Tropical Journal of Drug Research January 2025; 2 (1): 14 - 28
ISSN: 2476-8065 (Electronic)
Available online at http://www.tjdr.org
https://doi.org/10.26538/tjdr/v2i1.3

Original Research Article

Chemical Profiling, In-vitro and Computational analysis of the Ethanol Extract of *Mangifera indica* Bark in the search for new antidiabetic agents

Izevbigie EV.*[1], Akanbi AI.[1], Oyawaluja BO.[2]

[1] *African Center of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science (ACEDHARS), Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.*
[2] *Faculty of Pharmacy, University of Lagos, Nigeria.*

*For correspondence: Email: esosaizevbigie753@gmail, 239203032@live.unilag.edu.ng; +2347066895059

Sent for review: 18 January 2025 Revised accepted: 27 January 2025

Abstract

Purpose: Diabetes mellitus is a global health challenge requiring novel therapeutic agents. The inhibition of *α-amylase* and *α-glucosidase* enzymes is a key strategy for controlling postprandial hyperglycemia. While synthetic inhibitors exist, their adverse effects necessitate safer alternatives. This study evaluates the chemical composition and antidiabetic potential of *Mangifera indica* bark extract through *in vitro* and computational analyses.
Methods: High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) were used to identify phytochemicals in the extract. The *α-amylase* and *α-glucosidase* inhibitory activities were assessed using enzyme inhibition assays, with IC_{50} values determined. Molecular docking studies were conducted using AutoDock Vina to evaluate the binding affinity of key phytoconstituents, and pharmacokinetic properties were analyzed using SwissADME.
Results: HPLC and GC-MS identified gallic acid (0.58 g/g) and Mangiferin (0.03 g/g) as major bioactive compounds. The extract exhibited strong *α-amylase* inhibition (IC_{50} = 16.11 µg/mL) and *α-glucosidase* inhibition (IC_{50} = 6.96 µg/mL), outperforming Acarbose. Molecular docking revealed Mangiferin as the primary bioactive compound, with binding affinities of -9.1 kcal/mol and -7.8 kcal/mol for *α-glucosidase* and *α-amylase*, respectively. ADME analysis indicated favorable pharmacokinetics and drug-likeness properties.
Conclusion: *Mangifera indica* bark extract demonstrated potent antidiabetic activity through enzyme inhibition, with Mangiferin identified as a promising lead compound. These findings support its potential as a natural therapeutic agent for diabetes management, warranting further pharmacological and clinical investigations.

Keywords: *Mangifera indica*, Mangiferin, *α-glucosidase*, *α-amylase*, Diabetes, IC_{50}, Druglikeness.

Tropical Journal of Drug Research is indexed by Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals
(DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Diabetes mellitus is a long-term metabolic disease marked by high blood sugar levels due to either insulin resistance, insufficiency, or both. This disease affects millions of people globally thus becoming a major global health concern. As at 2019, the prevalence of diabetes was estimated to be 9.3% (463 million people), and is expected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.[1] Sedentary lifestyles, poor diets, and rising obesity rates are some of the reasons contributing to the rising prevalence of diabetes. The most prevalent kind of diabetes, type 2, which is mostly avoidable and is especially impacted by lifestyle decisions.[2] Diabetes has been associated with microvascular complications including retinopathy, nephropathy, and neuropathy, indicate the progressive and severe impact of the disease.[3] The disease also has a significant financial impact on people and healthcare systems around the world due to medical care expenses and lost productivity.[4] Mitigating this crisis requires effective management techniques, such as public health interventions, biomarker monitoring and management, lifestyle interventions, and pharmacological prevention.[5]

Herbal medicine is rapidly becoming a popular choice for managing diabetes mellitus with many individuals turning to herbal remedies as alternatives or complements to conventional treatments. This trend is particularly noticeable in regions where access to healthcare is limited or where cultural beliefs favor natural remedies as they are often seen as safer substitutes. Also, due to cultural familiarity of the plants, herbal therapy is a more enticing option, especially for people who are wary of the possible negative effects of conventional pharmaceuticals.[6] Nonetheless, caution should be exercised when using herbal medication in the treatment of diabetes. Herbal preparations often lack quality control, which could affect their safety and efficacy. It's also important to take into account possible interactions between orthodox drugs and natural therapies as such patients are advised to speak with medical professionals to prevent drug-herb interactions. Herbal treatments' significance in managing diabetes is probably going to grow as more studies confirm their effectiveness. A promising approach to improving the care of diabetes mellitus, especially in culturally diverse environments, is the nexus of contemporary scientific research and traditional practices.

Numerous herbs have been identified by research as having potential for managing diabetes among which is *mangifera indica*. The present study investigated the anti-diabetic activity of ethanol stem bark extract of *M. Indica* and the identification of natural chemical constituents which were responsible for the bioactivity. High Performance Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GC-MS) were used for the identification and quantification of phytochemical compounds.

The genus *Mangifera*, includes over 22 Genera of flowering plants in the Cashew family, Anacardiaceae with the best-known being the common mango.[7] Mangoes (*Mangifera indica)* are known by the following names across Africa; German plum (Liberia), Cepton or Laberu (Serria Leone), Amango in Twi (Ghana), máńgòrò (Yorùbá, Nigeria), and Mangwaro (Hausa, Nigeria). Mango bark is traditionally used to treat diarrhea, cancer, diabetes, prostatitis, toothache and cough and urinary tract infections.[8] In African communities, where herbal treatments are an essential part of health practices, these plants serve as examples of the wealth of ethnomedical knowledge. Further investigation into these plants may confirm their conventional applications and support more comprehensive diabetes care plans.

Numerous compounds, including Quercetin, Mangiferin Rutin, Isorhamnetin, Gallic acid, Benzoic acid, Kaempferol 3-glucoside, Quercetin 3-glucoside, and Cinnamic acid, have been isolated from various parts of *M. indica.* Strong antioxidant, anti-lipid peroxidation, immunomodulation, cardiotonic, hypertensive, wound healing, antidegenerative, and antidiabetic properties have been reported by Mangiferin, a polyphenolic antioxidant and glucosyl xanthone.[9] These phytochemical compounds have been linked to the prevention of degenerative diseases such as cancer, cardiovascular disorders and diabetes.[10] The *Mangifera indica* plant is therefore relevant for synthesis of pharmaceuticals, nutraceuticals, food supplements, and medicinal substances in both traditional and modern systems. Different components of the plant are used to treat diarrhea, dysentery, anaemia, asthma, and stomachic conditions. They are also used as a dentifrice, antiseptic, astringent, diaphoretic, laxative, and diuretic, Abscesses, broken horns, rabid dog or jackal bites, snakebite, datura poisoning, blisters, oral wounds, liver problems, excessive urination, tetanus, and asthma are all treated using all parts.[11] The antidiabetic effects of *Mangifera indica* can be understood through several mechanisms: Improvement of insulin sensitivity, antioxidant and anti-inflammatory activities, modulation of lipid metabolism and reduction of blood glucose levels. Various studies have shown that mango extracts can significantly lower blood glucose

levels in diabetic animal models. This effect is believed to be due to the inhibition of carbohydrate-digesting enzymes, such as *α-amylase* and *α-glucosidase*, which results in delayed carbohydrate absorption and improved glycaemic control.[12]

Molecular docking techniques are pivotal in the discovery of novel drug candidates, particularly in the context of antidiabetic agents targeting enzymes such as *alpha-amylase* and *alpha-glucosidase*. This computational method enables researchers to predict the binding affinity and orientation of bioactive compounds the active sites of these enzymes. By leveraging the insights gained from molecular docking studies, researchers can identify structural features that enhance inhibitory potency.

This study therefore uniquely highlights the antidiabetic potential of ethanolic bark extract of *Mangifera indica* by combining chemical profiling, *in vitro* enzyme inhibition assays, and computational molecular docking. The integration of SwissADME and molecular dynamics simulations is intended to evaluate the extracts phytoconstituents as safe and effective treatment options for diabetes management. To the best of our knowledge, this is the first study to comprehensively evaluate the pharmacological, computational, and structural properties of *M. indica* ethanolic bark extract for antidiabetic applications.

MATERIALS AND METHODS

Materials

The following instruments and equipment were used: High-Performance Liquid Chromatography (HPLC, Agilent® 1200 series), Gas Chromatography-Mass Spectrometry (GC-MS, Agilent® 5977B GC/MSD), analytical balance (Mettler Toledo®), sonicator (J.P. Selecta®), water bath (T8H1), funnel, beaker, volumetric flask, measuring cylinder, retort stands, vial bottles, micro syringe filters, Whatman® filter paper, and cotton wool.

Chemicals and Reagents

All the chemicals utilized in this study were of analytical grade and procured from Sigma-Aldrich® (St. Louis, MO, USA) and Supelco, Merck (Darmstadt, Germany). The reagents included 96% ethanol, nitric acid, hydrochloric acid, acetonitrile, methanol, 5% diphenyl/95% dimethyl polysiloxane (for GC-MS), 0.1% phosphoric acid in water, Mangiferin, gallic acid, dinitrosalicylic acid (DNSA), porcine pancreatic *α-amylase*, and sodium phosphate buffer.

Collection of Plant Materials

Bark of *Mangifera indica* (Linn.) was collected in August 2024 from the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria with location latitude 70 33′ 0″ N and longitude 40 34′ 0″ E, 271 m MSL (mean sea level) in the month and voucher specimen FPI 2529. Authentication of the specimen was conducted and the voucher specimen was deposited in the Medicinal Plants Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (FPI available on Index Herbariorum: https://sweetgum.nybg.org/science/ih/herbarium_list.php).

Extraction

The plant bark was air-dried in an open space, then ground to a fine powder using a milling machine. Extraction was carried out according to the method of Aderonke *et al*., [13] with slight modifications. A 400 g sample of the powdered bark was placed in an amber-colored bottle and 1.5 L of 96% ethanol was added as the solvent. The mixture was macerated for 72 hours with intermittent shaking, followed by filtration to yield 1100 mL of ethanolic extract. The filtrate was concentrated using a rotary evaporator (RS 3000, J.P. Selecta® Spain) and then further dried in a water bath to obtain the dry extract, which was stored in a sample bottle at room temperature, protected from light.

High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed based on the method described by Zhang.[14] with minor modifications for optimal detection of Mangiferin and gallic acid. The mobile phase consisted of methanol and 0.1% phosphoric acid (in a 31:69 v/v ratio). The analysis was carried out at room temperature with a flow rate of 1.0 mL/min, and ultraviolet (UV) detection at 258 nm.

Preparation of Standard Stock Solutions

Reference standards of Mangiferin and gallic acid (5 mg each) were dissolved in methanol to prepare 1000 µg/mL stock solutions in 5 mL volumetric flasks. A 10 µL aliquot of each standard was injected into the HPLC to determine retention times.

Preparation of Mixed Standards

About 1 mL of each 1000 µg/mL stock solution of Mangiferin and gallic acid was mixed in a 10 mL volumetric flask and diluted with methanol to achieve a final concentration of 100 µg/mL.

Preparation of Graded Concentrations for Calibration

Concentrations ranging from 5 to 100 µg/mL were prepared by serial dilution, sonicated for 20 minutes, filtered, and injected in 20 µL aliquots into the HPLC for UV detection at 258 nm.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed using an Agilent® 5977B GC/MSD system, fitted with an Elite-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness). The ionization system operated in electron impact mode at 70 eV, with helium (99.99%) as the carrier gas at a constant flow rate of 1 mL/min. The injection volume was 1 µL, using a 10:1 split ratio. The injector temperature was 300°C, and the ion source temperature was set to 250°C. The oven temperature program was as follows: initial temperature 110°C (hold for 1 min), ramped at 10°C/min to 310°C (hold for 2 min). Mass spectra were recorded from 45 to 450 Da with a scan interval of 0.5 s. The solvent delay was set to 0-3 minutes.

***α-Amylase* Inhibitory Assay**

The *α-amylase* inhibitory activity of the ethanolic bark extract of *Mangifera indica* was evaluated according to the method of Worthington.[15] Dilutions of the extract (0–200 µL) and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing porcine pancreatic *α-amylase* (0.5 mg/mL) were incubated at 25°C for 10 minutes. Subsequently, 500 µL of 1% starch solution in the same buffer was added, and the mixture was incubated for another 10 minutes. The reaction was terminated by adding 1.0 mL of dinitrosalicylic acid (DNSA), followed by incubation in a boiling water bath for 5 minutes. The mixture was then cooled and diluted with distilled water to 10 mL. Absorbance values were measured at 540 nm using a spectrophotometer [16] 6300 VIS, Labnet, Finland.

***α-Glucosidase* Inhibitory Assay**

α-Glucosidase inhibitory activity was assessed following modified methods from Matsui.[17] and Bräunlich.[18] The extract (0–200 µL) and 100 µL of *α-glucosidase* (0.5 mg/mL) in 0.1 M phosphate buffer (pH 6.9) were incubated at 25°C for 10 minutes. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside was added and the mixture was incubated for 5 minutes at 25°C. Absorbance was measured at 405 nm. The IC50 values were determined by plotting percentage inhibition versus log inhibitor concentration, and acarbose was used as a reference inhibitor.

The inhibitory activities were expressed as percentage inhibition using equation 1:

$$\text{Inhibition (\%)} = \frac{Abs\ ref - Abs\ sample}{Abs\ ref} \text{ X } 100 \quad \text{.....Equation 1}$$

Where Abs_{ref} is the absorbance of the reference and Abs_{sample} is the absorbance of the test sample.

Retrieval and Preparation of Proteins

The 3D structures of human pancreatic *α-amylase* (PDB ID: 5EMY) and human maltase-glucoamylase (PDB ID: 2QMJ) were retrieved from the Protein Data Bank (http://www.rcsb.org).[19] Native ligands and water molecules were removed using Biovia Discovery Studio,[20] and missing polar hydrogen atoms and charges were added using MGL-AutoDockTools (ADT, v1.5.7).[21] Grid dimension was set and the proteins were saved in PDBQT format for docking simulations.

Ligand Preparation

The 3D structures of bioactive compounds identified by HPLC and those with an abundance greater than 10% from GC-MS analysis were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov),[22] downloaded in SDF format. The insulin-mimetic ligands include Acarbose (CID 417744), Mangiferin (CID 5358385), Phenol (CID 996), Gallic acid (CID 370), Hexadecanoic acid methyl ester (CID 5362679), and 9-Octadecenoic acid (Z)-methyl ester (CID 5364509). The structures were converted to PDB format using OpenBabel (version 2.4.1),[23] with polar hydrogens added and Gasteiger charges assigned. The ligands were then saved as PDBQT prior to docking simulations.

Molecular Docking Studies

Docking validation was performed by redocking Acarbose into the active sites of *α-amylase* and *α-glucosidase*. The root mean square deviation (RMSD) was calculated after superimposing the binding pose with the co-crystallized inhibitor. Molecular docking was conducted using AutoDock Vina (version 4.2.6)[21] to assess the binding affinity of the bioactive compounds and reference inhibitors. The docking results were analyzed, and the docked complexes were visualized using PyMOL,[24] and Discovery Studio Visualizer.[20]

ADME and Physiochemical Properties

The physicochemical properties of the bioactive compound with the lowest binding energy were assessed using the SwissADME online server (https://swissadme.ch).[25]

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations were performed to evaluate the stability and physical movements of the docked protein-ligand complexes. The ligand with the lowest binding score was subjected to MD simulations to assess its impact on protein structure. Simulations were carried out using the Internal Coordinates Normal Mode Analysis (iMODS) online server (https://imods.iqfr.csic.es),[26] which employs the elastic network model (ENM) to approximate molecular flexibility and conformational changes efficiently. The slow dynamics of the docked complexes were examined, and their conformational variations were demonstrated using Eigenvalues to indicate the stiffness of each normal mode, B-factor mobility flexibility and Deformation Energy which represents the energy required to induce a specific conformational change.

Statistical Analysis

Data were analyzed using one sample t-test. Differences were considered statistically significant at $p < 0.05$. GraphPad Prism (version 10.4.0) was used for statistical analysis.

RESULT AND DISCUSSION

The increasing demand for new antidiabetic agents has led researchers to explore medicinal plants as sources of lead compounds. Profiling the chemical composition of plant extracts not only provides insight into key active constituents but also serves as an effective method for standardizing and ensuring the quality of herbal preparations.[27] In this study, we profiled the chemical composition of the ethanolic extract of *Mangifera indica* stem bark, utilizing High-Performance Liquid Chromatography (HPLC). The calibration curves for Gallic acid and Mangiferin are depicted in Figure 1 and 2, while Figure 3 and 4 show the Mangiferin and Gallic acid standard chromatograms. These provide the calibration equations and peak area values that were used to quantify the concentrations of Mangiferin and Gallic acid in the extract as 0.58 g and 0.03 g per gram of *M. indica* extract as shown in Table 1. Figure 5, depicts the *Mangifera Indica* ethanolic bark extract chromatogram, which is useful for quality control measures where the chromatogram's peaks are compared to reference standards to identify the compounds and detect adulterations. The chromatographic profile observed for this extract was consistent with the polar nature of its phytochemical constituents. Notably, Mangiferin, a C-glucoside xanthone, is abundantly present in *Mangifera indica*, as previously reported.[28] This compound is known for its potent antioxidant properties, which may contribute to the potential therapeutic benefits of *M. indica* stem bark in managing diabetes and other conditions associated with oxidative stress.

To further investigate the composition of the extract, Gas Chromatography-Mass Spectrometry (GC-MS) was employed, revealing several bioactive compounds total ion peaks as shown in Figure 6. Table 2 depicts the comprehensive list of identified compounds with the most abundant being phenol, hexadecenoic acid methyl ester, and 9-octadecenoic acid (Z)-methyl ester. These compounds have been previously associated with significant antioxidant, hypocholesterolemic, anti-inflammatory, antibacterial, and antiandrogenic properties.[29] Hexadecenoic acid methyl ester is a derivative of palmitoleic acid, a known lipokine (a lipid hormone) with metabolic regulation properties, including improved insulin sensitivity and anti-inflammatory effects.[30]

The enzymes *α-amylase* and *α-glucosidase* are known to play crucial roles in carbohydrate metabolism. While *α-amylase* hydrolyzes starch and glycogen into maltose and dextrins,[31] *α-glucosidase* breaks down disaccharides and oligosaccharides into glucose. The inhibition of these enzymes is a promising strategy for managing postprandial hyperglycemia.[32] In this study, the ethanolic extract of *M. indica* exhibited potent *α-amylase* inhibitory activity compared with reference standard Acarbose as shown in Figure 7. Figure 8 revealed that the *Mangifera indica* ethanolic extract had an IC_{50} value of 16.11 µg/mL, which was significantly lower than that of the reference standard, Acarbose (IC50 = 52.42 µg/mL). The extract and Acarbose both showed a statistically significant ($p < 0.0001$) inhibition greater than 90% at a concentration of 3.9 µg/mL. These results suggest that a lower dose of *M. indica* extract could achieve comparable *α-amylase* inhibition compared with Acarbose.

Table 1: HPLC profiling and bioactive concentration of ethanol extracts of *M. indica*

Plant Extract	Bioactive Compounds	Area (mAU*s)	Retention time (min)	Bioactive concentration (g/g) Calculated
Mangifera indica	Gallic acid	167,559	1.39	0.58
	Mangiferin	49,024	5.22	0.03

Table 2: GC-MS composition of the *Mangifera indica* extract

S/N	Compound	RT	% composition	Quality (%)
1	Phenol	3.45	14.86	91
2	Ethanol, TMS derivative	3.80	4.62	58
3	Benzoic acid, methyl ester	4.78	7.90	64
4	Tetracyclo [6.3.0.0(2,11).0(3,7)] undecan-10-one, 6-(2-methoxyethyloxymethyloxy)-7-methyl	5.03	1.79	43
5	Phenol, 4-[2-(5-nitro-2-benzoxazolyl) ethenyl]-	6.04	0.68	38
6	6H-Pyrazolo[3,4-b] pyridin-6-one, 1,7-dihydro-3,4-dimethyl-1-(1-phenylethyl)-	8.07	0.80	38
7	Phenol, 2,5-bis(1,1-dimethylethyl)-	9.59	0.74	83
8	Methyl 4-*O*-acetyl-2,3,6-tri-*O*-ethyl-alpha. -d-galactopyranoside	9.78	1.29	30
9	Butanoic acid, 3-hydroxy	10.90	2.62	35
10	Caryophyllene-(I1)	11.18	0.97	91
11	Acetic acid, chloro-, octadecyl ester	12.28	0.74	93
12	(4R*,5R*,9S*)-5,9-Dimethylspiro [3.5] nonan-1-one	12.66	2.36	83
13	Cyclohexanol, 3,3,5-trimethyl	13.17	0.87	72
14	Hexadecenoic acid, methyl ester	13.42	10.48	98
15	Dibutyl phthalate	13.71	3.94	78
16	1-Octadecene	13.99	1.38	50
17	9-Octadecenoic acid (Z)-, methyl ester	14.84	14.65	99
18	Methyl stearate	15.04	3.64	99
19	7-Pentadecyne	15.18	2.66	70
20	1-Methylbicyclo [3.2.1] octane	15.34	2.24	89
21	Thunbergol	15.55	2.52	49
22	Pregna-5,17(20)-dien-3-ol, (3. beta.,17E)-	16.76	0.60	89
23	1-Naphthalenecarboxylic acid, decahydro-1,4a-dimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-, methyl ester	16.96	0.69	78
24	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-	17.30	5.77	80
25	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl) but-2-en-1-ol	18.3	2.64	87
26	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a. alpha.,7. beta.,8a. beta.)-	18.86	0.63	53
27	1-Naphthalenecarboxylic acid, decahydro-1,4a-dimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-, methyl ester	19.44	0.96	64
28	(E)-15,16-Dinorlabda-8(17),11-dien-13-one	20.58	0.64	55

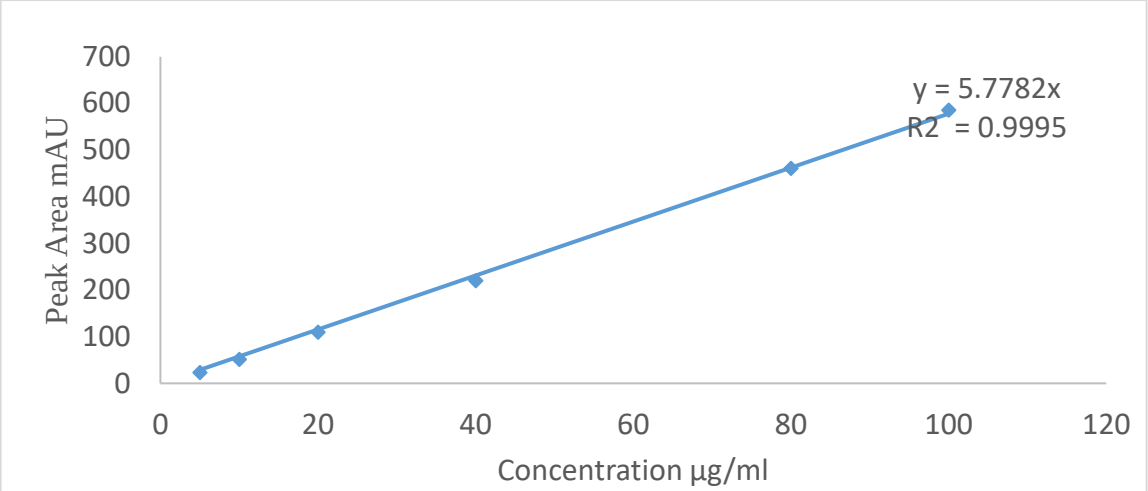

Figure 1: Calibration curve for Gallic acid

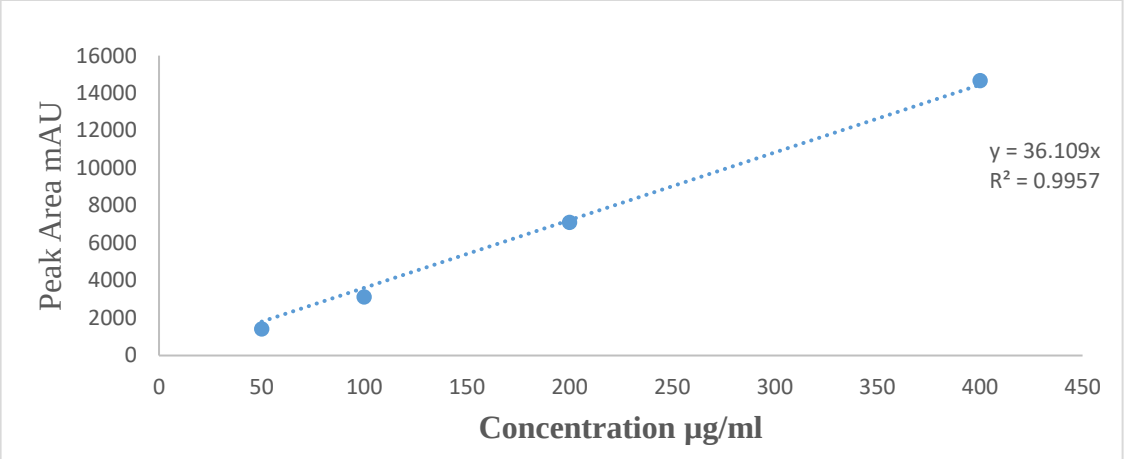

Figure 2: Calibration curve for Mangiferin

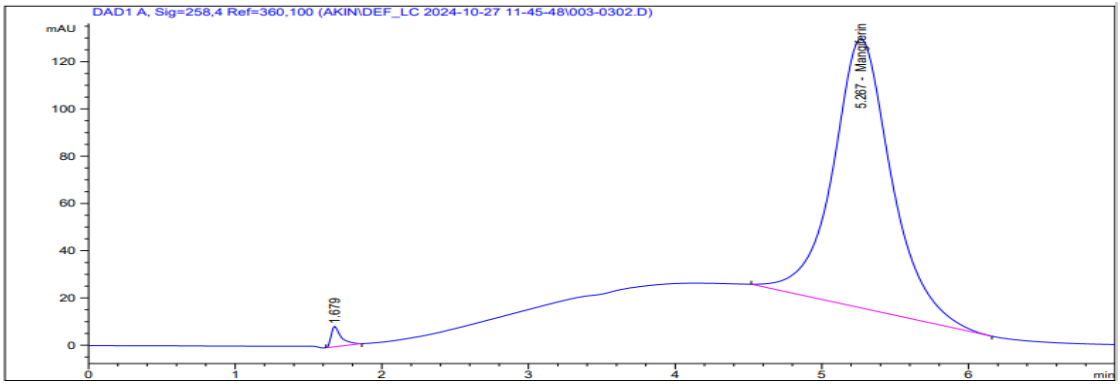

Figure 3: Mangiferin standard chromatogram

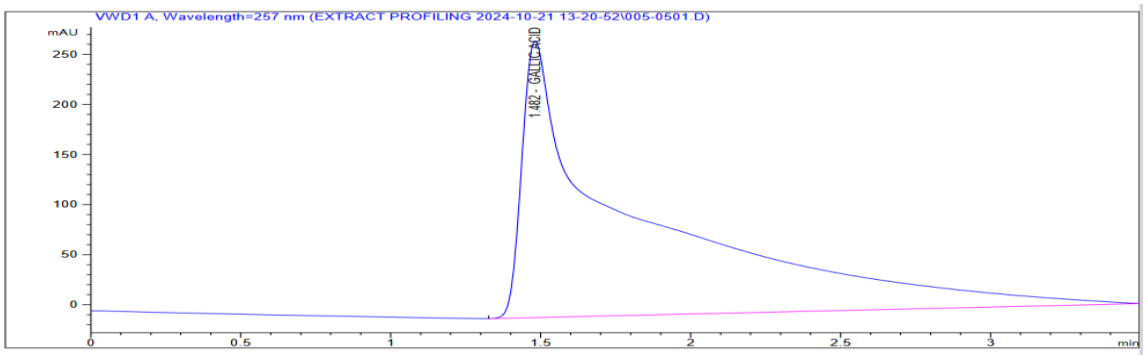

Figure 4: Gallic Acid Standard chromatogram

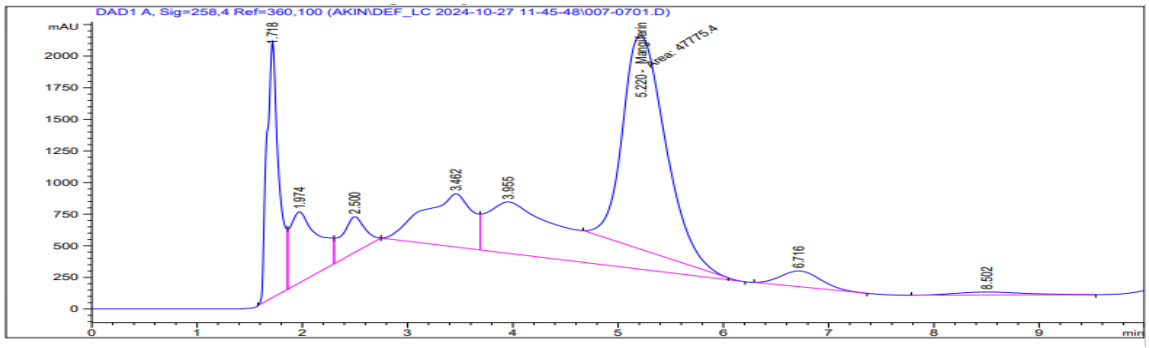

Figure 5: Chromatogram of *Mangifera Indica* ethanolic bark extract

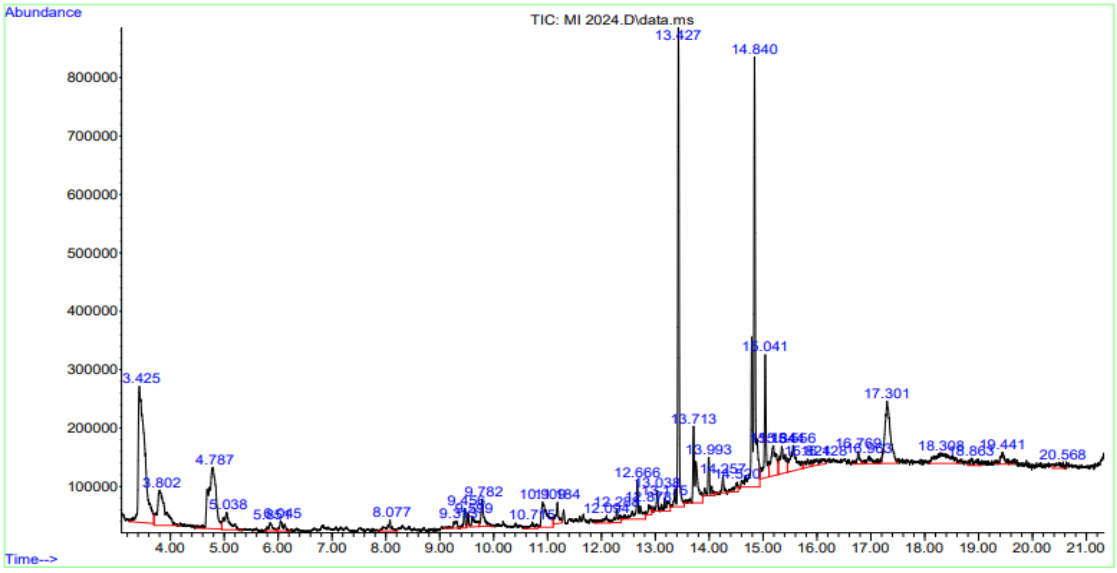

Figure 6: Total Ion chromatogram of *Mangifera Indica*

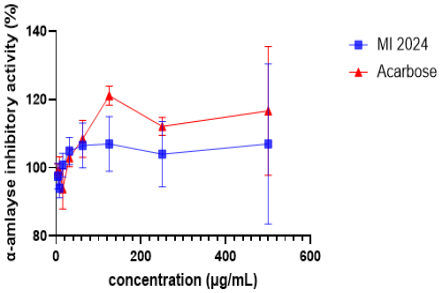

Figure 7: *α-amylase* inhibitory activity

Figure 9 shows the *α-glucosidase* inhibitory assay results for *M. indica* with a 73.2% inhibition of *α-glucosidase* at a concentration of 3.9 µg/mL, which was significantly higher than the 8.7% inhibition observed with Acarbose. In figure 10, we see that the IC_{50} value of *M. indica* (6.96 µg/mL) was notably lower than that of Acarbose (94.47 µg/mL), indicating superior inhibitory activity ($p = 0.0001$). The enhanced inhibitory effect of *M. indica* on *α-glucosidase* and *α-amylase* has been attributed to Mangiferin, a flavonoid known for its bioactive properties.[33]

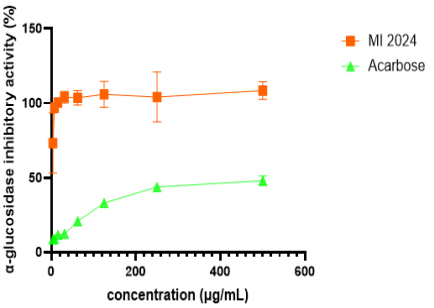

Figure 9: *α-glucosidase* inhibitory activity

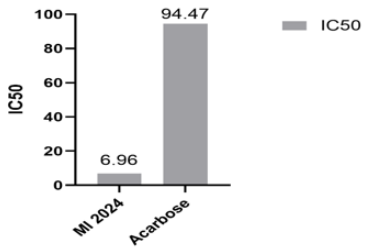

Figure 10: IC50 values of *Mangifera indica* and Acarbose against *α-glucosidase*

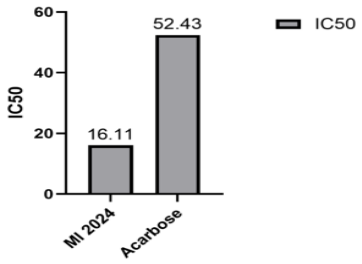

Figure 8: IC50 values of *Mangifera indica* and Acarbose against *α-amylase*

These findings suggest that Mangiferin could be a promising lead compound for managing type 2 diabetes by offering superior inhibitory activity against *α-glucosidase* and significant *α-amylase* inhibition. The results support the traditional use of *M. indica* in the management of hyperglycemia and underscore the therapeutic potential of its phytochemicals. Further studies, including investigations into the molecular mechanisms and clinical trials, are necessary to fully assess its efficacy and safety for diabetes management.

The chemical structures of Mangiferin, Gallic acid, Phenol, Hexadecenoic acid methyl ester, 9-octadecenoic acid (Z)-methyl ester and Acarbose used in docking studies are depicted in Figure 11 while Table 3 shows the molecular docking scores of selected phytoconstituents against *α-amylase* and *α-glucosidase* which revealed binding energies below -7.0 Kcal/mol for both Mangiferin and Acarbose, suggesting a favorable binding affinity at the enzymatic active sites. Notably, Mangiferin showed stronger binding interactions compared to Acarbose, likely due to its fewer hydroxyl functional groups, which form fewer hydrogen bonds at the binding pocket. This observation could explain the enhanced inhibitory activity of Mangiferin against these enzymes.

Aside from efficacy and toxicity, many drug development failures can be traced to poor pharmacokinetics and bioavailability. Table 4 shows the absorption, distribution, metabolism and excretion (ADME) profile analysis for Mangiferin and Acarbose using Swiss ADME. Mangiferin showed a similar Bioavailability Score with Acarbose at 17% but a lower Synthetic Accessibility indicating that it is easier to synthesize Mangiferin compared to Acarbose. This is particularly beneficial in low resource economies like Africa.

Table 3: Molecular docking Scores

Ligands	Docking scores (Kcal/Mol)	
	α-amylase (5EMY)	*α-glucosidase* (2QMJ)
Acarbose	-7.8	-7.1
Mangiferin	-9.1	-7.8
Gallic acid	-6.3	-6.3
Phenol	-4.8	-5.2
9 – Octadecenoic acid (Z) methyl ester	-5.2	-5.2
Hexadecenoic acid methyl ester	-5.3	-4.7

Table 4: SwissADME Analysis of Mangiferin and Acarbose

Ligand Name	Physiochemical Properties									
	logP	TPSA (Å²)	Natoms	MW (g/mol)	nHBA	nHBD	BS	Nrotb	MR	SA
Acarbose	-6.06	321.17	44	645.60	19	14	0.17	9	136.69	7.34
Mangiferin	-0.81	201.28	30	422.34	11	8	0.17	2	100.70	4.76

Abbreviations: logP – octanol/water partition coefficient, TPSA – Total polarizable surface Area, Natoms – Number of atoms, MW – Molecular weight, nHBA – Number of hydrogen bond acceptor, nHBD – Number of hydrogen bond donor, BS- Bioavailability Score, Nrotb – Number of rotatable bonds; MR – Molecular Refractivity, SA- Synthetic Accessibilit

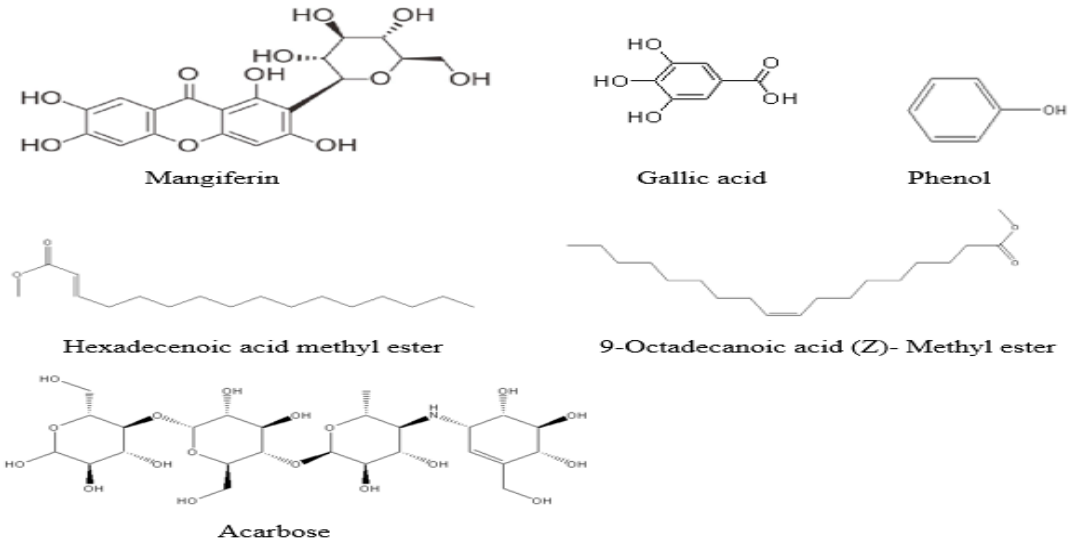

Figure 11: Chemical structures of ligands used in docking studies

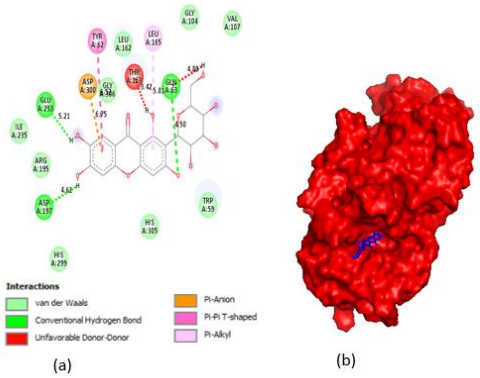

Figure 12: (a) *α-amylase*-Mangiferin 2D Interaction (b) *α-amylase*-Mangiferin 3D Interaction

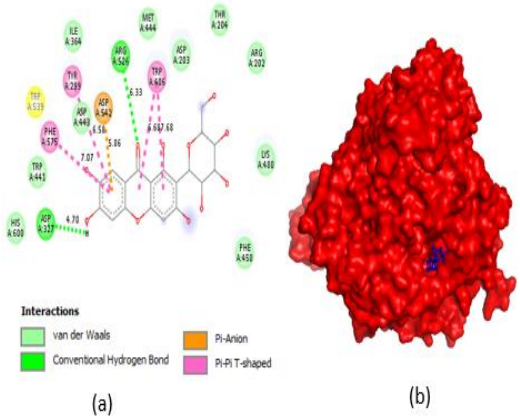

Figure 13 (a) *α-glucosidase*-Mangiferin 2D Interaction (b) *α-glucosidase*-Mangiferin 3D Interaction.

In Table 5, Mangiferin demonstrated a better drug-likeness profile according to Lipinski, Ghose, Veber, Egan, and Muegge, exhibiting fewer violations across these rule-based filters compared to Acarbose. These suggest that Mangiferin may serve as an excellent oral drug candidate for further structure-activity relationship studies aimed at optimizing its pharmacological properties.

In the molecular docking study shown in Figure 12, Mangiferin formed 16 interactions with key amino acids in the *α-amylase* binding pocket, primarily involving van der Waals forces with residues such as ASP197, ARG195, ILE235, GLU233, ASP300, GLY306, and others. Additionally, three hydrogen bond interactions were observed. In comparison, Acarbose formed 17 interactions with the *α-glucosidase* binding pocket as shown in Figure 13, involving more pi-pi T-shaped interactions and two hydrogen bond formations, with residues like ASP327, HIS600, TRP441, and PHE575.
These results suggest that both compounds effectively interact with the enzymatic active sites, but Mangiferin may exhibit more favorable binding characteristics in certain cases.

Protein flexibility is a key determinant of the interaction between biological macromolecules and their ligands.[34] Figure 14 shows result of molecular mobility defined by normal mode analysis (NMA) performed on the docked complexes of both *α-amylase* and *α-glucosidase* with Mangiferin revealed significant deformability, with both proteins showing a deformability index close to 1.0. The B-factor analysis of the 2QMJ-Mangiferin complex showed more hinge regions than the 5EMY-Mangiferin complex, indicating better flexibility in the former. The molecular dynamics simulations shown in Figure 15 indicated that both docked protein-ligand complexes exhibited low eigenvalues (2.4e-04 and 1.7e-04), suggesting that these complexes are stable and flexible during molecular motion. Additionally, covariance matrices revealed strong correlations, supporting the stability and plausibility of the interactions between Mangiferin and the target enzymes. Based on these promising molecular docking and dynamics findings, we propose that Mangiferin holds significant potential as a drug candidate for managing type 2 diabetes.

Table 5: Druglikeness Analysis

Ligand Name	Number of Violations					
	Lipinski	Ghose	Veber	Egan	Muegge	Leadlikeness
Acarbose	3	4	1	1	5	2
Mangiferin	2	1	1	1	3	1

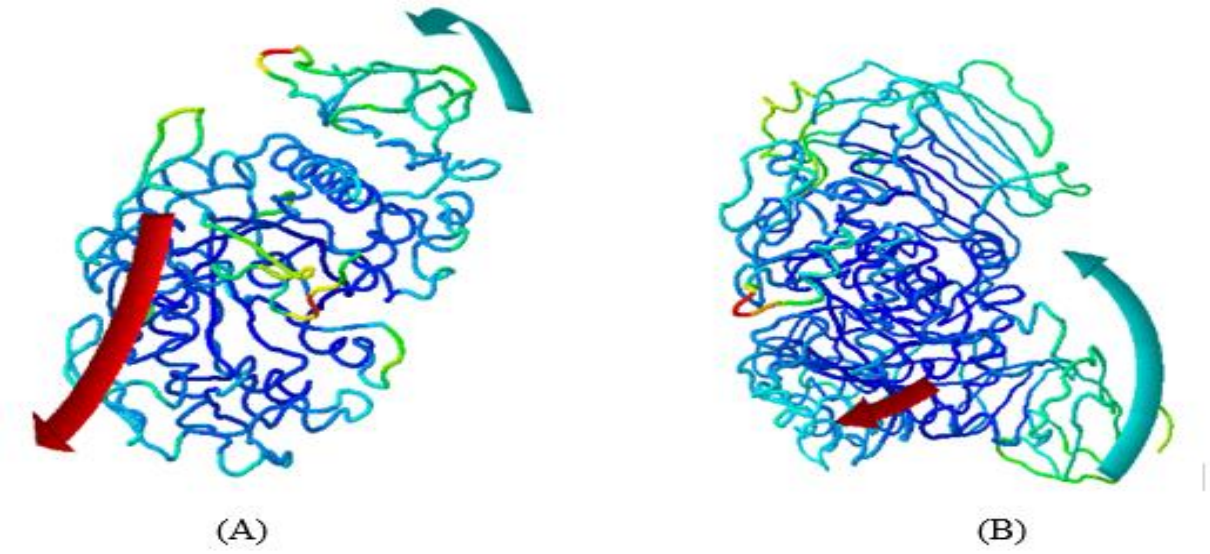

Figure 14: Molecular mobility evaluated by NMA of the docked complexes: (A) 5EMY-Mangiferin (B) 2QMJ-Mangiferin The two-colored affine-arrows display the mobility or the direction of motion, where the longer arrows indicate greater motion.

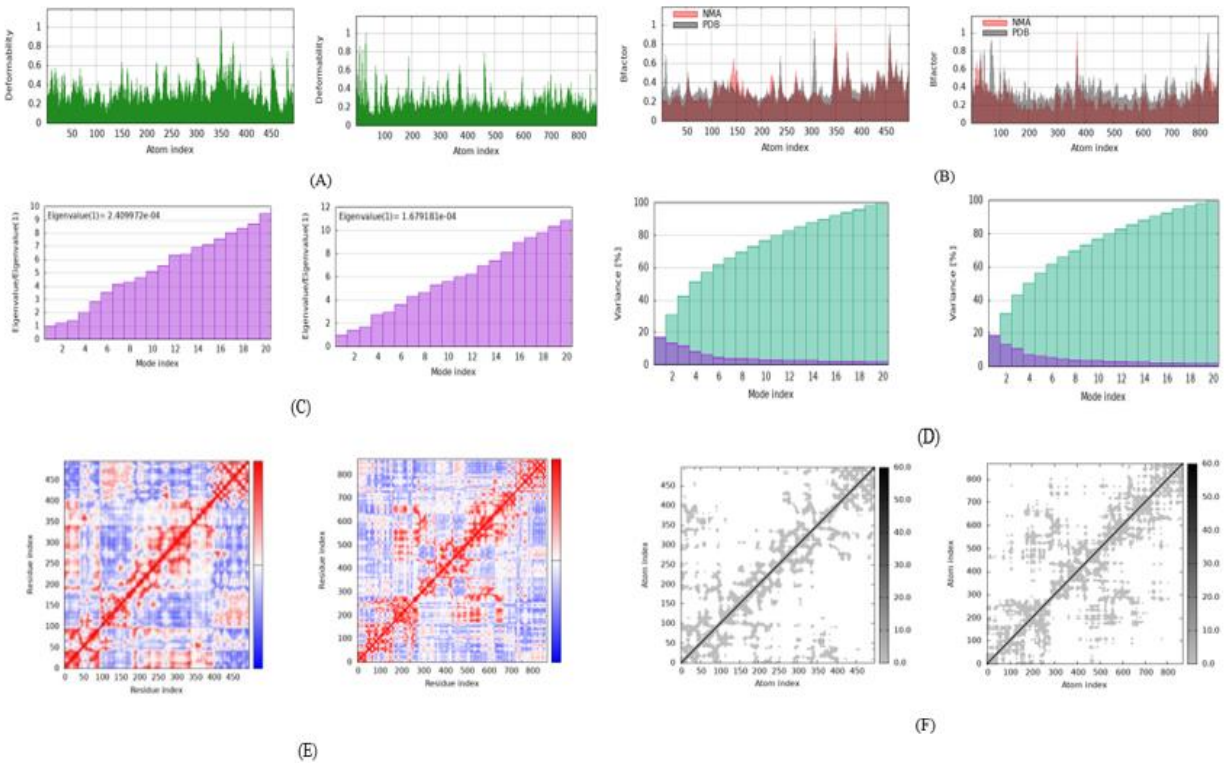

Figure 15: Outputs of molecular dynamics simulations through iMODS for 5EMY-Mangiferin and 2QMJ-Mangiferin: (A) deformability; (B) B-factor plot; (C) eigenvalue; (D) variance plot; (E) elastic network model; and (F) covariance map.

Future directions and recommendations following this study include lead optimization studies such as quantitative structure activity relationship (QSAR) analysis of Mangiferin and skeletal editing studies of the flavanone moiety to generate pharmacologically active derivatives are recommended to identify modifications that enhance potency, bioavailability, and pharmacokinetics, facilitating the design of optimized lead compounds.

An assessment of the comparative pharmacodynamics and toxicology studies of Mangiferin and its derivatives is also recommended to assess the *in-vivo* efficacy and safety profile in comparison with standard antidiabetic drugs like Acarbose. The acute and chronic toxicity of the compound should also be explored in animal models to ensure a comprehensive safety profile.

Following positive results from *in-vivio* studies, Phase I Studies should be initiated to evaluate the safety, tolerability, and pharmacokinetics of the Mangiferin derivatives followed by the development of formulations such as tablets or nanoparticles to improve Mangiferin's bioavailability and stability.

We also recommend assessing the Mangiferin's impact on related metabolic disorders such as dyslipidemia and obesity. Further investigation of the potential anti-inflammatory and cardiovascular protective effects linked to the compounds documented antioxidant properties is advised.

These recommendations aim to build a robust framework for validating the therapeutic efficacy of *M. indica* and accelerating its development as a novel antidiabetic agent.

CONCLUSION

This study highlights the significant potential of *Mangifera indica* stem bark as a therapeutic agent for managing type 2 diabetes. The chemical profiling of the ethanolic extract revealed the presence of bioactive compounds, including Mangiferin and Gallic acid, which are known for their antioxidant properties and may contribute to the plant's antidiabetic effects. The *M. indica* extract demonstrated potent inhibitory activity against both *α-amylase* and *α-glucosidase*, with superior *α-glucosidase* inhibition compared to the reference standard, Acarbose. The molecular docking studies further supported these findings, indicating favorable binding interactions of Mangiferin with the active sites of both enzymes. Moreover, Mangiferin exhibited a promising drug-likeness profile, suggesting its potential as a lead compound for further pharmacological development.

These results support the traditional use of *Mangifera indica* in managing hyperglycemia and suggest that its bioactive compounds, particularly Mangiferin, could serve as effective candidates for the development of new antidiabetic therapies. Further studies, including in vivo experiments and clinical trials, are necessary to confirm the efficacy, safety, and underlying molecular mechanisms of *M. indica* in diabetes management.

CONCLUSION

This study showed that Flavonoids had a superior inhibitory activity against *α-amylase* (5EMY) and *α-glucosidase* (2QMJ) with the trend being Flavonoids > Alkaloids > Terpenes based on superior binding affinities outperforming the known inhibitor acarbose following molecular docking of 383 secondary metabolites. Detailed SAR analysis revealed that structural features, such as glycosylation and presence of the flavone / flavanol moiety enhanced binding affinity. Amentoflavone emerged as a lead compound with high binding affinity, favorable ADME properties, and synthetic accessibility, indicating its strong potential as a novel antidiabetic agent.

The interactions of selected flavonoids with key amino acids in the binding sites of 5EMY and 2QMJ provided insights into their inhibitory mechanisms, emphasizing the role of functional groups like hydroxyl, glycosyl, and flavonoid ring systems in enhancing binding and activityfor patients with chronic medical conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

ACKNOWLEDGMENT

We wish to acknowledge staff of the African Center of Excellence for Drug Research, Herbal Medicine Development and Regulatory Science

(ACEDHARS) and the University of Lagos for their support in the conduct of this study.

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams R; IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019; 157:107843. doi: 10.1016/j.diabres.2019.107843.
2. Zimmet P, Alberti KG, Magliano DJ. Diabetes in 2015: Global estimates of the prevalence of diabetes and its burden. Diabetes Res Clin Pract. 2016; 117: 123-125. doi: 10.1016/j.diabres.2016.01.031.
3. Zakir M, Ahuja N, Surksha MA, Sachdev R, Kalariya Y, Nasir M, Kashif M, Shahzeen F, Tayyab A, Khan MSM, Junejo M, Manoj Kumar F, Varrassi G, Kumar S, Khatri M, Mohamad T. Cardiovascular Complications of Diabetes: From Microvascular to Macrovascular Pathways. Cureus. 2023; 15(9):e45835. doi: 10.7759/cureus.45835.
4. Park J, Zhang P, Wang Y, Zhou X, Look K, Bigman E. Diabetes management with phytochemicals: Bioactive plant compounds to reverse insulin resistance and glucose imbalance. Sci Rep. 2022; 12: 4159.
5. Guan H, Tian J, Wang Y, Niu P, Zhang Y, Zhang Y, Fang X, Miao R, Yin R, Tong X. Advances in secondary prevention mechanisms of macrovascular complications in type 2 diabetes mellitus patients: a comprehensive review. Eur J Med Res. 2024; 29:152.
6. Salm S, Rutz J, Van den Akker M, Blaheta RA, Bachmeier BE. Current state of research on the clinical benefits of herbal medicines for non-life-threatening ailments. Front Pharmacol. 2023; 14. doi 10.3389/fphar.2023.1234701
7. Deependra Y, Kulveer SY, Singh SP. Mango: Taxonomy and botany. J. Pharmacog Phytochem. 2018; 7(2):3253-3258. Available from: https://www.phytojournal.com/archives/2018.v7.i2.4027/mango-taxonomy-and-botany
8. Gupta R, Verma S, Kumar P. Antidiabetic activity of *Mangifera indica* bark extract in alloxan-induced diabetic rats. Pharmacognosy Res. 2012; 4(3):162-165. doi: 10.4103/0974-8490.100396.
9. Binu P, Abhilash S, Vinetha RC, Arathi P, Harikumaran NR. Evaluation of antioxidant potential of natural xanthone mangiferin in myocardium: A rat model study. Int J. Adv Res Biol Sci. 2016; 3(11):220-229. doi: 10.22192/ijarbs.2016.03.11.028.
10. Coelho E, Souza M, Corrêa L, Viana A, Azevêdo L. Bioactive Compounds and Antioxidant Activity of Mango Peel Liqueurs (*Mangifera indica* L.) Produced by Different Methods of Maceration. Antioxidants. 2019; 8. 102. 10.3390/antiox8040102.
11. Ali BA, Alfa AA, Tijani KB, Idris ET, Unoyiza US, Junaidu Y. Nutritional health benefits and bioactive compounds of *Mangifera indica* L. (Mango) leaves methanolic extracts. Asian Plant Res J. 2020; 6(2):41-51. doi: 10.9734/aprj/2020/v6i230126.
12. Akhter S. Low to no cost remedies for the management of diabetes mellitus: Global health concern. J. Diabetes Metab Disord. 2021;20(1):951-962. doi: 10.1007/s40200-021-00783-6.
13. Aderonke AA, Ejiofor J, Eniola OW, Akanji MA, Olatunbosun OO. Evaluation of the Safety Profile of Ethanol Stem Bark Extract of *Mangifera indica* on Reproductive Functions of Male Albino Rats. NIJOPHASR. 2020 Aug. 16;8 (2):44-8. Available from: https://www.nijophasr.net/index.php/nijophasr/article/view/289
14. Zhang X, Su B, Li J, Li Y, Lu D, Zhu K, Zhao M. Analysis by RP-HPLC of Mangiferin component correlation between medicinal *Loranthus* and their mango host trees. J. Chromatogr Sci. 2012; 52(1):1–4. doi: 10.1093/chromsci/bms196.
15. Worthington V, editor. *Alpha amylase*. In: Worthington enzyme manual. Freehold, NJ: Worthington Biochemical Corp; 1993. p. 36-41.

16. Selin B, Irfan T. Production of β-mannanase, inulinase, and oligosaccharides from coffee wastes and extracts. Int J. Biol Macromol. 2024; 261:129798. doi: 10.1016/j.ijbiomac.2024.129798.
17. Matsui TC, Yoshimoto C, Osajima K, Oki T, Osajima Y. *In vitro* survey of *α-glucosidase* inhibitory food components. Biosci Biotechnol Biochem. 1996; 60(12):2019-2022.
18. Bräunlich M, Slimestad R, Wangensteen H, Brede C, Malterud KE, Barsett H. Extracts, anthocyanins and procyanidins from *Aronia melanocarpa* as radical scavengers and enzyme inhibitors. Nutrients. 2013; 5(3):663-678. doi: 10.3390/nu5030663.
19. Burley SK, Berman HM, Bhikadiya C, Bi C, Chen L, Di Costanzo Z, Zardecki C. RCSB Protein Data Bank: Biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. 2018; 47(D1):D464–D474. doi: 10.1093/nar/gky1004.
20. Biovia DS, Systèmes D. Discovery Studio Modeling Environment. Version 53. Dassault Systèmes; 2016.
21. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput Chem. 2010; 31(2):455-461. doi: 10.1002/jcc.21334.
22. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Bryant SH. PubChem Substance and Compound databases. Nucleic Acids Res. 2015; 44(D1):D1202–D1213. doi: 10.1093/nar/gkv951.
23. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. J. Cheminform. 2011; 3(1):33. doi: 10.1186/1758-2946-3-33.
24. Schrödinger L, DeLano W. PyMOL. The PyMOL Molecular Graphics System, Version 2. Schrödinger, LLC: New York, NY, USA; 2020.
25. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7:42717. doi: 10.1038/srep42717.
26. López-Blanco JR, Aliaga JI, Quintana-Ortí ES, Chacón P. iMODS: Internal coordinates normal mode analysis server. Nucleic Acids Res. 2014; 42(W1):W271–W276. doi: 10.1093/nar/gku339.
27. Ning Z, Lu C, Zhang Y, Zhao S, Liu B, Xu X, Liu Y. Application of plant metabonomics in quality assessment for large-scale production of traditional Chinese medicine. Planta Med. 2013; 79(11):897-908.
28. Loan NTT, Long DT, Yen PND, Hanh TTT, Pham TN, Pham DTN. Purification process of mangiferin from *Mangifera indica* L. leaves and evaluation of its bioactivities. Processes. 2021; 9(5):852. doi: 10.3390/pr9050852.
29. Purushoth PT, Panneerselvam P, Suresh R, Clement AW, Balasubramanian S. GC-MS analysis of ethanolic extract of *Canthium parviflorum* Lamk leaf. J. Appl Pharm Sci. 2013;3(2):166-168.
30. Guo X, Jiang X, Chen K, Liang Q, Zhang S, Zheng J, Ma X, Jiang H, Wu H, Tong Q. The Role of Palmitoleic Acid in Regulating Hepatic Gluconeogenesis through SIRT3 in Obese Mice. Nutrients. 2022; 14(7):1482. https://doi.org/10.3390/nu14071482
31. Abhijit S, Rashmi S, Srivastava NS, Yashwant M, Bhagyashri C. Anti-diabetic activity of *Tridax procumbens*. J. Sci Innov Res. 2014; 3(2):221-226.
32. Raman BV, Naga Vamsi Krishna A, Narasimha Rao B, Pardha Saradhi M, Basaveswara Rao MV. Plants with antidiabetic activities and their medicinal values. Int Res J. Pharm. 2012; 3(3):11-15.
33. Sekar V, Chakraborty S, Mani S, Sali VK, & Vasanthi HR. Mangiferin from Mangifera indica fruits reduces post-prandial glucose level by inhibiting *α-glucosidase* and *α-amylase* activity. Sou. Afr, J. Bot. 2019; 120:129-134.
34. Ghosh P, Bhakta S, Bhattacharya M, Sharma AR, Sharma G, Lee SS, Chakraborty C. A novel multi-epitopic peptide vaccine candidate against *Helicobacter pylori*: In-silico identification, design, cloning and validation through molecular dynamics. Int J. Pept Res Ther. 2021; 27:1149–1166.