hardware and Software Utilized in the Study

The hardware used in this study includes a computer with a 2.10 GHz Intel Core i3-10110U processor, 4 GB of RAM, and a 64-bit operating system. Software used in this study includes AutoDock Vina 1.5.7, Discovery Studio Visualizer 25.1, PyMOL molecular visualizer 4.60, and Avogadro 1.2.0 and Open Babel version 3.1.1.

Ligand and Protein Preparation

Polyphenols identified by HPLC were selected as ligands for docking studies. The two-dimensional (2D) structures of the compounds were obtained from the PubChem database in Structure Data File (SDF) format. The ligands were converted from SDF format to Protein Data Bank (PDB) format using Open Babel GUI software (O'Boyle *et al*., 2011). Energy minimization of the ligands was subsequently performed using Avogadro software to obtain stable conformations and reduce steric hindrance prior to docking analysis (Hanwell *et al*., 2012).

The three-dimensional (3D) crystal structures of the target proteins used for this study were also obtained from Research Collaboratory for Structural Bioinformatics–Protein Data Bank (RCSB-PDB) (https://www.rcsb.com). The selected proteins included sugar beet AGL (PDB ID: 3W37) in complex with acarbose. This target was isolated from *Beta vulgaris*, classified as a carbohydrate hydrolase, with a resolution of 1.70 A and DPP-4 (PDB ID: 1X70) in complex with sitagliptin. This target was isolated from *Homo sapiens*, classified as a hydrolase, and resolved to 2.10 Å. Protein structure selections were performed to achieve a suitable level of crystallographic quality and improve docking reliability. Water molecules, unwanted chains, and co-crystallized ligands not involved in binding interactions were removed prior to docking using Discovery Studio Visualizer and PyMOL software (DeLano, 2002). The grid center coordinates required for docking simulations were determined using PyMOL molecular visualization software.

Protocol Validation

The best-ranked docking poses generated by AutoDock Vina were converted from PDBQT format to PDB format using Open Babel GUI (Al-Khodairy *et al.,* 2013). Subsequently, the docked ligands and their corresponding crystallographic ligands were imported into BIOVIA Discovery Studio Visualizer for structural superimposition. The docked ligand was aligned with the crystallographic ligand within the same coordinate space, and RMSD values were calculated using the RMSD analysis tool available in Discovery Studio. The calculated RMSD values were used to assess the docking protocol's ability to reproduce the experimentally observed binding orientation.

ADMET Study of Polyphenols from MDPN

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the identified polyphenol compounds were predicted using ADMETlab 3.0 (Fu *et al*., 2024). The polyphenol compounds subjected to ADMET analysis were identified from the HPLC profiling of MDPN. The canonical Simplified Molecular Input Line Entry System (SMILES) notations of the identified compounds were retrieved from the PubChem database and

compiled in a Microsoft Excel spreadsheet. These SMILES strings were copied from the Excel spreadsheet and pasted into the "Enter SMILES" field in the platform's screening module. The submitted compounds were processed to predict their pharmacokinetic and toxicity properties. Following completion of the analysis, the generated results were downloaded in comma-separated values (CSV) format. Relevant ADMET parameters were extracted from the downloaded dataset and organized for further analysis and interpretation.

The generated data were used to evaluate the oral bioavailability and drug-likeness properties of the compounds. Toxicity profiles of selected polyphenols were also provided, with the following parameters evaluated: hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity and median lethal dose (LD_{50}).

Antioxidant Assays

The antioxidant potential of the stem extract of *Picralima nitida* was evaluated using the DPPH radical-scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay, with ascorbic acid as the standard.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the extract was determined (Brand-Williams *et al.,* 1995). Different concentrations of the extract were prepared and mixed with DPPH solution. The reaction mixture was incubated in the dark for 30 min at room temperature.

The absorbance of the mixture was measured at 517 nm using a UV-visible spectrophotometer. The percentage inhibition of DPPH radicals was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where: A_0 = Absorbance of the control**;** A_1 = Absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power of the extract was determined (Benzie and Strain, 1996). The FRAP reagent was freshly prepared and mixed with different concentrations of the extract. The reaction mixture was incubated at room temperature, and absorbance was measured at 593 nm using a spectrophotometer. Increased absorbance indicated higher ferric-reducing antioxidant power.

Data Presentation and Statistical Analysis

Antioxidant study was carried out in triplicate and results are presented as the mean ± standard deviation. Student's t-test (SPSS version 25) was used to analyze the data, and the level of significance was set at $P<0.05$.

RESULTS

Polyphenolic Profile of MDPN

HPLC analysis of MDPN identified seven polyphenols. Kaempferol (136.01 ppm) and vanillic acid (134.54 ppm) showed the highest concentrations, whereas epicatechin (0.06 ppm) and epigallocatechin (0.25 ppm) were present in the lowest concentrations (Table 1).

Table 1: Polyphenols in MDPN from HPLC analysis.

Compound	Retention Time (min)	Concentration (ppm)
Gallic Acid	4.65	1.87
Epicatechin	5.10	0.06
Epigallocatechin	7.87	0.25
Kaempferol	8.47	136.01
Quercetin	11.57	14.80
Myricetin	12.01	24.19
Vanillic Acid	16.02	134.54

Antioxidant Activity Analysis

The DPPH radical scavenging activity of MDPN increased in a concentration-dependent manner, rising from 66.73 % (20 µg/mL) to 79.17 % (100 µg/mL), compared to the percentage inhibition of ascorbic acid (standard) from 70.14 % to 97.27% under similar changes in concentration. The IC_{50} values for MDPN and ascorbic acid were 89.37 µg/mL (R^2 = 0.962) and 47.74 µg/mL (R^2 = 0.929), respectively (Figure 1).

Similarly, FRAP percentage inhibition for MDPN varies from 55.40 % (20 µg/mL) to 82.970 % (100 µg/mL), compared to 59.69 % to 93.53 % for ascorbic at similar concentrations. The calculated IC_{50} values were 10.271 µg/mL (R^2 = 0.973) for MDPN, and 6.897 µg/mL (R^2 = 0.974) for ascorbic acid (Figure 2).

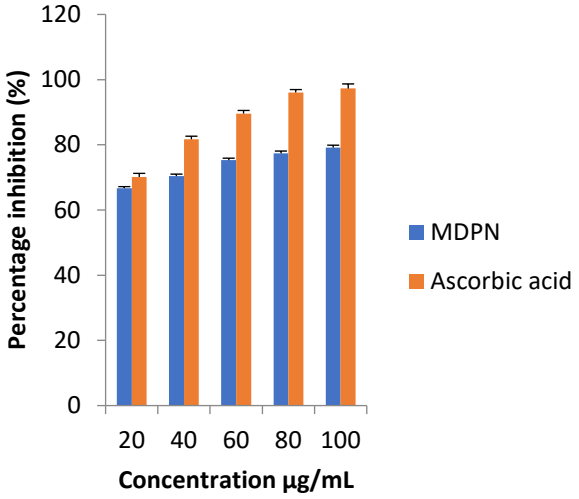

Figure 1: DPPH radical scavenging potential of MDPN.

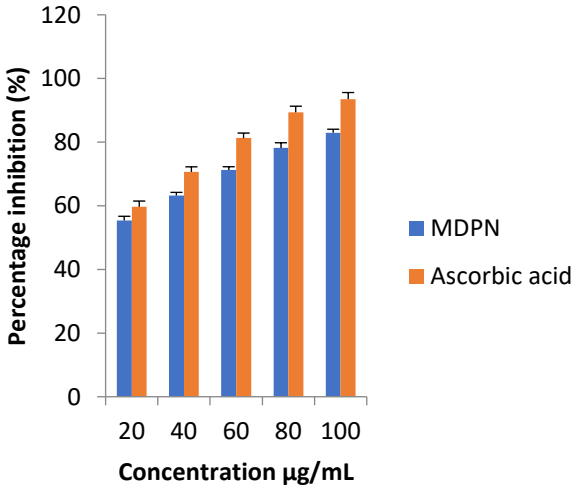

Figure 2: Ferric reducing antioxidant potential of MDPN.

Drug-likeness Properties

The molecular weights of the identified polyphenolic compounds ranged from 168 to 318 Dalton. The number of hydrogen bond donors (HBD) varied between 2 and 6, while the number of hydrogen bond acceptors (HBA) ranged from 4 to 8. Epigallocatechin and myricetin each possessed six HBD groups, above the maximum set range of 5. Partition coefficient (XLogP3) of the polyphenols varies from 0 to 1.9; kaempferol is the most hydrophobic compound among the polyphenols, while epigallocatechin is the least hydrophobic compound (Table 2). Lipinski's rule of five was violated once by Epigallocatechin and myricetin, thus making the compounds highly druggable.

Molecular docking scores

Molecular docking score and amino acid interaction of the polyphenols with AGL targets are presented in Table 3. The molecular docking score varies between -6.2 and -8.1 (acarbose -8.6) for AGL. Epigallocatechin and myricetin exhibited the strongest binding affinities and better inhibitory efficacy for AGL. Their interactions with the appropriate amino acids are provided in Figure 3. Hydrophobic interactions involving the amino acids residues Trp432, Trp329, Phe601, hydrogen bonds (Asp232, Asp568, His626, Asp357, Arg552), van der Waals (Trp467, Ile358, Trp565, Phe476, Ser474, Ile396), and unfavorable bonds (Arg552) were prevalent in the α-glucosidase complex.

Molecular docking score and amino acid interaction of the polyphenol with DPP-4 targets are presented in Table 4. The molecular docking score ranges from -5.9 to -8.2 (sitagliptin: -9.4) for DPP-4 targets. Myricetin, quercetin, and kaempferol displayed the highest binding affinities and efficacy for the DPP-4 target. Their interactions with the appropriate amino acids are provided in Figure 4. Hydrophobic interactions involving the amino acids residues Phe357, Tyr666, Tyr662, hydrogen bonds (Asn710) and Van der Waals (His740, Glu206, Ser209, Tyr631, Val656, Val711, Trp659) were prevalent in the DPP-4 complex.

Selectivity Analysis

Calculated selectivity indices for the polyphenols for DPP-4; AGL targets ranged from 0.43 to 3.26. Epigallocatechin, epicatechin, vanillic acid, and gallic acid exhibited strong selective preference towards DPP-4, whereas myricetin, quercetin, and kaempferol displayed greater selective preference for α-glucosidase (Table 5).

Docking Protocol Validation

The reliability of the molecular docking protocol was evaluated by re-docking the co-crystallized ligands of AGL (Acarbose) and DPP-4 (Sitagliptin). The standard ligands were extracted from their respective crystal structures, prepared using the same protocol applied to the polyphenols, and subsequently re-docked into the corresponding protein binding sites using AutoDock Vina (Trott and Olson, 2010).

For AGL, the co-crystallized ligand, acarbose, was re-docked into the active site using a grid box centered at x = 0.1445, y = −2.0662 and z = −23.0408 Å with dimensions of 30 × 30 × 30 Å. The re-docking process yielded a root-mean-square deviation (RMSD) of 2.03498 Å between the crystallographic and predicted binding conformations. Similarly, for DPP-4 (PDB ID: 1X70), the co-crystallized sitagliptin was re-docked using a grid box centered at x = 41.261, y = 51.052, and z = 35.558 Å with dimensions of 25 × 25 × 25 Å. The docking protocol produced an RMSD value of 0.2263 Å, indicating excellent agreement between the docked and experimentally determined binding poses (Table 6).

Table 2: Lipinski's rule of five for polyphenols from HPLC analysis (Standards are shown in brackets).

Name of Ligand	Molecular Weight in Dalton (≤500)	Number of Hydrogen Bond Donors (≤5)	Number of Hydrogen Bond Acceptors (≤10)	XLogP3 (≤5)
Gallic Acid	170	4	5	0.7
Epicatechin	290	5	6	0.4
Epigallocatechin	306	6	7	0
Kaempferol	286	4	6	1.9
Quercetin	302	5	7	1.5
Myricetin	318	6	8	1.2
Vanillic Acid	168	2	4	1.4

Table 3: Molecular docking score and amino acid interaction of polyphenols to AGL.

Name of Ligand	Docking Score (kcal/mol)	Hydrogen Bond	Hydrophobic Bond	van de Waals
Acarbose	-8.6	Asp568, Asp469, His626, Asp357, Arg552, Asp232, Asn237	Trp432, Trp329, Phe601 (Pi-Alkyl)	Ala602, Gly567, Trp565, Trp467, Arg624, Ile396, Ile358, Phe476, Ser474, Ile233, Phe236
Epigallocatechin	-8.1	Asp232, Asp568, His626, Asp357, Arg552	Trp432, Trp329, Phe601 (Pi-Alkyl) Met470 (Pi-Sulfur)	Trp467, Ile358, Trp565, Phe476, Ser474
Myricetin	-8.1	Asp357, Asp469		Asp232, Met470, Trp432, Trp565, Trp467, His626, Ile396, Ile358, Ala628, Phe601, Trp329, Asp630
Epicatechin	-7.8	Arg552, His626, Asp568	Trp432, Trp329, Phe601 (Pi-Alkyl) Met470 (Pi-Sulfur	Trp467, Trp565, Phe476, Ser474, Asp232, Asp357, Asp469,
Quercetin	-7.8	Asp469		Asp232, Met470, Trp432, Arg552, Trp565, Trp467, His626, Ile358, Ile396, Asp357, Phe601, Ala628, Trp329, Asp630

Table 3: Molecular docking score and amino acid interaction of polyphenols to AGL (Cont'd).

Name of Ligand	Docking Score (kcal/mol)	Hydrogen Bond	Hydrophobic Bond	van de Waals
Kaempferol	-7.5	Asp357	Trp432 (Pi-Pi T Shaped)	Met470, Asp469, His626, Trp467, Ile396, Phe601, Trp329, Ala628, Asp630
Gallic Acid	-6.4	Asp568		Phe601, Arg552, Trp565, His626, Asp469, Trp467, Ile396, Asp357, Ile358, Trp329, Trp432
Vanillic Acid	-6.2	Asp357	Trp432 (Pi-Pi Stacked), Trp467, Trp565, His626, Phe601 (Pi-Alkyl)	Asp568, Trp329, Arg552, Asp469, Ile396

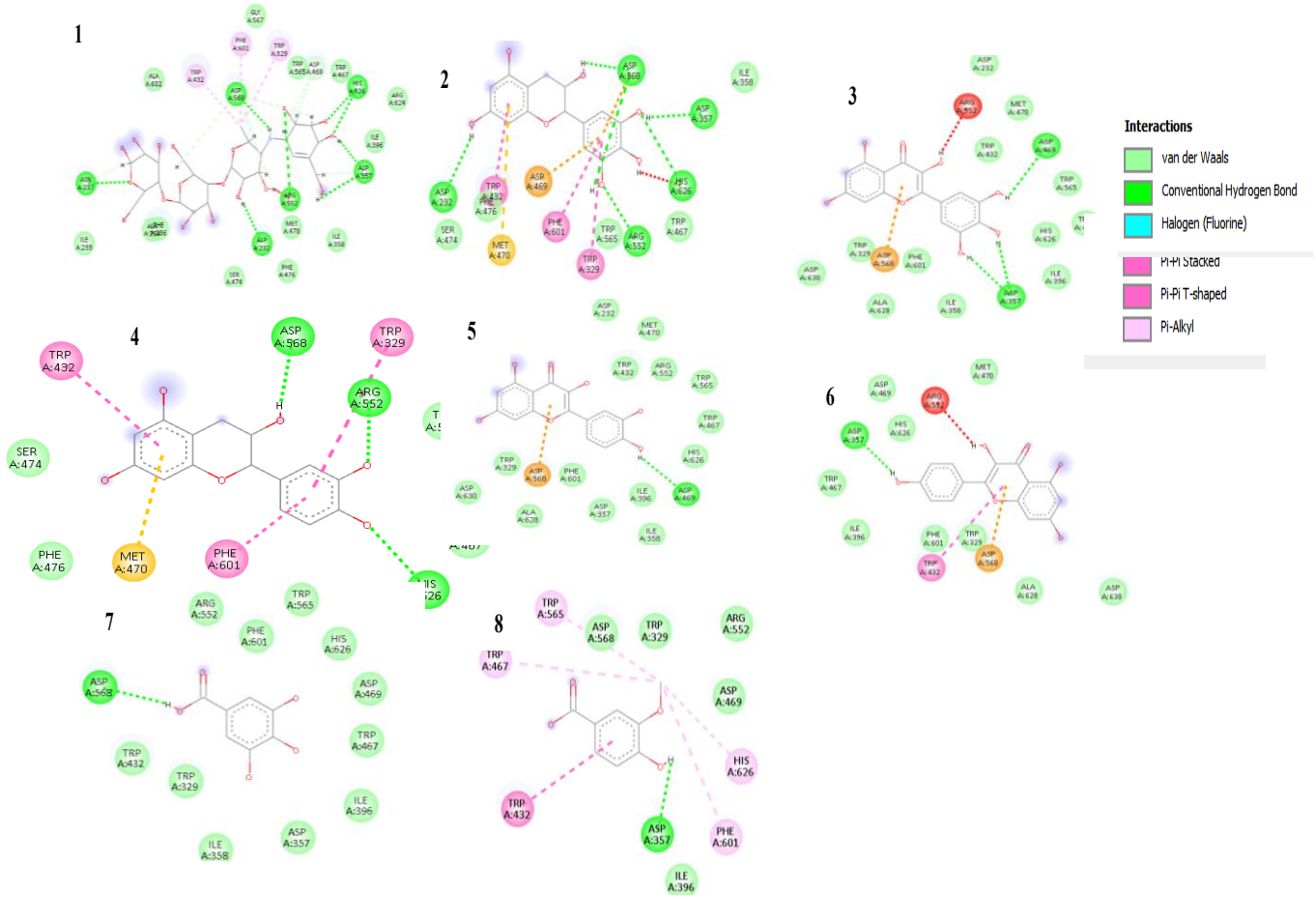

Figure 3: 2D interactions of the Polyphenols with α-Glucosidase Protein Target. 1. Acarbose, 2. Epigallocatechin, 3. Myricetin, 4. Epicatechin, 5. Quercetin, 6. Kaempferol, 7. Gallic Acid, 8. Vanillic Acid

Table 4: Molecular docking score and amino acid interaction of polyphenols to DPP-4.

Name of Ligand	Docking Score (kcal/mol)	Hydrogen Bond	Hydrophobic Bond	van de Waals
Sitagliptin	-9.4	Arg358, Tyr547, Arg125, Asn710	Phe357, Tyr666, Tyr662	Val207, Ser209, Glu206, Trp659, Tyr631, Val656, Val711, His740
Epigallocatechin	-7.6	Arg125, Asn710, Glu205, His740	His740, Arg125	Ser630, Trp629, Gly741, Trp201, Trp124, Gln123, Lys122, Asp739
Myricetin	-8.2	Glu205	Tyr666, Phe357, Tyr662	Ser630, His740, Asn710, Arg125, Glu206, Ser209, Tyr547, Trp659, Tyr631, Val656, Val711
Epicatechin	-7.4	Val207, Glu206	Phe357, Tyr666	Ser209, Glu205, Asp663, Asn710, Arg125, Ser630, Tyr547, Arg358, Phe208
Quercetin	-8.2		Tyr662, Phe357, Tyr666	Ser209, Glu206, Asn710, Trp659, Tyr631, Val656, Val711, Ser630, His740, Arg125, Tyr547
Kaempferol	-8.2	Glu205	Phe357, Tyr666, Tyr662	Ser209, Asn710, Arg125, His740, Ser630, Val711, Val656, Tyr631, Tyr547
Gallic Acid	-6.0	Arg125, Asn710	Tyr666, Tyr662	Glu206, Tyr547, Trp659, Val656, Tyr631, Ser630, Val711, His740
Vanillic Acid	-5.9	Arg125, Glu205	Tyr666, Tyr662 (Pi-Pi Stacked), His740 (Pi-Alkyl)	Trp659, Val656, Tyr631, Val711, Tyr547, Ser630, Asn710

Table 5: Selectivity index of the ligands for DPP-4 and AGL receptors.

Name of Ligand	Ki for DPP-4 (μM)	Ki for AGL (μM)	cSI (Ki for AGL/Ki for DPP-4)
Sitagliptin	0.03	-	-
Acarbose	-	0.49	-
Epigallocatechin	2.65	1.14	0.43
Myricetin	0.97	1.14	1.18
Epicatechin	3.73	1.90	0.51
Quercetin	0.97	1.90	1.95
Kaempferol	0.97	3.16	3.26
Gallic Acid	39.8	20.2	0.51
Vanillic Acid	47.1	28.4	0.60

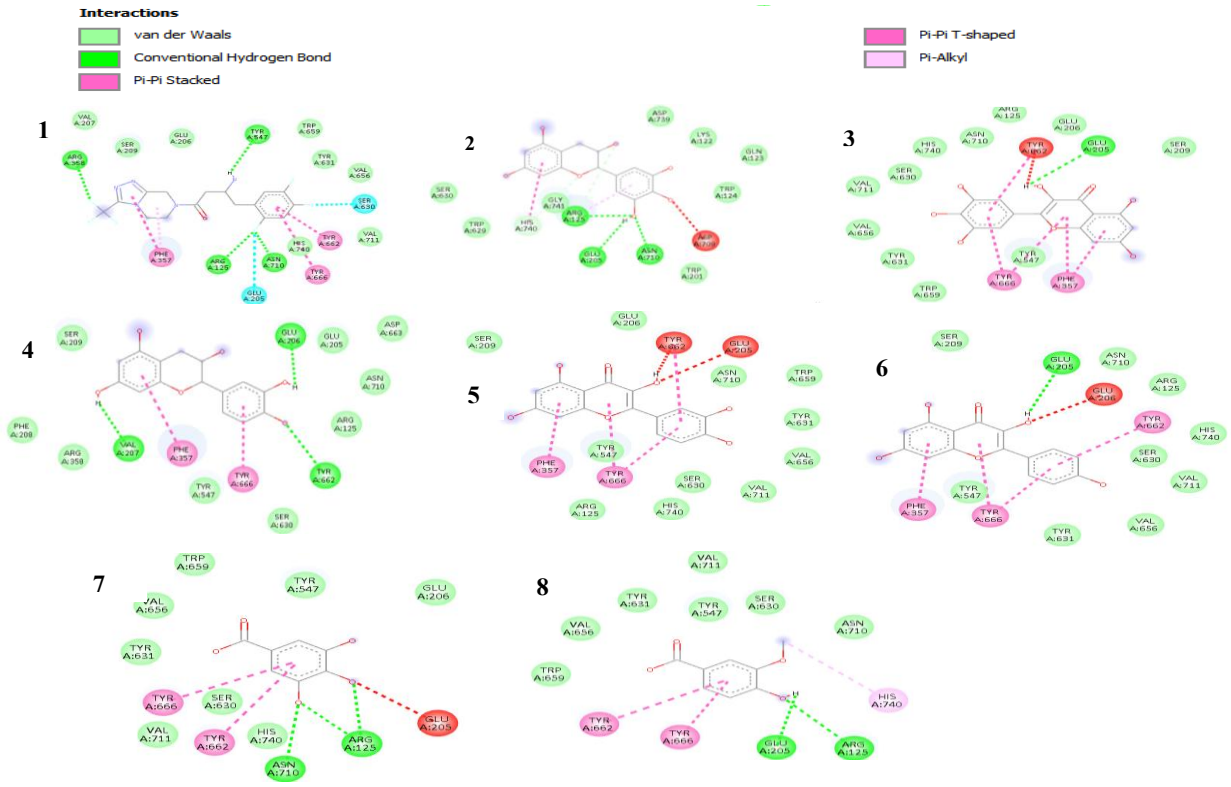

Figure 4: 2D amino acid interactions of the polyphenols with DPP-4 Protein Target. 1. Sitagliptin, 2. Epigallocatechin, 3. Myricetin, 4. Epicatechin, 5. Quercetin, 6. Kaempferol, 7. Gallic Acid, 8. Vanillic

Table 6: Root Mean Square Deviation of the co-crystallized ligands for DPP-4 and AGL targets

Target Protein	PDB ID	Co-crystallized Ligand	RMSD (Å)
AGL	3W37	Acarbose	2.035
DPP-4	1X70	Sitagliptin	0.226

Table 7: Predicted ADME properties of the polyphenols from MDPN.

Name of Ligand	Absorption			Distribution			Metabolism		Excretion		Drug Likeness
	F50 %	HIA	Caco-2 (log Papp in cm/s)	BBB	PPB (%)	Vdss (L/kg)	CYP2D6 Inhibitor	CYP3A4 Inhibitor	CL_{Plasma} (mL/min /kg)	$T_{1/2}$	QED
Gallic Acid	0.621	0.017	-5.604	0.002	37.57	-0.523	1.38E-06	0.0021	5.40	2.2	0.46
Epicatechin	0.996	0.016	-6.472	0.091	92.65	0.046	5.05E-07	0.0015	15.83	2.3	0.51
Epigallocatechin	0.999	0.025	-6.660	0.036	92.65	0.037	1.80E-09	0.0035	11.63	2.2	0.44
Kaempferol	0.983	0.015	-5.969	0.001	92.65	-0.812	0.00489	0.9745	5.69	1.4	0.54
Quercetin	0.999	0.134	-6.176	0.0004	92.65	-0.879	0.00018	0.9367	8.29	1.6	0.43
Myricetin	0.998	0.318	-6.453	0.0004	92.65	-0.851	0.00020	0.9985	6.77	1.6	0.37
Vanillic Acid	0.561	0.111	-5.096	0.002	92.65	-0.671	0.01677	0.0028	3.04	2.0	0.69

ADME Properties

The pharmacokinetic profile of the polyphenol compounds are presented in Table 7. Oral bioavailability (F50%) ranged from 0.561 to 0.999 (56.1% to 99.9%), and the estimated fraction of the polyphenols absorbed in the gut, determined by the Human Intestinal Absorption (HIA) parameter, varied from 0.015 to 0.318. Human intestinal permeability and effectiveness of polyphenols in crossing the intestinal barriers (caco-2) varied from -5.096 to -6.660. Crossing by polyphenol into the Blood Brain Barrier (BBB) ranges from 0.0004 to 0.036. Extent of binding of the polyphenol to the plasma protein (PPB) varied from 37.57 % to 92.65 %. Volume distribution at steady state (Vdss) varied from 0.037 L/kg to 0.879 L/kg. Inhibition of Cytochrome-P450; CYP2D6 varied from 1.80 E-09 to 0.0167, CYP3A4 varied from 0.0015 to 0.9985. Plasma clearance (CLplasma) varied from 3.04 mL/min/kg to 15.83 mL/min/kg. Half-life varied from 1.4 to 2.3 h.

Toxicity prediction

The toxicity profile for the polyphenols are shown in Table 8, the various assessing parameter are provided in range as follow; hepatotoxicity (0.325 – 0.611), neurotoxicity (0.011 – 0.303), ototoxicity (0.075 – 0.844), hematotoxicity (0.015 – 0.340), nephrotoxicity (0.003 – 0.429), genotoxicity (0.143 – 0.995), immunotoxicity (0.003 – 0.040), carcinogenicity (0.216 – 0.716), respiratory (0.508 – 0.857), cytotoxicity (0.023 – 0.790), LC50DM (3.517 – 4.485) and LC50FM (2.981 – 4.112).

Table 8: Prediction of toxicity properties of the polyphenols from MDPN

Name of Ligand	Hepato-toxicity	Neuro-toxicity	Oto-toxicity	Hemato-toxicity	Nephro-toxicity-DI	Genotoxi-city	RPMI-8226 Immunotoxicity	Carcino-genicity	Respira-tory	HEK293 Cytotoxicity	IGC50	LC50DM	LC50FM
Gallic Acid	0.397	0.011	0.821	0.081	0.056	0.760	0.003	0.219	0.508	0.023	2.511	3.517	3.385
Epicatechin	0.611	0.101	0.635	0.133	0.083	0.975	0.035	0.216	0.849	0.790	3.127	4.261	3.912
Epigallocatechin	0.515	0.008	0.844	0.072	0.024	0.995	0.013	0.226	0.857	0.758	3.130	4.122	3.501
Kaempferol	0.386	0.039	0.075	0.045	0.019	0.977	0.040	0.716	0.713	0.763	3.653	4.376	4.007
Quercetin	0.337	0.009	0.164	0.032	0.011	0.975	0.023	0.600	0.674	0.561	3.759	4.485	4.112
Myricetin	0.325	0.001	0.474	0.015	0.003	0.995	0.007	0.502	0.652	0.489	3.601	4.384	3.894
Vanillic Acid	0.466	0.303	0.317	0.340	0.429	0.143	0.030	0.413	0.672	0.043	2.602	3.670	2.981

DISCUSSION

A previous study on the stem bark of *Picralima nitida* identified and quantified naringin, naringenin, rutin, flavan-3-ol, and flavanone using a similar phytochemical profiling protocol (Erharuyi and Azubuike, 2025). However, that study employed methanol as the extraction solvent, whereas the present study utilized methanol-dichloromethane solvent mixture with a broader polarity spectrum. This solvent system bridges the gap between highly polar and moderately lipophilic phytochemicals by increasing extraction yields through lysis of parenchyma cell membranes, while simultaneously solubilizing diverse phytochemicals (Sasidharan *et al*., 2011).

Most of the phytochemicals identified in this study are polyphenolic compounds containing one to five hydroxyl groups attached to aromatic rings, some of which, such as vanillic acid and gallic acid, also possess carboxylic acid moieties. These structural characteristics enable them to donate electrons or hydrogen atoms to an unstable free radical, thereby mitigating oxidative stress (Xue *et al*., 2025). This resulted in the assaying of the antioxidant potential of MDPN.

DPPH assay is widely used to evaluate the antioxidant capacity of a plant extract (Baliyan *et al*., 2022). In its free radical state, it exhibits a deep purple coloration due to the presence of an unpaired electron. Upon accepting a hydrogen atom or electron from phenolic group or flavonoids, DPPH is reduced to a non-reactive form, resulting to a color change from deep purple to pale yellow (Molyneux, 2004). The conversion of reactive oxygen species to unreactive species suggests that the accumulation of these unstable reactive radicals can be inhibited, and ailments due to oxidative stress can be reduced. Although this assay is fast and highly reproducible, it is usually employed as a first-line screening tool for radical scavenger, and often complemented by other antioxidant assays like FRAP for a more confirmatory result (Kedare and Singh, 2011).

FRAP is used as a second-line screening assay to complement a primary analysis. It reduces ferric ions to ferrous ions, as indicated by a change in color from pale yellow-straw brown to bright violet-blue. Pale yellow straw brown occur as a coordination complex of ferric-2,4,6-tris(2-pyridyl)-1,3,5-triazine (Fe^{3+}-TPTZ) to bright violet blue color of ferrous-2,4,6-tris(2-pyridyl)-1,3,5-triazine (Fe^{2+}-TPTZ). The increased intensity of the chromogen (TPTZ) color indicates the antioxidant strength of the extract. Ascorbic acid was used as the reference standard due to its well-documented antioxidant activity, high water solubility, and rapidly reacting free radical scavenging potential, making it an appropriate benchmark for antioxidant capacity measurement (Gęgotek and Skrzydlewska, 2022).

Ascorbic acid exhibited a higher percentage inhibition over MDPN at all concentrations. Comparing their IC_{50} values further indicate higher potency of ascorbic acid over MDPN, this implies that lower concentration of ascorbic acid will be required to achieved similar antioxidant potential by higher concentration of MDPN. A similar pattern was observed with FRAP. However, the potency was more seen in the FRAP assay than the DPPH assay; this could be due to the superior solubility in water and organic solvents of the FRAP radical over the DPPH radical (Bibi Sadeer *et al*., 2020). Ideally, the combination of polyphenols with documented antioxidant properties would be expected to produce additive or synergistic effects that could surpass ascorbic acid (Stagos, 2019). However, MDPN is a crude extract containing numerous phytochemicals, some of which may attenuate the overall antioxidant potential.

The potential of DMPN to act as radical scavengers via electron donation from polyphenols prompted the subsequent evaluation of the drug-like properties of these polyphenols. This was checked with Lipinski's Rule of Five to assess the likelihood of the identified polyphenols being orally active drugs in humans. According to the rule, poor oral absorption or membrane permeability is more likely when a molecule violates more than one of its criteria (Benet *et al*., 2016). In this study, these rules were violated only once, indicating that the polyphenols have high drug-likeness due to optimal predictive physicochemical properties for passive absorption and oral bioavailability. Assessment of XLogP3 further indicates sufficient aqueous solubility and lipid permeability in the polyphenols, allowing them to pass through the gastrointestinal lining into the bloodstream.

Auto Dock Tools software suite is commonly used for molecular modeling (Forli *et al*., 2016). In the context of the AGL-polyphenol or DPP-4-polyphenol complex, it likely offers innovative visualization and analysis capabilities, enabling researchers to produce detailed 2D interaction diagrams that visually represent the specific interactions between AGL and polyphenols or DPP-4 and polyphenols (Figures 3 and 4). These aid in a more comprehensive understanding of the complex's binding pattern (Chang *et al*., 2022). Overall, the description of the AGL-polyphenols or DPP-4-polyphenol complex's interactions and the use of these computational tools reflect a systematic and in-depth approach to studying the interactions (Silwoski *et al.,* 2014). This information may be essential for remodeling and optimizing likely drug candidates and potentially developing new therapeutics based on the target protein-polyphenol interactions.

The ability of polyphenol molecules to bind with selected protein targets, AGL and DPP-4, was studied. These targets were selected based on the documented use of *Picralima nitida* for the treatment of type 2 diabetes (T2D) (Teugwa

et al., 2013). The molecular docking scores for AGL and DPP-4 targets indicated moderate to strong binding affinities, with the identified polyphenols exhibiting docking scores comparable to those of the reference standards. These findings suggest favorable structural complementarity and the potential to compete effectively at the enzyme's active sites. Epigallocatechin and myricetin exhibited docking scores indicating interaction stability comparable to that of acarbose. Quercetin, myricetin, and kaempferol also displayed docking-score interaction stability similar to that of sitagliptin. Their high binding affinities suggest that lower concentrations may be required to inhibit AGL and DPP-4 activities.

AGL is an important target for regulating carbohydrate metabolism, and inhibiting it is a proven strategy for managing T2D by controlling postprandial hyperglycemia (Dimitriadis *et al*., 1985). Also, a multifunctional enzyme such as DPP-4 is involved in the breakdown of incretin hormones. Its inhibition prolongs incretin action, signaling the pancreas to release insulin and lowering blood sugar levels in T2D (Godinho *et al*., 2015). Inhibiting these enzymes simultaneously with polyphenols in DMPN provides powerful control of post-meal blood sugar in T2D without increasing the risk of hypoglycemia or weight gain (Min *et al*., 2018). High affinities indicate potent inhibitory activity of polyphenols at the enzyme's active or allosteric sites, often comparing very favorably with acarbose and sitagliptin.

The target-ligand complex interaction procedure, involving hydrogen bonding, van der Waals interactions, and hydrophobic interactions, is important for a proper understanding of the molecular origin of their binding and possible antidiabetic properties. Hydrogen bonding may contribute to the stability of the complexes formed, providing a bonding pattern and revealing key binding interactions. The electrostatic interaction arises from the attraction or repulsion of charged particles, such as cationic and anionic species from molecules. In this study, this interaction may result from charged groups on both sides of the molecules that come in close proximity with each other. Understanding these interactions is essential for elucidating the van der Waals contributions to the complex's function and furthering its stability. Hydrophobic interactions can occur between nonpolar or hydrophobic regions of molecules, potentially contributing to increased stability in protein-ligand complexes. Polyphenol with a hydrophobic core is likely to engage in hydrophobic interactions with hydrophobic patches on the protein targets. These interactions can facilitate the binding of polyphenols to α-glucosidase or DPP-4 and influence understanding of T2D (Zhao *et al*., 2021; Alzahrani, 2023).

The active-site pocket of AGL is formed by a TIM-barrel domain and a unique N-terminal bulging loop (N-loop). This N-loop forms the enzyme's extended, pocket-shaped active site cleft and is important for stabilizing long-chain malto-oligosaccharides (Tagami *et al*., 2014). The entrance and binding pocket are fortified by hydrophobic and aromatic amino acids, including Trp329, vital for catalytic specificity towards α-1,6-glucosidic bonds, Trp432, and Phe-601via hydrophobic forces and π-π interactions (Ahmed *et al*., 2024). In hydrophobic interactions**,** the aromatic and lipophilic rings of the polyphenolic ligand are stabilized by non-polar van der Waals contacts with residues, while π-π stacking involves electron-rich indole and phenyl rings of Trp329 and Trp432, which engage in edge-to-face or face-to-face π-π interactions with the conjugated phenolic rings of the ligand. In addition to gatekeeper interactions, the hydroxyl groups of the polyphenol typically form key hydrogen bonds with active-site catalytic residues, Asp232, Asp469, or Asp568, to inhibit enzymatic activity (Corkovic *et al*., 2022). These residues form a lipophilic barrier at the entrance to the active-site pocket. Targeting Trp329, Trp432, and Phe601 allows the polyphenolic compound to anchor properly, thereby blocking the normal access of complex sugars to the enzyme (Artanti *et al*., 2023).

Docking analyses of DPP-4 with polyphenol show that ligands predominantly interact through two main structural regions: the S_1 and S_2 pockets. S_i pocket interactions occur with hydrophobic and catalytic residues such as Tyr547, Ser630, Tyr631, Val656, Trp659, Tyr666, Asn710, Val711, and His740. The hydrophobic polyphenol core embeds deeply within this tight space, forming stable π-π stacking and hydrophobic interactions. Interaction in the S_2 pocket occurs through key residues, Glu205, Glu206, and Tyr662, forming strong hydrogen bonds between the hydroxyl (-OH) groups and the sugar moieties via the negatively charged surfaces of these residues, effectively blocking the degradation of incretin hormones like GLP-1 (Kolhotra et al., 2018).

The actions of these polyphenols indicate their high potential as natural, multi-target therapeutic agents for managing T2D. The strong selective preference implies that the compounds specifically target the active catalytic site of DPP-4 rather than other related enzymes, thereby maximizing therapeutic benefits while minimizing off-target side effects. Epigallocatechin is often identified as the most potent among polyphenols in some oral infusions; it forms highly stable hydrogen and aromatic bonds with key DPP-4 residues: Arg125, Glu206, and Phe357. Its binding affinity frequently rivals or exceeds standard synthetic DPP-4 inhibitors. Epicatechin frequently shows stable binding interactions with both DPP-4 and other diabetes-related receptors, positioning it as a key scaffold for drug development. Vanillic acid and gallic acid not only bind effectively to DPP-4 but also confer strong antioxidant and anti-inflammatory properties, providing comprehensive metabolic protection. The *in silico* finding that myricetin,

quercetin, and kaempferol exhibit greater selectivity for AGL suggests that these polyphenols act as potent, targeted inhibitors of carbohydrate digestion, a highly significant finding for T2D management and glycemic control. This is significant because AGL is an enzyme in the intestinal lining that breaks down complex carbohydrates into absorbable glucose. By inhibiting this enzyme, these polyphenols delay carbohydrate digestion, thereby preventing sudden post-meal blood sugar spikes. Clinically approved AGL inhibitors often cause uncomfortable side effects because they also heavily inhibit pancreatic α-amylase, leading to fermentation in the lower gut. The *in silico* preference for AGL over α-amylase suggests these polyphenols could offer a safer alternative with fewer digestive side effects.

These three compounds belong to the *flavonol* subclass of flavonoids, which naturally exhibit strong competitive binding and hydrogen-bonding interactions with the active sites of the AGL enzyme (Mahmud *et al*., 2023). The selective preference is largely driven by the number of phenolic hydroxyl groups on their B-ring. Myricetin (with 3 hydroxyl groups) generally demonstrates the highest binding affinity and inhibition capacity, followed by quercetin (2 hydroxyl groups) and kaempferol (1 hydroxyl group). In computational analyses, it is consistently observed that the aglycone forms of these flavonoids bind securely to the enzyme's catalytic cavity without violating drug-likeness parameters. This paves the way for their use as natural nutraceuticals or lead compounds for designing safer anti-hyperglycemic drugs (Hu *et al.,* 2025).

Comparing the spatial deviation of the polyphenols to the standard used in this study, acceptable and successful docking poses were observed, with close alignment with the experimentally solved crystal structure, confirming a reliable and highly predictive docking protocol.

Awodele and co-workers (2019) implicated aqueous extract from the seed in causing teratogenicity, genotoxicity, hepatic damage, and depletion of glutathione storage following chronic oral (100 mg/kg to 400 mg/kg) administration in rats. Also, extracts (aqueous and 80% ethanol) from the seeds had previously been shown to be safe at 5000 mg/kg when administered orally in mice (Aina *et al*., 2021). The ethyl acetate fraction of the methanol extract protects liver enzymes against carbon tetrachloride-induced hepatotoxicity in Swiss rats (Olalade *et al*., 2023). In a predictive *in silico* toxicity study by Erharuyi and Azubuike (2025), most of the screened phytochemicals exhibited low to moderate toxicity, except for rutin, which was associated with ototoxicity. This study confirms some of these parameters as moderate to high, especially for hepatotoxicity, ototoxicity, genotoxicity, cytotoxicity, carcinogenicity, and respiratory toxicity. Conversely, low to moderate toxicity was induced by the polyphenols in nephrotoxicity, neurotoxicity, hematotoxicity, and immunotoxicity. Hepatotoxicity prediction is important in assessing the risk of drug-induced liver injury. The toxicity scores of the studied compounds were distributed around the standard decision threshold of 0.50, indicating a borderline-to-moderate toxicological risk profile. Quercetin, myricetin, kaempferol, and gallic acid had values below 0.40 and were therefore classified as safe or non-hepatotoxic with higher confidence. Epigallocatechin and vanillic acid showed values around 0.50, which are considered a gray area since these compounds cannot be clearly classified as safe or unsafe. Epicatechin showed a value greater than 0.60, which is deemed a potential structural alert or definite hepatotoxin requiring close experimental scrutiny. The effect of the polyphenols on neurons was evaluated by a neurotoxicity study; most of the compounds show very low (epigallocatechin, gallic acid, myricetin, kaempferol, and quercetin) to moderate (vanillic acid and epicatechin) neurotoxic liability. The ototoxicity study showed low (kaempferol), moderate (quercetin, myricetin, and vanillic acid), and high (epigallocatechin, epicatechin, and gallic acid) structural alerts for toxicity. Toxicity effects on blood and bone marrow indicate that gallic acid, epigallocatechin, myricetin, kaempferol, and quercetin are highly safe compounds, while vanillic acid and epicatechin are mildly to moderately safe with respect to polyphenol-induced hematologic toxicity. Most of the studied polyphenols are nephrotoxicity-free, except for vanillic acid, which showed a moderate nephrotoxic effect. The genotoxicity results showed the opposite: most compounds exhibited high genotoxicity, except for vanillic acid, thus providing high confidence that vanillic acid is non-mutagenic or lacks structural alerts for DNA reactivity. The compounds were predicted to possess an extremely low risk of causing adverse immune reactions in the RPMI-8226 multiple myeloma cell line.

Environmental toxicity profiles of the polyphenols were also evaluated. Quercetin exhibits elevated IGC50 (Inhibition Growth Concentration), LC50DM (Lethal Concentration Daphnia Model), and LC50FM (Lethal Concentration Fish Model) values, indicating reduced toxicity to microbes, fish, and Daphnia. Conversely, vanillic acid and gallic acid show a marginal increase in aquatic toxicity, as evidenced by reduced IGC50, LC50DM, and LC50FM values. There may be a need to further evaluate their potential environmental implications, and potential use as drugs needs careful formulation.

A moderate level of hepatotoxicity was noted with epicatechin and epigallocatechin; these compounds, along with gallic acid, caused moderate to high ototoxicity. Most polyphenols exhibit respiratory toxicity and genotoxicity, except for vanillic acid, which shows low genotoxicity. Myricetin and quercetin exhibited moderate carcinogenicity, whereas kaempferol exhibited high

carcinogenicity. Similarly, high cytotoxicity was exhibited by kaempferol, epicatechin, and epigallocatechin.

CONCLUSION

This study validates the traditional use of *Picralima nitida* stem in African communities for managing diabetes mellitus, demonstrating that its therapeutic efficacy is mediated by a multi-target mechanism of action. HPLC profiling successfully identified seven key polyphenols within the methanol-dichloromethane extract of *P. nitida* (MDPN), with kaempferol and vanillic acid occurring in the highest concentrations.

The extract exhibits strong, concentration-dependent antioxidant activity via hydrogen atom and electron-donation mechanisms, as evidenced by DPPH and FRAP assays. This antioxidant capacity is vital for neutralizing metabolic free radicals and alleviating diabetes-induced oxidative stress.

Furthermore, *in silico* molecular docking and selectivity analysis revealed that these identified polyphenols act as dual-target inhibitors against key blood glucose regulatory enzymes. Epigallocatechin and myricetin demonstrated the highest binding affinities and inhibitory potentials against α-glucosidase. Myricetin, quercetin, and kaempferol displayed superior binding orientations and affinities toward Dipeptidyl peptidase-4 (DPP-4). Selectivity index evaluation classified epigallocatechin, epicatechin, vanillic acid, and gallic acid as preferentially inhibiting DPP-4, whereas myricetin, quercetin, and kaempferol showed greater preference for α-glucosidase.

Complementary ADMET evaluations confirmed favorable drug-likeness profiles, oral bioavailability, and established baseline pharmacokinetic parameters for these bioactive compounds. Taken together, these findings indicate that the polyphenols in *P. nitida* stems offer a synergistic, multi-target therapeutic approach to managing blood glucose. This positions MDPN as a promising natural source for developing novel dual-action antidiabetic agents and functional therapeutics.

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AUTHORS' CONTRIBUTION

Conceptualization, writing, editing and supervision: EEO. Data collection, writing and editing: KPA, APU, TCU, PEE.

CONFLICT OF INTEREST

The authors declare no conflicts of interest throughout the study.

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REFERENCES

Aguwa CN, Ukwe CV, Inya-Agha S, Okonta JM (2001). Antidiabetic effect of *Picralima nitida* aqueous seed extract in experimental rabbit model. *Journal of Natural Remedies* 1(2):135-139. DOI: 10.18311/jnr/2001/22.

Ahmed ZB, Mahammed TH, Chegma T, Seidel V, Yousfi M (2024). Alpha-glucosidase and α-amylase inhibitory activity of *Pistacia atlantica* Desf. gall extracts and identification of putative bioactives using a combined UPLC fingerprinting and molecular docking approach. *Journal of Diabetes and Metabolic Disorder* 23(2):2081-2094. DOI: 10.1007/s40200-024-01470-y.

Aina OO, Okoyenta OC, Kareem KO, Bamgbose DJ, Ajibaye O, Afolabi BM (2021). Toxicological studies of *Picralima nitida* aqueous and ethanolic extracts in animal model. *London Journal of Medical and Health Research* 21(6):21-30.

Akabassi GC, Padonou EA, Assogbajo AE, Zirihi-Guede N (2021). Prices and sold amount dynamics, endogenous knowledge and distribution of *Picralima nitida* (Stapf) T. Durand and H. Durand across in the Dahomey Gap and Guinea Congolese regions. *AAS Open Research.* 3:29. DOI: 10.12688/aasopenres.13087.3.

Al-Khodairy FM, Khan MK, Kunhi M, Pulicat MS, Akhtar S, Arif JM (2013). In silico prediction of mechanism of erysolin-induced apoptosis in human breast cancer cell lines. *American Journal of Bioinformatics Research* 3:62-71.

Alzahrani A (2023). *In-silico* study of four Alkaloids as Dipeptidyl Peptidase-4 (DPP4) inhibitors to generate anti-diabetics effect. *Egyptian Journal of Chemistry* 66(2):471-477.

Artanti N, Dewijanti ID, Muzdalifah D, Windarsih A, Suratno S, Handayani S (2023). Alpha glucosidase inhibitory activity of combination of *Caesalpinia sappan* L. and *Garcinia mangostana* extract. *Journal of Applied*

Pharmaceutical Science 13(05):189-198. DOI: 10.7324/JAPS.2023.117478.

Awodele O, Coulidiaty AGV. Afolayan GO, Agagu S, Omoseyindemi B, Busia K (2019). Toxicological evaluation of *Picralima nitida* in rodents. *Journal of Ethnopharmacology* 236:205-219. DOI: 10.1016/j.jep.2019.03.008.

Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules* 27(4):1326. DOI: 10.3390/molecules27041326.

Baumel-Alterzon S, Katz LS, Brill G, Garcia-Ocaña A, Scott DK (2021). Nrf2: The master and captain of beta cell fate. *Trends Endocrinology and Metabolism* 32(1):7-19. DOI: 10.1016/j.tem.2020.11.002.

Baumel-Alterzon S, Katz LS, Brill G, Jean-Pierre C, Li Y, Tse I, Biswal S, Garcia-Ocaña A, Scott DK (2022). Nrf2 regulates β-cell mass by suppressing β-cell death and promoting β-cell proliferation. *Diabetes* 71(5):989-1011. DOI: 10.2337/db21-0581.

Benet LZ, Hosey CM, Ursu O, Oprea TI (2016). BDDCS, the rule of 5 and drugability. *Advanced Drug Delivery Reviews* 101:89-98.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry* 239(1):70-76. DOI: 10.1006/abio.1996.0292.

Bibi Sadeer N, Montesano D, Albrizio S, Zengin G, Mahomoodally MF (2020). The versatility of antioxidant assays in food science and safety-chemistry, applications, strengths, and limitations. *Antioxidants (Basel)*. 9(8):709. DOI: 10.3390/antiox9080709.

Chang Y, Hawkins BA, Du JJ, Groundwater PW, Hibbs DE, Lai F (2022). A guide to In-silico drug design. *Pharmaceutics*. 15(1):49. DOI: 10.3390/pharmaceutics15010049.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* 28(1):25-30, DOI: 10.1016/S0023-6438(95)80008-5.

Ćorković I, Gašo-Sokač D, Pichler A, Šimunović J, Kopjar M (2022). Dietary polyphenols as natural inhibitors of α-Amylase and α-Glucosidase. *Life (Basel)*. 12(11):1692. DOI: 10.3390/life12111692.

Creed SM, Gutridge AM, Argade MD, Hennessy MR, Friesen JB, Pauli GF, van Rijn RM, Riley AP (2021). Isolation and pharmacological characterization of six Opioidergic *Picralima nitida* alkaloids. *Journal of Natural Product* 84(1):71-80. DOI:10.1021/acs.jnatprod.0c01036.

Dimitriadis GD, Tessari P, Go VL, Gerich JE (1985). Alpha-Glucosidase inhibition improves postprandial hyperglycemia and decreases insulin requirements in insulin-dependent diabetes mellitus. *Metabolism* 34(3):261-265. DOI: 10.1016/0026-0495(85)90010-1.

Dodson M, Shakya A, Anandhan A, Chen J, Garcia JGN, Zhang DD (2022). NRF2 and diabetes: The good, the bad, and the complex. *Diabetes* 71(12):2463-2476. DOI: 10.2337/db22-0623.

Erharuyi O, Azubuike CC (2025). HPLC analysis, *in-vitro* and *in-silico* evaluation of antioxidant activity of methanol stem bark extract of *Picralima nitida* (Apocynaceae). *Tropical Journal of Phytochemistry and Pharmaceutical Sciences* 4 (5):230-242. DOI: doi.org/10.26538/tjpps/v4i5.6.

Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ (2016). Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nature Protocols* 11(5):905-919. DOI: 10.1038/nprot.2016.051.

Fu L, Shi S, Yi J, Wang N, He Y, Wu Z, Peng J, Deng Y, Wang W, Wu C, *et al* (2024). ADMETlab 3.0: An updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. *Nucleic Acids Research* 52(W1):W422-W431. DOI: 10.1093/nar/gkae236.

Kotkar GD, Shetgaonkar AD, Tilve SG (2023). Diversity of chemical skeletons: A practical strategy to benefit. In: Meena SN, Nandre V, Kodam K, Meena RS (eds). Progress in Biochemistry and Biotechnology, New Horizons in Natural Compound Research, Academic Press. Pp75-132. DOI: 10.1016/B978-0-443-15232-0.00023-0.

Gęgotek A, Skrzydlewska E (2022). Anti-oxidative and anti-inflammatory activity of ascorbic acid. *Antioxidants (Basel)* 11(10):1993. DOI: 10.3390/antiox11101993.

Gnabre J, Bates R, Huang RC (2015). Creosote bush lignans for human disease treatment and prevention: Perspectives on combination therapy. *Journal of Traditional and Complementary Medicine* 5:119e126. DOI: 10.1016/j.jtcme.2014.11.024.

Godinho R, Mega C, Teixeira-de-Lemos E, Carvalho E, Teixeira F, Fernandes R, Reis F (2015). The place of dipeptidyl peptidase-4 inhibitors in Type 2 diabetes therapeutics: A "me too" or "the special one" antidiabetic class? *Journal of Diabetes Research* 2015:806979. DOI: 10.1155/2015/806979.

Gonzatti MB, Monteiro Júnior JE, Rocha AJ, de Oliveira JS, Evangelista AJJ, Fonseca FMP, Ceccatto VM, de Oliveira AC, Freire JEC (2023). Mechanism of molecular interaction of sitagliptin with human DPP4 enzyme - New Insights, *Advances in Medical Sciences* 68(2): 402-408. DOI: 10.1016/j.advms.2023.10.002.

Gour A, Creed S, Riley AP, Sharma A (2025). Comprehensive pharmacokinetics and ADME evaluation of Akuamma alkaloids, *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences* 1263:124713. DOI: 10.1016/j.jchromb.2025.124713.

Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR (2012). Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics* 4(1):17. DOI: 10.1186/1758-2946-4-17.

Hu L, Luo Y, Yang J, Cheng C (2025). Botanical flavonoids: Efficacy, absorption, metabolism and advanced pharmaceutical technology for improving bioavailability. *Molecules* 30(5):1184. DOI: 10.3390/molecules30051184.

Jomova K, Alomar SY, Valko R, Liska J, Nepovimova E, Kuca K, Valko M (2025). Flavonoids and their role in oxidative stress, inflammation, and human diseases. *Chemico-Biological Interactions* 413:111489. DOI: 10.1016/j.cbi.2025.111489.

Kedare SB, Singh RP (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology* 48(4):412-22. DOI: 10.1007/s13197-011-0251-1.

Kumar S, Pandey AK (2013). Chemistry and biological activities of flavonoids: An overview. *Scientific World Journal* 2013:162750. DOI: 10.1155/2013/162750.

Laryea MK, Abotsi KMW, Woode E, Dickson RA, Webster L, Ekuadzi E (2026). Antiplasmodial and anti-trypanosomial monoterpene indole alkaloids from *Picralima nitida*. *Natural Product Research* 40(6):1548-1557. DOI: 10.1080/14786419.2024.2434710.

Lemanowicz J, Gawlińska K, Jaskulska I, Jaskulski D, Sar M (2026). Flavonoids in plants and human health: From biosynthesis to neurodevelopmental and neurodegenerative Disorders. *Molecules.* 31(1):66. DOI: 10.3390/molecules31010066.

Lima K, Malmir M, Sabiha S, Pinto R, Silva IMD, Figueira ME, Rocha J, Duarte MP, Silva O (2025). Effects of *Periploca chevalieri* Browicz on postprandial glycemia and carbohydrate-hydrolyzing enzymes. *Pharmaceuticals (Basel)* 18(6):913. DOI: 10.3390/ph18060913.

López-Herrador S, Corral-Sarasa J, González-García P, Morillas-Morota Y, Olivieri E, Jiménez-Sánchez L, Díaz-Casado ME (2025). Natural hydroxybenzoic and hydroxycinnamic acids derivatives: mechanisms of action and therapeutic applications. *Antioxidants (Basel)* 14(6):711. DOI: 10.3390/antiox14060711.

Mahmud AR, Ema TI, Siddiquee MF, Shahriar A, Ahmed H, Mosfeq-Ul-Hasan M, Rahman N, Islam R, Uddin MR, Mizan MFR (2023). Natural flavonols: Actions, mechanisms, and potential therapeutic utility for various diseases. *Beni Suef University Journal of Basic and Applied Science* 12(1):47. DOI: 10.1186/s43088-023-00387-4.

Mathur V, Alam O, Siddiqui N, Jha M, Manaithiya A, Bawa S, Sharma N, Alshehri S, Alam P, Shakeel F (2023). Insight into structure-activity relationship of DPP-4 inhibitors for development of antidiabetic agents. *Molecule* 28(15):5860. DOI: 10.3390/molecules28155860.

Mihaylova D, Dimitrova-Dimova M, Popova A (2024). Dietary phenolic compounds-wellbeing and perspective applications. *International Journal of Molecular Science* 25(9):4769. DOI: 10.3390/ijms25094769.

Min SH, Yoon JH, Hahn S, Cho YM (2018). Efficacy and safety of combination therapy with an α-glucosidase inhibitor and a dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes mellitus: A systematic review with meta-analysis. *Journal of Diabetes Investigation* 9(4):893-902. DOI: 10.1111/jdi.12754.

Molyneux P (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology* 26:211-219.

Odion EE, Favour JC, Osigwe CC, Umeji TC, Uwaeme FU (2025). *Boerhavia diffusa* (linn.) Nyctaginaceae: Phytochemical profiling and sub-chronic toxicity study of methanolic leaf extract in rodents. *Cameroon Journal Experimental Biology* 19(1):9-16. DOI: 10.4314/cajeb.V19i1.2.

Ogbeide OK, Sajere O, Obukuru IS, Akedayi P, Omorefe NO, Unachukwu IC, Mbonu R, Aghedo O, Iyekowa O, Uadia JO (2024). Comparative studies on the bioactive chemical composition, antioxidant and antimalarial potentials of *Picralima nitida* leaf and stem bark extracts. *Benin Journal of Physical Sciences* 1(1): 231-250.

Ololade AM, Anowi FC, Anwuchaepe AA, IfedibaluChukwu EI (2023). Pharmacognostic study and hepatoprotective activity of the methanolic extract and fractions of leaves of *Picralima nitida* (Apocynaceae). *Sciences of Phytochemistry* 2(1):88-98. DOI: 10.58920/sciphy02010114.

Sampath C, Raju AV, Freeman ML, Srinivasan S, Gangula PR (2022). Nrf2 attenuates hyperglycemia-induced nNOS impairment in adult mouse primary enteric neuronal crest cells and normalizes stomach function. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 322(3):G368-G382. DOI: 10.1152/ajpgi.00323.2021.

Sarkar D, Christopher A, Shetty K (2022). Phenolic bioactives from plant-based foods for glycemic Control. Frontiers in Endocrinology (Lausanne) 12:727503. DOI: 10.3389/fendo.2021.727503.

Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga LL (2011). Extraction, isolation and characterization of bioactive compounds from plants extracts. *African Journal of Traditional Complementary and Alternative Medicine* 8(1):1-10.

Sliwoski G, Kothiwale S, Meiler J, Lowe EW (2014). Computational methods in drug discovery. *Pharmacological Reviews* 66:334-395. DOI: 10.1124/pr.112.007336.

Stachelska MA, Karpiński P, Kruszewski B (2025). A comprehensive review of biological properties of flavonoids and their role in the prevention of metabolic, cancer and neurodegenerative diseases. *Applied Science* 15:10840. DOI: 10.3390/app151910840.

Stagos D (2019). Antioxidant activity of polyphenolic plant extracts. *Antioxidants (Basel)* 9(1):19. DOI: 10.3390/antiox9010019.

Suraweera L, Rupasinghe T, Dellaire HPV, Xu G (2020). Regulation of Nrf2/ARE pathway by dietary flavonoids: A friend or foe for cancer management? *Antioxidants (Basel)* 9(10):973. DOI: 10.3390/antiox9100973.

Tagami T, Yamashita K, Okuyama M, Mori H, Yao M, Kimura A (2015). Structural advantage of sugar beet α-glucosidase to stabilize the Michaelis complex with long-chain substrate. *Journal of Biological Chemistry* 290(3):1796-803. DOI: 10.1074/jbc.M114.606939.

Teugwa CM, Mejiato PC, Zofou D, Tchinda BT, Boyom FF (2013). Antioxidant and antidiabetic profiles of two African medicinal plants: *Picralima nitida* (Apocynaceae) and *Sonchus oleraceus* (Asteraceae). *BMC Complementary and Alternative Medicine* 13:175. DOI: 10.1186/1472-6882-13-175.

Tsai HY, Ho CT, Chen YK (2017). Biological actions and molecular effects of resveratrol, pterostilbene, and 3'-hydroxypterostilbene. *Journal of Food and Drug Analysis* 25(1):134-147. DOI: 10.1016/j.jfda.2016.07.004.

Waffo-Teguo P, Krisa S, Richard T, Mérillon J-M (2008). Grapevine Stilbenes and their biological effects. In: Ramawat KG, Merillon JM, (eds). Bioactive Molecules and Medicinal Plants. Springer; Berlin/Heidelberg, Germany. Pp 25-54.

Xue S, Tan W, Mao S, Pan H, Ye X, Donlao N, Tian J (2025). Polyphenol-based functional materials: Structural insights, composite strategies, and biomedical applications. *Advanced Science (Weinh)* 12(39):e08924. DOI: 10.1002/advs.202508924.

Yao KJN, Assamoi A, Antoine A, Ouattara HD, N'dri KED (2023). Antidiabetic activities of *Picralima nitida* powder, an indigenous Ivorian edible plant in Type II diabetes mellitus rat model. *Biomedicine and Biotechnology* 8(1):14-23. DOI: 10.12691/bb-8-1-2.

Zahra M, Abrahamse H, George BP (2024). Flavonoids: Antioxidant powerhouses and their role in nanomedicine. *Antioxidants (Basel)* 13(8):922. DOI: 10.3390/antiox13080922.

Zhao L, Zhang M, Pan F, Li J, Dou R, Wang X, Wang Y, He Y, Wang S, Cai S (2021). *In silico* analysis of novel dipeptidyl peptidase-IV inhibitory peptides released from *Macadamia integrifolia* antimicrobial protein 2 (MiAMP2) and the possible pathways involved in diabetes protection. *Current Research in Food Science* 4:603-611. DOI: 10.1016/j.crfs.2021.08.008.