International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 1, 2025

Original Article

GC-MS ANALYSIS AND MOLECULAR DOCKING STUDIES OF *LASERPITIUM LATIFOLIUM L* EXTRACT FOR ANTI-DIABETIC ACTIVITY

DIVYA MUKKA, KRISHNA PRASAD DEVARAKONDA*

School of Pharmacy, Department of Pharmacology, Anurag University, Venkatapur (M), Ghatkesar, Medchal (D), Hyderabad-500088, Telangana, India

***Corresponding author: Krishna Prasad Devarakonda; *Email: krishnaprasadpharmacy@anurag.edu.in**

Received: 13 Aug 2024, Revised and Accepted: 02 Dec 2024

ABSTRACT

Objective: To assess the anti-diabetic activity of *Laserpitium latifolium l* extract through computational docking studies.

Methods: Crude extract of *Laserpitium latifolium l* whole plant was obtained using a rotary evaporator. The extract was analyzed for bioactive compounds using Gas chromatography/Mass spectrometry (GC-MS). The extract was examined for their anti-diabetic effects through computational docking studies and *in vitro*. For docking studies, proteins of diabetes mellitus, α-amylase and α-glucosidase, were selected from the literature.

Results: Binding affinity assessed by score function identified 2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-(highest docking score of-7.8 kcal/mol) and cycloartenol acetate (docking score of-7.5kcal/mol) are the most promising compounds that showed strong affinity to target proteins of diabetes mellitus. These compounds have the highest docking scores, suggesting that they are potential candidates for anti-diabetic drug development. Extract of *L. latifolium* exhibited 68.1% potent activity at 250 µg/ml.

Conclusion: In the anti-diabetic potential of crude extracts, multiple pancreatic and extra-pancreatic mechanisms may occur synergistically to achieve a strong anti-diabetic effect.

Keywords: *Laserpitium latifolium L*, Alpha-amylase and alpha-glucosidase, 2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-, Cycloartenol acetate

DOI: https://dx.doi.org/10.22159/ijap.2025v17i1.52343 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

In many recent years, the effect and affection that Diabetes Mellitus (DM) has generated inside the global community has been alarming. In 2019, the range of people with diabetes is 463 million and is projected to obtain 700 million by 2045 [1]. In Mexico, it is taken into consideration the second biggest reason of mortality [2]. DM is a persistent degenerative disease associated with accelerated blood sugar ranges, which results in damage to cells, organs, and structures [3]. Greater glucose inside the blood results in the increment of reactive oxygen species [4], inducing the proliferation of oxidative strain [5], which induces the formation of advanced glycation end products [6], the development of dyslipidemia [7], as well as a chain of metabolic troubles associated with the sickness. There are notable forms of diabetes, which consist of Type 1 diabetes, that's as a result of insulin dependence. There may be additionally Type II DM related to insulin resistance or sensitivity [8]. DM is complicated and tough to treat. These days, new alternatives that are able to control diabetes is still in research. Medicinal vegetation has traditionally been used as therapeutic systems [9] and ethnobotanical extracts were analysed for richness in phytochemical compounds [10] due to their useful biological activities [11]. Those bioactive compounds had been isolated from exclusive elements of flowers (bark, lumen, branches, leaves, flora, fruits, roots, and seeds) [12].

Laserpitium latifolium L. (*L. latifolium*), belonging to family Apiaceae has long been used as a diuretic, purgative, and tonic in belly sicknesses, pulmonary tuberculosis, and fever; in gynecology; for treating coronary heart and liver and in rheumatism; and externally in toothache and puritic dermatomycoses; and also in brewing and veterinary medicine. It is widely recognized that quercetin has been isolated from *L. latifolium*. Some research has pronounced the biologically active additives of *L. latifolium*, in particular sesquiterpene lactones.

Chemical investigations have discovered the presence of daucane derivatives such as laserpitin, laserpitinol, isolaserpitine, and deoxodehydrolasepitine. Tests on essential oils obtained from leaves and fruits of *L. latifolium* confirmed the presence of monoterpenoids like sabinene, α-pinene, and limonene. From the leaves of *L. latifolium,* phenolic compounds were isolated and recognized with the assistance of a paper chromatograph and showed with High-Performance Liquid Chromatography (HPLC). The consequences confirmed the presence of quercetin 3-zero-arabinoside (avicularin); quercetin 3-O-glucoside (isoquercitrin); quercetin O-rhamnoside (quercitrin); and quercetin 3-O-rutinoside (rutin). The aim of the study was to assess the anti-diabetic activity of *L. latifolium l* extracts through computational docking and *in vitro* anti-diabetic studies.

MATERIALS AND METHODS

Materials0

Ethanol, sodium phosphate, NaCl, starch, dinitro salicylic acid, acarbose, yeast, and glucose were purchased from SD. Fine Chemicals, Hyderabad. α-amylase solution was acquired from Signa and Metronidazole was acquired from M. S. N. Labs, Hyderabad, India.

Plant collection and authentication

L. latifolium L. whole plant had been acquired from the neighborhood places of Tirupati, AP. *L. latifolium L.* Plant (Voucher No. 0211) was authenticated by Dr. Madhava Shetty, Asst. Professor, Department of Botany, Sri Venkateshwara University, Thirupathi, Andhra Pradesh, India.

Extraction by maceration

The whole plant of *L. latifolium L.* washed with water to get rid of contaminants like dirt and other impurities and was shade-dried. These dried wholes were ground and sieved to get uniform, coarse powder. Powdered plant material was weighed (1 kg) and immersed in ethanol and kept for maceration for a period of 7 d with occasional stirring. On the eighth day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40 °C. The resultant extract was put in a desiccator to remove any solvent left in it. The dried ethanolic extract of *L. latifolium (EELL)* was packed in an airtight bottle and put in a dry place for further studies [13].

Qualitative evaluation of phytoconstituents

The extract was screened for the presence of various phytoconstituents like carbohydrates, amino acids, proteins, alkaloids, cardiac glycosides, triterpenoids, saponins, flavonoids, phenolic compounds, tannins, and steroids [14].

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS evaluation is completed in an Agilent 8890 gas chromatograph device and mass spectrophotometer [15], equipped with a HP-5 MS fused silica column (5% phenyl methyl siloxane, 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with a mass selective detector. Helium fuel was used as carrier gas and adjusted to column pace with the flow of 1.2 ml/min. Different GC-MS specifications are ion-source temperature, 250 °C; interface temperature, 300 °C; pressure, 11.367 psi; holdup time, 1.2376 min; and split ratio 15:1 with injection temperature of 250 °C. The column temperature began at 75 °C for 5 min and changed to 150 °C at a rate of 4 °C/min. The temperature was raised to 250 °C at a rate of 20 °C/min and held for 5 min. The entire elution became 53.5 min.

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of the mass spectrum was conducted using the database of the National Institute of Standards and Technology (NIST). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *L. latifolium* fraction obtained were compared with the standard mass spectra of known components stored in NIST library.

Molecular docking

As a result of structural molecular biology and structure-based drug discovery, the area of molecular docking has arisen during the last three decades. Automated molecular docking software attempts to comprehend and anticipate molecular recognition by estimating binding affinity and detecting probable binding patterns. The most common molecular docking partners are a small molecule and a target macromolecule. Although ligand-protein docking is more widely used, protein-protein docking is gaining prominence. In this study, virtual screening tool like AutoDock Vina was used for docking program, and Biovia Discovery studio 2024 was used for visualization [16].

Selection of target protein

The target protein of DM was found from literature are Alpha-Amylase and Alpha-Glucosidase. The 3D structure of this target protein having Protein Data Bank (PBD) ID: 2QV4 for alpha-amylase and PBD ID: 2QMJ for alpha-glucosidase were retrieved from PDB database in. pdb format.

Ligand selection

The initial 3D structures of the selected ligands were retrieved in. sdf from Pubchem. PubChem and ChemSpider are repositories for chemical substances and their related biological activities. The optimized structures were then converted into. pdb format using Open Babel software.

Docking technique

Protein preparation

After opening the protein molecule in AutoDock Vina, the water molecules must be deleted and polar hydrogens added.

The PDB files of both ligands and proteins were converted in an extended PDB format, termed PDBQT, to perform molecular docking analysis using AutoDock Vina.

Data of target protein, ligand, and size of grid were saved in the. txt file and named as config. Grid box size was set up at 40×40×40 points for XYZ with a grid spacing of 0.5 Å.

Alpha-glucosidase

The conversion of oligosaccharides and disaccharides into glucose by the enzyme alpha-glucosidase is needed for carbohydrate hydrolysis. The resultant monosaccharides may be absorbed by the small intestine and raise blood glucose levels [17]. This enzyme may originate from a bacterial plant, fungus or mammal [18]. Acarbose, miglitol, nojirimycin, and other compounds should be assessed for their capacity to avoid or treat Type-II DM (fig. 1).

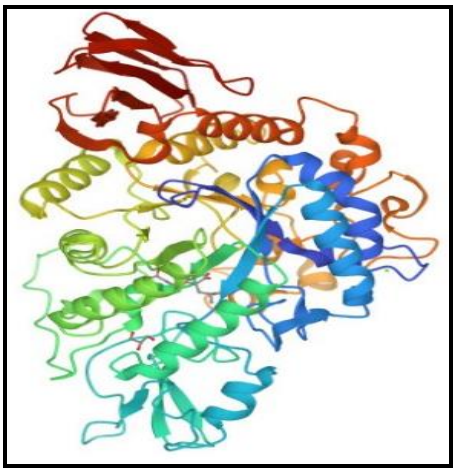


Fig. 1: 3D structure of alpha-glucosidase (2QMJ)

Alpha-amylase

The alpha-1-4 glycosidic linkages seen in the endo-region of amylase or amylopectin chains can be broken down by endo-amylase. A well-known endo-amylase is alpha-amylase, and it may be found in a wide variety of microbes, including bacteria and archaea. Because it's a calcium metalloenzyme, a metal cofactor must be present for it to function [18, 19]. Alpha configuration and alpha-limit dextrin are the end products of alpha-amylase activity, which results in branched oligosaccharides. Depending on how much the substrate has been hydrolyzed, alpha-amylase has 2 types. Endo-and exo-hydrolases, where endo-hydrolases work on the terminal non-reducing ends while endo-hydrolases function inside the substrate molecule (fig. 2).

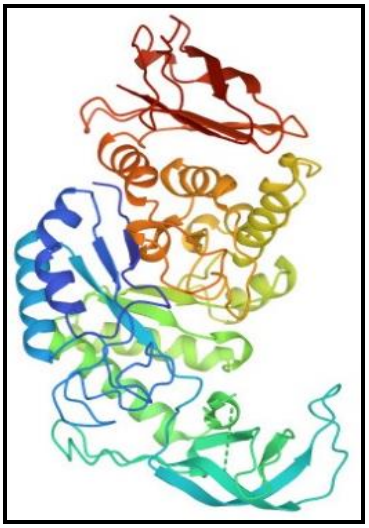


Fig. 2: 3D Structure of Alpha-amylase (2QV4)

***In vitro* anti-diabetic assay**

α-Amylase inhibition assay

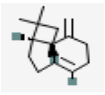
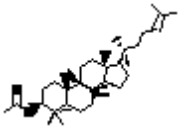
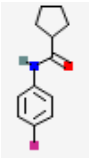
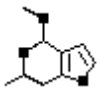
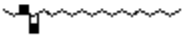
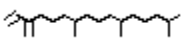
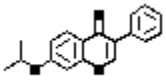
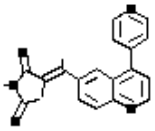
Around 0.5 ml of extract was mixed with 0.5 ml of α-amylase solution (0.5 mg/ml) with 0.02 M of sodium phosphate buffer (pH-

6.9) with 0.006 M NaCl. The mixture was incubated at room temperature for 10 min and 0.5 ml of starch solution (1%) in 0.02 M sodium buffer was added. Resultant mixture was incubated at room temperature for 10 min and the reaction was terminated using 1 ml of dinitro salicylic acid color agent. At this time, the test was placed in water bath (100 °C for 5 min) and cooled until room temperature was obtained. The mixture was then diluted with 10 ml of deionized water and absorbance was determined at 540 nm. The adsorbent of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) sample was also determined. Acarbose was used as standard drug. The inhibition of α-amylase was calculated using the following equation.

$$\% \text{ Inhibition of } \alpha\text{-amylase} = 1 - \frac{\text{Abs Sample}}{\text{Abs control}} \times 100$$

Where Abs controls correspond to the absorbance of the solution without extract (buffer instead of extract) and with α-amylase solutions and abs sample corresponds to the solution with extract and α-amylase solution [19, 20].

Table 1: Bioactive compounds found in Ethanolic extract of *Laserpitium Latifolium L*

S. No.	Compound name	Retention time	Molecular formula	Molecular structure
1.	Benzeneacetonitrile, α-hydroxy-	3.475 min	C_8H_7N0	
2.	Tyr-Ala	5.694 min	$C_{12}H_{16}N_2O_4$	
3.	2(4H)-Benzofuranone , 5, 6, 7, 7a-tetrahydro-4,4,7a-trimethyl-	15.821 min	$C_{11}H_{16}O_2$	
4.	Caryophyllene	17.084 min	$C_{15}H_{24}$	
5.	Cycloartenol acetate	17.084 min	$C_{32}H_{52}O_2$	
6.	Cyclopentanecarboxamide,N-(4-fluorophenyl)-	15.821 min	$C_{12}H_{14}FNO$	
7.	3-O-Methyl-d-glucose	18.421 min	$C_7H_{14}O_6$	
8.	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1α,2β,5α)-	22.603 min	$C_{10}H_{18}$	
9.	4-Methoxy-6-methyl-6,7-dihydro-4H-furo [3,2-c] pyran	22.747 min	$C_9H_{12}O_3$	
10.	Hexadecanoic acid, ethyl ester	26.835 min	$C_{18}H_{36}O_2$	
11.	Phytol	29.630 min	$C_{20}H_{40}O$	
12.	Neophytadiene	29.630 min	$C_{20}H_{38}$	
13.	Ipriflavone	37.000 min	$C_{18}H_{16}O_3$	
14.	2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-	38.481 min	$C_{18}H_{11}N_3O_2S$	

Glucose uptake by yeast cells

The yeast suspended in distilled water was subjected to repeated centrifugation (3000 G, 5 min) until clear supernatant fluid was obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extract (50 to 250 µg/ml) were added to 1 ml of glucose solution (5 mm) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µl of yeast suspension, followed by vertexing and further incubating at 37 °C for 60 min. After 60 min the tubes were centrifuged (2500 G, 5 min) and amount of glucose was estimated in supernatant. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using following formula.

$$\text{Increase in glucose uptake} = \frac{\text{Abs sample - Abs control}}{\text{Abs sample}} \times 100$$

Where Abs sample is the absorbance of test samples and abs control is the absorbance of control reaction (containing all regions except the test sample). All the experiments were carried out in triplicates [21].

Statistical analysis

Data were analysed and compared by one-way analysis of variance followed by post-hoc Tukey's test. $P<0.05$ was considered to be statistically significant. Data are presented as a mean±SEM

RESULTS AND DISCUSSION

In phytochemical screening of ethanolic extraction of *L. Latifolium l* carbohydrates, amino acids, proteins, alkaloids, cardiac glycosides, triterpenoids, saponins, flavonoids, phenolic compounds, tannins, and steroids showed positive results, whereas gums showed negative results.

GC-MS evaluation carried on the ethanolic fraction of *L. Latifolium l* confirmed bioactive compounds like Glycerin, Benzeneacetonitrile, α-hydroxy-, Tyr-Ala, 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, cycloartenol acetate, 4-Methoxy-6-methyl-6,7-dihydro-4H-furo[3,2-c] pyran, Phytol, Neophytadiene, Ipriflavone, 2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-.(table 1).

Molecular docking study is done to identify the significant binding interactions at the active pocket of targeted protein alpha-amylase with PDB ID: 2QV4. A docking study of standard drug acarbose was conducted along with 10 selected molecules for comparing the interactions. The standard drug showed a binding affinity of-8.5 kcal/mol and was found stabilized by hydrogen bonding with ARG-A: 252 and vander Waals bond with GLY-A: 334, ASP-A: 402, PHE-A: 335, PRO-A: 4, THR-A: 11 (fig. 3).

Among 10 compounds, 2,4-Thiazolidinedione,5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-was found with highest docking score of-7.8 kcal/mol and found stabilized by hydrogen bonding with ARG-A: 421, ARG-A: 398 and vander Waals bonds GLY-A: 403, PRO-A: 332, ASP-A: 402, THR-A: 11 (fig. 4).

The compound has formed similar interactions in comparison with standard interactions with GLY-A: 334, ASP-A: 402, PHE-A: 335, PRO-A: 4, THR-A: 11, ARG-A: 252, which might be significant and contribute to the docking score.

Similarly, cycloartenol acetate has demonstrated a docking score of 7.5kcal/mol. Interaction with PHE-A: 335, THR-A: 6, THR-A: 11, ASP-A: 402, GYL-A: 334, ARG-A: 252, ARG-A: 10, PRO-A: 4 was found common for both the compounds (fig. 5).

Further experimental investigation might help us to understand significant interactions and correlate docking results (table 2 and table 3).

Table 2: Docking scores of compounds with alpha-amylase (PDB: 2QV4)

S. No.	Name of the compound	Docking score (K. Cal/mol)
1.	Tyr-Ala	-6.4
2.	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	-6.1
3.	Caryophyllene	-6.1
4.	Cycloartenol acetate	-7.5
5.	Cyclopentanecarboxamide,N-(4-fluorophenyl)-	-5.9
6.	3-O-Methyl-d-glucose	-5.4
7.	4-Methoxy-6-methyl-6,7-dihydro-4H-furo [3,2-c] pyran	-5.2
8.	Hexadecanoic acid, ethyl ester	-4.0
9.	Neophytadiene	-4.8
10.	2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-	-7.8
11.	Acarbose	-8.5

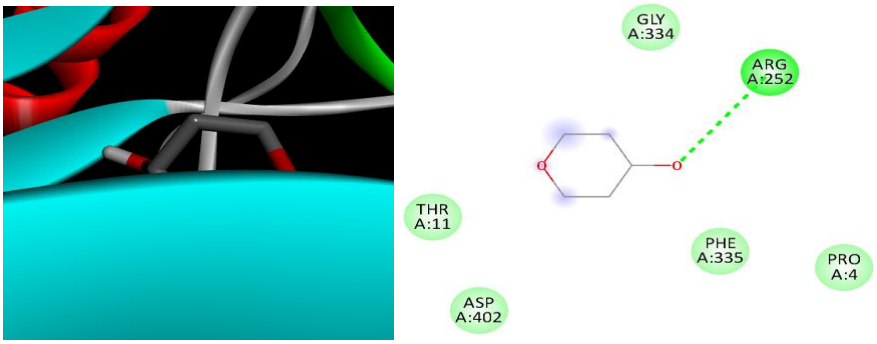


Fig. 3: Standard-acarbose (-8.5)

Docking with Alpha-Amylase (PDB: 2QV4)

A molecular docking study is also done to identify the significant binding interactions at the active pocket of targeted protein alpha-amylase with PDB ID 2QMJ. The standard drug showed a binding affinity of-8.5 kcal/mol and was found stabilized by hydrogen bonding with LYS-A: 765, TYR-A: 605, and vander waals bond with GLU-A: 767, PRO-A: 676, LEU-A: 640, TYR-A: 636, TYR-A: 733, GLY-A: 766, ILE-A: 734 (fig. 6).

Among 10 compounds, 2,4-Thiazolidinedione,5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-was found with the highest docking score of-8.1 kcal/mol and found stabilized by

hydrogen bonding with GLU-A: 767, PRO-A: 676, GLU-A: 788, vander Waals bond with TYR-A: 733, TYR-A: 636, ARG-A: 643, LEU-A: 640, ARG-A: 647, ARG-A: 653, ASP-A: 649. The compound has formed similar interactions in comparison with standard interactions with TYR-A: 733, TYR-A: 636, LEU-A: 640, PRO-A: 676, and GLU-A: 767, which might be significant and contribute to the docking score (fig. 7).

Similarly, cycloartenol acetate has demonstrated a docking score of-7.6 kcal/mol. Interactions with GLU-A: 767, LYS-A: 765, PRO-A: 676, LEU-A: 640, TYR-A: 636, TYR-A: 733, and GLY-A: 766 were found common for both compounds (fig. 8).

Further experimental investigation might help us to understand significant interactions and correlate docking results (table 4 and table 5).

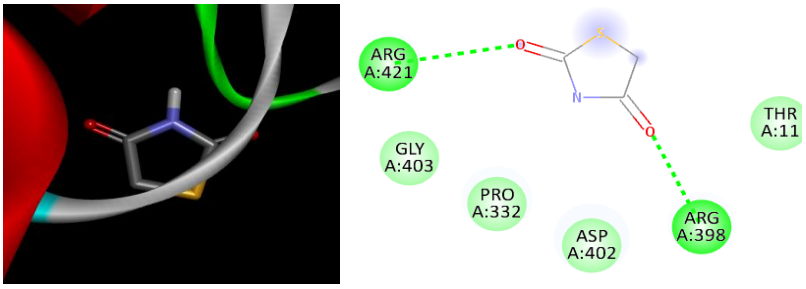


Fig. 4: 2, 4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z) (-8.1)

Table 3: Types of interactions obtained with the molecules and the target proteins

Name of the compound	Type of interaction	Interacting residues
Tyr-Ala	Van der waals	ILE-A: 312, ASP-A: 317, TRP-A: 316, TRP-A: 539, PHE-A: 348
	Conventional-Hydrogen bond	ARG-A: 346, THR-A: 314
2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Van der waals	ASN-A: 352, ALA-A: 310, GLY-A: 304, GLY-A: 351, GLY-A: 309, ASP-A: 353, ARG-A: 303, TRP-A: 344, GLN-A: 302
	Conventional-Hydrogen bond	ARG-A: 346
	Pi-Alkyl	PHE-A: 348
Cyclopentanecarboxamide, N-(4-fluorophenyl)-	Van der waals	ASP-A: 212, LEU-A: 211
	Conventional-Hydrogen bond	ASN-A: 216, HIS-A: 215, LEU-A: 214
	Pi-Alkyl	PRO-A: 228, LYS-A: 227
3-O-Methyl-d-glucose	Van der waals	PHE-A: 348
	Carbon-Hydrogen bond	ARG-A: 346
4-Methoxy-6-methyl-6,7-dihydro-4H-furo [3,2-c] pyran	Van der waals	ASP-A: 353, ARG-A: 303, GLY-A: 304
	Pi-Alkyl	TRP-A: 344
	Conventional-Hydrogen bond	ARG-A: 346
	Alkyl	ALA-A: 310, PHE-A: 348
Hexadecanoic acid, ethyl ester	Van der waals	TRP-A: 406, GLN-A: 603, PHE-A: 575, GLY-A: 602, ALA-A: 576, ASP-A: 542, MET-A: 444, ASP-A: 203, PHE-A: 450, LYS-A: 480
	Pi-Alkyl	TYR-A: 299
	Conventional-Hydrogen bond	TYR-A: 605
Caryophyllene	Van der waals	ARG-A: 303, ASN-A: 301, GLY-A: 309, GLY-A: 304, ALA-A: 310, GLN-A: 302, ILE-A: 312, ARG-A: 346, ARG-A: 267, THR-A: 314
	Conventional-Hydrogen bond	PHE-A: 450
	Alkyl	PHE-A: 348
Cycloartenol acetate	Van der waals	PHE-A: 335, THR-A: 6, THR-A: 11, ASP-A: 290, ASP-A: 402, GYL-A: 334, ARG-A: 252, ARG-A10, GLY-A: 403, SER-A: 289
	Alkyl	TYR-A: 333, HIS-A: 331, PRO-A: 332, PRO-A: 4
Neophytadiene	Van der waals	GLY-A: 249, ASN-A: 250, ASP-A: 212, PCA-A: 1
	Alkyl	LYS-A: 208, LYS-A: 227, LEU-A: 211, LEU-A: 214, ILE-A: 230, PRO-A: 228
2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-	Van der waals	GLY-A: 403, PRO-A: 332, ASP-A: 402, THR-A: 11
	Conventional-Hydrogen bond	ARG-A: 421, ARG-A: 398
Acarbose	Van der waals	GLY-A: 334, ASP-A: 402, PHE-A: 335, PRO-A: 4, THR-A: 11
	Conventional-Hydrogen bond	ARG-A: 252
	Carbon-Hydrogen bond	TYR-A: 605

Table 4: Docking scores of compounds with alpha-glucosidase (PDB: 2QMJ)

S. No.	Name of the compound	Docking score (KCal/mol)
1.	Tyr-Ala	-7.5
2.	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	-5.8
3.	caryophyllene	-7.3
4.	Cycloartenol acetate	-7.6
5.	Cyclopentanecarboxamide,N-(4-fluorophenyl)-	-6.5
6.	3-O-Methyl-d-glucose	-5.2
7.	4-Methoxy-6-methyl-6,7-dihydro-4H-furo [3,2-c] pyran	-5.8
8.	Hexadecanoic acid, ethyl ester	-4.6
9.	Neophytadiene	-5.0
10.	2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-	-8.1
11.	Acarbose	-8.5

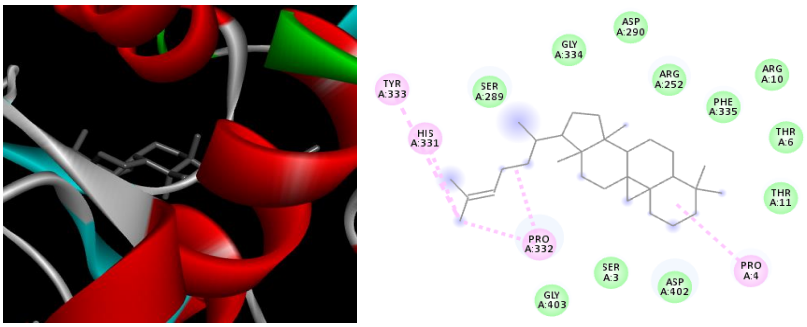


Fig. 5: Cycloartenol acetate docking score(-7.6)

Table 5: Types of interactions obtained with the molecules and the target proteins

Name of the compound	Type of interaction	Interacting residues
Tyr-Ala	Van der waals	ASP-A: 649, ARG-A: 647
	Carbon hydrogen bond	PRO-A: 676
	Conventional-Hydrogen bond	ARG-A: 653
2(4H)-Benzofuranone ,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Van der waals	GLY-A: 766, GLU-A: 767, TYR-A: 636 TYR-A: 733, ASP-A: 649, ARG-A: 643, ARG-A: 653, LEU-A: 640
	Conventional-Hydrogen bond	ARG-A: 647
	Alkyl	PRO-A: 676
Cyclopentanecarboxamide,N-(4-fluorophenyl)-	Van der waals	GLU-A: 767, GLU-A: 788, GLY-A: 766, TYR-A: 733
	Pi-Alkyl	LYS-A: 765
3-O-Methyl-d-glucose	van der waals	ARG-A: 643, THR-A: 639, LEU-A: 640, TYR-A: 636
	Pi-Alkyl	PRO-A: 676
	Conventional Hydrogen bond	GLU-A: 767
4-Methoxy-6-methyl-6,7-dihydro-4H-furo [3,2-c] pyran	Van der waals	LYS-A: 534, ALA-A: 509
	Pi-Alkyl	ARG-A: 520
	Carbon Hydrogen bond	PHE-A: 535, LEU-A: 286
	Pi-Carbon	LYS-A: 776
	Pi-Sigma	ALA-A: 285
Hexadecanoic acid, ethyl ester	van der waals	GLU-A: 767, THR-A: 639
	Pi-Alkyl	TYR-A: 636
	Alkyl	LEU-A: 605
Caryophyllene	Van der waals	TYR-A: 733, TYR-A: 636, LYS-A: 765, GLY-A: 766, GLU-A: 767, ILE-A: 734, ARG-A: 643, ARG-A: 647, LEU-A: 640
	Alkyl	PRO-A: 676
Cycloartenol acetate	Van der waals	LYS-A: 765, ARG-A: 647, TYR-A: 733, ARG-A: 653, GLN-A: 272, THR-A: 269, TYR-A: 660, GLU-A: 767, GLY-A: 766,
	Pi-Alkyl	PRO-A: 676
	Alkyl	LEU-A: 640, TYR-A: 636
Neophytadiene	Van der waals	ARG-A: 643, ARG-A: 647, ARG-A: 653, ASP-A: 649, THR-A: 639, TYR-A: 636, GLU-A: 767, GLY-A: 766, TYR-A: 733
	Alkyl	LEU-A: 640, PRO-A: 676
2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-	Van der waals	TYR-A: 733, TYR-A: 636, ARG-A: 643, LEU-A: 640, ARG-A: 647, ARG-A: 653, ASP-A: 649
	Carbon Hydrogen bond	GLU-A: 788
	Pi-Alkyl	PRO-A: 676
	Pi-Anion	GLU-A: 767
Acarbose	Van der waals	GLU-A: 767, PRO-A: 676, LEU-A: 640, TYR-A: 636, TYR-A: 733, GLY-A: 766, ILE-A: 734
	Conventional-Hydrogen bond	LYS-A: 765
	Carbon-Hydrogen bond	TYR-A: 605
	Unfavorable donar-donar	ASP-A: 542, ARG-A: 526
	Unfavorable aceptor-aceptor	ASP-A: 542, ARG-A: 526

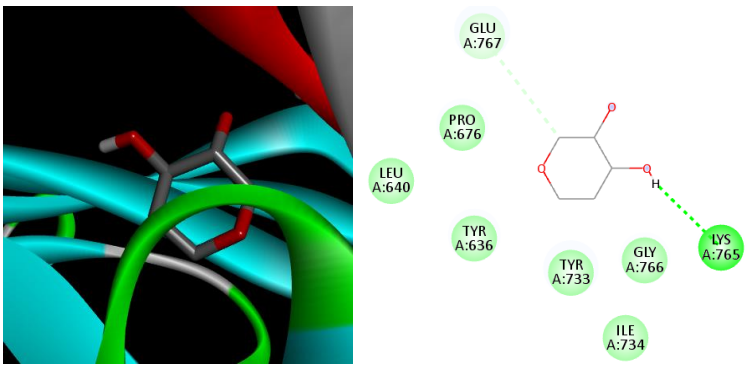


Fig. 6: Standard-acarbose (-8.5)

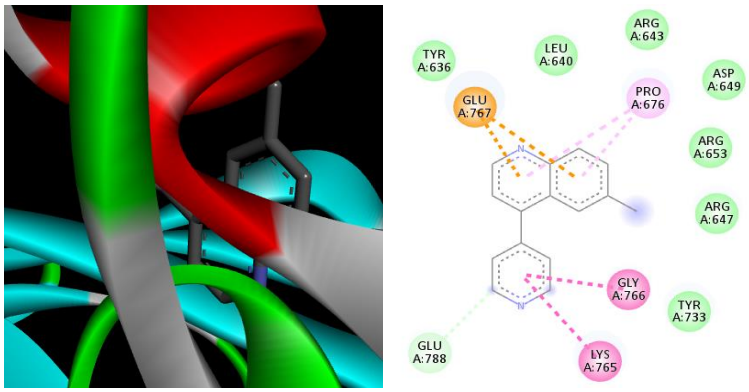


Fig. 7: 2, 4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z)-docking score(-8.9)

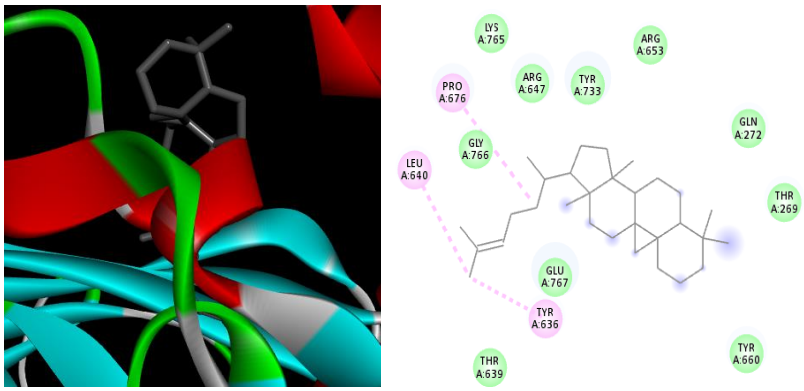


Fig. 8: Cycloartenol acetate docking score(-8.9)

***In vitro* anti-diabetic assay**

L. latifolium extract of different concentrations was assessed for *in vitro* anti-diabetic activity by glucose uptake assay using a yeast model. EELL exhibited potent activity (68.1% at 250 µg/ml). A dose-dependent rise in the % of glucose uptake with increasing concentration (50–250 µg/ml) of *L. latifolium* extract was observed (table 6 and table 7).

Table 6: Glucose uptake by yeast cells

Sample	Concentration (µg/ml)	Increase in glucose uptake%
Extract (EELL)	50	25.1±3.3
	100	30±4.6
	150	51.9±5.7
	200	66.5±8.9
	250	68.1±10.1
Standard (Metronidazole)	50	89.0±13.7
	100	89.9±12.9
	150	89.5±12.7
	200	89.5±14.3
	250	89.5±20.1

All the values were expressed in (n=3) Mean±SD

Table 7: Alpha amylase inhibition assay

Sample	Concentration	IC_{50} (µg/ml)
Ethanol (Extract)	0.5 mg/ml	745±45.8
Standard (Acarbose)	0.5 mg/ml	458±58.2

All the values were expressed in (n=3) mean±SD

DISCUSSION

Molecular docking studies are frequently employed in drug layout to predict interactions between ligands and proteins. This is performed with the aid of calculating the binding affinity and visualizing the amino acid interactions contributing to it. Docking permits the prediction of anti-diabetic pastime with the aid of assessing the binding affinity of isolated compounds for proteins involved in glucose metabolism. Anti-diabetic treatment plans are commonly advanced to target numerous mechanisms of glucose metabolism related to more than one pathway. In this study, molecular docking was done against proteins α-amylase α-glucosidase to decide the

efficacy of the extract. The binding conformation of the compounds is assessed totally on the scoring characteristic and predicting the power of the compound-receptor interaction. Ten docking interactions had been selected due to the fact they'd the great (lower ratings) free power of binding (ΔG kcal/mol). All the compounds interacted with the receptors to various degrees. The binding affinities are evaluated through scoring functions. The compounds 2,4-Thiazolidinedione, 5-[[4-(4-pyridinyl)-6-quinolinyl] methylene]-(5Z), cycloartenol acetate exhibited advanced binding to more than one receptor, suggesting their capacity as applicants for anti-diabetic drug development. In diabetes, high postprandial blood glucose leads to microvascular complications, including retinopathy, nephropathy, and neuropathy, and macrovascular complications refer to increased atherosclerosis-related events such as myocardial infarction and stroke [22, 23]. One of the therapeutic approaches for controlling postprandial hyperglycemia in diabetic patients is to prevent or decrease absorption of carbohydrate after food intake. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides by α-amylase and α-glucosidases before they are absorbed in the duodenum and upper jejunum [24]. Recent advances in understanding the activity of intestinal enzymes helped in the development of newer pharmacological agents [25]. α-glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α-glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates from the GI tract and decreases the rate of rise of postprandial glucose (PP hyperglycemia). This delay in digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes [26]. Acarbose is an α-glucosidase inhibitor that reduces digestion of complex carbohydrates and slows their absorption from the gut. These drugs also increase the release of the glucoregulatory hormone glucagon-like peptide-1 into the circulation, which may contribute to their glucose-lowering effects [27–29]. However, they may cause side effects such as malabsorption, abdominal pain, flatulence, and diarrhea, which lead to a high discontinuation rate [30, 31]. Acarbose and miglitol should not be prescribed in individuals with renal impairment. Acarbose should be used with caution in patients with hepatic diseases because it may cause reversible elevation of hepatic enzymes. Experimental results showed that EELL inhibited the α-glucosidase and α-amylase enzymes.

CONCLUSION

Although there are many treatments for diabetes, the disease is inevitable, and its epidemiological impact is rapidly worsening. The identification of new anti-diabetic drugs has been greatly accelerated by protein-ligand docking and simulation techniques. This study provided evidence that *L. latifolium L.* a plant rich in important terpenoids, flavonoids and phenolic compounds, could be used as an anti-diabetic drug. The *in vitro* and docking studies are in fairly good agreement. The docking calculations revealed the importance of binding energy, electrostatic energy and van der Waals energy. Several aspects need to be considered when developing new amylase and ⍰-glucosidase inhibitors. Further studies are needed to elucidate the exact pharmacodynamic effects and demonstrate efficacy in the treatment of diabetes.

FUNDING

This research received no external funding.

AUTHORS CONTRIBUTIONS

MD completed the research work, execution, and writing. DKP did the work plan, review, and corrections of the publication. Both authors agree with the submission and publication. Both authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTERESTS

No conflict of interest

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N. 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. 9th ed. 2019 Nov;157:107843. doi: 10.1016/j.diabres.2019.107843, PMID 31518657.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB. IDF diabetes atlas: global regional and country level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022 Jan;183:109119. doi: 10.1016/j.diabres.2021.109119, PMID 34879977.
3. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005 Jun;54(6):1615-25. doi: 10.2337/diabetes.54.6.1615, PMID 15919781.
4. Prior RL, Cao G. Antioxidant capacity and polyphenolic components of teas: implications for altering *in vivo* antioxidant status. Proc Soc Exp Biol Med. 1999 Apr;220(4):255-61. doi: 10.1046/j.1525-1373.1999.d01-44.x, PMID 10202399.
5. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763, PMID 28819546.
6. Yamagishi SI, Matsui T. Advanced glycation end products oxidative stress and diabetic nephropathy. Oxid Med Cell Longev. 2010 Mar-Apr;3(2):101-8. doi: 10.4161/oxim.3.2.11148, PMID 20716934.
7. Jones JG. Hepatic glucose and lipid metabolism. Diabetologia. 2016 Jun;59(6):1098-103. doi: 10.1007/s00125-016-3940-5, PMID 27048250.
8. Stumvoll M, Goldstein BJ, Van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005 Apr;365(9467):1333-46. doi: 10.1016/S0140-6736(05)61032-X, PMID 15823385.
9. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction isolation and characterization of bioactive compounds from plants extracts. Afr J Tradit Complement Altern Med. 2011 Jan;8(1):1-10. doi: 10.4314/ajtcam.v8i1.60483, PMID 22238476.
10. Sahoo SK, Bansal M. MPPT techniques a review. AMR. 2014;1055:182-7. doi: 10.4028/www.scientific.net/AMR.1055.182.
11. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: extraction isolation and identification of bioactive compounds from plant extracts. Plants (Basel). 2017 Sep;6(4):42. doi: 10.3390/plants6040042, PMID 28937585.
12. Kumar V, Yadav SK. Synthesis of different-sized silver nanoparticles by simply varying reaction conditions with leaf extracts of *Bauhinia variegata* L. IET Nanobiotechnol. 2012 Mar;6(1):1-8. doi: 10.1049/iet-nbt.2010.0015, PMID 22423864.
13. Allard PM, Bisson J, Azzollini A, Pauli GF, Cordell GA, Wolfender JL. Pharmacognosy in the digital era: shifting to contextualized metabolomics. Curr Opin Biotechnol. 2018 Dec;54:57-64. doi: 10.1016/j.copbio.2018.02.010, PMID 29499476.
14. Harborne JB. Phytochemical methods: a guide to modern technique of plant analysis champmam and hall. Vol. 3. London; 1988. p. 60-6.
15. Olivia NU, Goodness UC, Obinna OM. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. Futur J Pharm Sci. 2021;7(1):59. doi: 10.1186/s43094-021-00208-4.
16. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG. Important flavonoids and their role as a therapeutic agent. Molecules. 2020 Nov;25(22):5243. doi: 10.3390/molecules25225243, PMID 33187049.
17. Lin J, Opoku AR, Geheeb Keller M, Hutchings AD, Terblanche SE, Jager AK. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol. 1999 Dec;68(1-3):267-74. doi: 10.1016/s0378-8741(99)00130-0, PMID 10624887.
18. Zhang X, LI G, WU D, YU Y, HU N, Wang H. Emerging strategies for the activity assay and inhibitor screening of alpha-glucosidase. Food Funct. 2020 Jan;11(1):66-82. doi: 10.1039/c9fo01590f, PMID 31844870.
19. Akaho E. An overview of epigenetic drugs and their virtual screening study retrieved from zinc database along with an auto dock study of the best inhibitor. Int J App Pharm. 2021 May;13(5):122-31. doi: 10.22159/ijap.2021v13i5.42275.

20. Lebovitz HE. Alpha-glucosidase inhibitors. Endocrinol Metab Clin North Am. 1997 Sep;26(3):539-51. doi: 10.1016/s0889-8529(05)70266-8, PMID 9314014.
21. Shettar AK, Vedamurthy AB. Studies on *in vitro* antidiabetic activities of *Hopea ponga* and *Vitex leucoxylon*. Int J Pharm Pharm Sci. 2017 Feb;9(2):263. doi: 10.22159/ijpps.2017v9i2.16280.
22. Ramachandran A, Snehalatha C, Shetty AS, Nanditha A. Trends in prevalence of diabetes in Asian countries. World J Diabetes. 2012 Jun 15;3(6):110-7. doi: 10.4239/wjd.v3.i6.110, PMID 22737281.
23. Pothireddy S, Chukka S, Shankaraiah P. Evaluation of antidiabetic antidyslipidemic and hepatoprotective activity of *Homalium zeylanicum* in alloxan-induced diabetic rats. Int J Res Dev Pharm Life Sci. 2014 Mar;3(3):1004-10.
24. Mohamed EA, Siddiqui MJ, Ang LF, Sadikun A, Chan SH, Tan SC. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from orthosiphon stamineus benth as anti-diabetic mechanism. BMC Complement Altern Med. 2012 Oct;12:176. doi: 10.1186/1472-6882-12-176, PMID 23039079.
25. Narkhede MB, Ajimire PV, Wagh AE, Mohan M, Shivashanmugam AT. *In vitro* antidiabetic activity of *Caesalpina digyna* (R.) methanol root extract. Asian J Plant Sci Res. 2011 Feb;1(2):101-6.
26. Uddin N, Hasan MR, Hossain MM, Sarker A, Hasan AH, Islam AF. *In vitro* α-amylase inhibitory activity and *in vivo* hypoglycemic effect of methanol extract of *Citrus macroptera Montr*. fruit. Asian Pac J Trop Biomed. 2014 Jun;4(6):473-9. doi: 10.12980/APJTB.4.2014C1173, PMID 25182949.
27. Wenning L, Pillai GC, Knepper TC, Ilic K, Ali AM, Hibma JE. Clinical pharmacology worldwide: a global health perspective. Clin Pharmacol Ther. 2021 Oct;110(4):946-51. doi: 10.1002/cpt.2274, PMID 33893656.
28. Morganti S, Tarantino P, Ferraro E, D Amico P, Duso BA, Curigliano G. Next generation sequencing (NGS): a revolutionary technology in pharmacogenomics and personalized medicine in cancer. Adv Exp Med Biol. 2019;1168:9-30. doi: 10.1007/978-3-030-24100-1_2, PMID 31713162.
29. Kurian T. Molecular docking study of epigallocatechin gallate on flt3 in complex with gilteritinib for anticancer activity. Asian J Pharm Clin Res. 2024 Jan;17(1):5-7. doi: 10.22159/ajpcr.2024.v17i1.48733.
30. Kurian T. In silico screening by molecular docking of heterocyclic compounds with furan or indole nucleus from database for anticancer activity and validation of the method by redocking. Int J Pharm Pharm Sci. 2024 Apr;16(4):42-5. doi: 10.22159/ijpps.2024v16i4.50478.
31. Aiswariya, BVS, Satya MS. Molecular docking and admet studies of benzotriazole derivatives tethered with isoniazid for antifungal activity. Int J Curr Pharm Sci. 2022;14(4):78-80. doi: 10.22159/ijcpr.2022v14i4.2004.