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Research Article

Exploring the Antidiabetic Potential of *Antidesma alexiteria* L.: *In-vitro* GLUT-4 Expression and Molecular Docking Studies

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ABSTRACT

GLUT-4, an insulin-regulated glucose transporter primarily located in skeletal muscle and adipose tissue, plays a crucial role in the insulin-stimulated uptake of glucose uptake. Impaired GLUT-4 transcription and translocation lead to insulin resistance and hyperglycemia. Hence, this study investigates the role of bioactive compounds from plant sources using gene-level and *in-silico* interaction studies. In the present investigation, *in-silico* and *in-vitro* gene expression analysis of the GLUT-4 gene with acetone bark extract of *Antidesma alexiteria* was conducted. GLUT-4 gene expression was analyzed using 3T3-L1 adipose cells and insulin as standard. The results demonstrated a dose-dependent increase in GLUT-4 expression, with the highest expression observed at a concentration of 100 µg/mL. According to molecular docking research, the compound juziphine interacted with the catalytic residues Glu177 and Gly400 of the GLUT-4 protein, satisfying ADMET properties. The stability of the protein-ligand complex was further validated by molecular dynamics simulations. The study suggested that the extract has insulin-mimetic properties, enhancing GLUT-4 expression and that juziphine could serve as a promising antidiabetic agent.

Introduction

Antidesma alexiteria L. belongs to the family Phyllanthaceae and is endemic to Sri Lanka and southern India. This little evergreen tree can reach a height of 5 to 8 meters tall. Its bark is smooth, thin, and pale brown. Traditional medicine used several species of *Antidesma* to treat human ailments. *Antidesma ghaesembilla* and *A. bunius* reported with antioxidant activity.[1,2] Research indicates that *A. madagascariense* and *A. menasu* were significantly reduced inflammation.[3,4] Research has shown that *A. celebium*[5] and *A. bunius,*[6] as well as their bioactive compounds, have the potential to function as antidiabetic medicines. However, no detailed analysis on phytoconstituents and their antidiabetic property of *A. alexiteria* has been reported.

In the majority of nations worldwide, diabetes mellitus (DM) is the leading cause of death in human beings. Numerous health issues, including problems with the liver, kidneys, and heart, are linked to diabetes mellitus.[7] Insufficient production and operation of insulin by the pancreas results in diabetes. Insulin resistance, also referred to as type II diabetes, occurs when our bodies produce enough insulin but are unable to use it effectively.[8,9] Finding new pharmacological agents from natural sources that have few side effects and may be able to cure diabetes mellitus is, therefore more important.

Insulin-dependent glucose transport, primarily occurring in fat tissues and, skeletal muscle, plays a crucial role in regulating glucose balance. Insulin promotes the transport

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of glucose into cells in response to rising blood glucose levels after a meal, where it is either stored as glycogen or oxidized for energy production. Impairments in this process are among the earliest detectable abnormalities in the development of type II diabetes. The metabolic disease known as type II diabetes is typified by hyperglycemia and insulin resistance.[10] An essential element of insulin-mediated glucose absorption is the glucose transporter type-4 (GLUT-4), one of 13 sugar transport proteins encoded in the human genome.[11] GLUT-4 is predominantly expressed in skeletal muscle and adipose tissue, where its intracellular localization is tightly regulated. Under basal conditions, GLUT-4 remains sequestered in intracellular vesicles, preventing glucose entry into cells. However, upon insulin stimulation, a signaling cascade is initiated that promotes the translocation of GLUT-4, facilitating glucose uptake and restoring blood glucose homeostasis.[12, 13]

Understanding the molecular mechanisms that govern GLUT-4 trafficking and insulin signaling is critical for developing targeted therapeutic interventions. The current study examines the regulation of GLUT-4 translocation, its impact on insulin resistance and type 2 diabetes, as well as its role in glucose metabolism.

An overview of the literature reveals that scientific research on *A. alexiteria* L. is limited, with only a few studies reporting on its preliminary phytochemical screening and antioxidant activities.[14] The study included both qualitative and quantitative screening of phytocompounds, describing the phytochemical analysis utilizing *A. alexiteria* leaf extract. The presence of bioactive substances, including phenols, phytosterols, glycosides, coumarins, saponins, and carbohydrates, has been confirmed through various tests. According to the study, hydroethanolic preparations of *A. alexiteria* leaf extract demonstrated the best free radical scavenging ability. The study found that *A. alexiteria* contains significant amounts of antioxidant chemicals, making it a promising option for medical purposes. Anthocyanin extraction from its fruit as a natural food colorant[15] and propagation methods.[16] These findings indicate a significant gap in the comprehensive understanding of its biopotential and reputed beneficial effects. Therefore, this study aims to bridge this gap by exploring the plant's phytochemical composition, pharmacological activities, and potential applications in medicine and industry.

Materials And Methods

GLUT-4 Expression Analysis

For the gene expression studies of antidiabetic activity, three concentrations of crude acetone bark extract of *A. alexitera* with GLUT-4 (Glucose Transport Gene) were treated with 3T3-L1 adipocyte cells procured from NCCS, Pune, India.

Gene Identification and Primer Designing

For the antidiabetic gene expression studies GLUT-4 gene is selected. This gene is responsible for glucose transport to fat and muscle tissues in response to exercise and insulin. This is the primary process involved in maintaining glucose homeostasis in the body. The housekeeping gene was the β-actin.

RNA Isolation

The cells were rinsed with cold 1X sterile phosphate-buffered saline (PBS) to extract total RNA. Washed cells were further subjected to the addition of buffer RZ (provided in the kit). The cell lysate was further treated with 200 µL chloroform for phase separation. The aqueous phase obtained was precipitated with 70% ethanol and transferred to the RNA binding columns. The RNA binding columns were later washed with wash buffers RD and RW and the final RNA was eluted in 30 uL of RNase-free water. The obtained RNA was subjected to DNase treatment for the removal of any DNA molecules. The following mixture (Total RNA-20–50 µg; 10X buffer-5 µL; DNase I–2 µL; DEPC treated water- up to 50 µL) was prepared and incubated at 37°C for 30 minutes.

Assessment of RNA Concentration and Quality

The concentration and purity of the extracted total RNA were assessed using a spectrophotometer (Multiskan Skyhigh-Thermo Scientific) by measuring the absorbance at 230, 260, and 280 nm. The absorbance ratio of the sample is greater than or between 2 and 2.25 indicating the high purity of RNA, which was chosen for further experiments.

Quantitative Real Time PCR

In accordance with the guidelines provided by the manufacturer, the CFX Connect Real-Time system (Bio-Rad) was used to perform quantitative PCR (qPCR) for the GLUT-4 gene. The PCR reactions were performed in a final of 10 µL containing 5 µL 2X SYBR green qPCR Mix (GBiosciences), 1-µL of 10 µM sense and antisense primer, 1-µL of template DNA and 2 µL of Nuclease free water. Polymerase chain reaction primer of GLUT-4 sense 5`-GTAACTTCATTGTCGGCATGG-3` and antisense 5`-AGCTGAGATCTGGTCAAACG-`3 and for β-actin sense 5`-GATTACTGCTCTGGCTCCTAG-`3 and antisense 5`-GACTCATCGTACTCCTGCTTG-`3[17] were designed with National Centre for Biotechnology Information (NCBI) database. 40 PCR cycles were conducted after 3 minutes of initial denaturation under thermocycling conditions at 95°C. As an internal standard, the β-actin primer gene PCR results were employed. All samples underwent duplicate amplifications. The data were analyzed, and further calculations were performed using the software Bio-Rad CFX Maestro.

Selection of Target

The FASTA sequence of the human GLUT4 protein (accession number: P14672) was retrieved from the UniProt database and used as the template for homology modeling. The SWISS-MODEL server performed homology modeling of GLUT4. Furthermore, the model generated by SWISS-MODEL was analyzed using a Ramachandran plot in PROCHECK.

Selection of Ligands

The selected ligands (anomuricine, retrofractamide A, juziphine and norrubrofusarin 6-beta-gentiobioside) were identified using HR-LCMS analysis of acetone extract. The structures of the ligands were retrieved from PubChem in .sdf format.

Molecular Docking

MD studies were conducted using AutoDock Vina in PyRx 0.8 software. PyRx is a free and open-source software tool featuring two key components: AutoDock Vina for docking and Open Babel for file conversion. The protein structure underwent pre-processing with Discovery Studio Visualizer before the docking procedure. Water molecules that were co-crystallized along with other non-essential components were removed, and polar hydrogen atoms were included. Only the chain of interest with active site residues is selected for docking studies. Similarly, ligands energy minimization is performed and changed to .pdbqt format. The active site of the protein was enclosed in a grid box configured using AutoDock Vina, with an exhaustiveness parameter of 8.

Pharmacokinetic Properties

ADMET properties of ligands were analyzed using the pkCSM online server. ADMET analysis (Absorption, Distribution, Metabolism, Excretion, and Toxicity) is critical in drug discovery and development. It evaluates how a drug behaves within a biological system and its potential to be both effective and safe.

Molecular Dynamic Simulations

A molecular simulation of the docked complex was performed using a GROMACS 2024.3 package using a CHARMM 36 force field. Energy minimization was done using the Verlet cut-off algorithm over a total of 50,000 steps. RMSD was performed at a constant temperature of 300 K and a constant pressure of 1 atm. Protein stability was analyzed using RMSD, RMSF, Rd of gyration, and hydrogen bonds. Root mean square deviation (RMSD) measures the average distance between the backbone atoms of a protein as it moves from its original structural conformation to its final position. Root mean square fluctuation (RMSF) measures the average fluctuations of the backbone atoms of a protein over time from its reference position. The structural compactness and conformational stability of the protein can be quantitatively measured by the radius of gyration.

Results

Assessment of RNA Concentration and Quality

Total RNA was extracted from both the control and the treated cells (3T3-L1) in the current study. The result of purity evaluation were indicated in Table 1, which was found to be between 312 to 354 µg/mL. Additionally, the purity was verified by determining the absorbance ratio at 260 and 280 nm. In the present study, the obtained RNA has an A260/A280 ratio of 2.09 to 2.15.

Quantitative Real-time PCR

β-actin was confirmed to be a housekeeping gene under various treatment conditions using qPCR analysis. The qPCR reaction mix included 5 µL of 2X SYBR Green qPCR Mix, 1-µL of 10 µM sense and antisense primers, 1-µL of template cDNA, and 2 µL of nuclease-free water. β-actin›s threshold values (cq) were measured and showed consistent amplification and stability. The melt curve analysis ensured primer specificity, verifying a single, distinct amplification product. Additionally, to account for sample variability and enable precise relative quantification of target gene expression, the β-actin expression was utilized for normalization in the GLUT-4 gene expression study. The results indicated that GLUT-4 expression rises dose-dependently, according to the normalized expression graph at various concentrations (Fig. 1). The maximum expression was seen at 100 µg/mL, suggesting that the extract may help improve insulin action and glucose uptake.

Selection of Target

The Ramachandran plot provides a visualization of the permissible phi and psi dihedral angles in protein structures (Fig. 2). The selected monomeric protein structure, with a resolution of 3.25 Å, is bound to its native ligand, cytochalasin B. It has a molecular weight

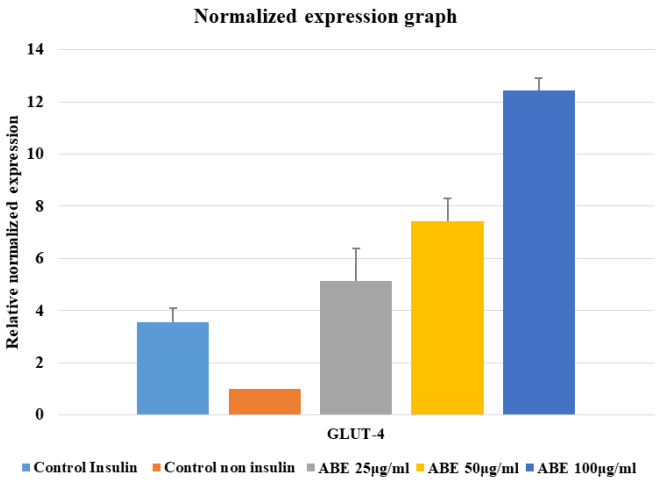

Fig. 1: Normalized expression graph of GLUT-4 gene

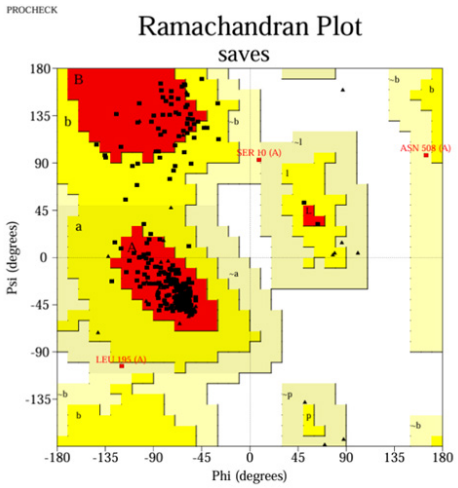

Fig. 2: Ramachandran plot of the modeled GLUT4 protein analyzed using the PDBSum online server

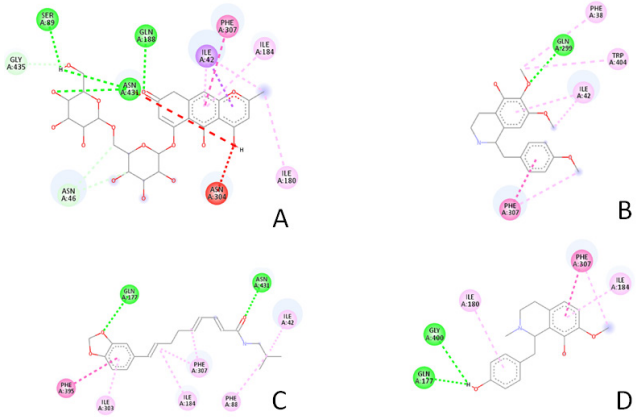

Fig. 3: 2D interactions of ligands A) Norrubrofusarin 6-beta-gentiobioside B) Anomuricine C) Retrofractamide A D) Juziphine with GLUT4 receptor

of 57.01 kDa and consists of 520 amino acid residues. Ramachandran plot analysis revealed that 93.3% of the residues were positioned within the most favored regions, while the remaining 6.7% were positioned in additionally allowed regions (Fig. 2).

Molecular Docking

In this study, four ligands derived from the bark of *A. alexiteria* were screened against the GLUT4 receptor using AutoDock Vina. The results showed that the lowest binding affinity, -9.6 kcal/mol, was shown by norrubrofusarin 6-β-gentiobioside. The strength of interaction between a docked complex is indicated by binding affinity, with lower values indicating stronger interactions within the protein-ligand complex. Various interactions influence binding affinity, including covalent interactions (hydrogen bonding) and non-covalent interactions (hydrophobic, electrostatic, and van der Waals forces). Among these, hydrophobic interactions and hydrogen bonding are essential for maintaining the stability of the protein-ligand complex. (Table 2 and Fig. 3).

Among the compounds that were evaluated, Juziphine exhibited hydrophobic interactions with Phe307 and Ile180 and interactions of hydrogen bonds with the catalytic residues including Gln177 and Gly400. Norrubrofusarin 6-β-gentiobioside demonstrated a higher number of hydrogen bond interactions; however, these did not involve the active site residues of the GLUT4 receptor. The compound Anomuricine established a single hydrogen bond with the catalytic residue Gln299, whereas Retrofactamide A exhibited two hydrogen bond interactions involving the active site residue Gln177. From the molecular docking and interaction studies, it is evident that the compound juziphine shows good interaction with the active site residues Glu177 and Gly400. The native ligand cytochalsin B exhibited -10 kcal/mol as the binding score and was involved in two hydrogen bond formations with Asn46 and Phe307.

Pharmacokinetic Properties

ADMET properties comprising absorption, distribution, metabolism, excretion, and toxicity are critical components in drug discovery, as they help in determining a drug's pharmaceutical profile (Table 3). Absorption parameters influence a compound's bioavailability, permeability, and solubility. The analysis of absorption properties revealed that the selected ligands exhibited water solubility values ranging from -3.67 to -2.85. All compounds, except Norrubrofusarin 6-β-gentiobioside, demonstrated good intestinal absorption rates exceeding 90%. $CaCO_2$ cells, derived from human colon carcinoma, are widely utilized as *in-vitro* models to evaluate human intestinal absorption because they form a monolayer with tight junctions that mimic the intestinal epithelial barrier. P-glycoprotein, an ATP-binding cassette transporter, plays a key role in drug transport across biological membranes. Among the screened compounds, retrofractamide A functions as both a P-glycoprotein substrate and an inhibitor, while juziphine and norrubrofusarin 6-β-gentiobioside act solely as substrates and do not inhibit P-glycoprotein I and II. A fundamental pharmacokinetic variable, the volume of distribution (Vd), quantifies the hypothetical volume necessary to distribute a drug evenly throughout the body to achieve the observed plasma concentration. All substances met the requirements for central nervous system (CNS) permeability and blood-brain barrier (BBB) penetration on the basis of distribution. The liver serves as the principal site for cytochrome P450 enzymes, which are essential for drug metabolism and detoxification. Inhibitors of these enzymes can interfere with drug metabolism, rendering them unsuitable for further drug development. Norrubrofusarin 6-β-gentiobioside does not act as either a substrate or an inhibitor of cytochrome P450 enzymes, indicating favorable metabolic stability. Regarding excretion, total clearance measures the body's ability to remove a drug. A negative total clearance value suggests

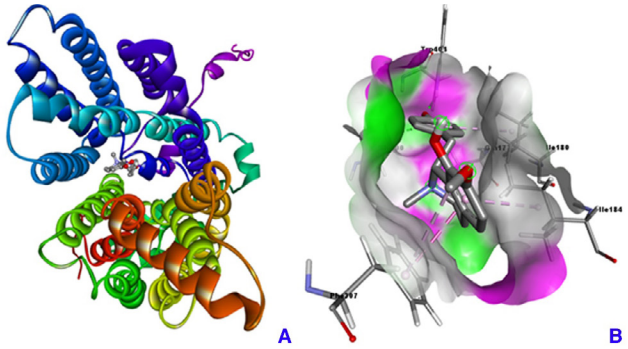

Fig. 4 (A) & (B): 3D view of lead compound juziphine complexed with the GLUT-4 receptor

incomplete drug elimination. All compounds, except norrubrofusarin 6-β-gentiobioside, exhibited good total clearance values. In terms of toxicity, all hit compounds were found to be non-mutagenic. However, anomuricine and retrofractamide A were identified as hepatotoxic, which may require further evaluation for potential liver-related side effects. Overall pharmacokinetic property analysis reveals that the compounds anomuricine and retrofractamide A possess hepatotoxic properties, while norrubrofusarin 6-β-gentiobioside exhibits negative total clearance. The compound juziphine showed good druggable properties. Thus, based on both docking studies and pharmacokinetic properties, the compound juziphine can be considered as a lead molecule for further studies (Fig. 4).

Molecular Dynamic Simulations

In the present study, to examine the dynamic behavior and estimate the stability of GLUT-4 during its interaction with the lead compound, Juziphine, molecular dynamics simulations were performed. Critical dynamic parameters, including RMSD, root mean square fluctuation (RMSF), radius of gyration (Rg), and the number of hydrogen bonds within the protein-ligand complex, were meticulously analyzed to gain deeper insights into the binding stability and conformational dynamics (Figs 5 and 6).

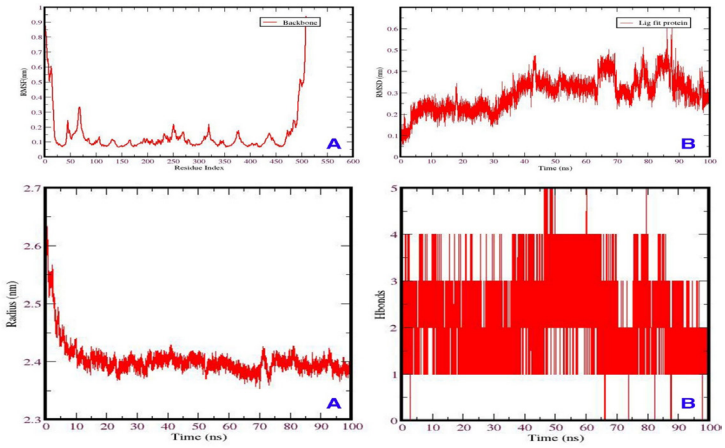

Fig. 5: (A) Root mean square deviation (RMSD) and (B) Root mean square fluctuation (RMSF) plots of the molecular dynamic simulations of the M^{pro}-ligand complexes generated over 100 ns

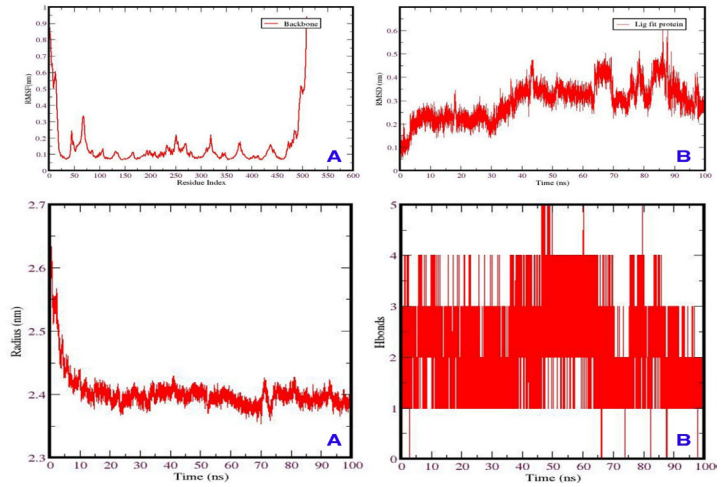

Fig. 6: (A) Radius of gyration and (B) Number of hydrogen bonds plots of the molecular dynamic simulations of the M^{pro}-ligand complexes generated over 100 ns

Table 1: The concentration and purity of isolated RNA from 3T3-L1 cells

Sample	*RNA Conc. (µg/mL)*	*A260/280*	*A260/230*
control	340	2.12	2.19
25 µg/mL	312	2.11	2.03
50 µg/mL	326	2.15	2.09
100 µg/mL	354	2.09	2.09

[A- Absorbance]

Root mean square deviation analysis

By comparing the protein's initial conformation and final structure, RMSD analysis assesses the extent of structural change in the backbone. Protein stability is evaluated based on the magnitude of these deviations observed throughout the simulation, with minimal fluctuations indicating a stable structure. In this study, the RMSD values of the C-alpha backbone for the lead compound and GLUT4 were analyzed over a 100 ns simulation period. The average RMSD for the GLUT4-Juziphine complex was found to be 0.29 nm, signifying that the protein-ligand complex remained structurally stable with only minor deviations from its initial conformation. The consistent RMSD values throughout the simulation suggest that juziphine binds stably to GLUT4, inducing minimal conformational changes. These findings indicate that Juziphine may effectively interact with GLUT4 without causing significant structural perturbations.

Root mean square fluctuation

Residue-based RMS fluctuations were determined for the GLUT4-Juziphine complex to gain a comprehensive understanding of the stability of the ligand binding sites. The dynamic areas of the protein-binding complex have been identified by RMSF analysis. Areas with less ordered structures (loops, coils, and twists) have higher RMSF values, while well-defined secondary structures (α-helices and β-sheets) have relatively lower fluctuations. RMSF analysis was employed in this work to evaluate the structural changes induced by ligand binding, providing insights into the stability and conformational flexibility of the binding complex. The average RMSF value of GLUT-4 Juziphine is 0.14, indicating lower flexibility in the binding

Table 2: Interaction analysis of phytochemicals from acetone bark extract against GLUT4 receptor

Phytochemical	*Pubchem ID*	*Binding affinity (kcal/mol)*	*Hydrogen bond*	*Distance (A^0)*	*Hydrophobic interaction*
Norrubrofusarin 6-beta-gentiobioside	137185916	-9.6	GLN 188	3.10	ILE 42
			GLN 189	3.21	PHE 307
			GLN 190	2.50	ILE 180
			GLN 191	1.91	ILE 184
			GLN 192	3.59	
			GLN 193	3.56	
			GLN 194	3.20	
Anomuricine	157209	-8.2	GLN 299	3.02	PHE 307
					PHE 308
					PHE 309
					PHE 310
Retrofractamide A	11012859	-8.1	GLN 177	2.87	PHE 395
			ASN 431	3.17	ILE 184
					ILE 42
					PHE 88
					PHE 307
					ILE 303
Juziphine	14526072	-8	GLN 177	2.32	PHE 307
			GLY 400	2.88	ILE 184
					ILE 180
Cytochalsin B (Native ligand)	5311281	-10	ASN46	3.30	PHE307
			PHE307	3.08	VAL85

Table 3: Pharmacokinetic properties of the selected phytochemicals

Property	*Model Name*	*Anomuricine*	*Retrofractamide A*	*Juziphine*	*Norrubrofusarin 6-beta-gentiobioside*	*Unit*
Absorption	Water solubility	-3.077	-5.755	-3.675	-2.853	Numeric (log mol/L)
	Caco2 permeability	1.221	1.394	1.22	-1.226	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	90.876	93.054	91.827	26.541	Numeric (% Absorbed)
	Skin permeability	-2.761	-2.735	-2.885	-2.735	Numeric (log Kp)
	P-glycoprotein substrate	Yes	Yes	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Yes	No	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	Yes	Yes	No	No	Categorical (Yes/No)
Distribution	VDss (human)	0.996	0.142	0.919	1.727	Numeric (log L/kg)
	Fraction unbound (human)	0.221	0	0.172	0.238	Numeric (Fu)
	BBB permeability	-0.332	-0.441	-0.176	-1.886	Numeric (log BB)
	CNS permeability	-2.484	-0.972	-2.098	-4.86	Numeric (log PS)
Metabolism	CYP2D6 substrate	Yes	No	Yes	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Yes	Yes	No	Categorical (Yes/No)
	CYP1A2 inhibitior	Yes	No	Yes	No	Categorical (Yes/No)
	CYP2C19 inhibitior	No	Yes	No	No	Categorical (Yes/No)
	CYP2C9 inhibitior	No	No	No	No	Categorical (Yes/No)
	CYP2D6 inhibitior	Yes	No	Yes	No	Categorical (Yes/No)
	CYP3A4 inhibitior	No	No	No	No	Categorical (Yes/No)
Excretion	Total Clearance	1.003	0.347	0.987	-0.034	Numeric (log mL/min/kg)
	Renal OCT2 substrate	No	No	No	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	No	No	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.587	-0.315	-0.084	0.48	Numeric (log mg/kg/day)
	hERG I inhibitor	No	No	No	No	Categorical (Yes/No)
	hERG II inhibitor	Yes	Yes	Yes	Yes	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.902	2.989	2.481	2.453	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	0.806	2.311	0.924	4.634	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	Yes	Yes	No	No	Categorical (Yes/No)
	Skin Sensitisation	No	No	No	No	Categorical (Yes/No)
	T.Pyriformis toxicity	0.345	1.615	1.126	0.285	Numeric (log ug/L)
	Minnow toxicity	0.998	-0.116	1.262	9.254	Numeric (log mM)

site residues. The compound's binding has stabilized the target protein's interaction site, as indicated by the lower RMSF value.

Radius of gyration

Both the unbound protein and the protein–ligand complex were evaluated for structural compactness using the radius of gyration (Rg), providing insights into their conformational stability alongside root mean square deviation (RMSD) and root mean square fluctuation (RMSF). A higher Rg value indicates a loosely packed structure, while a lower Rg value reflects a more tightly packed configuration. The radius of gyration (Rg) is used in molecular dynamic research to show how a ligand molecule's binding causes structural changes in a protein. To assess the impact of hit binding on the conformational packing of GLUT-4, the radius of gyration (Rg) of the GLUT-4-Juziphine complex was analyzed over a 100 ns simulation. The average Rg value was found to be 2.4, indicating the lowest degree of conformational packing.

Hydrogen bond analysis

The strength of the docked complex is mostly dictated by hydrogen bonding interactions. In the GLUT4-juziphine complex, approximately 0 to 4 hydrogen bonds were observed throughout the simulation. This range of

hydrogen bonds suggests that while some key interactions support the binding of juziphine to GLUT4, it is not heavily reliant on hydrogen bonding alone. The presence of a few hydrogen bonds indicates a moderately stable complex, with the ligand able to maintain its binding in the active site. The GLUT4-juziphine complex displays around 0–4 hydrogen bonds, indicating its stability as a complex.

Discussion

In 1942, Warburg and Christian established a standard spectrophotometric technique to detect nucleic acid contamination in molecular biology research. For gene expression studies, RNA samples with an A260/A280 ratio of 1.8 to 2.0 are regarded as pure.[18] Polysaccharides absorb light at 230 nm, proteins at 280 nm, and nucleic acids at 260 nm.[19] The RNA samples used in the GLUT-4 gene expression study fall within the accepted ratio range, confirming their purity.

Genes for insulin and glucose transporters are closely linked in glucose metabolism.[20] Insulin production is triggered when blood glucose levels rise, and it activates GLUT genes, facilitating the transfer of glucose from cytoplasmic vesicles to the plasma membrane.[21] Disruption of insulin signaling in type 2 diabetes results in impaired GLUT-4 translocation.[22] According to a study, *Catharanthus roseus* markedly increased the expression of GLUT-4 mRNA, indicating better absorption of glucose. By upregulating GLUT genes, the plant extract stimulates glucose transport and functions as an insulin mimic. The GLUT-4 level was raised and the blood glucose level was lowered more successfully with the larger dose (200 mg/kg). This study demonstrates how plant extracts can alter the expression of GLUT-4.[23]

This study demonstrates that GLUT-4 expression increases in a dose-dependent manner when treated with *A. alexiteria* acetone-bark extract, with the maximum expression found at a concentration of 100 µg/mL. This suggests that the extract may enhance insulin action and support glucose uptake, potentially aiding in improved glycemic control. Consistent with previous studies, insulin treatment significantly upregulated GLUT-4 expression in the control group, underscoring its crucial role in maintaining glucose homeostasis. However, the lack of insulin in the non-insulin control group resulted in reduced GLUT-4 expression, underscoring the importance of insulin signaling for optimal GLUT-4 activation. Interestingly, *A. alexiteria* acetone-bark extract also increased GLUT-4 expression, suggesting that bioactive compounds in the extract may mimic insulin's effects on glucose transporter regulation. An *in-vitro* study was validated using *in-silico* molecular docking studies, where four ligands—viz., anomuricine, retrofractamide A, juziphine, and norrubrofusarin 6-beta-gentiobioside — derived from the bark of *A. alexiteria* were screened against the GLUT4 receptor. Overall docking studies showed the compound juziphine interacted with catalytic residues Glu177 and Gly400 of GLUT4 receptor and exhibited acceptable ADMET properties. Furthermore, molecular dynamics simulation studies were performed for 100 ns to validate the structural stability of the complex. Interestingly, the protein showed less deviation and fluctuation with a stable interaction with the ligand molecule. Thus, it can be deduced that the *in-vitro* antidiabetes property was exhibited by the compound juziphine.

These findings highlight the potential therapeutic role of *A. alexiteria* in modulating glucose metabolism. The extract's ability to enhance GLUT-4 expression even in the absence of insulin suggests that it may contain compounds that activate insulin-signaling pathways or promote GLUT-4 translocation through alternative mechanisms. Further research is needed to confirm the extract's insulin-mimetic properties and its potential use in diabetes management. The present study covers *in-silico* and *in-vitro* screening of the antidiabetic effect of *A. alexiteria* bark extract. The validation of the above results can be performed using *in-vivo* studies. However, *in-vivo* studies are required to confirm both the results since they provide higher value information compared to *in-silico* and *in-vitro* studies. Further, *in-silico* studies have confirmed jujube as the lead compound showing the best antidiabetic activity in the extract. The antidiabetic property of the compound isolated from the crude sample could have substantiated the study, providing a correlation between the lead compound and the crude extract.

Conclusion

Overall results indicate that *A. alexiteria* bark extract possessed antidiabetic properties. The dose-dependent study of *A. alexiteria* acetone bark extract showed increased GLUT-4 expression at a concentration of 100 µg/mL, suggesting an increased insulin activity and glucose uptake. It can be deduced that phytochemicals in the extract may mimic the insulin's effects on glucose transporter regulation. It is interesting to note that the *in-vitro* antidiabetic activity of *A. alexiteria* extract is due to the presence of juziphine. The compound juziphine showed good interactions with Glu177 and Gly400 catalytic residues of GLUT-4 receptor. Hence, the present study validates the therapeutic activity of *A. alexiteria* bark as a potential antidiabetic agent.

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