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Original Research Article

6-methyl-2-(3-nitrophenyl) imidazo [1,2-a] from Methanol Extract of Mangifera Indica as Potential Novel alpha-amylase inhibitor and Chemical Inducer of Glut 4 translocation: A Molecular Docking Computational Study

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Abstract

Diabetes, a chronic metabolic disorder characterised by hyperglycaemia has become a major global health concern, with an increasing prevalence worldwide (Mukhtar, Galalain & Yunusa, 2020). Despite the availability of various anti-diabetic drugs, the search for natural remedies to manage diabetes has gained significant attention. *Mangifera indica* extracts have been studied for their anti-oxidant, anti-diabetic, lipid-lowering and anti-obesity potentials (Kumar *et al*., 2021). This study was designed to investigate the therapeutic effects of Mangifera indica methanol extract against Diabetes Mellitus, using *in silico* methods, Molecular docking simulations were performed to assess the binding affinities and interactions of the identified compounds from the Gas Chromatography/Mass Spectometry analysis result with key enzymes, proteins and hormones involved in glucose metabolism, such as alpha-amylase, insulin receptors, Glucose Transporter type 4 and Glucagon-like peptide 1. 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine had the highest binding affinity with Insulin receptors(-7.6kcal/mol), alpha amylase(-8.0kcal/mol) and Glucose transporter type 4(-8.5kcal/mol). Oxime-, methoxy-phenyl had the highest binding affinity with Glucagon-like peptide 1(-6.5kcal/mol). These findings suggest that mango leaves could serve as a source of natural anti-diabetic agent, which could lead to the development of new and effective treatments for diabetes. Further in vitro and in vivo studies are warranted to validate the bioactivity of these compounds and their mechanisms of action. Overall, this project contributes to the growing body of evidence supporting the use of in silico approaches for the discovery of novel antidiabetic agents from natural products.

Keywords: Mangifera indica, Diabetes, Molecular Docking, alpha amylase.

INTRODUCTION

The global rise in type 2 diabetes mellitus (T2DM) has become a pressing public health concern, affecting millions of individuals and placing a significant burden on healthcare systems worldwide (Opreh *et al.*, 2024). This condition is characterized by insulin resistance and impaired glucose metabolism, leading to elevated blood sugar levels, particularly after meals (Adeoye *et al*., 2023). Managing postprandial hyperglycemia—an increase in blood sugar following food intake—is crucial for preventing the long-term complications associated with diabetes, such as cardiovascular disease, neuropathy, and kidney damage (Adeoye *et al*., 2022a).

One effective strategy for controlling postprandial blood sugar levels involves inhibiting α-amylase, an enzyme that plays a key role in the digestion of carbohydrates (Adeoye, *et al*., 2022b). By blocking this enzyme's activity, it is possible to slow down the breakdown of starches into glucose, thereby reducing the amount of sugar that enters the bloodstream (Olajide *et*

al., 2023). While several pharmaceutical agents are available to inhibit α-amylase, they often come with side effects that can deter patients from adhering to their treatment regimens (Oyedemi *et al*., 2017). This has sparked a growing interest in exploring natural products as safer alternatives that not only provide therapeutic benefits but also minimize adverse effects.

Among the various natural sources being investigated, Mangifera indica—commonly known as mango—stands out due to its rich history in traditional medicine and its widespread popularity as a delicious fruit (Shah *et al*., 2010). Beyond its culinary appeal, mango has been recognized for its potential health benefits, including anti-inflammatory and antioxidant properties (Lauricella *et al.*, 2017). Recent research has highlighted the presence of diverse phytochemicals in mango, such as polyphenols, flavonoids, and triterpenoids, which may contribute to its beneficial effects on metabolic health (Kumar *et al.*, 2021). Notably, the different extracts of Mangifera indica has shown promising results in preliminary studies for its ability to inhibit α-amylase activity (Irondi *et al*., 2014; Sekar *et al*., 2019). This suggests that specific compounds within this extract could serve as effective agents for managing blood sugar levels.

In our study, we aim to delve deeper into this promising area by focusing on a particular compound: 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a]. This compound was isolated from the methanol extract of Mangifera indica and has piqued our interest due to its structural characteristics and potential biological activities. Using advanced molecular docking techniques, we will explore how this compound interacts with α-amylase at a molecular level. Our goal is to determine whether it can act as a novel inhibitor of this enzyme. Additionally, we will investigate another critical aspect of glucose metabolism: GLUT4 translocation. GLUT4 is a glucose transporter that plays a vital role in facilitating glucose uptake into cells, particularly in muscle and adipose tissues (Stöckli *et al*., 2011). Enhancing GLUT4 translocation can significantly improve insulin sensitivity and help lower blood sugar levels (Wang *et al*., 2020). By examining whether 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] can induce GLUT4 translocation alongside its α-amylase inhibitory activity, we hope to uncover new insights into its potential as a dual-action therapeutic agent.

By bridging traditional knowledge with cutting-edge scientific techniques, our research aims not only to advance our understanding of how natural compounds can be utilized in diabetes management but also to contribute to the development of new therapeutic options derived from plant sources. We believe that exploring these natural alternatives could lead to safer and more effective strategies for controlling diabetes, ultimately improving the quality of life for those affected by this chronic condition. Through this study, we hope to shine a light on the potential of Mangifera indica and its bioactive compounds in combating one of the most significant health challenges of our time.

MATERIALS

Collection and Identification of the plant

Health beneficial properties of Mangifera indica are well recognized. Different parts of this plant possess significant potential to treat various diseases. The leaves of *Mangifera indica* were collected and was identified by Mrs Ukangwa, from the Department of Biochemistry, School of Basic Medical Sciences, Benjamin S. Carson (Snr.) College of Health and Medical Sciences, Babcock University. The leaves were washed with distilled water then dried in an oven at 60°Cfor three days.

Chemicals and Reagents

Distilled water was used to wash the mango leaves prior to drying. The extraction process was carried out using Methanol (Sigma-Aldrich) and Filter paper. Analytical-grade chemicals were used.

Computer software

Open Babel GUI, PyRx, Data warrior and Discovery studio 2021 client are the softwares used in the docking process. The x-ray crystallographic 3D structures of Insulin receptor (PDB ID), Glut transporter 4 (PDB ID), alpha amylase (PDB ID 4GQR) and beta glucosidase (PDB ID 2JFE) were downloaded from online Protein Data Bank(https://www.rcsb.org/). Discovery studio was used to prepare the protein and view the ligand-target reactions of the proteins. Open Babel GUI was used to concatenate the ligand and standard drug structures. PyRx is used in setting the grid for the docking process.

METHODS

Methanol extract

The leaves of *Mangifera indica* were washed to remove debris then they were oven dried at 40°C for three days. The leaves were blended to powdered form. 100g of the ground sample was soaked in 800mL of 70% methanol in the ratio 1g: 8mL. The mixture was placed in a container with a tight seal for three days and was shook at regular intervals. The mixture was filtered using Whatmann No.1 filter paper and funnel. The solvent was removed from the filterate by evaporation using a stuart rotary evaporator. Afterwards, the concentrated sample was kept in the oven for three days at 25°C.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC-MS was carrryout in the toxicology laborator biochemistry department in the Nigerian Institute of Medical Research (NIMR), Yaba Lagos state. GC-MS analysis of the methanolic plant extract was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas

Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused a capillary column (30 × 0.25µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed (a split ratio of 10:1). The injector temperature was maintained at 300 °C, and the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280°C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 3 min, and the total GC/MS running time was 21.33min. Interpretation of mass spectrum GC-MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns and the National Center for Biotechnology Information. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

In silico Identification of Biological markers of Diabetes

There have been several in silico studies aimed at identifying potential biomarkers of diabetes. Several targets have been used to identify potential drugs for the treatment of this disease. Insulin receptors, Glucose transporter 4, alpha amylase and Glucagon-like peptide 1 will be targeted in this study. Targetting the insulin receptors by identifying small molecules that enhance insulin signalling, targeting glucose transporter type 4 by identifying small molecules that will enhance glucose uptake. Inhibiting alpha amylase activity, will slow the breakdown of complex carbohydrates and reduce the amount of glucose entering the bloodstream. The inhibition of beta-glucosidase activity will lead to decreased glucose absorption and reduced postprandial hyperglycemia.

Preparation of glut 4 transporter

Docking analysis of the chemical compounds of *Mangifera indica* against Glucose transporter type 4 was performed in order to compare the relative affinity of the ligands against GLUT 4. Discovery studio 2021 client is used to determine the ligand interactions and remove bound ligands and water molecules. The 3D structure of the protein (PDB ID: 7WSM) was retrieved from the RCSB Protein Data Bank Site and saved in .pdb format.

Preparation of alpha amylase

Docking analysis of the chemical compounds of *Mangifera indica* against alpha amylase was performed in order to compare the relative affinity of the ligands against alpha amylase. Discovery studio 2021 client is used to determine the ligand interactions and remove bound ligands and water molecules. The 3D structure of the protein (PDB ID: 4GQR) was retrieved from the RCSB Protein Data Bank Site and saved in .pdb format.

Preparation of ligands

The 3D structures of the compounds of *Mangifera indica* obtained from the GC/MS analysis as well as the structure of acarbose, the standard drug in the treatment of diabetes were downloaded from Pubchem in SDF format, the structures of the ligand were saved in .pdb format.

Setting of grid dimensions for autodock calculations of Glucose transporter type 4

For the docking of ligand molecules to Glucose transporter type 4, search space coordinates were provided using PyRx. The dimensions of the grid box were set to ensure the ligand could bind to all the potential binding sites of the protein to provide the best conformation. The settings were: X:100.237, Y: 103.485 Z: 104.9427; dimensions (Angstrom)X:22.4646, Y: 26.5910, Z:28.2355.

Setting of grid dimensions for autodock calculations of alpha amylase

For the docking of ligand molecules to alpha amylase, search space coordinates were provided using PyRx. The dimensions of the grid box were set to ensure the ligand could bind to all the potential binding sites of the protein to provide the best conformation. The settings were: X:7.2930, Y:20.4541, Z:45.1245 dimensions (Angstrom)X:22.6913,Y:17.8941, Z: 23.2879

Docking analysis and visualization of binding conformations

During docking analysis, the ligand molecule is screened against a library of receptor conformations to identify the most energetically favorable binding conformation. This is typically achieved using algorithms such as AutoDock, GOLD, or Glide, which calculate the binding free energy of different ligand-receptor conformations. Once the binding conformation has been predicted, it can be visualized using molecular visualization software such as PyMOL, or Chimera. Visualization of the binding conformation allows the analysis of the interactions between the ligand and receptor, and to identify potential areas for chemical modification to improve binding affinity or specificity.

Statistical Analysis

Based on data obtained from PyRx, tables were created using Excel spreadsheets, and rating algorithms were used to determine which compound had the highest binding score.

RESULTS

Gas Chromatography/ Mass Spectrophotometry (GC/MS) Result of *Mangifera indica* methanol extract

Table 1 shows that the GC/MS analysis shows the presence of 8 compounds in *Mangifera indica*

methanol extract. The structures are shown in Figure 4.1, the compounds were characterized as terpenes, phenolic compounds, alkaloids, fatty acids and flavonoids. Cyclohexanone, 3-ethyl-3,5,5- trimethyl-, Octadecene, (E)- and Pentadecanoic acid, 14-methyl-, methyl ester are classified as fatty acids. Oxime-, methoxy- phenyl- is classified as an alkaloid which has been previously reported to possess antibacterial properties. Alkaloids have been found to have a variety of therapeutic benefits, including anti-diabetic ones, their chemical backbones have the potential to interact with a wide range of proteins involved in glucose homeostasis.6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine is known to exhibit anti-tumor and anti-inflammatory bioactivities. Cyclohexane 1,1'-(2-methyl-1,3-propanediyl) bis and Octadecene, (E) both possess antimicrobial and antifungal activities. Pentadecanoic acid, 14-methyl-, methyl ester has been reported to exhibit anti-oxidant, anti-microbial and anti fungi. Cyclohexanone, 3-ethyl-3,5,5-trimethyl- and Cyclohexane, 1,1'-(2-propyl-1,3-propanediyl) bis both exhibit anti-bacterial activities while no reported bioactivities for Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-. Figure 4.2 and Table 4.1 show that Pentadecanoic acid, 14-methyl-, methyl ester(19.813min) had the highest retention while Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-(5.645min) had the lowest. The most abundant compound in *Mangifera indica* is Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-(1.33%) while the least abundant is Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- (0.47%) and Cyclohexane 1,1' – (2-methyl-1,3-propanediyl)bis-(0.47%).

Table 1: Gas Chromatography/ Mass Spectometry result of Mangifera *indica* methanol extract

S/N	Retention Time (min)	% Peak area	Library/ID	Reported Bioactivity
1	6.360	0.86	Oxime-, methoxy-phenyl-	Antibacterial Antidiabetes
2	8.872	0.49	6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine	Anti-inflammatory Anti-tumor
3	13.456	0.47	Cyclohexanone,3-ethyl-3,5,5-trimethyl-	Antibacterial
4	13.679	0.52	Cyclohexane ,1,1'-(2-methyl-1,3-propanediyl) bis-	Anti microbial Anti fungi
5	13.456	0.47	Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis-	Antibacterial
6	13.759	1.21	Octadecene, (E)-	Anti microbial Anti fungi
7	5.645	1.33	Benzene,1-methoxy-4-methyl-2-(1-methylethyl)-	Not reported
8	19.813	0.64	Pentadecanoic acid, 14-methyl-, methyl ester	Anti oxidant Anti fungi Anti microbial

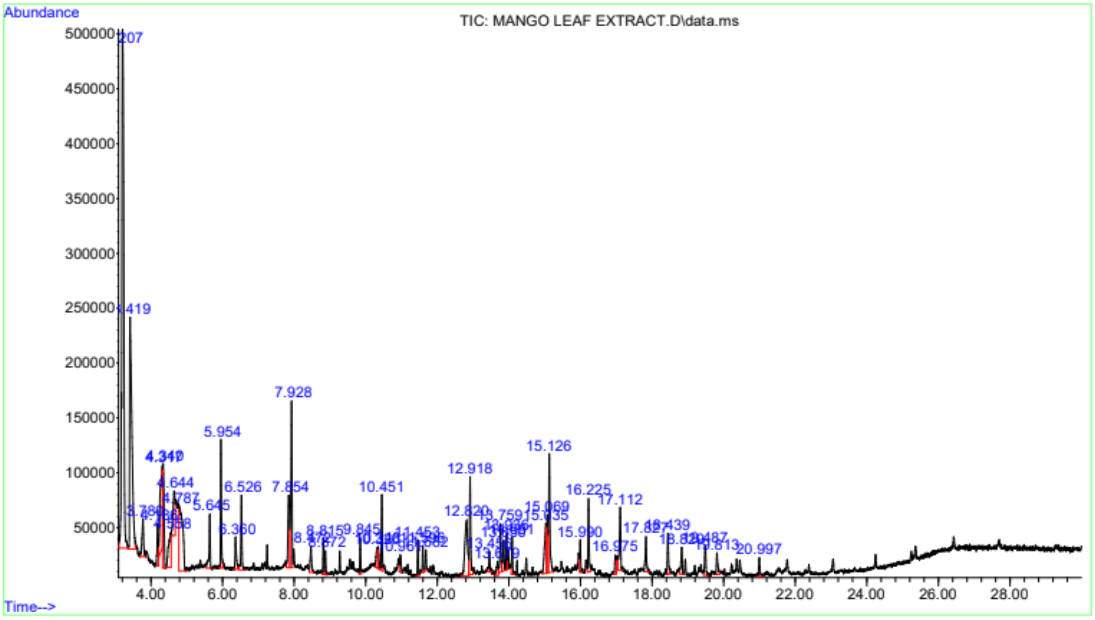

Figure 1: GC/MS chromatogram of Mangifera indica methanol extract

Binding energy of Mangifera indica compounds and standard ligand with Glucose Transporter type 4

The data in Table 2 shows that Octadecene(E)- had the lowest binding energy (5.1kcal/mol) with insulin receptors, while Metformin had the highest binding energy(-8.7kcal/mol) with insulin receptors. Among the 9 compounds of Mangifera indica methanol extract 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine had

the highest binding affinity (-8.5kcal/mol) for Insulin receptors.

Table 2: Binding energy of the compounds in Mangifera indica and standard ligand with Glucose Transporter Type 4

S/N	Compounds	Binding energy(kcal/mol)
1	cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis-	-7.2
2	cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis-	-6.4
3	Benzene,1-methoxy-4-methyl-2-(1-methylethyl)-	-5.7
4	Pentadecanoic acid, 14-methyl-, methyl ester	-6.1
5	Metformin	-8.6
6	Octadecene(E)-	-5.1
7	Cyclohexanone,3-ethyl-3,5,5-trimethyl-	-6.0
8	6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine	-8.5
9	Oxime-, methoxy-phenyl	-7.2

Binding energy of *Mangifera indica* compounds and standard ligand with alpha amylase

The data in Table 3 shows that Octadecene(E)- had the lowest binding energy(-4.9kcal/mol) with the active site of alpha amylase Among the 9 compounds of Mangifera indica methanol extract, 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine had the highest binding affinity (-8.0kcal/mol) with alpha amylase.

Table 3: Binding energy of the Compounds in Mangifera indica and standard ligand with Alpha amylase

S/N	Compounds	Binding energy (kcal/mol)
1	cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis-	-6.8
2	cyclohexane,1,1'-(2-propyl-1,3-propanediyl) bis-	-6.7
3	Benzene,1-methoxy-4-methyl-2-(1-methylethyl)-	-5.3
4	Pentadecanoic acid, 14-methyl-, methyl ester	-5.4
5	Myricetin	-7.7
6	Octadecene(E)-	-4.9
7	Cyclohexanone,3-ethyl-3,5,5-trimethyl-	-5.8
8	6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine	-8.0
9	Oxime-, methoxy-phenyl	-6.6

Binding Interactions between ligands and Diabetes targets

Binding Interactions between ligands and Glucose transporter Type 4 (PDB ID: 7WSM)

Table 4 shows that Metformin which had the highest binding energy(-8.6kcal/mol) formed 2 hydrogen bonds with Glucose transporter Type 4 while 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine with the highest binding affinity(-8.5kcal/mol) formed 8 hydophobic bonds and 2 hydrogen bonds with Glucose transporter type 4, cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis- (-7.2kcal/mol) formed 3 hydrophobic bonds with Glucose transporter type 4, cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis-(-6.4kcal/mol) formed 2 hydrophobic bonds with Glucose transporter type 4, Pentadecanoic acid, 14-methyl-, methyl ester(-6.1kcal/mol) formed 8 hydrophobic bonds and 2 hydrogen bonds with Glucose transporter type 4 and Oxime-, methoxy-phenyl(-7.2kcal/mol) formed 5 hydrogen bonds and 3 hydrophobic bonds with Glucose transporter type 4.

Table 4: Binding Interactions between ligands and Glucose Transporter type 4(PDB ID: 7WSM)

S/N	LIGAND	PubChem CID	Binding Energy(kcal/mol)	H-bond interaction	Hydrophobic interaction	Electrostatic	Pi-sulfur bond	Halogen bond
1	Metformin	4091	-8.6	2	0	0	0	0
2	6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine	620074	--8.5	2	8	0	0	0
3	cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis-	137756	-7.2	0	3	0	0	0
4	cyclohexane,1,1'-(2-propyl-1,3-propanediyl) bis-	143244	-6.4	0	2	0	0	0
5	Pentadecanoic acid, 14-methyl-, methyl ester	21205	-6.1	2	8	0	0	0
6	Oxime-, methoxy-phenyl	86193537	-7.2	5	3	0	0	0

Binding interaction of Oxime-, methoxy-phenyl with Glucose transporter type 4(PDB ID: 7WSM)

(A) 2D binding pose (B) 3D binding pose

Figure 2A shows that the amino acid residues involved in the binding of Metformin to Glucose transporter type 4 are GLU 396, GLN 177, VAL 181, ASN 333, PHE 307, ILE 184, PHE 395, ILE 42, ILE 180, GLN 298, GLY 400 and PRO 401. Figure 4.4 B shows that Metformin was fitted into the binding pocket of Glucose transporter type 4.

Figure 4 shows the amino acids residues involved in the binding of 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine to Glucose transporter type 4 are GLY 424, ASN 427, GLN 299, GLN 298, ILE 42, PHE 307, PHE 88, TYR 308, ASN 431, ASN 304, TRP 404, TRP 428, PHE 38 and SER 153. Figure 4.5B shows that 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine was fitted into the binding pocket of Glucose transporter type 4.

Figure 5 shows the amino acids residues involved in the binding of cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis- to Glucose transporter type 4 are TYR 428, ASN 427, ILE 180, PHE 38, ILE 184, PHE 395, GLN 298, TRP 404, SER 153, TYR 308, PHE 88, A SN 431, PHE 307 and ASN 304. Figure 6 shows that cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis- was fitted into the binding pocket of Glucose transporter type 4.

Figure 7 shows the amino acids residues involved in the binding of cyclohexane,1,1'-(2-propyl-1,3-propanediyl) bis- to Glucose transporter type 4 are ASN 427, TRP 404, GLY 400, GLU 396, GLN 177, PRO 401, GLN 298, PHE 395, ILE 303, PHE 307, ILE 42, GLN 299, ASN 304, ILE 184, VAL 181, ILE 180 and PHE 38. Figure 4.7B shows that cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- was fitted into the binding pocket of Glucose transporter type 4.

Figure 8 shows the amino acids residues involved in the binding of Pentadecanoic acid, 14-methyl-, methyl ester to Glucose transporter type 4 are TRP 404, GLY 424, SER 153, GLY 154, SER 96, ASN 427, ILE 99, TRP 428, PHE 38, ASN 431, GLN 299, PHE 307, ASN 304, ILE 42, ILE 184, VAL 181, ILE 180 and PHE 395. Figure 4.8B shows that Pentadecanoic acid, 14-methyl-, methyl ester was fitted into the binding pocket of Glucose transporter type 4.

Figure 9 shows the amino acids residues involved in the binding of Oxime-, methoxy-phenyl to Glucose transporter type 4 are GLY 424, SER 96, TRP 404, ASN 427, PHE 38, ASN 431, SER 153, TRP 428, GLN 299, ILE 99, GLY 150, ILE 42 and GLY 154. Figure 4.9B shows that Oxime-, methoxy-phenyl was fitted into the binding pocket of Glucose transporter type 4.

Binding Interactions between ligands and alpha amylase (PDB ID: 4GQR)

Table 5 shows that 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine which had the highest binding affinity(-8.0kcal/mol) formed 2 hydrogen bonds and 6 hydrophobic bonds with alpha amylase, Oxime-, methoxy-phenyl (-6.6kcal/mol) formed 1 hydrogen bond, 3 hydrophobic bonds and 4 halogen bonds with alpha amylase. Myricetin(-7.7 kcal/mol) formed 3 hydrogen bonds, 5 hydrophobic bonds and 1 electrostatic bond with alpha amylase. Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- (-6.7kcal/mol) formed 9 hydrogen bonds and 2 electrostatic bonds with alpha amylase. Cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis- (-6.8kcal/mol) formed 3 hydrophobic bonds with alpha amylase.

Table 5: Binding Interactions between ligands and alpha amylase (PDB ID: 4GQR)

S/N	LIGAND	PubChem CID	Binding energy(kcal/mol)	H bond interaction	Hydrophobic interactions	Electrostatic bonds	Pi-sulfur Bonds	Halogen bond
1	Oxime-, methoxy-phenyl	86193537	-6.6	1	3	0	0	4
2	Myricetin	5281672	-7.7	3	5	1	0	0
3	Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis-	143244	-6.7	9	0	2	0	0
4	Cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis-	137756	-6.8	0	3	0	0	0
5	6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine	620074	-8.0	2	6	0	0	0

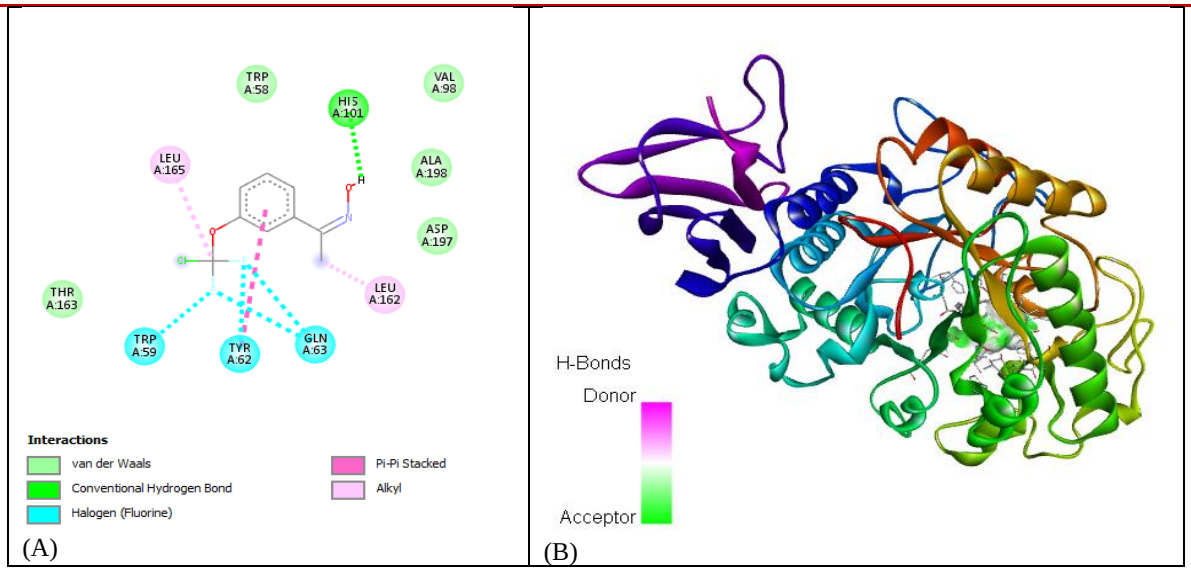

Figure 5.5: Binding interactions of Oxime-, methoxy-phenyl with Alpha Amylase (PDB ID: 4GQR), (A) 2D Binding pose; (B) 3D Binding pose

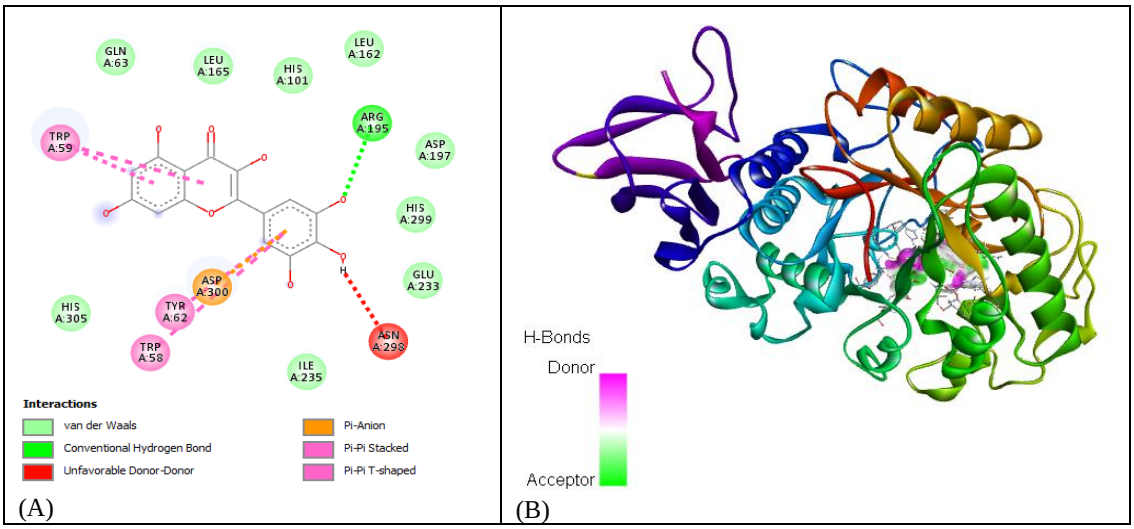

Figure 5: Binding interactions of Myricetin with Alpha Amylase (PDB ID: 4GQR), (A) 2D Binding pose; (B) 3D Binding pose

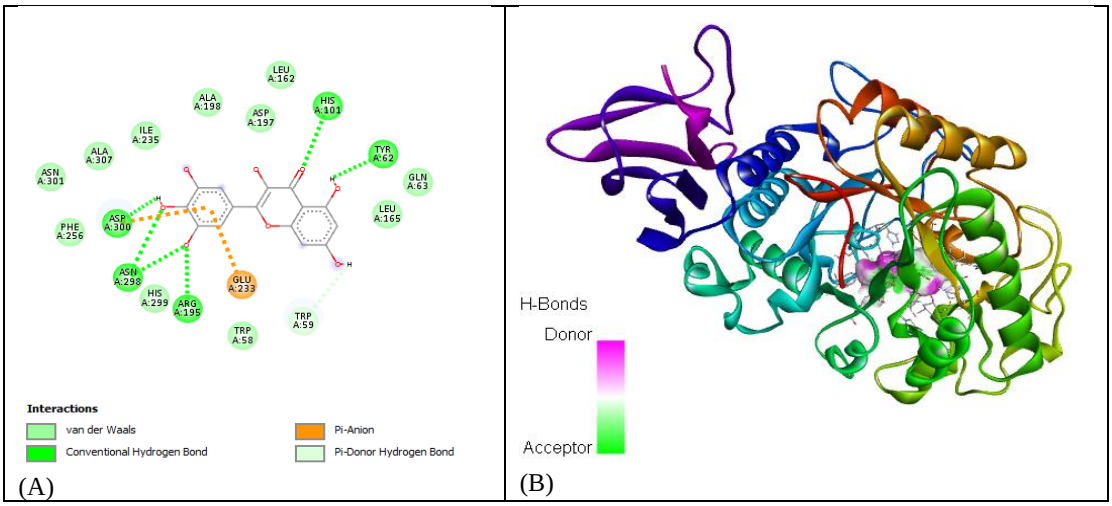

Figure: Binding interactions of Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- with Alpha Amylase (PDB ID: 4GQR), (A) 2D Binding pose; (B) 3D Binding pose

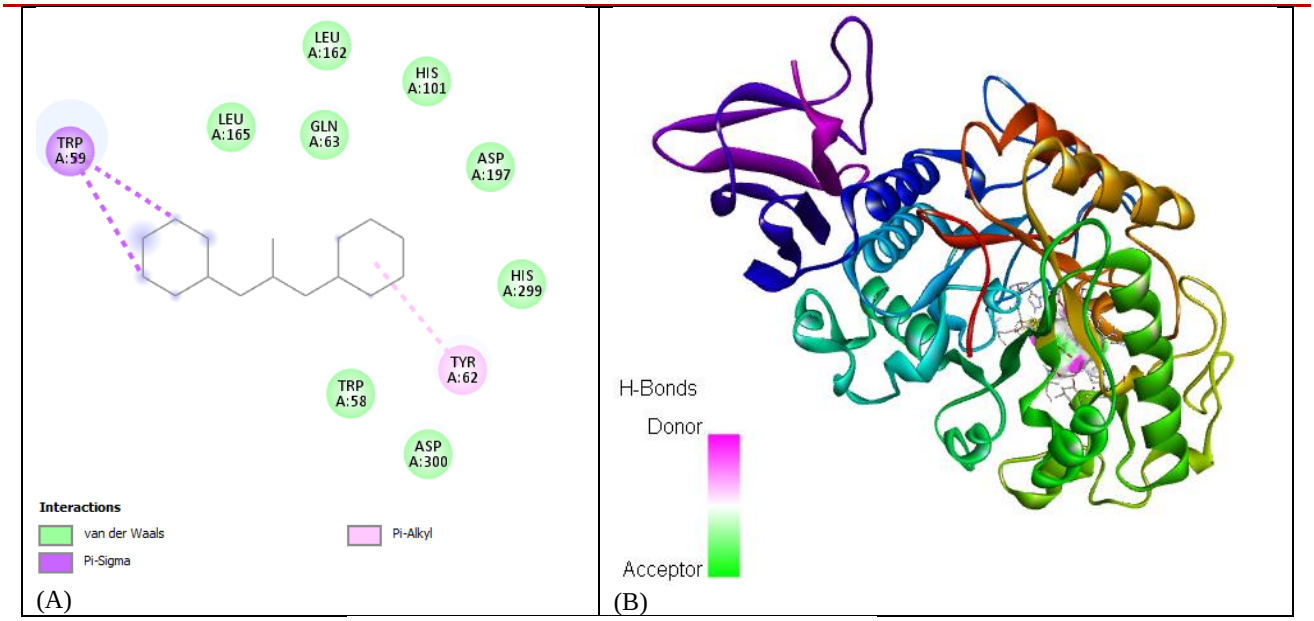

Figure: Binding interactions of Cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis- with Alpha Amylase (PDB ID: 4GQR), (A) 2D Binding pose; (B) 3D Binding pose

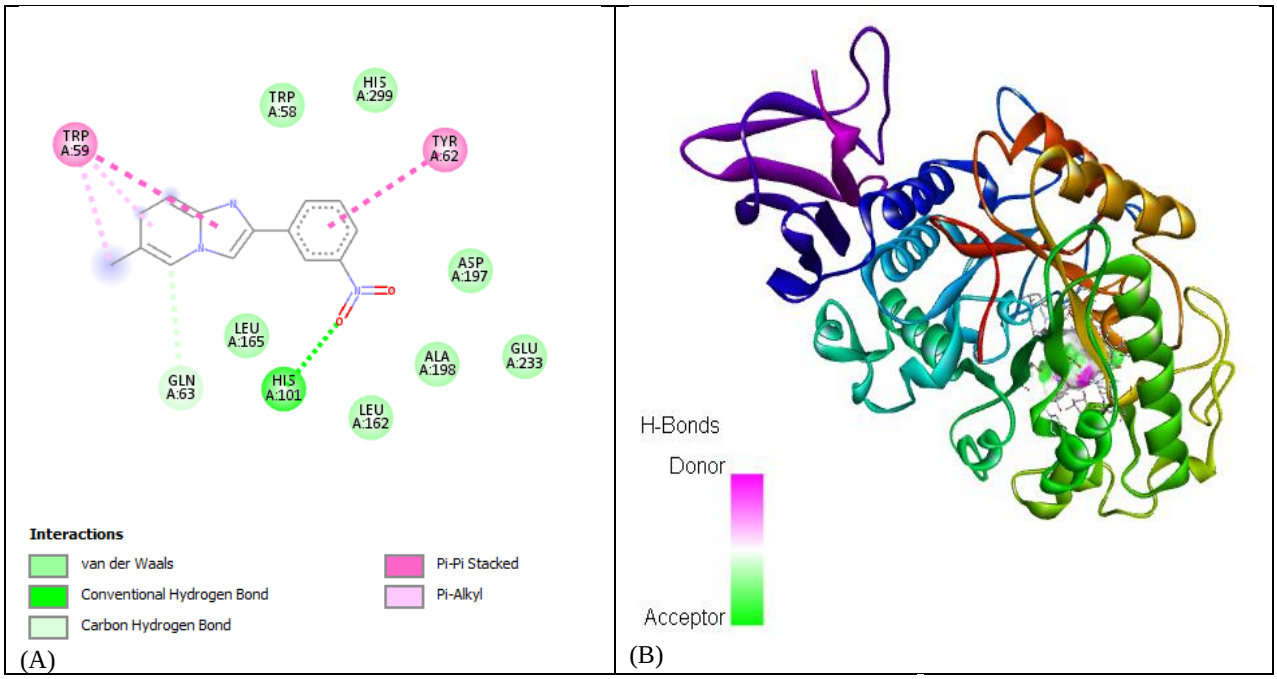

Figure: Binding interactions of 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine with Alpha Amylase (PDB ID: 4GQR), (A) 2D Binding pose; (B) 3D Binding pose

Shows the amino acid residues involved in the binding of Oxime-, methoxy-phenyl to alpha amylase are TRP 58, HIS 101, VAL 98, ALA 198, ASP 197, LEU 162, GLN 63, TYR 62, TRP 59, THR 163 and LEU 165. Figure 5.5B shows that Oxime-, methoxy-phenyl was fitted into the binding pocket of Alpha Amylase.

Figure shows that the amino residues involved in the binding of Myricetin to alpha amylase are TRP 59, GLN 63, LEU 165, HIS 101, LEU 162, ARG 195, ASP 197, HIS 299, GLU 233, ASN 298, ILE 235, ASP 300, TYR 62, TRP 58 and HIS 305. Figure 5.6B shows that Myricetin was fitted into the binding pocket of Alpha Amylase.

Figure shows that the amino acid resides involved in the binding of Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- are ASN 301, ALA 307, ILE 235, ALA 198, ASP 197, LEU 162, HIS 101, TYR 62, GLN 63, LEU 165, TRP 59, GLU 233, TRP 58, ARG 195, HIS 299, ASN 298, ASP 300, PHE 256 and ASP 300.Figure 5.7B shows that Cyclohexane,1,1'-(2-propyl-1,3-propanediyl)bis- was fitted into the binding pocket of Alpha Amylase.

Figure shows that the amino acid residues involved in the binding of Cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis- are TRP 59, LEU 165, LEU 162, GLN 63, HIS 101, ASP 197, HIS 299, TYR 62, TRP 58 and ASP 300.Figure 5.8B shows that Cyclohexane,1,1'-(2-methyl-1,3-propanediyl)bis- was fitted into the binding pocket of Alpha Amylase.

Shows that the amino acid residues involved in the binding of 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine are TRP 59, TRP 58, HIS 299, TYR 62, ASP 197, GLU 233, ALA 198, LEU 162, HIS 101, LEU 165 and GLN 63. Figure 5.9B shows that 6-methyl-2-(3-nitrophenyl) imidazo[1,2-a] pyridine was fitted into the binding pocket of Alpha Amylase.

DISCUSSION

A wide variety of secondary metabolites are biosynthesized by plants, which function as living chemical factories (Adeoye *et al*., 2023; Asiyanbola *et al*., 2024). These secondary metabolites are the building blocks of many commercial pharmaceutical medications as well as herbal cures derived from therapeutic plants (Adeoye *et al*., 2022; Adebola, *et al*., 2024). Traditional medicinal practices have often relied on the use of plants and herbs to treat various diseases (Adewole *et al*., 2023; Adebola *et al.*, 2024; Adebola *et al*., 2024). The use of *in silico* analysis in the field of medicinal plants has gained significant attention in recent years. One such plant of interest is *Mangifera indica*, which has been traditionally used in some cultures for its potential anti-diabetic properties (Minniti *et al.*, 2023). This project aimed to explore the therapeutic potential of mango leaves in treating diabetes, utilizing computational tools and databases to predict and analyze various bioactive compounds.

Biological markers are measurable indicators of the severity or presence of a disease state. This study showed the potential of Mangifera indica methanol extract with various biological markers *in silico.* In this study, molecular docking softwares were used to investigate the potential interactions between the bioactive compounds present in *Mangifera indica* and key molecular targets involved in diabetes, such as alpha-amylase, Glucagon-like peptide 1, Insulin and Glucose transporter type 4.

The binding energies detected from docked simulation with Insulin receptor using AutoDock Vina ranged from -4.6kcal/mol to -7.8kcal/mol. 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine had the highest binding affinity for Insulin receptor among the 8 compounds detected. 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine had a binding energy of -7.6kcal/mol which was lower than that of the standard ligand, Metformin(-7.8kcal/mol). The results indicate that 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine may improve insulin sensitivity.

The binding energies detected from the docked simulations with Glucose transporter type 4, ranged from -5.1kcal/mol to -8.6kcal/mol. 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine had a binding energy of -8.5kcal/mol which was lower than that of the Standard ligand, Metformin(-8.5kcal/mol).The results indicate that 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine may induce Glucose transporter type 4 translocation. Metformin is a better inducer than 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine.

The binding energies detected from the docked simulations with Alpha Amylase ranged from -4.9kcal/mol to -8.0kcal/mol. 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] pyridine had a binding energy of -8.0kcal/mol which was higher than that of the Standard ligand, Myricetin(-7.7kcal/mol). The results indicate that 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] may inhibit alpha amylase, leading to a reduction in the rate of glucose absorption.

The binding energies detected from the docked simulations with Glucagon-like peptide 1 ranged from -4.4kcal/mol to -6.5kcal/mol. Oxime-, methoxy-phenyl had a binding energy of -6.5kcal/mol. The results indicate that Oxime-, methoxy-phenyl may increase glucagon-like peptide 1 (GLP-1) secretion.

CONCLUSION

Mangifera indica methanol extract showed inhibitory effects against the biological markers of Diabetes, *in silico.* The study showed that 6-methyl-2-(3-nitrophenyl)imidazo[1,2-a] may likely be alpha amylase inhibitor, inducer of Glut 4 translocation and improves insulin sensitivity. Oxime-, methoxy-phenyl is an enhancer of Glucagon-like peptide 1 secretion. Medication developed utilizing Mangifera indica extract may be beneficial for treating a variety of disorders linked to hyperglycemia.

REFERENCES

- Adebola, A., Adeogun, A., Akano, O., Adeoye B. O. A., Ayodeji, A., Kolawole, O., Oluwadunsin, A., Olatinwo, G., Ogunsanya, S., Bangsi, A., Linda, N., Nwobi, J., Oluwseun, O., Aderonke, A., Temitope, A., Moore, A., & Olubukola, A. (2024). Cytokeratin-7 Expressions and the Protective Roles of Theobroma cacao Seed Extract Following Reserpine Induced Hepatotoxicity in Wistar Rats. 9. https://doi.org/10.36348/sjbr.2024.v09i06.00X
- Adebola, A., Adeoye B. O., A., Akano, O., Ayodeji, A., Oluwadunsin, A., Adeogun, A., Ogunsanya, S., Nwobi, J., Nnenna, N., Olubukola, A., Oluwole, O., & Micheal, A. (2024). fmrfamide mitigated mdma (3, 4- methylenedioxymethamphetamine)/tramadol-induced testicular toxicity in wistar rat. *Tropical Journal of Natural Product Research, 8.*
- Adebola, A., Oluwafunmbi, A., Olufunso, A., Nwobi, J., Sunday, F., Akano, O., Ogunsanya, S.,

Oluwole, O., Linda, N., Ayodeji, A., & Olayinka, O. (2024). Chronic stress induced – neurodegenerative changes in wistar rats: hippocampal evidence and the mitigating effects of allium sativum. *Babcock University Medical Journal*, *7*. https://doi.org/10.38029/babcockuniv.med.j..v7i1

- Adeoye, B. O., Ayobola, I., Daniyan, M., Victor, O, E., David, A., Abijo, A., & Bimbola, A. A. (2022). Ameliorative effects of Nigerian bitter honey on streptozotocin-induced hepatorenal damage in Wistar rats. *Journal of Krishna Institute of Medical Sciences University*, *11*, 65–76.
- Adeoye, B. O., Iyanda, A., Michael, O., Oyebimpe, O., & Fadeyi, B. (2022). Inhibitory effects of Nigerian Sweet and Bitter Honey on Pancreatic Alpha Amylase Activity. *Nigerian Journal of Nutritional Sciences*, *43*, 19–24.
- Adewole, A. E., Shittu, M. D., & Adeoye, B. O. (2023). Additive Effect of Polyalthia Longifolia Leaf Meal on Growth Performance and Blood Profile of Growing Rabbits (Oryctolagus Cuniculus). *European Journal of Biomedical*, *10*(2), 412-417.
- Asiyanbola, I. D., Daniyan, M. O., Opoggen, T. S., Olayemi, I. O., Adeoye, O. B., Ekundina, V. O., & Oyemitan, I. A. (2024). Neuroprotective potentials of the essential oil of Curcuma aeruginosa Roxburg rhizomes in mice with cerebral malaria. *Phytomedicine Plus*, *4*(3), 100581. https://doi.org/10.1016/j.phyplu.2024.100581
- Irondi, E. A., Oboh, G., Akindahunsi, A. A., Boligon, A. A., & Athayde, M. L. (2014). Phenolic composition and inhibitory activity of *Mangifera indica* and *Mucuna urens* seeds extracts against key enzymes linked to the pathology and complications of type 2 diabetes. *Asian Pacific Journal of Tropical Biomedicine*, *4*(11), 903–910. https://doi.org/10.12980/APJTB.4.201414B364
- Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., Maheshwari, C., Prajapati, U., Singh, S., Prajapat, R. K., Dhumal, S., Punia, S., Amarowicz, R., & Mekhemar, M. (2021). Mango (Mangifera indica L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Antioxidants*, *10*(2), 299. https://doi.org/10.3390/antiox10020299
- Lauricella, M., Emanuele, S., Calvaruso, G., Giuliano, M., & D'Anneo, A. (2017). Multifaceted Health Benefits of Mangifera indica L. (Mango): The Inestimable Value of Orchards Recently Planted in Sicilian Rural Areas. *Nutrients*, *9*(5), 525. https://doi.org/10.3390/nu9050525
- Minniti, G., Laurindo, L. F., Machado, N. M., Duarte, L. G., Guiguer, E. L., Araujo, A. C., Dias, J. A., Lamas, C. B., Nunes, Y. C., Bechara, M. D., Júnior, E. B., Gimenes, F. B., & Barbalho, S. M. (2023). Mangifera indica L., By-Products, and Mangiferin on Cardio-Metabolic and Other Health Conditions: A Systematic Review. *Life*, *13*(12). https://doi.org/10.3390/life13122270
- Olajide, L., Ayomikun, L., Rafiu, S., Adeoye Bayo O., A., Olajide, O., Orodele, K., & Tosin, O. (2023). Anti-Inflammatory Property of Costus afer Ker Gawl Ethanol Leaf Extract in STZ-Induced Diabetic Rats. *Scholars International Journal of Biochemistry*, *6*, 129–137. https://doi.org/10.36348/sijb.2023.v06i10.002
- Adeoye Bayo O., A., Iyanda, A., Daniyan, M., Ayodeji, A., Michael, O., & Olatinwo, G. (2022). *Botanical and Bioactive Markers of Nigerian Bitter Honey*, 1848–1853. https://doi.org/10.26538/tjnpr/v6i11.17
- Adeoye Bayo O., A., Iyanda, A., Daniyan, M., Ayodeji, A., Olajide, L., Akinnawo, O., Adebola, A., Oluwseun, O., & Olatinwo, M. (2023). Anti-dyslipidaemia and cardio-protective effects of nigerian bitter honey in streptozotocin induced diabetic rats. *8*, 10–18. https://doi.org/10.22270/ujpr.v8i2.920
- Opreh, O. P., Adeoye Bayo O., A., Akintomiwa, O., Angela, U., Oyeleke, I., & Moore, A. (2024). An Impactful Health Fair in a Semi-Urban Community: Awolowo Town Case Study. *Saudi Journal of Medicine*, *9*. https://doi.org/10.36348/sjm.2024.v09i06.00X
- Oyedemi, S. O., Oyedemi, B. O., Ijeh, I. I., Ohanyerem, P. E., Coopoosamy, R. M., & Aiyegoro, O. A. (2017). Alpha-Amylase Inhibition and Antioxidative Capacity of Some Antidiabetic Plants Used by the Traditional Healers in Southeastern Nigeria. *TheScientificWorldJournal*, *2017*, 3592491. https://doi.org/10.1155/2017/3592491
- Sekar, V., Chakraborty, S., Mani, S., Sali, V. K., & Vasanthi, H. R. (2019). Mangiferin from *Mangifera indica* fruits reduces post-prandial glucose level by inhibiting α-glucosidase and α-amylase activity. *South African Journal of Botany*, *120*, 129–134. https://doi.org/10.1016/j.sajb.2018.02.001
- Shah, K. A., Patel, M. B., Patel, R. J., & Parmar, P. K. (2010). Mangifera Indica (Mango). *Pharmacognosy Reviews*, *4*(7), 42–48. https://doi.org/10.4103/0973-7847.65325
- Stöckli, J., Fazakerley, D. J., & James, D. E. (2011). GLUT4 exocytosis. *Journal of Cell Science*, *124*(24), 4147–4159. https://doi.org/10.1242/jcs.097063
- Wang, T., Wang, J., Hu, X., Huang, X.-J., & Chen, G.-X. (2020). Current understanding of glucose transporter 4 expression and functional mechanisms. *World Journal of Biological Chemistry*, *11*(3), 76–98. https://doi.org/10.4331/wjbc.v11.i3.76