RESEARCH PAPER

Phytochemical Investigation, Molecular Docking and Antidiabetic Study of Selected Medicinal Plant

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Abstract

Background:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, and altered glucose metabolism. Multi-target therapeutic agents from medicinal plants have gained considerable attention due to their ability to regulate multiple pathways involved in diabetes. *Justicia tranquebariensis* is traditionally used for diabetes management and contains lignans with potential antidiabetic activity. The present study aimed to isolate cubebin from *Justicia tranquebariensis* and evaluate its antidiabetic potential through integrated in vitro, in silico, network pharmacology, and in vivo approaches.

Methods:

Cubebin was isolated from the petroleum ether extract and characterized by TLC, HPTLC, and FTIR analysis. In vitro antidiabetic activity was evaluated using α-amylase, α-glucosidase, and β-galactosidase inhibition assays. Molecular docking was performed to determine the binding affinity of cubebin against α-amylase. Network pharmacology analysis was conducted to identify potential protein targets and signaling pathways associated with diabetes. In vivo antidiabetic activity was evaluated in streptozotocin-induced diabetic rats by assessing fasting blood glucose, HbA1c, plasma insulin, biochemical parameters, and pancreatic histopathology.

Results:

Cubebin was isolated with a yield of 0.72% from the petroleum ether extract. The extract showed significant α-amylase inhibitory activity with an IC_{50} value of 146.73 µg/mL. Molecular docking revealed strong binding affinity of cubebin toward α-amylase with a binding energy of −9.0 kcal/mol. Network pharmacology analysis identified key target proteins including AKT1, IL6, TNF, NOS3, and CDK2 and revealed involvement in major diabetes-related pathways such as PI3K–AKT, AMPK, AGE–RAGE, and TNF signaling pathways. In vivo studies demonstrated that cubebin (40 mg/kg) significantly reduced fasting blood glucose levels to near-normal levels, improved insulin levels, reduced HbA1c, normalized renal and hepatic biomarkers, and restored pancreatic β-cell architecture in diabetic rats.

Conclusion:

Cubebin exhibits significant multi-target antidiabetic activity through enzyme inhibition, insulin modulation, and pathway regulation, suggesting its potential as a natural therapeutic candidate for diabetes management.

Keywords: Justicia tranquebariensis; Cubebin; Diabetes mellitus; Molecular docking; Network pharmacology; α-Amylase; α-Glucosidase; STZ-induced diabetes

How to cite this article: Rekha B, Natarajan V, Prathap B. Phytochemical Investigation, Molecular Docking and Antidiabetic Study of Selected Medicinal Plant. Int J Drug Deliv Technol. 2026;16(17s): 695-706. DOI: 10.25258/ijddt.16.17s.81

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] The global prevalence of diabetes has increased significantly in recent decades, making it a major public health concern.[2] Chronic hyperglycemia leads to severe complications such as nephropathy, neuropathy, retinopathy, cardiovascular diseases, and hepatic dysfunction.[3] Therefore, effective glycemic control and prevention of diabetic complications remain major therapeutic goals.

Currently available antidiabetic drugs, including insulin and oral hypoglycemic agents, are effective in controlling blood glucose levels but are often associated with adverse effects such as hypoglycemia,

gastrointestinal disturbances, hepatotoxicity, and drug resistance.[4] Moreover, diabetes is a multifactorial disease involving oxidative stress, inflammation, insulin resistance, and impaired insulin secretion; therefore, multi-target therapeutic agents are required for effective management.[4]

One of the important therapeutic strategies in type 2 diabetes management is the inhibition of carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, which delay glucose absorption and reduce postprandial hyperglycemia.[5] Although synthetic inhibitors such as acarbose are available, they often cause gastrointestinal side effects, leading to increased interest in plant-derived enzyme inhibitors.[4]

Justicia tranquebariensis Linn. (Acanthaceae) is a medicinal plant traditionally used in Siddha medicine for the treatment of diabetes and liver disorders. Phytochemical studies have reported the presence of flavonoids, alkaloids, phenolic compounds, terpenoids, and lignans, which possess antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities.[6] Cubebin, a dibenzylbutyrolactone lignan isolated from *Justicia tranquebariensis*, has been reported to exhibit antioxidant, anti-inflammatory, and enzyme inhibitory properties and may act as a potential antidiabetic compound.[7]

Recent advances in computational biology, particularly molecular docking and network pharmacology, have enabled the identification of multi-target mechanisms of bioactive compounds involved in insulin signaling, inflammation, oxidative stress, and glucose metabolism.[8] In addition, streptozotocin-induced diabetic animal models are widely used to evaluate the antidiabetic potential of plant extracts and isolated compounds.[9]

However, there is limited scientific evidence integrating phytochemical isolation, enzyme inhibition, molecular docking, network pharmacology, and in vivo antidiabetic evaluation of cubebin from *Justicia tranquebariensis*. Therefore, the present study aimed to isolate and characterize cubebin and evaluate its antidiabetic activity through in vitro enzyme inhibition, molecular docking, network pharmacology, and in vivo experimental studies to provide scientific validation for its traditional use and to explore its potential as a multi-target antidiabetic agent.

2. Materials and Methods (Concise Journal Version)

2.1 Plant Material Collection and Extraction

The aerial parts of *Justicia tranquebariensis* were collected from Tamil Nadu, India, authenticated by a qualified taxonomist, and a voucher specimen was deposited for reference. The plant material was shade-dried, powdered, and subjected to sequential Soxhlet extraction using petroleum ether, ethanol, and ethyl acetate. The extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4°C for further analysis.

2.2 Phytochemical Screening and GC–MS Analysis

Preliminary phytochemical screening of the extracts was carried out using standard qualitative tests to detect alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phenols, glycosides, carbohydrates, proteins, amino acids, and fixed oils. GC–MS analysis of the ethanolic extract was performed using a Shimadzu GC–MS-QP2010 Ultra system, and compounds were identified by comparing mass spectra with the NIST library database.

2.3 Isolation and Characterization of Cubebin

Cubebin was isolated from the petroleum ether extract using alkaline partitioning followed by solvent purification. The isolated compound was characterized by thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), and Fourier transform infrared spectroscopy (FTIR) to confirm its purity and functional groups.

2.4 In Vitro Enzyme Inhibition Assays

The inhibitory activity of the extracts was evaluated against α-amylase, α-glucosidase, and β-galactosidase enzymes using standard protocols. Acarbose was used as the reference drug. Percentage inhibition was calculated, and IC_{50} values were determined.

2.5 Molecular Docking Studies

Molecular docking studies were performed to evaluate the binding affinity of selected phytoconstituents against α-amylase (PDB ID: 1HNY). Ligand structures were obtained from PubChem and docking was carried out using CB-Dock2 integrated with AutoDock Vina. Binding affinity was expressed as binding energy (kcal/mol), and interactions with active site residues were analyzed.

2.6 Network Pharmacology Analysis

Potential targets of cubebin were predicted using SwissTargetPrediction, and diabetes-related genes were obtained from the GeneCards database. Common targets were identified and used to construct a protein–protein interaction network using STRING and Cytoscape. Gene Ontology and KEGG pathway enrichment analyses were performed to identify associated biological processes and signaling pathways.

2.7 Experimental Animals and Induction of Diabetes

Male Wistar albino rats (180–200 g) were maintained under standard laboratory conditions. Experimental diabetes was induced by intraperitoneal injection of streptozotocin (55 mg/kg) dissolved in citrate buffer (pH 4.5). Rats with fasting blood glucose levels above 250 mg/dL were considered diabetic and included in the study.

2.8 Experimental Design

Animals were divided into seven groups (n = 6): normal control, diabetic control, metformin (100 mg/kg), plant extract (200 and 400 mg/kg), and cubebin (20 and 40 mg/kg). Treatments were administered orally for 21 days, and fasting blood glucose levels were measured at regular intervals.

2.9 Biochemical and Histopathological Analysis

At the end of the experimental period, blood samples were collected for estimation of fasting blood glucose, HbA1c, plasma insulin, liver function markers (AST, ALT, ALP), and renal function markers (urea and creatinine). Pancreatic tissues were collected and examined histopathologically after hematoxylin and eosin staining.

2.10 Statistical Analysis

All data were expressed as mean ± SEM (n = 6) and analyzed using one-way ANOVA followed by Dunnett's post hoc test. A value of $p < 0.05$ was considered statistically significant.

Results

3.1 Phytochemical Screening and GC-MS Analysis

Qualitative phytochemical screening revealed a solvent-dependent distribution of secondary metabolites (Table 1). The ethanol extract showed the broadest phytochemical profile, containing alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, carbohydrates, proteins, and amino acids. The petroleum ether extract was rich in non-polar constituents such as steroids, terpenoids, flavonoids, and fixed oils, while the ethyl acetate extract showed intermediate phytochemical composition. GC–MS analysis of the ethanolic extract identified 35 compounds (Figure 1), with major constituents including 2-furancarboxaldehyde, stigmasta derivatives, catechol, hexadecanoic acid, and hydroquinone. Cubebin was not detected in GC–MS due to its non-volatile nature and was confirmed separately by chromatographic and spectroscopic methods.

Table 1. Qualitative phytochemical screening of different extracts of *Justicia tranquebariensis*

S. No	Phytochemical Test	Petroleum Ether Extract	Ethanol	Ethyl Acetate
1	Alkaloids	–	+	+
2	Flavonoids	+	+	+
3	Tannins	–	+	+
4	Saponins	–	+	–
5	Steroids	+	–	–
6	Terpenoids	+	+	+
7	Phenols	–	+	+
8	Glycosides	–	+	+
9	Carbohydrates	–	+	+
10	Proteins	–	+	–
11	Amino acids	–	+	–
12	Resins	+	+	+
13	Fixed oils and fats	+	–	+

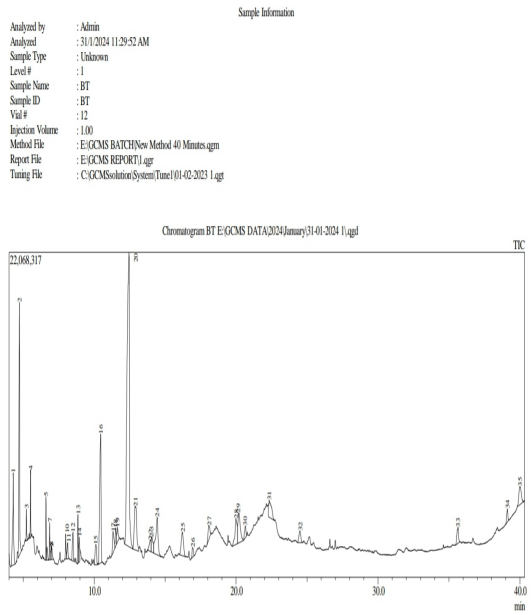

Figure 1. GC–MS total ion chromatogram (TIC) of ethanolic extract of *Justicia tranquebariensis* showing the presence of multiple phytoconstituents at different retention times.

3.2 Isolation and Characterization of Cubebin

Cubebin was isolated from the petroleum ether extract with a yield of 0.72% w/w. TLC showed a single spot with an Rf value of approximately 0.45. HPTLC analysis identified cubebin at Rf 0.413 with consistent peak patterns across samples. FTIR analysis confirmed characteristic functional groups including O–H stretching, lactone carbonyl (C=O), C–O–C ether linkage, and aromatic ring vibrations, confirming the dibenzyl butyrolactone structure (Figure 2–4, Table 2).

Table 2. FTIR spectral data of isolated cubebin

S. No	Wavenumber (cm^{-1})	Functional Group	Assignment
1	3436.50	O–H stretching	Phenolic hydroxyl group
2	2924.09	C–H stretching	Aliphatic C–H
3	1637.93	C=O stretching	Lactone carbonyl group
4	1512.45	C=C stretching	Aromatic ring
5	1456.32	C–H bending	Aromatic C–H bending
6	1113.51	C–O–C stretching	Ether linkage
7	1026.18	C–O stretching	Alcohol/ether group
8	605.85	C–H bending	Aromatic ring

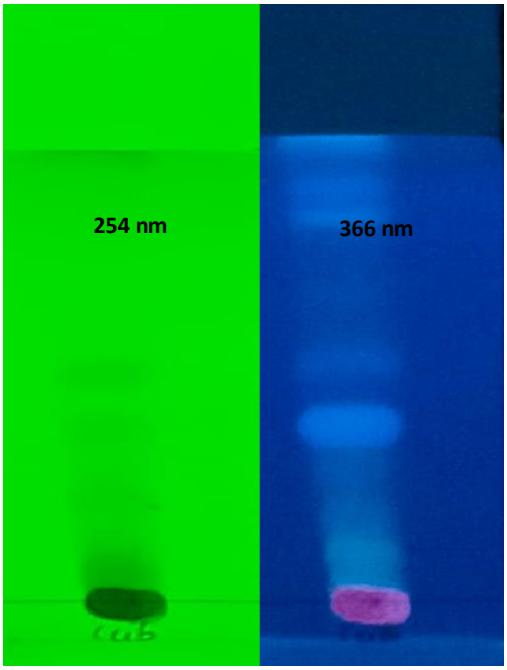

Figure 2. Thin layer chromatography (TLC) chromatogram of isolated cubebin developed using toluene:ethyl acetate (7:3 v/v) as the mobile phase and visualized under UV light at 254 nm and 366 nm. A single prominent spot with an Rf value of approximately 0.45 was observed, indicating the presence of cubebin and confirming the purity of the isolated compound.

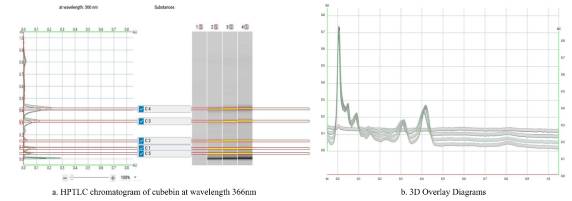

Figure 3. High performance thin layer chromatography (HPTLC) analysis of isolated cubebin developed using toluene:ethyl acetate (7:3 v/v) solvent system. (a) HPTLC chromatogram scanned at 366 nm showing distinct peaks corresponding to different phytoconstituents, where peak C4 at Rf ≈ 0.413 was identified as cubebin. (b) Three-dimensional overlay densitogram showing peak intensity and band distribution confirming the purity and consistency of cubebin across different tracks.

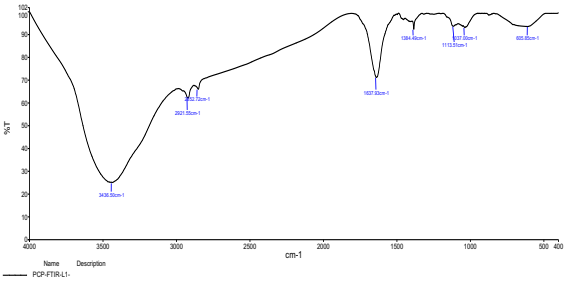

Figure 4. Fourier transform infrared (FTIR) spectrum of isolated cubebin recorded using the KBr pellet method in the range of 4000–400 cm^{-1}. The spectrum showed characteristic absorption bands corresponding to O–H stretching, lactone carbonyl (C=O) stretching, C–O–C ether linkage, and aromatic ring vibrations, confirming the presence of a dibenzylbutyrolactone lignan structure.

3.3 In Vitro Enzyme Inhibition

All extracts showed concentration-dependent inhibition of α-amylase, α-glucosidase, and β-galactosidase. The petroleum ether extract exhibited the strongest inhibitory activity with the lowest IC_{50} values, particularly against α-glucosidase (IC_{50} = 138.84 µg/mL) and α-amylase (IC_{50} = 146.73 µg/mL). The ethyl acetate extract showed the weakest activity. Although acarbose showed higher potency than the extracts, the petroleum ether extract showed comparable inhibition, particularly against α-glucosidase.

Table 3. IC_{50} values of *Justicia tranquebariensis* extracts against carbohydrate-digesting enzymes

Enzyme	Petroleum Ether Extract (µg/mL)	Ethanol Extract (µg/mL)	Ethyl Acetate Extract	Acarbose (Standard) (µg/mL)

			(µg/mL)	
α-Amylase	146.73 ± 2.11	214.21 ± 3.42	242.22 ± 4.10	110.64 ± 1.98
α-Glucosidase	138.84 ± 1.87	233.65 ± 2.95	268.14 ± 3.76	102.44 ± 1.65
β-Galactosidase	169.53 ± 2.54	219.54 ± 3.12	252.91 ± 3.88	95.87 ± 1.42

3.4 Molecular Docking Against α-Amylase

Molecular docking studies showed that cubebin had the highest binding affinity (−9.0 kcal/mol), which was higher than acarbose (−8.0 kcal/mol) (Table 4). Other compounds such as sesamin and β-sitosterol also showed strong binding affinity. Cubebin formed stable interactions with active site residues, indicating strong enzyme inhibition potential (Figure 5).

Table 4. Molecular docking scores of selected phytoconstituents against α-amylase (PDB ID: 1HNY)

S. No	Compound	Binding Energy (kcal/mol)	No. of H-bonds	Key Interacting Residues
1	Cubebin	−9.0	3	Asp197, Glu233, Asp300
2	Sesamin	−8.9	2	Asp197, His305
3	β-Sitosterol	−8.6	1	Glu233
4	Lariciresinol	−8.1	2	Asp300, His299
5	Acarbose (Standard)	−8.0	4	Asp197, Glu233, Asp300, His305
6	Catechol	−4.8	1	Glu233
7	Hydroquinone	−4.6	1	Asp197
8	4-Aminoresorcinol	−5.1	1	Asp300
9	2-Furancarboxaldehyde	−5.0	0	Hydrophobic interactions

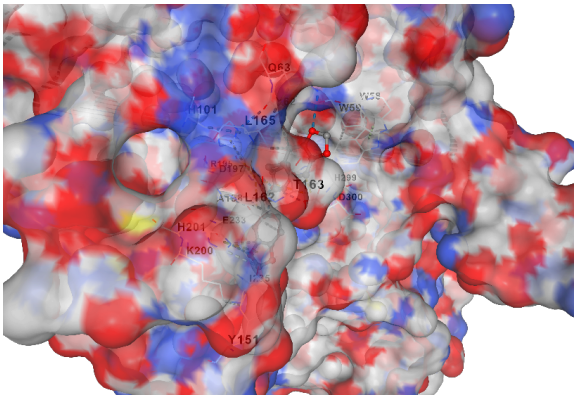

Figure 5. Molecular docking interaction of cubebin with α-amylase enzyme showing binding pose within the active site and interaction with key amino acid residues

3.5 Network Pharmacology and Multi-Target Analysis

In silico ADMET analysis indicated that cubebin possesses favorable drug-likeness properties, including high gastrointestinal absorption, blood–brain barrier permeability, and acceptable topological polar surface area, suggesting good membrane permeability and oral bioavailability (Table 5). Toxicity prediction revealed an LD_{50} value of 1190 mg/kg with negative results for AMES toxicity, carcinogenicity, and mutagenicity, although mild hepatotoxicity and immunotoxicity risks were predicted.

Target prediction identified 100 potential protein targets for cubebin, of which 17 overlapped with high-relevance diabetes-associated genes from GeneCards (Table 6, Figure 6). PPI network construction via STRING and Cytoscape produced a densely connected network of 17 nodes and 74 edges. CytoHubba analysis by maximum clique centrality identified nine hub genes: IL6, TNF, AKT1, PPARG, MMP9, CCND1, CDK2, CDK4, and NOS3 (Figure 7). GO enrichment analysis identified cellular response to hormone stimulus, regulation of programmed cell death, and positive regulation of signaling as the most significantly enriched biological processes, while NF-κB complex was the predominant cellular component and nitric oxide synthase activity represented the strongest molecular function enrichment (Figure 8). KEGG pathway analysis revealed that the most significantly enriched pathways included the AGE–RAGE signaling pathway, insulin resistance pathway, TNF signaling pathway, AMPK signaling pathway, and PI3K–AKT signaling pathway (Figure 9). Targeted molecular docking of cubebin against the five hub pathway proteins — TNF-α (3G10), IL6 (5SFK), MMP9 (1ITV), AKT1 (1P6S), and NOS3 (8UFS) — demonstrated stable and favorable binding affinities

across all targets, confirming the multi-target pharmacological profile of cubebin.

Table 5. ADMET and toxicity prediction profile of cubebin (ProTox-III)

Category	Parameter	Result	Probability
Acute toxicity	Predicted LD_{50}	1190 mg/kg	—
Mutagenicity	AMES toxicity	Negative	—
	Ames mutagenicity	Negative	0.92
Carcinogenicity	Carcinogenicity	Negative	0.71
Skin toxicity	Skin sensitization	Sensitizer	—
Cardiotoxicity	hERG blockers	Non-blocker	—
	Cardiotoxicity	Inactive	—
Organ toxicity	Hepatotoxicity	Active	0.74
	Neurotoxicity	Active	0.77
	Nephrotoxicity	Inactive	0.59
	Respiratory toxicity	Active	0.57
Immunotoxicity	Immunotoxicity	Active	0.86
Cytotoxicity	Cytotoxicity	Inactive	0.98
Blood–brain barrier	BBB permeability	Inactive	0.55
Ecotoxicity	Ecotoxicity	Active	0.55
Clinical toxicity	Clinical toxicity	Inactive	0.60
Molecular initiating events	Thyroid receptor α	Inactive	0.90
	Thyroid receptor β	Inactive	0.78
	Transthyretin	Inactive	0.97
	GABA receptor	Inactive	0.96
	Kainate receptor	Inactive	0.99
	Acetylcholinesterase	Active	0.74
	Pregnane X receptor	Inactive	0.92
	Na^+/I^- symporter	Inactive	0.98

Table 6. Common target genes of cubebin associated with diabetes identified through network pharmacology analysis

S. No	Gene Symbol	Target Protein	UniProt ID	Role in Diabetes
1	MMP9	Matrix metalloproteinase 9	P14780	Tissue remodeling, inflammation
2	FGFR1	Fibroblast growth factor receptor 1	P11362	Glucose and lipid metabolism
3	JAK3	Tyrosine-protein kinase JAK3	P52333	Cytokine signaling
4	CDK2	Cyclin-dependent kinase 2	P24941	Cell cycle regulation
5	JAK1	Tyrosine-protein kinase JAK1	P23458	Inflammatory signaling
6	CDK4	Cyclin-dependent kinase 4	P11802	β-cell proliferation
7	SYK	Tyrosine-protein kinase SYK	P43405	Immune and inflammatory response
8	CYP17A1	Cytochrome P450 17A1	P05093	Steroid metabolism
9	CCND1	Cyclin D1	P24385	Cell cycle progression
10	PPARGC1B	PPAR gamma coactivator 1 beta	Q86YN6	Energy metabolism
11	AKT1	AKT serine/threonine kinase 1	P31749	Insulin signaling pathway

12	IRS1	Insulin receptor substrate 1	P35568	Insulin receptor signaling
13	SLC2A4	Glucose transporter type 4 (GLUT4)	P14672	Glucose transport
14	NOS3	Nitric oxide synthase 3	P29474	Endothelial function
15	TNF	Tumor necrosis factor	P01375	Inflammation, insulin resistance
16	VEGFA	Vascular endothelial growth factor A	P15692	Angiogenesis
17	IL6	Interleukin 6	P05231	Inflammatory response

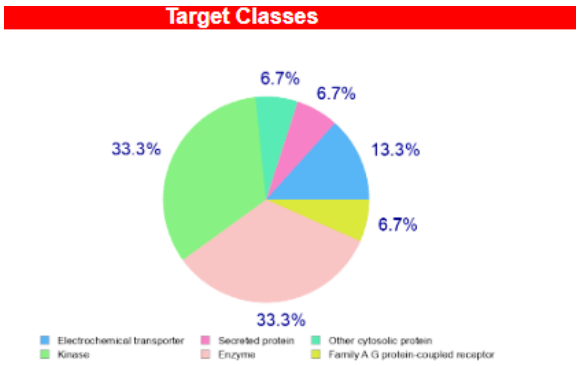

Figure 6. Classification of potential target proteins of cubebin based on protein class.

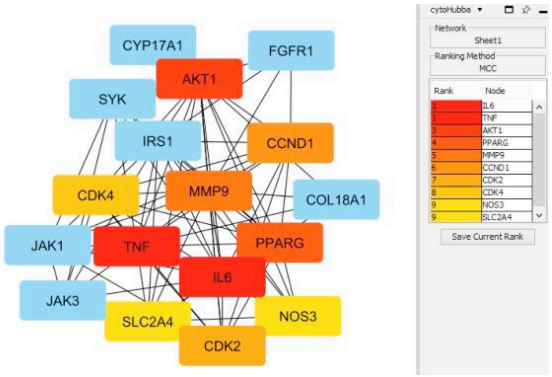

Figure 7. Protein–protein interaction (PPI) network of the 17 overlapping targets between cubebin and diabetes-related genes constructed using STRING and visualized in Cytoscape.

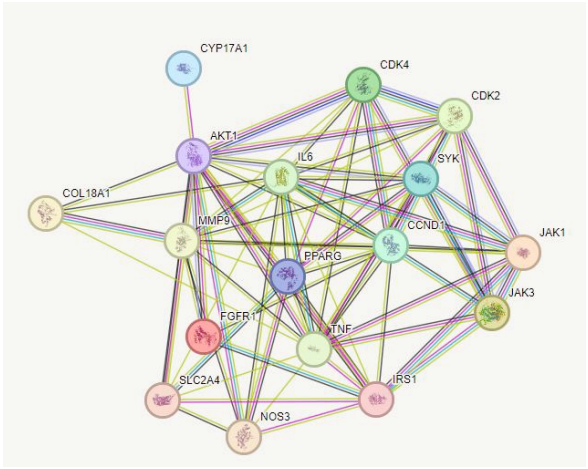

Figure 8. Gene Ontology (GO) enrichment analysis of the 17 common targets. The bubble plot represents enriched biological processes, cellular components, and molecular functions. Bubble size indicates gene count, while color represents statistical significance.

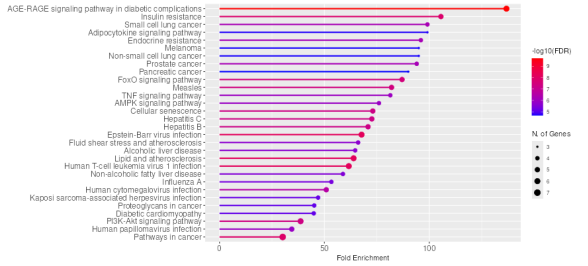

Figure 9. KEGG pathway enrichment analysis of cubebin targets associated with diabetes. The figure highlights key pathways including AGE–RAGE signaling, insulin resistance, TNF signaling, AMPK signaling, and PI3K–AKT signaling pathways.

3.6 In Vivo Antidiabetic Activity

3.6.1 Fasting Blood Glucose

STZ-induced rats showed significant hyperglycemia (>250 mg/dL). Cubebin treatment significantly reduced blood glucose levels from day 4 onwards ($p < 0.001$), comparable to metformin. By day 21, cubebin (40 mg/kg) reduced blood glucose to near-normal levels, while the plant extract showed dose-dependent but slower reduction (Table 7).

Table 7. Effect of *Justicia tranquebariensis* extract and cubebin on fasting blood glucose levels (mg/dL) in STZ-induced diabetic rats [Values are expressed as mean ± SEM (n = 6). ** $p < 0.01$, *** $p < 0.001$ compared with diabetic control group.]

Group	Before STZ	After STZ	Day 0	Day 4	Day 7	Day 14	Day 21

Normal control (0.1% CMC)	95.24 ± 5.20	92.54 ± 7.93	94.67 ± 6.93	89.73 ± 6.20	117.59 ± 8.25	106.34 ± 7.89	91.88 ± 8.04
Diabetic control (STZ 55 mg/kg)	101.72 ± 6.30	257.21 ± 8.42	263.71 ± 7.50	270.37 ± 8.55	286.07 ± 7.95	284.26 ± 8.27	289.85 ± 8.75
Standard (Metformin 100 mg/kg)	98.05 ± 4.33	260.30 ± 7.22	261.31 ± 7.63	121.60 ± 6.80***	117.33 ± 8.25***	107.37 ± 8.39***	110.64 ± 6.53***
JT extract (200 mg/kg)	106.67 ± 8.30	259.53 ± 7.56	271.37 ± 7.10	234.77 ± 8.31	190.79 ± 5.22**	184.60 ± 4.80**	154.97 ± 5.37***
JT extract (400 mg/kg)	96.69 ± 6.20	266.33 ± 6.08	274.05 ± 6.39	189.72 ± 6.25**	135.20 ± 7.97***	113.76 ± 8.27***	109.07 ± 7.71***
Cubebin (20 mg/kg)	90.37 ± 7.25	255.80 ± 7.99	253.74 ± 6.03	120.57 ± 6.74***	107.20 ± 5.90***	82.26 ± 4.20***	84.38 ± 5.07***
Cubebin (40 mg/kg)	94.88 ± 7.35	269.37 ± 8.30	251.22 ± 7.19	117.64 ± 6.20***	96.37 ± 6.85***	80.77 ± 4.12***	83.06 ± 3.24***

3.6.2 Body Weight and Oral Glucose Tolerance

Diabetic rats showed progressive body weight loss, whereas treated groups showed significant weight gain by day 21. In OGTT, cubebin significantly reduced blood glucose at 60 and 120 min, indicating improved glucose tolerance and insulin sensitivity (Table 8–9).

Table 8. Effect of *Justicia tranquebariensis* extract and cubebin on body weight (g) in STZ-induced diabetic rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01 compared with diabetic control group.]

Group	Day 0	Day 4	Day 7	Day 14	Day 21
Normal control (0.1% CMC)	182.34 ± 8.20	186.27 ± 6.77	198.55 ± 8.91	207.32 ± 7.38	223.82 ± 8.92
Diabetic control (STZ 55 mg/kg)	185.37 ± 7.36	187.57 ± 7.30	188.70 ± 6.82	179.51 ± 6.27	175.17 ± 7.24
Standard (Metformin 100 mg/kg)	187.25 ± 6.10	190.64 ± 8.23	196.25 ± 7.60	204.63 ± 5.24	215.24 ± 5.22***
JT extract (200 mg/kg)	185.55 ± 6.94	186.24 ± 6.32	191.76 ± 6.87	199.53 ± 6.57	208.37 ± 6.40*
JT extract (400 mg/kg)	180.27 ± 7.05	185.72 ± 6.81	193.70 ± 8.95	202.84 ± 7.20	217.64 ± 8.22***
Cubebin (20 mg/kg)	187.77 ± 7.95	191.37 ± 8.17	197.57 ± 7.92	206.21 ± 7.52	221.30 ± 7.54***
Cubebin (40 mg/kg)	186.05 ± 6.21	190.29 ± 6.57	199.40 ± 6.93	208.85 ± 7.55	227.34 ± 6.30***

Table 9. Effect of *Justicia tranquebariensis* extract and cubebin on Oral Glucose Tolerance Test (OGTT) in experimental rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01 compared with normal control group.]

Group	0 min	30 min	60 min	120 min
Normal control (0.1% CMC)	94.71 ± 4.22	181.23 ± 5.87	189.43 ± 4.44	198.24 ± 5.20

Standard (Metformin 100 mg/kg)	101.35 ± 8.77	171.57 ± 6.27	152.40 ± 8.22*	121.81 ± 7.12**
JT extract (200 mg/kg)	93.54 ± 6.67	182.50 ± 6.92	195.78 ± 6.32	194.24 ± 7.24
JT extract (400 mg/kg)	96.24 ± 6.14	177.32 ± 8.22	188.37 ± 6.20	173.75 ± 7.34
Cubebin (20 mg/kg)	98.34 ± 7.34	175.52 ± 8.05	159.85 ± 8.60*	132.85 ± 7.85*
Cubebin (40 mg/kg)	94.23 ± 7.12	173.05 ± 7.55	151.85 ± 6.37*	122.37 ± 7.23**

3.6.3 Hematological and Glycemic Control Markers

Diabetic control rats showed increased HbA1c and urine glucose with reduced WBC count. Cubebin significantly reduced HbA1c, restored WBC levels, and eliminated urine glucose, indicating improved long-term glycemic control (Table 10).

Table 10. Effect of *Justicia tranquebariensis* extract and cubebin on HbA1c, WBC, and urine glucose levels in STZ-induced diabetic rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01 compared with diabetic control group]

Group	WBC (×10³ cells/mm³)	HbA1c (%)	Urine glucose (mg/dL)
Normal control (0.1% CMC)	7.73 ± 0.54	5.67 ± 0.21	Nil
Diabetic control (STZ 55 mg/kg)	5.02 ± 0.27	8.93 ± 0.37	32.21 ± 1.22
Standard (Metformin 100 mg/kg)	8.14 ± 0.61**	6.02 ± 0.43**	Nil
JT extract (200 mg/kg)	6.84 ± 0.38	7.96 ± 0.55	5.24 ± 0.40
JT extract (400 mg/kg)	7.17 ± 0.48*	6.25 ± 0.60*	Nil
Cubebin (20 mg/kg)	7.46 ± 0.44*	5.90 ± 0.42**	Nil
Cubebin (40 mg/kg)	7.99 ± 0.67**	5.84 ± 0.22**	Nil

3.6.4 Kidney and Liver Function Parameters

Diabetic rats showed increased urea, creatinine, SGOT, SGPT, and ALP levels with decreased albumin levels. Cubebin significantly normalized renal and hepatic markers, indicating protective effects against diabetic complications (Tables 11–12).

Table 11. Effect of *Justicia tranquebariensis* extract and cubebin on renal function markers in STZ-induced diabetic rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01, *** p < 0.001 compared with diabetic control group.]

Group	Blood urea nitrogen (mg/dL)	Creatinine (mg/dL)	Albumin (g/dL)
Normal control (0.1% CMC)	20.43 ± 1.77	0.67 ± 0.03	3.93 ± 0.15
Diabetic control (STZ 55 mg/kg)	28.60 ± 1.06	2.08 ± 0.01	2.26 ± 0.21
Standard (Metformin 100 mg/kg)	21.37 ± 0.97**	0.69 ± 0.02***	3.87 ± 0.30**
JT extract (200 mg/kg)	23.24 ± 1.40	1.20 ± 0.05*	3.14 ± 0.24*
JT extract (400 mg/kg)	22.95 ± 1.24*	0.92 ± 0.06**	3.72 ± 0.31**
Cubebin (20 mg/kg)	21.88 ± 1.52**	0.69 ± 0.06***	3.77 ± 0.22**
Cubebin (40 mg/kg)	20.93 ± 1.27**	0.65 ± 0.04***	3.90 0.19**

Table 12. Effect of *Justicia tranquebariensis* extract and cubebin on liver function markers in STZ-induced diabetic rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01 compared with diabetic control group.]

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal control (0.1% CMC)	83.72 ± 6.22	40.55 ± 3.91	209.22 ± 11.24
Diabetic control (STZ 55 mg/kg)	117.50 ± 7.81	65.54 ± 2.97	263.47 ± 8.33
Standard (Metformin 100 mg/kg)	86.87 ± 6.08**	41.56 ± 3.30**	211.70 ± 9.97**

JT extract (200 mg/kg)	97.27 ± 4.35	48.86 ± 2.27*	232.44 ± 8.04
JT extract (400 mg/kg)	91.23 ± 6.63*	43.75 ± 3.08**	221.04 ± 8.60*
Cubebin (20 mg/kg)	87.90 ± 7.68**	42.49 ± 2.20**	210.36 ± 10.87**
Cubebin (40 mg/kg)	84.06 ± 7.22**	41.67 ± 1.95**	208.14 ± 9.20**

3.6.5 Plasma Insulin Levels

Plasma insulin levels were significantly reduced in diabetic rats. Cubebin treatment significantly increased insulin levels, comparable to metformin, indicating pancreatic β-cell protective and insulin secretagogue activity (Table 13).

Table 13. Effect of *Justicia tranquebariensis* extract and cubebin on plasma insulin levels in STZ-induced diabetic rats. [Values are expressed as mean ± SEM (n = 6). *p < 0.05, ** p < 0.01, *** p < 0.001 compared with diabetic control group.]

Group	Plasma insulin (µIU/mL)
Normal control (0.1% CMC)	20.47 ± 0.93
Diabetic control (STZ 55 mg/kg)	8.54 ± 0.44
Standard (Metformin 100 mg/kg)	18.63 ± 0.87***
JT extract (200 mg/kg)	12.50 ± 1.01*
JT extract (400 mg/kg)	14.67 ± 0.73**
Cubebin (20 mg/kg)	17.62 ± 1.12***
Cubebin (40 mg/kg)	18.70 0.92***

3.6.6 Histopathological Examination

Histopathological analysis showed severe pancreatic β-cell damage in diabetic rats, whereas cubebin-treated groups showed restoration of islet architecture and normal pancreatic structure, confirming pancreatic protective effects (Figures 10).

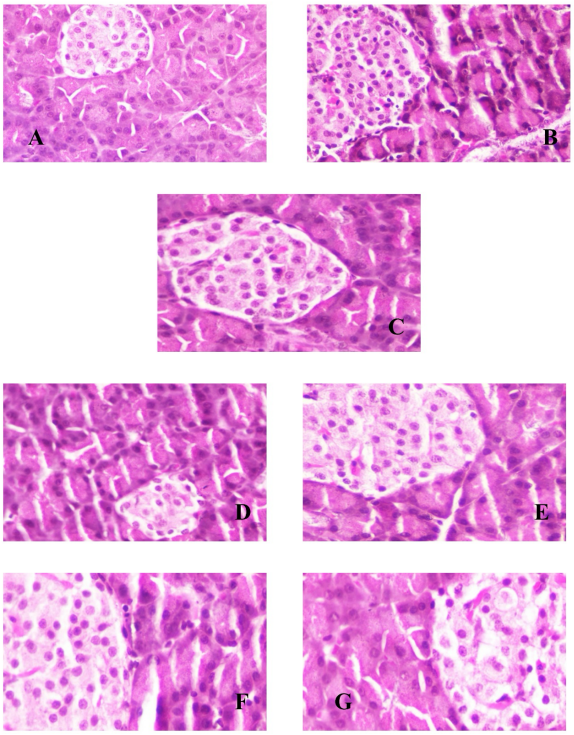

Figure 10. Histopathological examination of pancreatic tissue sections stained with hematoxylin and eosin (H&E) (400×). (A) Normal control showing normal pancreatic architecture; (B) Diabetic control showing destruction of islets of Langerhans and damaged acinar cells; (C) Metformin-treated group showing restoration of pancreatic islets; (D) *Justicia tranquebariensis* (200 mg/kg) treated group showing mild regeneration; (E) *Justicia tranquebariensis* (400 mg/kg) treated group showing improved pancreatic architecture; (F) Cubebin (20 mg/kg) treated group showing regeneration of islet cells; (G) Cubebin (40 mg/kg) treated group showing near-normal pancreatic architecture.

Discussion

The present study demonstrated that cubebin isolated from the petroleum ether extract of *Justicia tranquebariensis* exhibited significant antidiabetic activity through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, modulation of insulin signaling pathways, anti-inflammatory activity, and pancreatic β-cell protection. The petroleum ether extract showed notable α-amylase inhibitory activity (IC_{50} 146.73 µg/mL), while molecular docking revealed strong binding affinity of cubebin (−9.0 kcal/mol) toward α-amylase, indicating effective inhibition of carbohydrate digestion and reduction of postprandial hyperglycemia. Network pharmacology analysis further demonstrated that cubebin targets key diabetes-associated proteins such as AKT1, IL6, and TNF and modulates major signaling

pathways including PI3K–AKT and AMPK pathways, suggesting its role in improving insulin sensitivity, reducing inflammation, and regulating glucose metabolism. In vivo studies showed that cubebin (40 mg/kg) significantly reduced fasting blood glucose and increased insulin levels, along with improvement in HbA1c, renal and hepatic biomarkers, and restoration of pancreatic islet architecture, indicating β-cell regeneration and pancreatic protective effects.

The findings of the present study are consistent with previous reports on medicinal plants and lignans with antidiabetic activity. Kim et al. (2025) reported that plant extracts improved pancreatic β-cell function and insulin secretion in streptozotocin-induced diabetic rats by reducing oxidative stress and apoptosis, leading to β-cell regeneration and improved glycemic control.[10] Similarly, Okpe et al. (2014) demonstrated that treatment with plant extracts promoted regeneration of pancreatic islets and improved liver biochemical parameters in diabetic rats, indicating pancreatic protective and hepatoprotective effects.[11] The antihyperglycemic activity observed in the present study is also supported by Daniel et al. (2022), who reported that extracts from *Nelsonia canescens* significantly reduced blood glucose and oxidative stress in diabetic rats, which was attributed to the presence of bioactive phytochemicals such as flavonoids and phenolic compounds.[12] In addition, inhibition of carbohydrate-digesting enzymes such as α-amylase and α-glucosidase is a well-established therapeutic strategy for controlling postprandial hyperglycemia, as these inhibitors delay carbohydrate digestion and glucose absorption, thereby reducing blood glucose levels, as reported by Khan et al. (2023).[13] Akshatha et al. (2021) demonstrated through molecular docking studies that natural compounds with strong binding affinity toward α-amylase can act as effective enzyme inhibitors and potential antidiabetic agents.[14] Syed et al. (2025) reported that cubebin reduced inflammatory cytokines such as TNF-α and NF-κB and improved biochemical and histological parameters in streptozotocin-induced diabetic models, indicating its anti-inflammatory and organ-protective effects.[15]

The present study is novel in demonstrating the integrated antidiabetic potential of cubebin isolated from *Justicia tranquebariensis* through a combination of phytochemical isolation, enzyme inhibition, molecular docking, network pharmacology, and in vivo evaluation. While previous studies have reported antidiabetic activity of plant extracts and the anti-inflammatory and antioxidant properties of cubebin, the present study provides comprehensive evidence that cubebin acts through a multi-target mechanism involving insulin signaling pathways, inflammatory mediators, and pancreatic β-cell regeneration. The identification of IL6, TNF, and AKT1 as hub targets and the involvement of PI3K–AKT and AMPK pathways further support the role of cubebin as a multi-target antidiabetic compound. These findings suggest that cubebin may serve as a promising natural therapeutic candidate for diabetes management and provide scientific validation for the traditional use of *Justicia tranquebariensis* in the treatment of diabetes, thereby supporting further pharmacological and clinical investigations.

Limitations

The present study was subject to certain limitations. The in vivo experiments were conducted exclusively in male Wistar albino rats, and sex-specific pharmacological differences were not evaluated. The study period of 21 days, while sufficient for acute antidiabetic assessment, did not permit evaluation of long-term safety or efficacy. The predicted hepatotoxicity and neurotoxicity signals from ProTox-III, though not manifested in the in vivo hepatic enzyme data, warranted further investigation through dedicated chronic toxicity studies. Additionally, the precise molecular interactions of cubebin with pancreatic β-cell regeneration pathways were not directly investigated and remain to be elucidated through cell-based mechanistic studies.

Conclusion

Taken together, the findings of the present study established that cubebin, isolated from *Justicia tranquebariensis*, operated through a well-defined multimodal antidiabetic mechanism encompassing intestinal enzyme inhibition, multi-target modulation of inflammatory and metabolic signaling pathways, β-cell protection, and systemic organ preservation. Its superior potency relative to the crude extract and near-equivalence to metformin across multiple in vivo endpoints, supported by robust in silico pharmacological evidence, identified cubebin as a scientifically validated, pharmacologically promising lead compound warranting further preclinical development and formulation studies as a phytotherapy for type 2 diabetes mellitus.

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