Trop J Nat Prod Res, August 2024; 8(8): 8168-8175 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of the *In vitro* and *In silico* Pancreatic Lipase Inhibitory Activity of Ethanol Leaf Extract of *Tapinanthus cordifolius* and its Effect on Oral Glucose Tolerance in Mice

Amarachi Chike-Ekwughe[1,2]*, Lucy B. John-Africa[3], Abiodun H. Adebayo[1], Olubanke O. Ogunlana[1]

[1]*Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria*
[2]*Department of Life Sciences, Faculty of Computing and Applied Sciences, Baze University, Abuja, Nigeria*
[3]*Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Abuja, Nigeria*

ARTICLE INFO

Article history:
Received:9 March 2024
Revised: 24 July 2024
Accepted: 14 August 2024
Published online 01 September 2024

ABSTRACT

The rising prevalence of diabetes and the high cost of treatment necessitate the search for natural, safe, and effective treatment methods. This study examined the *in vitro* and *in silico* pancreatic lipase inhibitory activity as well as the hypoglycemic effect of the ethanol extract of *Tapinanthus cordifolius* (TC) leaves in mice. TC leaves were extracted with ethanol by Soxhlet extraction technique. The pancreatic lipase inhibitory activity of TC extract at various concentrations (50, 100, 200, 400, 800, and 1600 µg/mL) was evaluated using spectrophotometric method. The effect of bioactive compounds from TC extract on pancreatic lipase was assessed *in silico* using molecular docking approach. The hypoglycaemic effect of TC extract was evaluated *in vivo* by an oral glucose tolerance test (OGTT) in normoglycaemic mice. Glibenclamide (5 mg/kg) was used as the positive control. TC extract showed significant pancreatic lipase (PL) inhibitory activity with IC_{50} value of 294.30 ± 0.67 µg/mL, which was comparable to that of orlistat (IC_{50} = 195.70 ± 4.60 µg/mL). *In silico* studies identified campesterol, heptadecafluorononanoic acid, *α*-tocopherol-*β*-d-mannoside, clionasterol, and *β*-tocopherol as potent pancreatic lipase inhibitors. TC extract (200, 400, and 800 mg/kg) significantly reduced hyperglycemia in glucose-challenged mice. It exhibited a significant ($p<0.05$) increase in glucose absorption, similar to glibenclamide, with the most notable effect at 60 minutes post-glucose load. These findings suggest that *Tapinanthus cordifolius* leaf can effectively inhibit pancreatic lipase and reduce blood glucose levels, and may serve as a natural treatment alternative for diabetes mellitus.

***Keywords*:** Pancreatic lipase, *Tapinanthus cordifolius*, Hypoglycaemic activity, Oral glucose tolerance test.

Introduction

Glucose intolerance, the hallmark of type 2 diabetes, is due to peripheral insulin resistance and islet *β*-cell malfunction.[1] The glucose tolerance test can be used as a diagnostic tool for type 2 diabetes, and an assessment criteria for glucose tolerance in clinical practice and research. It is important to note that the glucose tolerance test is the only way to diagnose impaired glucose tolerance. The control of postprandial blood glucose is essential for the prompt treatment of diabetes and the mitigation of long-term consequences.[2] One of the proposed treatment approaches for hyperglycemia and hyperlipidemia is the inhibition of digestive enzymes, and delay the absorption of fats and carbohydrates, thereby lowering plasma levels of fatty acids and glucose.[3] Lipase is involved in the breakdown of triglycerides into glycerol and free fatty acids.

*Corresponding author. E mail: amarachi.chike-ekwughe@bazeuniversity.edu.ng
Tel: +2348034146534

Citation: Chike-Ekwughe, A, John-Africa, LB, Adebayo, AH, Ogunlana, OO. Evaluation of the *In vitro* and *In silico* Pancreatic Lipase Inhibitory Activity of Ethanol Leaf Extract of *Tapinanthus cordifolius* and its Effect on Oral Glucose Tolerance in Mice. Trop J Nat Prod Res. 2024; 8(8): 8168-8175. https://doi.org/10.26538/tjnpr/v8i8.37

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Therefore, the inhibition of this enzyme can cause a delay in the breakdown of fats, and eventually lead to less absorption and reduced postprandial blood sugar levels. Nowadays, anti-diabetic medications cannot restore normal blood sugar homeostasis without undesirable side effects. For this reason, various enzyme inhibitors derived from plants have been investigated for the control of postprandial blood glucose.[2]

Diabetes mellitus needs urgent attention due to its high endemic proportions globally. Several herbal remedies have been explored in the treatment of diabetes.[4] The only peripherally-acting medication authorized by the FDA (Food and Drug Administration) for treating obesity is orlistat, a pancreatic lipase inhibitor.[5,6] Pancreatic Lipase (PL) is a crucial enzyme in the hydrolysis and absorption of lipids. As a result, pancreatic lipase inhibition lowers fat absorption and is advantageous for controlling obesity and metabolic diseases. PL inhibitors might not have any adverse effects linked to other centrally-acting anti-obesity medications.[7]

In recent years, the use of herbal medicines for the treatment of various diseases, including diabetes has increased significantly. Herbal medicine, often called phytomedicine, is a crucial component of primary healthcare systems in developed and developing nations because it has fewer adverse effects and its less expensive compared to synthetic pharmaceuticals.[8-10] *Tapinanthus cordifolius* (TC), a heart-leaved tapinanthus, is a tiny evergreen shrub with oval-shaped leaves and yellow blooms. It thrives in forests, scrublands, and other tree-filled regions. *Tapinanthus* contains 40 species, including TC.[11] TC has a worldwide distribution, but endemic to Africa. It belongs to a distinct genus in the Loranthaceae family. In Nigeria, it is known by various vernacular names like "Ohumagana" in Igbo, "Kauci" in

Hausa, and "Afomo Onisana" in Yoruba. TC has been shown to possess various pharmacological effects including hypotensive, anti-lipidaemic, anti-oxidant, anti-inflammatory, and antimicrobial effects.[12,13]

The current study was designed to investigate the possible benefits of TC leaf extracts in the management of diabetes mellitus. The study examined the inhibitory effect of TC extract on pancreatic lipase both *in vitro* and *in silico*, and its possible hypoglycaemic effect through an oral glucose tolerance test (OGTT).

Materials and Methods

Chemicals

All chemicals were of analytical grade, and were purchased from local suppliers. Insulin, glibenclamide, glucose strips, and glucose were of Solarbio Science and Technology, Beijing, China.

Plant collection and identifiction

Fresh leaves of *Tapinanthus cordifolius* (TC) were collected from Awka, Anambra state, Nigeria. The plant material was identified and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. A specimen was was kept in the institute's herbarium, and assigned a voucher reference number NIPRD/H/7203.

Extraction of plant material

TC leaves were carefully cleaned, and air-dried at room temperature (25°C) for 28 days. The dried leaves were ground into a fine powder using a mortar and pestle.

The powdered leaves (2.8 kg) was extracted by maceration in 85% ethanol (2 L) for 72 h. The extract was filtered through a muslin cloth, and then concentrated in a rotary evaporator at 78°C. The concentrated extract was weighed, the percentage yield calculated, and then stored in the refrigerator until ready for use.[13,14]

Experimental animals

Twenty-five Swiss albino mice of either sex weighing between 23 and 30 g were acclimatized to the laboratory conditions at Nigeria Institute for Pharmaceutical Research and Development (NIPRD) for two weeks with unlimited access to food and drinking water. Ethical approval was granted by the Research Ethics Committee, NIPRD, Abuja, with reference number: NIPRD/05:03:05-24. Animals were handled following the international guidelines for the use and care for experimental animals.

Evaluation of pancreatic lipase inhibitory activity

The pancreatic lipase inhibitory activity of TC extract was evaluated *in vitro* according to the method described by Nabil-Adam *et al.*[15] with modification. Briefly, 1.6 mM paranitrophenyl laurate was dissolved in 0.5% Triton X-100-containing Tris-HCl buffer (66 mM, pH 7.4) to create the substrate stock. Pancreatic lipase (50 µL of 150 U/mL) was also dissolved in the same buffer, and incubated with equal volume of the extract at varied concentrations (50 - 1600 g/mL) for 15 min. The reaction was stopped by 1% sodium carbonate. A negative control reaction mixture was produced following the same procedure highlighted above, but substituting the extract with distilled water. Orlistat was used as the reference standard. Additionally, a blank solution was prepared to remove interference. The absorbance of the reaction mixture was measured at 405 nm using a spectrophotometer. The percentage lipase inhibitory activity was estimated using the equation below.

The IC_{50} value (g/mL) was calculated using regression analysis on GraphPad Prism version 6.0 software.

In silico evaluation of pancreatic lipase inhibitory activity

Hardware and software: Autodock tools v 1.5.4, PyMOL 1.3, and ChemDraw Ultra 11 were installed on a laptop.

Protein preparation

The x-ray diffraction crystal structure of pancreatic Lipase with PDB ID: 2OXE (R-value Free: 0.261, 2.80 Ao and R-value Work: 0.222) was retrieved from the Protein Data Bank (PDB) archive. The protein was prepared using the protein preparation wizard panel of Glide, a part of the Maestro Molecular Modeling Suite (Schrödinger Suite 2021-2). The receptor grid was defined using the co-crystallized ligand (OXE). Bond order was determined, hydrogen atoms were added, disulfide bonds were created, and missing side chains or loops were added using *prime*. Water molecules located beyond 3.0 Å from the heteroatoms were removed. The OPLS4 force field and *PROPKA* were used to optimize the structure. The binding pocket of the ligands was then detailed in a receptor grid file.[16]

Ligand preparation

Appropriately chiralized low-energy three-dimensional structures of the compounds identified from the GC-MS analysis of TC were constructed. The Schrödinger Suite 2021-2 Ligprep module was used to prepare the co-crystallized ligand. The potential ionization states were calculated at a physiological pH of 7.2 for each ligand. all possible stereoisomers associated with the chiral centers were generated manually and added to the ligands.[17]

Receptor grid generation

For ligand docking, the location and dimensions of the protein's active site was ascertained using Schrödinger Maestro 12.8 receptor grid-building tool. 1S, 2S, 3R, 4S, and 5S stereoisomers of the co-crystallized ligands were used to form the scoring grid. Nonpolar receptor atoms were subjected to this procedure with a partial charge cut-off of 0.25 and a van der Waals (vdW) radius scaling factor of 1.0.[18]

Protein-Ligand docking

The Glide tool in the Schrödinger Maestro 12.8 induced fit docking panel was used to perform the molecular docking interaction between the lipase enzyme and the compounds with the highest score. The previously prepared receptor grid file was used throughout the docking procedure. With flexible ligand sampling, the ligands produced were docked in standard precision (SP) mode, refined alone with no further ligand sampling. The van der Waals (vdW) radius scaling factor for the ligand atoms were 0.80 and the partial charge cut-off throughout the docking process was 0.15.

Receptor-ligand complex pharmacophore modelling

An Auto (E-) pharmacophore model of the receptor-ligand complex of fthe ive compounds with the most significant binding affinity for the target protein was built using the PHASE software. The hypothesis was limited to producing a maximum of seven distinct features. Two was the minimal distance between features of different types, whereas four was the minimum distance between features of the same type. The term "vector" was used to describe donors when the pharmacophore model was developed.

Prediction of pharmacokinetics parameters

SwissADME and PROTOX-II servers' *in silico* integrated model predictions were used to evaluate the test ligands. The pharmacokinetic properties of absorption, distribution, metabolism, excretion, and toxicity (ADMET) were assessed using these computational methods.

Oral Glucose Tolerance Test (OGTT)

OGTT was conducted using the method described by Noora *et al*. (2020) with minor modifications.[8] Twenty-five mice were randomly divided into five groups of 5 mice each. The animals were fasted for 12 hours, after which they were treated as follows; Group 1 mice (negative control) were given 10 mL/kg body weight of the vehicle (10% DMSO). Group 2 mice (positive control) received glibenclamide (5 mg/kg body weight). Mice in Groups 3, 4, and 5 received TC extract at doses of 200, 400, and 800 mg/kg body weight, respectively. One hour later, all animals received glucose (2000 mg/kg) orally. Blood glucose level was measured at time zero, 15

minutes, 30 minutes, 60 minutes, and then hourly for 6 hours post oral glucose challenge. Glucose area under the curve was calculated by trapezoidal approximation of plasma glucose levels.

Statistical analysis

Data were presented as mean ± standard error of mean (SEM). The Statistical Package for Social Sciences (SPSS) for Windows version 21.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Differences between means were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. Significant difference was set at p-value < 0.05.

Results and Discussion

Pancreatic lipase inhibitory activity

The effect of TC extract on pancreatic lipase is presented in Figure 1. TC extract exhibited a concentration-dependent increase in pancreatic lipase inhibitory activity. The half maximal inhibitory concentration (IC_{50}) for TC extract was found to be 294.30 ± 0.67 µg/mL, which was comparable to that of the standard compound orlistat with an IC_{50} of 195.70 ± 4.60 µg/mL (Table 1).

Lipase is one of the main digestive enzymes that convert fats into readily absorbable fatty acids.[19] Reduction of post-prandial hyperglycaemia and hyperlipidaemia is one of the strategies in the effective management of diabetes mellitus. The inhibition of pancreatic lipase represents an approach in reducing post-prandial fatty acids absorption by inhibiting the breakdown of fats, cholesterol, and triglycerides. In the present study, the anti-diabetic activity of TC was investigated *in vitro*, *in vivo*, and *in silico*. The *in vitro* investigation was focused on the inhibition of pancreatic lipase. As highlighted in Figure 1 and Table 1, TC extract showed a significant *in vitro* inhibitory effect on pancreatic lipase. This result is consistent with that of Chike-Ekwughe *et al*.[20,21,] who studied the effect of TC extract on other digestive enzymes. According to their findings, the crude ethanol extract of TC showed a significant inhibition of alpha-glucosidase enzyme, with an IC_{50} value of 26.88 µg/mL.

Table 1: IC_{50} values for pancreatic lipase inhibitory activity of ethanol leaf extract of *Tapinanthus cordifolius*

Sample	IC_{50} value (µg/mL)
ETC	294.30 ± 0.67
Orlistat	195.70 ± 4.60

Values are Mean ±SEM (n = 3). ETC: Ethanol leaf extract of *Tapinanthus cordifolius*.

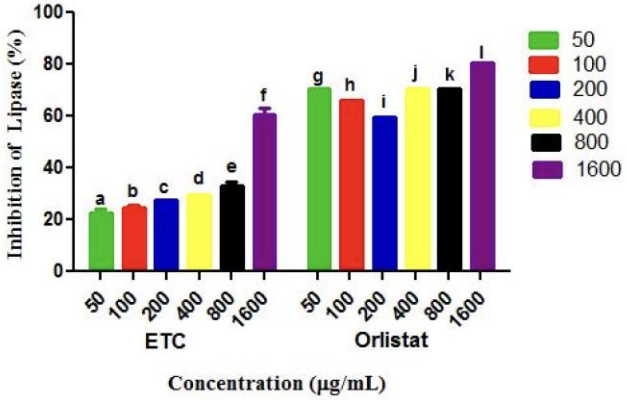

Figure 1: Pancreatic lipase inhibitory activity of ethanol leaf extract of *Tapinanthus cordifolius*

Data represents Mean ± SEM (n = 3). Values with different lower case letters are significantly different (p<0.05).

In silico pancreatic lipase inhibitory activity of TC

Docking score/Binding affinity of the ligands

Table 2 shows the docking scores of the top five hit compounds from the molecular docking analysis of the bioactive compounds of TC with pancreatic lipase. The compounds showed different degrees of binding affinities for pancreatic lipase. The highest docking score was recorded for campesterol (-4.51 kcal/mol), followed by heptadecafluorononanoic acid (-4.468 kcal/mol), and alpha-tocopherol (-4.391 kcal/mol). This indicated that the bioactive compounds from TC have strong affinity for the amino acid residues in the active site of the protein (pancreatic lipase). The two-dimensional (2D) and three-dimensional (3D) analyses of these interactions are presented in Figures 2 - 6. Among its other significant molecular interactions, campesterol formed hydrophobic interactions with TYR 133, PRO 199, CYS 200, ILE 228, VAL 229, PHE 234, ILE 274, PHE 277, PHE 96, and LEU 97, as well as hydrogen bond interaction with GLN 39. Heptadecafluorononanoic acid interacted by forming hydrophobic interactions with PHE234, LEU 232, ILE 228, LEU 97, PHE 96, TRP 271, ILE 274, PHE 277, PRO 199, CYS 200, and TYR 133. Alpha-tocopherol-*β*-D-mannoside interacted through hydrogen bonding with ARG 209, GLU 206, and GLU 341, and hydrophobic interactions with PRO 205, LEU 38 and PRO 33. Clionasterol interacted through hydrophobic interactions with TYR 133, and hydrogen bond formation with GLN 39, PRO 199, CYS 200, ILE 228, VAL 229, PHE 234, ILE 274, PHE 277, PHE 96, LEU 97, TRP 271, ALA 131, MET 132, and TYR 133. *β*-tocopherol showed interaction through the formation of hydrogen bonds with GLN 39 and hydrophobic interactions with LEU 97, PHE 96, ALA 131, MET 132, TRY 133, PHE 234, ILE 228, PRO 41, ILE 274, PHE 277, PRO 199, and CYS 200. The reference substance, OXE (10-Amino-10,11 dihydrodibenz(b,f)oxepine hydrochloride) interacted via hydrogen bonding with GLU 341, SER 213, GLU 206 and GLU 207, and by hydrophobic interactions with VAL 340 and PRO 212. It is important to note that many lipases have their active sites in the N-terminal domain and are controlled by a lid formed by a surface loop, β5 loop, and β9 loop. There is a catalytic triad, Ser152-His263-Asp176, at the bottom of this crevice.[22]

Table 2: Selected bioactive compounds of TC and their binding affinity (kcal/mol) against pancreatic lipase

Compound name	PubChem CID	Docking score
OXE	24755467	-5.166
Campesterol	173183	-4.51
Heptadecafluorononanoic acid	91692466	-4.468
α-Tocopherol-*β*-D-mannoside	597057	-4.391
Clionasterol	457801	-3.894
β-Tocopherol	6857447	-3.859

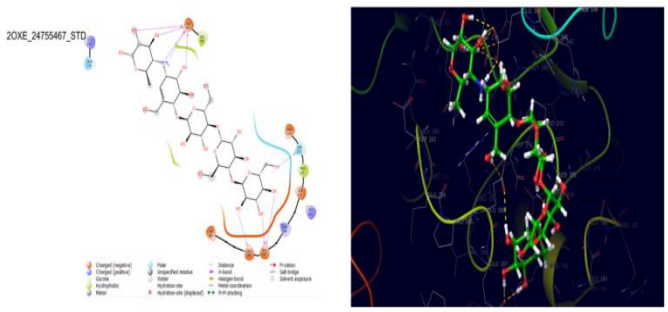

Figure 2: 2D and 3D depictions of the molecular interactions between pancreatic lipase and campesterol

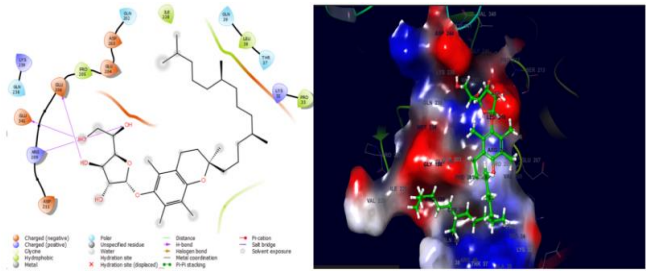

Figure 3: 2D and 3D depictions of molecular interactions between pancreatic lipase and heptadecafluorononanoic acid

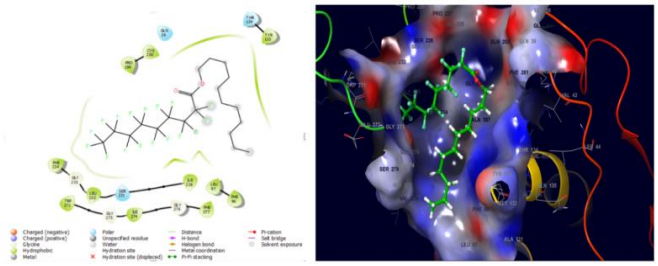

Figure 4: 2D and 3D depictions of molecular interactions between pancreatic lipase and α-tocopherol-*β*-D-mannoside

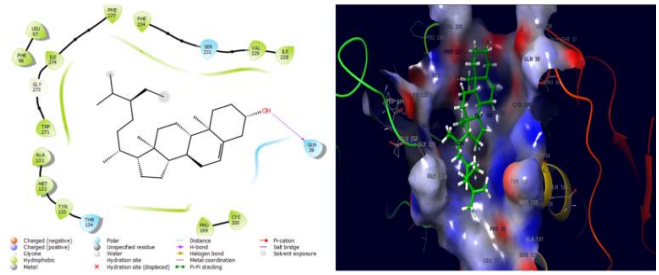

Figure 5: 2D and 3D depictions of molecular interactions between pancreatic lipase and Clionasterol

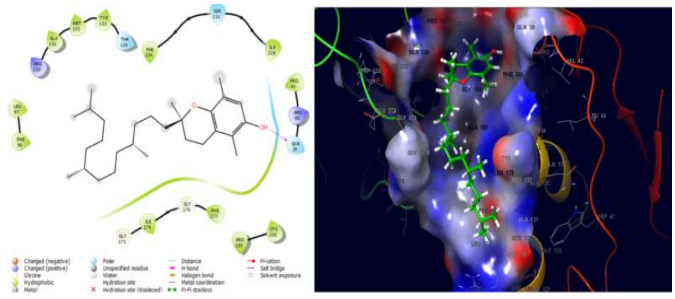

Figure 6: 2D and 3D representations of molecular interactions between pancreatic lipase and *β*-tocopherol

Pharmacophore model of receptor-ligand complex

The pharmacophore models of the receptor-ligand complex for the five selected compounds of TC are displayed in Figures 7 - 9. Hydrogen bond acceptors (A), hydrogen bond donors (D), hydrophobic interactions (H), and aromatic ring (R) are represented in the models. Heptadecafluorononanoic acid and campesterol displayed one hydrophobic interaction and one hydrogen bond donor. Clionasterol on the other hand, showed an aromatic ring, hydrogen bond donor, and hydrophobic interaction, while alpha-tocopherol-*β*-D-mannoside has similar interactions as clionasterol together with hydrogen bond acceptor. Beta-tocopherol interacted mainly through hydrophobic interaction. *In silico* pharmacophore modeling is used to explain the structural properties of a test ligand, and also identify the bonding interactions between the ligand and the target protein. In this study, the pharmacophore modeling was carried out to confirm the pancreatic lipase inhibitory effect of TC bioactive compounds. The models showed that the bioactive compounds from TC has the ability to inhibit pancreatic lipase by forming several non-covalent bond interactions, including hydrogen bonding, and hydrophobic interactions.[23]

ADMET properties of selected ligands

Tables 3 - 7 display the ADMET properties of the five selected compounds from TC extract. Information on their lipophilicity, water solubility, drug-likeness, bioavailability, metabolism, and toxicity are provided. The consensus Log P value for campesterol was 6.59, while that of heptadecafluorononanoic acid was found to be 10.08. All the compounds, however, obeyed the Lipinski rule. Campesterol, clionasterol, β-tocopherol, and *α*-tocopherol-β-D-mannoside was predicted to be immunotoxic, while heptadecafluorononanoic acid was predicted as carcinogenic. The prediction did not show any other toxic effect in the compounds. Hydrogen bonds are essential interactions in biochemistry because they are required for drug partition, enzyme catalysis, permeability, structure stability, and molecular recognition, among other functions. The solubility of a drug and its capacity to make significant interactions with its biomolecular targets can be enhanced by the presence of functional groups that can form hydrogen bonds, leading to strong binding and selectivity.

However, excessive hydrogen bond donors or acceptors may negatively impact the partitioning, and cell membrane permeability of a ligands. These polar groups can enhance the aqueous solubility of the drug, while lowering its transport through hydrophobic membrane.[24] The number of hydrogen bond donors and acceptors is a crucial molecular parameter for predicting the oral bioavailability of low molecular weight drug candidates. The most widely used oral bioavailability criteria (such as Veber and Lipinski rule of five) use this molecular parameter. It is well known that hydrogen bond donors and acceptors affect passive diffusion through cell membranes, an essential step in the absorption and distribution of drugs.

The docking pose and molecular interaction of the most promising compound, alpha-tocopherol in the flexible binding pockets of pancreatic lipase is shown in Figure 10. The fingerprint depiction of the interaction of alpha-tocopherol with the ten main amino acid residues of pancreatic lipase is presented in Figure 11. The nearly continuous coloured lines in the figure graphic illustrate how specific protein amino acid residues, notably GLY 233, ASP 224, and TRP 271, constantly interacted with the ligand.[25]

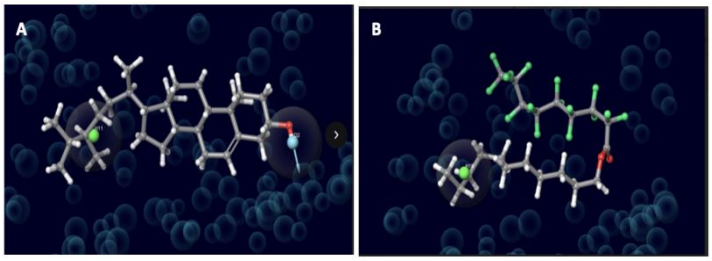

Figure 7: Pharmacophore model of receptor-ligand complex of A: Campesterol and B: Heptadecafluorononanoic acid with lipase

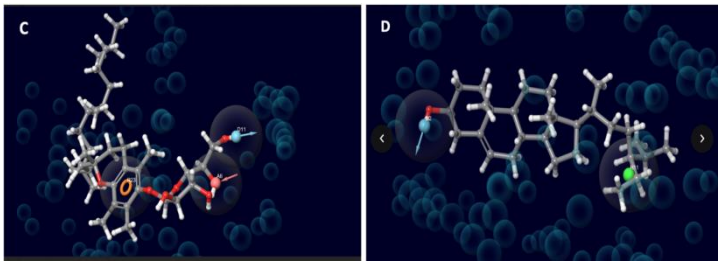

Figure 8: Pharmacophore model of receptor-ligand complex of C: Alpha-tocopherol-*β*-D-Mannoside and D: Clionasterol with lipase

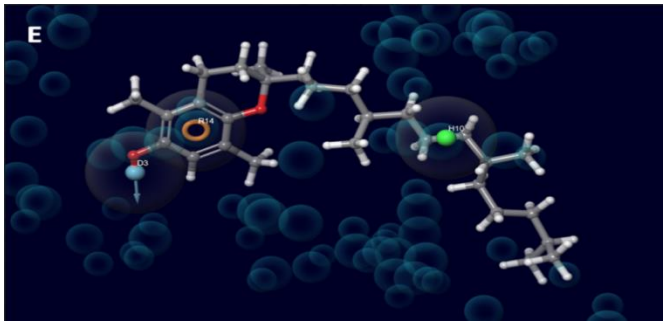

Figure 9: Pharmacophore model of receptor-ligand complex of E: Beta-tocopherol with lipase

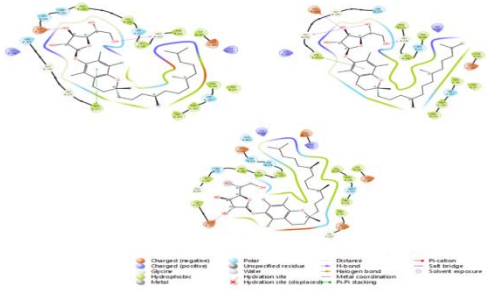

Figure 10: The binding pose of alpha-tocopherol with pancreatic lipase binding site after induced fit docking

Effect of TC on oral glucose tolerance in mice

The antidiabetic activity of TC was investigated *in vivo* using oral glucose tolerance test (OGTT) in mice. Results showed a significant reduction in glucose area under the curve (AUC) in mice following TC extract treatment at different doses (Table 8). A lower AUC for OGTT in diabetic mice indicates a hypoglycaemic effect. Similarly, there was a significant reduction in blood glucose levels following TC extract treatment compared to the untreated group ($P < 0.05$). Increased blood glucose levels is the hallmark of diabetes, leading to potentially severe consequences, including organ damage, particularly affecting the kidneys and the eyes, as well as numerous cardiovascular complications.[26,27,28] Treatment strategies, including the use of medicinal plants aim to minimize the variations in blood sugar levels and associated complications. One of such therapeutic approaches include curbing post-meal high blood sugar levels by slowing down glucose absorption by suppressing carbohydrate breakdown, as well as a reduction in the breakdown of fats through inhibition of digestive enzymes such as the pancreatic lipase.[19]

The oral glucose tolerance test (OGTT) is the most widely used technique for assessing glucose tolerance *in vivo*.[28] According to the WHO (2006),[29] OGTT must confirm or rule out an aberration of glucose tolerance in asymptomatic individuals. Fasting blood glucose alone is not sufficient to diagnose diabetes in about thirty percent of cases. Insulin release and sensitivity can also be evaluated with OGTT. It is also employed in assessing insulin resistance and apparent insulin release.[30] Once diabetes is diagnosed, it becomes necessary to institute proper treatment strategy using drugs with little or no unbearable side effects. In the present study, the hypoglycaemic activity of TC extract using the oral glucose tolerance test (OGTT) is being reported for the first time. In animal studies, some pancreatic lipase inhibitors have been shown to modestly enhance insulin sensitivity and glucose tolerance, independent of their effects on body weight.[31,32] The hypoglycaemic effect of the ethanol extract of TC at doses of 200, 400, and 800 mg/kg was comparable to that of glibenclamide (positive control) at 5 mg/kg body weight. A significant decrease in blood glucose level was observed from 15 min onwards for all the treatment groups (Figure 12). The rapid onset of action exhibited by TC extract mimics the hypoglycaemic mechanism involved in insulin-like agents, probably acting via increased secretion of insulin and/or increased sensitivity of tissues to the effect of insulin.[33,34,35]

Table 3: Lipophilicity of selected ligands from ethanol extract of *Tapinanthus cordifolius*

Molecule	MW	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
Campesterol	400.68	4.92	8.8	7.63	6.54	6.63	6.9
Heptadecafluorononanoic acid	464.08	6.06	10.97	16.21	6.91	10.27	10.08
α-Tocopherol-β-D-mannoside	592.85	6.14	8.89	6.31	3.49	8.12	6.59
Clionasterol	414.71	4.79	9.34	8.02	6.73	7.04	7.19
Beta -Tocopherol	416.68	4.94	10.33	8.53	5.94	9.2	7.79

Table 4: Water solubility of selected ligands from ethanol extract of *Tapinanthus cordifolius*

Molecule	ESOL Log S	ESOL Solubility (mg/ml)	ESOL Solubility (mol/l)	ESOL Class
Campesterol	-7.54	1.16E-05	2.90E-08	Poorly soluble
Heptadecafluorononanoic acid	-9.33	2.89E-07	4.67E-10	Poorly soluble
α-Tocopherol-β-D-mannoside	-8.17	4.04E-06	6.82E-09	Poorly soluble
Clionasterol	-7.9	5.23E-06	1.26E-08	Poorly soluble
Beta -Tocopherol	-8.29	2.15E-06	5.16E-09	Poorly soluble

Table 5: Drug-likeness of selected ligands from ethanol extract of *Tapinanthus cordifolius*

Molecule	Bioavailability Score	Lipinski violations
Campesterol	0.55	1
Heptadecafluorononanoic acid	0.17	2
α-Tocopherol-β-D-mannoside	0.55	1
Clionasterol	0.55	1
β-Tocopherol	0.55	1

Table 6: Pharmacokinetic profile of selected compounds from ethanol extract of *Tapinanthus cordifolius*

Molecule	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Campesterol	Low	No	No	No	No	No	No	No
Heptadecafluorononanoic acid	Low	No	Yes	No	No	No	No	No
α-Tocopherol-β-D-mannoside	Low	No	No	No	No	No	No	Yes
Clionasterol	Low	No	No	No	No	No	No	No
Beta -Tocopherol	Low	No	Yes	No	No	No	No	No

Table 7: Toxicity profile of selected ligands from ethanol leaf extract of *Tapinanthus cordifolius*

Target	A	B	C	D	E
Hepatotoxicity	-	-	-	-	+
Carcinogenicity	-	+	-	-	-
Immunotoxicity	+	-	+	+	+
Mutagenicity	-	-	-	-	-
Cytotoxicity	-	-	-	-	-
Predicted LD_{50} (mg/kg)	890	518	3000	890	1190
Predicted toxicity class	4	4	5	4	4

Table 8: Glucose Area Under the Curve (AUC) of oral glucose tolerance test (OGTT) in mice

Treatment Group	Glucose-AUC (mgh/dL)
Untreated + Glucose	1538
Glibenclamide (5 mg/kg) + Glucose	861.2
ETC (200 mg/kg) + Glucose	1134
ETC (400 mg/kg) + Glucose	984.9
ETC (800 mg/kg) + Glucose	1119

ETC = Ethanol leaf extract of *Tapinanthus cordifolius*

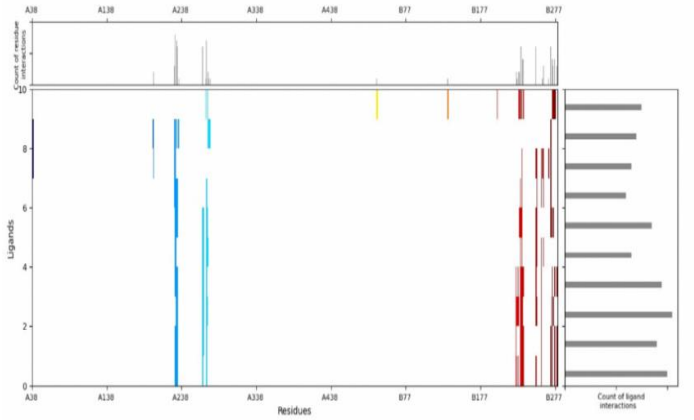

Figure 11: An induced fit docking fingerprint depicting the interactions between the amino acid residues of pancreatic lipase binding site and alpha-tocopherol

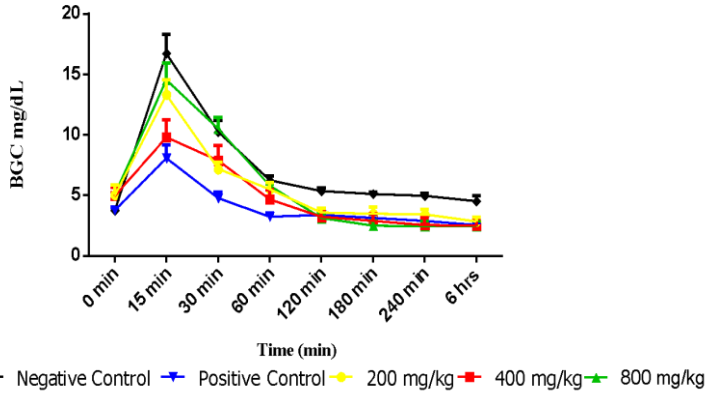

Figure 12: Effect of *Tapinanthus cordifolius* leaf extract and glibenclamide on blood glucose level after oral glucose tolerance test.
Data represent Mean ± SEM. [a] indicates statistical significant difference at $P < 0.05$ compared to negative control.

Conclusion

The present study evaluated the effect of the ethanol extract of *Tapinanthus cordifolius* (TC) leaf on glucose tolerance using *in vitro*, *in silico*, and *in vivo* methods. TC extract inhibited pancreatic lipase *in vitro*. Among the compounds identified in TC extract, campesterol, heptadecafluorononanoic acid, α-tocopherol-*β*-D-mannoside, clionasterol, and *β*-tocopherol showed significant pancreatic lipase inhibitory activity *in silico*, with α-tocopherol-*β*-D-mannoside exhibiting the most significant potential as pancreatic lipase inhibitor. *In vivo* studies revealed that TC extract prevented hyperglycemia in mice following an oral glucose tolerance (OGTT). These findings suggest that TC can effectively reduce blood glucose levels and may be a viable natural treatment for diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgments

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

References

1. Udia PM, Ogbonna OJ, Antai AB, Mbatutung IF, Eyo SE. Oral glucose tolerance test and some haematological effects of aqueous leaf extract of *Rothmannia hispida* (K. Schunn) fargel on normoglycaemic albino rats. Res J Pharmacogn Phytochem. 2013; 5(6):300-305.
2. Benrahou K, Naceiri MH, Bouyahya A, Daoudi NE, Bnouham M, Mezzour H, Mahmud S, Alshahrani MM, Obaidullah AJ, Cherrah Y, Faouzi MEA. Inhibition of α-Amylase, α-Glucosidase, and Lipase, Intestinal Glucose Absorption, and Antidiabetic Properties by Extracts of *Erodium guttatum*. Evid Based Complement Alternat Med. 2022; (1):5868682.
3. Awosika TO and Aluko RE. Inhibition of the *in vitro* activities of *α*-amylase, *α*-glucosidase and pancreatic lipase by yellow field pea (*Pisum sativum* L.) protein hydrolysates. Int J Food Sci Technol. 2019; 54(6):2021–2034.
4. Abdel-Moneim A and Fayez HA. Review on medication of diabetes mellitus and antidiabetic medicinal plants. Int J Bioassays. 2015; 4(6):4002-4012.
5. Cignarella A, Busetto L, Vettor R. Pharmacotherapy of obesity: An update. Pharmacol Res. 2021; 169:105649.
6. Pilitsi E, Farr OM, Polyzos SA, Perakakis N, Nolen-Doerr E, Papathanasiou AE, Mantzoros CS. Pharmacotherapy of obesity: available medications and drugs under investigation. Metabolism. 2019; 92:170-192.
7. Kim BY, Kang SM, Kang JH, Kim KK, Kim B, Kim SJ, Kim,YH., Kim JH, Kim JH, Nam GE, Park JY. Current long-term pharmacotherapies for the management of obesity. J Obes Metab Syndr. 2020; 29(2):99.
8. Noora RR, Deb P, Jahan R, Mahamud AI, Jannat K, Rahmatullah M. "Oral Glucose Tolerance Test with Methanolic Extract of *Homalomena aromatica* (Araceae) Whole Plant". EC Pharmacol Toxicol. 2020; 11(8):26-30.
9. Pal D and Lal P. Herbal Drugs and Medicinal Plants in Controlling and Treatment of Diseases Caused by Dengue Virus (DEN-1 & 2): Ethnopharmacology, Chemistry, and Clinical and Preclinical Studies. In: Anti-Viral Metabolites from Medicinal Plants. Cham: Springer Int. Publ. 2023; 683-746 p.
10. Niero R, Cechinel FV, Yunes RA. Natural Products as Source of Molecules with Therapeutic Potential: Research & Development, Challenges and Perspectives. Med Plants Phytomed. 2018; 1-33.
11. Ibrahim JA and Ayodele AE. Taxonomic significance of leaf epidermal characters of the family Loranthaceae in Nigeria. World Appl Sci J. 2013; 24(9):1172-1179.
12. Ahmed SA, Salau S, Khan A, Saeed M, Ul-Haq Z. Inhibitive Property of Catechin and Chlorogenic Acid Against Human Pancreatic Lipase: Molecular Docking and Molecular Dynamics Simulation Investigations. Adv J Chem Sect A. 2022; 5:226.
13. Adesina SK, Illoh HC, Johnny II, Jacobs IE. African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: an update. Afr J Tradit Complement Alternat Med. 2013; 10(4):161-170.
14. Benchaachoua A, Bessam HM, Saidi I. Effects of different extraction methods and solvents on the phenolic composition and antioxidant activity of *Silybum marianum* leaves extracts. Int J Med Sci Clin Invent. 2018; 5(3):3641-3647.
15. Nabil-Adam, A., Ashour, M.L., Tamer, T.M., Shreadah, M.A. and Hassan, M.A., 2023. Interaction of Jania rubens polyphenolic extract as an antidiabetic agent with α-amylase, lipase, and trypsin: *in vitro* evaluations and *in silico* studies. Catal. 13(2): 443.
16. Navjot SM, Manpreet K, Ashu J, Manu R, Divya B, Aaron, S. Efficacy of Various Irrigants Used with Self-Adjusting File System on Smear Layer: An *in vitro* study. J. Pharm. Res. Int. 2021; 33(36A): 52-61.
17. Ragoza M, Masuda T, Koes DR. Generating 3D molecules conditional on receptor binding sites with deep generative models. Chemical science, 2022; 13(9): 2701-2713.
18. Saleem Y, Crespi N, Rehmani MH, Copeland R. Internet of things-aided smart grid: technologies, architectures, applications, prototypes, and future research directions. IEEE Access. 2019; 7: 62962-63003.
19. MacFarlane NG. Digestion and absorption. Anaesth. Intensive Care Med. 2018; 19(3), 125-127.
20. Chike-Ekwughe A, Adegboyega AE, Johnson TO, Adebayo AH, Ogunlana OO. *In vitro* and in-silico inhibitory validation of *Tapinanthus cordifolius* leaf extract on alpha-amylase in the management of type 2 diabetes. Inform. Med. Unlocked. 2023; 36: 101148.
21. Chike-Ekwughe A, Adegboyega AE, Johnson TO, Adebayo AH, Ogunlana OO. *In vitro* and *in silico* inhibitory validation of *Tapinanthus cordifolius* leaf extract on alpha-glucosidase in the management of type 2 diabetes. J Biomol. Struct. Dyn. 2024; 42(5): 2512-2524.
22. Zhu G, Fang Q, Zhu F, Huang D, Yang C. Structure and function of pancreatic lipase-related protein 2 and its relationship with pathological states. Frontiers in Genetics. 2021; 12: 693538.
23. Coimbra JT, Feghali R, Ribeiro RP, Ramos MJ, Fernandes PA. The importance of intramolecular hydrogen bonds on the translocation of the small drug piracetam through a lipid bilayer. RSC *advances*. 2021; 11(2): 899-908.
24. Meyer JD, Manning MC. Hydrophobic ion pairing: altering the solubility properties of biomolecules. Pharmaceutical research, 1998; 15: 188-193.
25. Li SR, Tan YM, Zhang L, Zhou CH. Comprehensive insights into medicinal research on imidazole-based supramolecular complexes. Pharmaceutics, 2023; 15(5): 1348.
26. Apriandini L, Hanafi M, Djamil R, Desmiaty Y, Lotulung PD, Artanti, N. Isolation and Characterization of Flavonol glycoside as Antioxidant and Antidiabetic Compounds from Ebony (*Diospyroscelebica Bakh.*) Leaf Ethanol Extract.Trop. J. Nat. Prod. Res. , 2024; 8(6), 7368–7373.
27. McNulty SJ. Modern insulin therapies. Diabetes Int. 2000; 10(2): 38-41.
28. Matsuda M, DeFrenzo RA. Insulin Sensitivity Indices Obtained from Oral Glucose Tolerance Testing – Comparing with Euglycemic Insulin Clamps. Diabetes Care. 1999; 22(9): 1462-1470.
29. World Health Organization (WHO). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia. Report of a WHO/IDF Consultation, WHO, Geneva. 2006; 1-26.
30. Ajilore BS, Oluwadairo TO, Olorunnisola OS, Fadahunsi OS, Adegbola PI. GC–MS analysis, toxicological and oral

glucose tolerance assessments of methanolic leaf extract of *Eucalyptus globulus.* Futur. J. of Pharm. Sci. 2021; 7(1): 1-9.
31. Chike-Ekwughe A, John-Africa LB, Adebayo AH, Ogunlana OO. Evaluation of The Toxic Effects of Ethanolic Leaf Extract of *Tapinanthus cordifolius* in Mice and Rats. Trop. J. Nat. Prod. Res. 2023; 7(10): 4965-4972.
32. Udia PM, Ogbonna OJ, Antai AB, Mbatutung IF, Eyo SE. Oral glucose tolerance test and some haematological effects of aqueous leaf extract of *Rothmannia hispida* (K. Schunn) fargel on normoglycaemic albino rats. Res. J. Pharmacogn. Phytochem. 2013; *5*(6): 300-305.
33. Haile S. Management of diabetic ketoacidosis with lente insulin. Diabetes Int. 2000; 10(2): 61.
34. McNulty SJ. Modern insulin therapies. Diabetes Int. 2000; 10(2): 38-41.
35. De La Garza, AL, Milagro FI, Boque N, Campión J, Martínez JA. Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta med. 2011; 77(08): 773-785.