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RESEARCH ARTICLE

Phytochemical profiling, molecular docking and GLUT4 and PPAR-γ mRNA Expression study of *Tamarindus indica* seeds fraction for antidiabetic activity in Rats

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Abstract:
Background: Diabetes mellitus is a global health crisis characterized by chronic hyperglycemia, leading to severe complications and increased mortality. *Tamarindus indica* seeds have been traditionally used for medicinal purposes, and recent studies suggest their potential antidiabetic properties.
Methods: *Tamarindus indica* seeds were collected and processed to obtain chloroform and ethanol fractions. Phytochemical profiling was conducted using high-resolution liquid chromatography-mass spectrometry (HR-LCMS), identifying bioactive compounds. Molecular docking studies were performed to evaluate the interactions of these compounds with GLUT4 (PDB ID: 7WSM) and PPAR-γ (PDB ID: 6Y3U). In vivo antidiabetic activity was assessed in streptozotocin-induced diabetic rats, with blood glucose levels monitored over 12 hours. Gene expression analysis of GLUT4 and PPAR-γ was performed using RT-qPCR.
Results: HR-LCMS analysis identified compounds such as apigenin, epicatechin, and β-sitosterol. Molecular docking showed high binding affinities, with β-sitosterol having a docking score of -11.7 for GLUT4. In vivo studies demonstrated significant reductions in blood glucose levels: chloroform fraction (105.80 ± 6.72 mg/dL at 6 hours) and ethanol fraction (115.70 ± 6.82 mg/dL at 6 hours) compared to the diabetic control (260.15 ± 2.14 mg/dL). Gene expression analysis revealed increased GLUT4 protein levels: chloroform fraction (3.8-fold) and ethanol fraction (2.7-fold) versus diabetic control (0.5-fold), and increased PPAR-γ levels: chloroform fraction (3.5-fold) and ethanol fraction (2.5-fold) versus diabetic control (0.4-fold).
Conclusion: *Tamarindus indica* seed fractions exhibit significant antidiabetic properties, reducing blood glucose levels and enhancing GLUT4 and PPAR-γ expression. These findings support the therapeutic potential of Tamarindus indica seeds as natural antidiabetic agents, warranting further research and development.

Keywords: Diabetes mellitus, *Tamarindus indica*, HR-LCMS, Molecular docking, GLUT4, PPAR-γ, Antidiabetic activity.

Introduction

Diabetes mellitus, a long-term metabolic disease marked by persistently high blood sugar, is becoming a major global health concern. Diabetes is becoming more commonplace at an alarming rate, impacting millions of people globally.[1] The International Diabetes Federation estimates that 463 million adults worldwide have diabetes in 2019, and by 2045, there will be 700 million more.[2] Diabetes is influenced by a number of factors, such as environmental factors, lifestyle modifications, and genetic predisposition. The most prevalent kind of diabetes, type 2, makes up 90–95% of cases and is frequently linked to obesity and sedentary lifestyles. Diabetes is dangerous not just because it is so common but also because of its serious side effects, which include nephropathy, neuropathy, retinopathy, and cardiovascular illnesses.[3] Due to these consequences, diabetes is one of the leading causes of illness and early death worldwide, greatly reducing quality of life and raising mortality risk. Diabetes has a significant financial impact as well, including both direct medical expenses and indirect costs from disability and lost productivity.[4]

The tropical fruit-bearing tree *Tamarindus indica*, popularly known as tamarind, is native to Africa and is extensively grown in tropical and subtropical areas worldwide. The seeds of Tamarindus indica have been traditionally used in various medicinal practices, attributed to their rich phytochemical composition.[5] These seeds contain a plethora of bioactive compounds, including flavonoids, polyphenols, alkaloids, and tannins, which have demonstrated various therapeutic properties. Recent scientific investigations have focused on the antidiabetic potential of *Tamarindus indica* seeds, driven by the need for alternative and complementary therapies for diabetes

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management. Phytochemical profiling of these seeds reveals a diverse array of compounds that may contribute to their hypoglycemic effects.[6] Furthermore, molecular docking studies provide insights into the interaction of these phytochemicals with specific protein targets involved in glucose metabolism and insulin signaling.[7] Gene expression studies complement these findings by elucidating the molecular mechanisms underlying the antidiabetic effects of *Tamarindus indica* seeds. Collectively, these studies underscore the potential of *Tamarindus indica* seeds as a natural therapeutic agent for diabetes, warranting further research and development.[8]

Phytochemical profiling, molecular docking, and gene expression studies are pivotal in understanding the therapeutic potential of natural compounds.[9] High-resolution liquid chromatography-mass spectrometry (HR-LCMS) is a sophisticated analytical technique used for the comprehensive profiling of phytochemicals.[10] HR-LCMS enables the precise identification and quantification of bioactive compounds in complex plant matrices, providing a detailed chemical fingerprint of *Tamarindus indica* seeds.[11] Molecular docking studies involve the computational simulation of the interaction between these phytochemicals and target proteins associated with diabetes, such as enzymes involved in glucose metabolism and insulin receptors.[12] These studies help in predicting the binding affinity and inhibitory potential of the compounds, thus identifying promising candidates for antidiabetic drug development.[13] Gene expression analysis further elucidates the molecular mechanisms by which these compounds exert their effects. By examining changes in the expression levels of genes involved in key metabolic pathways, researchers can gain insights into the biological processes influenced by *Tamarindus indica* seed extracts.[14] Together, these approaches provide a robust framework for evaluating the antidiabetic potential of Tamarindus indica seeds at a molecular level.[15]

The objectives of the current research are to investigate the antidiabetic properties of *Tamarindus indica* seed fractions through a multidisciplinary approach. Specifically, this study aims to conduct a detailed phytochemical profiling using HR-LCMS to identify and quantify the bioactive compounds present in the seeds.[16] The research seeks to perform molecular docking studies to predict the interaction of these compounds with key diabetic protein targets, thereby elucidating their potential mechanisms of action. Finally, the study will involve gene expression analysis to understand the impact of *Tamarindus indica* seed extracts on the regulation of genes associated with glucose metabolism and insulin signaling. This comprehensive investigation is intended to provide a scientific basis for the use of Tamarindus indica seeds as a natural antidiabetic agent and to identify potential lead compounds for further drug development.

2. Materials and Methods

2.1. Materials and chemicals

The leaves of Tamarind indica seeds were obtained in October 2022, near the end of the rainy season, at an average temperature of 38°C, from a field that was in fallow in Maharashtra, India (18.5204° N, 73.8567° E). The collection and research of the leaves did not require any permits or approvals. A qualified taxonomist from the Department of Botanical Survey of India, India, identified them (Ref. No. AUTH 22-122). We bought the following from Merck, India: methanol, n-hexane, n-butanol, chloroform, a-naphthol, copper sulphate, potassium sodium tartrate, potassium hydroxide, hydrochloric acid, sulfuric acid, and tetrachloromethane. Purchased gallic acid from Pune, India's Sciquaint Innovations OPC Private Limited.

2.2. Methods

2.2.1. Preparation of Plant Extract

Healthy *Tamarind indica* seeds was collected from India region. The leaves were grounded using a sterilized mortar and pestle followed by soxhlet extraction. 50g of grounded powder was placed in the soxhlet and extraction was carried out by using 300ml of ethanol. The extraction process lasted for 6 -7 hrs and repeated until the solution was clear. The resultant extract was subjected for evaporation to obtain dried product under reduced pressure at 60^{o}C. Further, the dried extract was stored in sterilized tubes to carry out phytochemical analysis.[17]

2.2.2. Fractionation by column chromatography:

The *Tamarind indica* seeds extract was fractionated using column chromatography with a silica gel column. A gradient of chloroform and methanol was used as the mobile phase, starting with 100% chloroform and gradually increasing the methanol proportion (Abubakar and Haque, 2020). The fractions were collected, and their composition was analyzed using thin-layer chromatography (TLC).[18]

2.2.3. Phytochemical composition

2.2.3.1. Total Phenolic Content (TPC) Determination

Various amounts of gallic acid (0.05 mg/mL to 0.5 mg/mL in methanol) were employed to create a standard calibration curve. Each of the different Adiantum philippines fractions was included in a 0.5 mg/mL sample solution. 0.1 mL of Folin-Ciocalteu's reagent and 0.1 mL of the prepared sample solution were mixed together in a test tube. A few minutes later, 2.8 mL of 10% Na2CO3 was added, and the mixture was then allowed to sit in the dark for half an hour. The solution's absorbance was calculated at 765 nm. The total gallic acid equivalent (TPC) was reported as milligrams (mg GAE/g E. ± SD) for each gram of dry extract. Galactic acid concentrations between 20 and 100 mg/mL were used to generate the experiment's calibration curve.[19]

2.2.3.2. Total Flavonoid Content (TFC) Determination

The sample solution was transferred into a volumetric flask containing four milliliters of distilled water and one milliliter (0.5 mg) of the solution. After mixing it with 0.3 milliliters of 5% NaNO2, it was incubated for five minutes. The final amount was adjusted to 10 mL using distilled water after adding 2 mL of 1 M NaOH and 0.3 mL of 10% AlCl3. At 510 nm in wavelength, the sample's absorbance was measured. The quercetin equivalent (mg QE/g DE±SD) for each gram of extract was used to calculate TFC. The TFC was calculated by creating a calibration curve with different quercetin concentrations (20–100 mg/mL in methanol).[20]

2.2.4. Metabolites screening by HR-LCMS:

High-resolution liquid chromatography–mass spectrometry (HR-LCMS) was used to examine the Tamarindus indica for metabolites. After being injected into a C18 reverse-phase column, the samples were eluted using a gradient comprising 0.1% formic acid and acetonitrile and water. There were two ionization modes observed for the mass spectra: positive and negative.[21]

Identification of compounds using the Metlin library:

The comprehensive database of known metabolites, the Metlin library, was compared with the HR-LCMS data from the metabolite screening to identify the compounds. Based on precise mass measurements and fragmentation patterns, the chemicals were identified. Higher abundance chemicals from Tamarindus indica's ethanolic and chloroform fraction were chosen for the HR-LCMS report.[22]

2.2.5. Selection of Protein for Molecular Docking

The protein structures for molecular docking were meticulously chosen based on their critical roles in glucose metabolism and diabetes management. GLUT4, (PDB ID- 7WSM), a transporter essential for insulin-regulated glucose uptake into cells, and PPAR gamma (PDB ID- 6Y3U), a nuclear receptor pivotal in lipid metabolism and glucose homeostasis, were selected. These selections were retrieved from the Protein Data Bank, emphasizing the importance of high-resolution structures and their biological relevance to the mechanisms underlying diabetes. The focus on these proteins stems from their significant involvement in metabolic pathways that are dysregulated in diabetes, thereby providing a targeted approach to understanding and potentially mitigating the disease's molecular basis through the hypoglycemic effects of bioactive compounds found in Tamarindus indica seeds.[23]

2.2.6. Molecular Docking

Molecular docking studies were conducted to elucidate the potential interactions between the selected compounds identified from the methanolic fraction and chloroform fraction, by HR-LCMS analysis and the target proteins GLUT4 and PPAR gamma. The three-dimensional structures of the proteins were retrieved from the Protein Data Bank (https://www.rcsb.org/). The identified compounds were prepared and optimized for docking using BIOVIA Discovery Visual Studio v.21.1. Docking simulations were performed using AutoDock Vina v.1.1.2, The active site of the protein was defined using a grid box encompassing the catalytic site residues with dimensions of 20x20x20 Å and a grid co-ordinate for GLUT4 (PDB ID -7WSM) selected as x= 18.241000, y= -19.022000, z=- 43.049000 and PPAR gamma (PDB ID- 6Y3U) selected as x= 4.000000, y= -5.283000, z=-29.370000. The best-docked poses were selected based on the docking scores. The binding interactions, between the ligands and the target proteins, were visualized and analyzed using Ligplot v.2.2.[24]

2.2.7. *In-Vivo* antidiabetic activity

2.2.7.1. Experimental animals

This study was conducted on forty healthy adult male Sprague-Dawley (SD) rats weighing 170-200 gm procured from Animal House, S.N Institute of Pharmacy, Pusa, Maharashtra, India. The animals were kept in big, roomy cages made of polyacrylic, with a 12-hour light/dark cycle and ambient room temperature. The environmental and laboratory settings were conventional. The rats were fed regular rat diet and had unrestricted access to water. The Institutional Animal Ethics Committee of IAEC gave its approval to the study. (Approval Number: PL/16/2023 CPCSEA/IAEC).[25]

2.2.7.2. Induction of diabetes in Rats

The study employed male Sprague-Dawley rats, 16 weeks of age, whose initial blood glucose levels after fasting ranged from 90 to 110 mg/dL.[26] In rats that had fasted the night before, diabetes was created by a single intraperitoneal injection of 55 mg/kg body weight of streptozotocin (STZ), dissolved in 0.1M citrate buffer at pH 4.5. After injection, rats were given access to 10% glucose water to prevent hypoglycemia. A glucometer (Accu-Chek) was used to monitor blood glucose levels 72 hours later. Rats with levels ≥ 250 mg/dL were considered diabetic and enrolled in the study. The rats were then allocated into five groups: Group I (Normal Control) received no treatment; Group II (Diabetic Control) also received no treatment; Group III received the chloroform fraction of *Tamarindus indica* seeds (200 mg/kg); Group IV was administered the ethanol fraction of *Tamarindus indica* seeds (200 mg/kg); and Group V was given a marketed antidiabetic (Glimenclamide). Blood glucose levels were monitored at intervals of 0, 1, 2, 4, 6-, 8-, 10-, and 12-hours post-treatment. This methodological approach enabled the evaluation of the hypoglycemic effects of Tamarindus indica seed fractions compared to a standard antidiabetic treatment, highlighting the potential therapeutic benefits of these natural compounds in diabetes management.[27]

2.2.8. Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR) and mRNA Isolation

The mRNAs were isolated from collected blood samples of the treated rats using the Aurum Total RNA mini kit (Biorad, 7326820). These mRNAs were then reverse transcribed into cDNAs using iScript reverse transcriptase (Biorad, 1708840). Real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was performed using SsoFast Evergreen Supermix (Biorad, 1725200) according to the manufacturer's instructions. The pre-incubation cycle was set at 95°C for 30 seconds, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 20 seconds, and extension at 72°C for 10 seconds. The sequences of the primers were used as shown in Table 1. The RT-qPCR was interpreted using the real-time PCR system (Pikoreal 96, Thermo Scientific, TCR0096).[28,29]

Table 1: Primers used in this study for real time PCR

Sr. No.	Gene	Primers
1	GLUT4	F: 5´GAGCCTGAATGCTAATGGAG3
2	GLUT4	R: GAGAGAGAGCGTCCAATGTC3
3	PPAR-γ	F: 5´TTATCAAGGGTCCCAGTTTC3
4	PPAR-γ	R: 5´TTATTCATCAGGGAGGCCAG3

2.2.9. Statistical analysis

GraphPad Prism 9.0 was used for statistical analysis. The data were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to compare groups, and Dunnette's multiple comparison test was used to determine whether there were any significant differences. A p-value of less than 0.05 was deemed statistically significant. The results were graphically represented to illustrate the differences in blood glucose levels and gene expression among the various experimental groups. Additionally, correlation analysis was conducted to explore the relationship between the bioactive compounds identified in Tamarindus indica seed fractions and their antidiabetic effects.

3. Results and Discussion

3.1. Results

3.2. TPC and TFC in *Tamarindus indica* seeds

Table 2: TPC and TFC in *Tamarindus indica* seeds

Sr. No.	Fractions	TPC (mg GAE/g E.)	TFC (mg QE/g E.)
1	CHTI	128.67 ± 5.90	66.32 ± 2.32
3	ETTI	89.22 ± 3.78	78.3 ± 3.41

For each value, the mean ± standard deviation is used (n = 3). Gallic acid equivalent is GAE, and quercetin equivalent is QE, E.: extract; CHTI: chloroform fraction; ETTI: Ethanolic chloroform fraction.

3.3. Results of HR-LCMS analysis

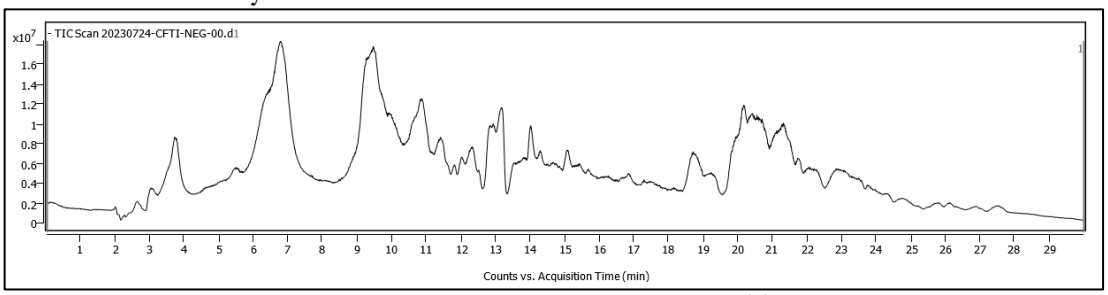

Figure 1: HR-LCMS report of chloroform fraction of *Tamarindus indica*

Table 3: List of the compounds identified in the chloroform fraction of *Tamarindus indica.*

Sr. No.	Compound Name	R.T.	M.W.	Formula	Class	Biological Activities	References
1	Apigenin	2.619	270.05	$C_{15}H_{10}O_5$	Flavonoid	Antidiabetic, antioxidant, anti-inflammatory, enhances GLUT4 translocation	[30]
2	Epicatechin	3.042	290.26	$C_{15}H_{14}O_6$	Flavonoid	Antidiabetic, improves insulin secretion, enhances insulin sensitivity, antioxidant	[30]
3	Eicosanoic Acid	3.120	312.53	$C_{20}H_{40}O_2$	Fatty Acid	Anti-inflammatory, beneficial for lipid metabolism	[31]
4	Catechin	2.988	354.09	$C_{15}H_{14}O_6$	Flavonoid	Antidiabetic, antioxidant,	[32]

						improves insulin sensitivity	
5	Medicagenic acid	3.758	502.69	$C_{30}H_{46}O_5$	Saponin	Antidiabetic, anti-inflammatory, antioxidant	[33]
6	Tetradecanedioic acid	5.945	258.35	$C_{14}H_{26}O_4$	Dicarboxylic Acid	Anti-inflammatory, metabolic regulator	[34]
7	Palmitoyl glucuronide	10.038	418.56	$C_{22}H_{38}O_8$	Glucuronide	Metabolic regulator, anti-inflammatory	[35]
8	Elaidic Acid Methyl Ester	8.152	296.49	$C_{19}H_{36}O_2$	Fatty Acid Ester	Metabolic regulator, impacts lipid metabolism	[36]
9	Taxifolin	10.977	304.25	$C_{15}H_{12}O_7$	Flavonoid	Antidiabetic, antioxidant, anti-inflammatory	[37]
10	Naringenin	11.001	272.25	$C_{15}H_{12}O_5$	Flavonoid	Antidiabetic, antioxidant, insulin-mimetic, inhibits α-glucosidase, activates AMPK	[38]
11	4,11,13,15-Tetrahydroridentin B	12.047	268.34	$C_{15}H_{20}O_4$	Flavonoid	Antioxidant, anti-inflammatory	[39]
12	Tectorigenin	7.628	300.26	$C_{16}H_{12}O_5$	Flavonoid	Antidiabetic, antioxidant, anti-inflammatory	[40]

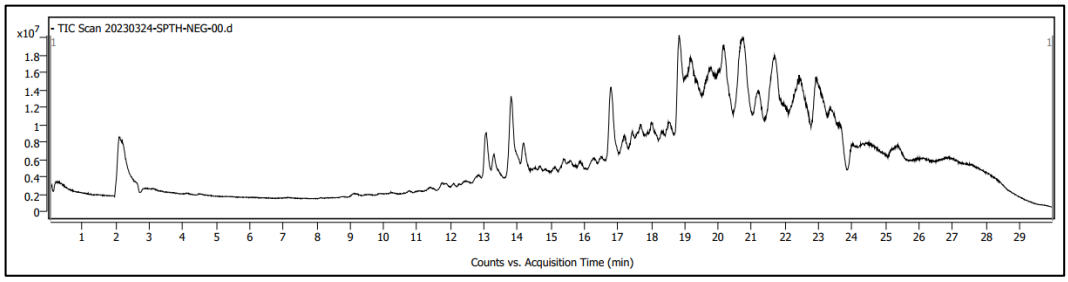

Figure 2: HR-LCMS report of ethanolic fraction of *Tamarindus indica*

Table 4: List of the compounds identified in the ethanolic fraction of *Tamarindus indica.*

Sr. No.	Compound Name	R.T.	M.W.	Formula	Class	Biological Activities	References
1	E-15-Heptadecenal	2.997	252.24	$C_{17}H_{32}O$	Aldehyde	Anti-inflammatory, antimicrobial	[41]
2	10-Octadecenoic Acid, Methyl Ester	2.997	296.48	$C_{19}H_{36}O_2$	Fatty Acid Ester	Metabolic regulator, impacts lipid metabolism	[41]
3	1,1-Dichloro-2,2,3,3-Tetramethylcyclopropane	5.081	166.03	$C_7H_{12}C_{l2}$	Cyclopropane Derivative	Antimicrobial, anti-inflammatory	[42]

4	3-(2-Furanyl)-2-propenal	3.042	122.03	$C_7H_6O_2$	Aldehyde	Antimicrobial, antioxidant	[42]
5	Elaidic Acid Methyl Ester	8.152	296.49	$C_{19}H_{36}O_2$	Fatty Acid Ester	Metabolic regulator, impacts lipid metabolism	[43]
6	L-1-Pyrroline-3-hydroxy-5-carboxylate	6.211	128.10	$C_5H_7NO_3$	Pyrroline Derivative	Antioxidant, anti-inflammatory	[44]
7	Eriodictyol	5.500	302.27	$C_{15}H_{12}O_6$	Flavonoid	Antidiabetic, antioxidant, anti-inflammatory	[45]
8	β-Sitosterol	7.42	414	$C_{29}H_{50}O$	Benzothiazole Derivative	Antidiabetic, antioxidant	[46]
9	3-Cyclopentylpropionic Acid, 2-Dimethylaminoethyl Ester	9.624	131.18	$C_{13}H_{25}NO_2$	Ester Derivative	Anti-inflammatory, metabolic regulator	[47]
10	Ethyl(E,Z)-decadienoate	9.910	196.29	$C_{12}H_20O_2$	Ester Derivative	Antimicrobial, antioxidant	[48]

3.4. Molecular Docking study

Molecular docking studies were conducted using twenty-three compounds derived from the HR-LCMS analysis of CHTI and ETTI These compounds were docked against three key proteins involved in Diabetes mellitus GLUT4 and PPARgamma. The detailed interactions and binding affinities of these compounds are presented in Tables 5 and Table 6 and visualized in Figs. 3-6.

Table 5: Molecular Docking Results of fractions of *Tamarindus indica* against GLUT4 and PPARgamma.

Sr. No.	Compound Name	GLUT4 (PDB ID: 7WSM)	PPARgamma (PDB ID: 6Y3U)
ch1	Apigenin	-10.6	-9.1
ch2	Epicatechin	-10.1	-10.3
ch3	Eicosanoic Acid	-6.3	-6.3
ch4	Catechin	-8.5	-8.2
ch5	Medicagenic acid	-8.1	-8.4
ch6	Tetradecanedioic acid	-6.0	-7.3
ch7	Palmitoyl glucuronide	-6.4	-6.7
ch8	Elaidic Acid Methyl Ester	-6.9	-7.2
ch9	Taxifolin	-8.8	-7.3
ch10	Naringenin	-9.8	-11.7
ch11	4,11,13,15-Tetrahydroridentin B	-8.4	-7.6
ch12	Tectorigenin	-8.6	-6.8
et13	E-15-Heptadecenal	-9.2	-8.6
et14	10-Octadecenoic Acid, Methyl Ester	-6.0	-6.3
et15	1,1-Dichloro-2,2,3,3-Tetramethylcyclopropane	-4.9	-5.3
et16	3-(2-Furanyl)-2-propenal	-4.9	-5.9
et17	Elaidic Acid Methyl Ester	-6.4	-5.7
et18	L-1-Pyrroline-3-hydroxy-5-carboxylate	-4.7	-5.7
et19	Eriodictyol	-10.1	-10.3
et20	β-Sitosterol	-11.7	-10.2
et21	3-Cyclopentylpropionic Acid, 2-Dimethylaminoethyl Ester	-5.7	-6.6
et22	Ethyl(E,Z)-decadienoate	-5.8	-6.2

Table 6: The active amino residues, bond category and bond type of selected compounds.

Compound	Amino Acid Interactions	Bond Type
Apigenin	GLN299, ASN431, ILE42, TRP428	Conventional Hydrogen Bond, Conventional Hydrogen Bond, Pi-Sigma, Pi-Pi Stacked
Naringenin	ILE326, HIS449, CYS285, ARG288, ALA292, LEU330	Pi-Sigma, Pi-Pi T-shaped, Pi-Alkyl, Pi-Alkyl, Pi-Alkyl, Pi-Alkyl
Eriodictyol	ILE326 , GLN286, ILE326, HIS449, CYS285, ARG288, ALA292,	Conventional Hydrogen Bond, Conventional Hydrogen Bond, Pi-Sigma, Pi-Pi T-shaped, Pi-Alkyl, Pi-Alkyl, Pi-Alkyl, Pi-Alkyl

	LEU330	
Beta-Sitosterol	PRO74, ALA442, ALA442, ALA442, PRO74, ILE313, PHE438, PHE438, PHE450	Alkyl, Alkyl, Alkyl, Alkyl, Alkyl, Alkyl, Pi-Alkyl, Pi-Alkyl, Pi-Alkyl

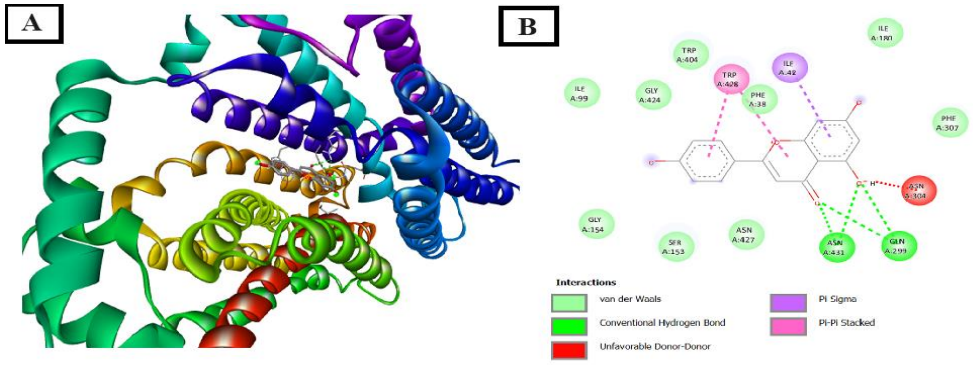

Fig. 3: 3D (A) and 2D (B) interaction of Apigenin against GLUT4 (PDB ID: 7WSM)

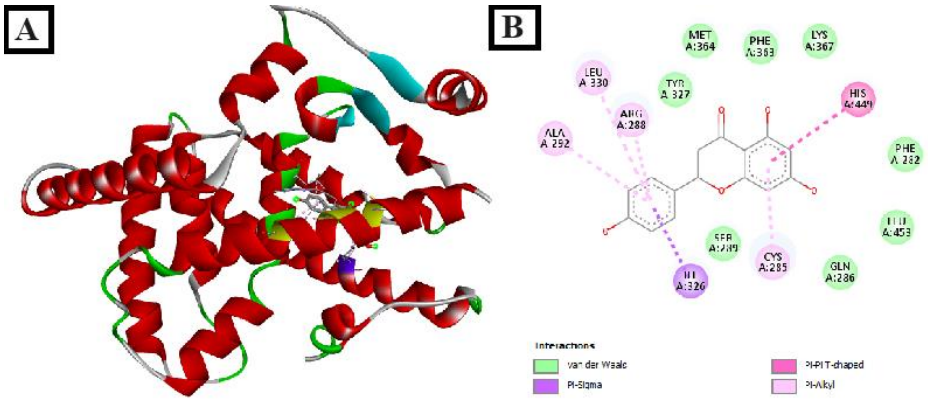

Fig. 4: 3D (A) and 2D (B) interaction of Naringenin against PPARgamma (PDB ID: 6Y3U)

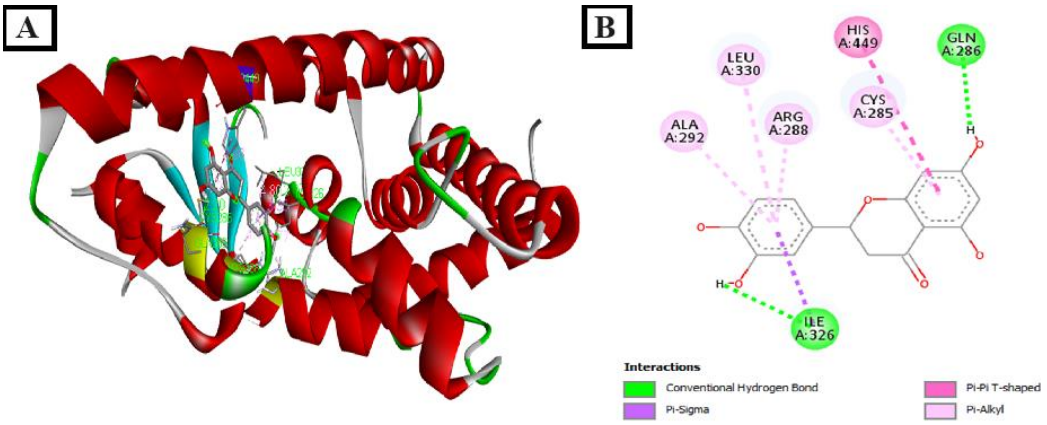

Fig. 5: 3D (A) and 2D (B) interaction of Eriodictyol against PPARgamma (PDB ID: 6Y3U)

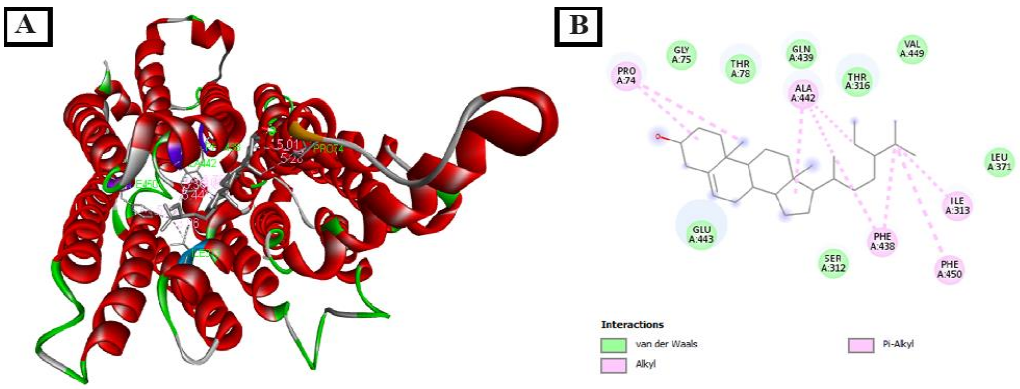

Fig. 6: 3D (A) and 2D (B) interaction of Beta-Sitosterol against GLUT4 (PDB ID: 7WSM)

3.5. *In-Vivo* antidiabetic activity

Table 7: Blood glucose levels in rats

Time (Hr)	Group-I (NC)	Group-II (DC)	Group-III (Chloroform Fraction)	Group-IV (Ethanol Fraction)	Group-V (Marketed tablet)
0	81.68±5.12	270.28±5.34$^{###}$	245.83±3.25***	255.80±11.13***	240.12±6.10***
1	80.43±3.08	273.02±6.66$^{###}$	230.63±5.07***	240.43±5.10***	210.28±11.85***
2	82.32±4.31	265.12±6.77$^{###}$	200.25±5.64***	220.15±5.44***	170.21±13.8***
4	84.53±6.36	264.13±5.22$^{###}$	150.40±5.17***	170.20±09.07***	100.43±5.10***
6	85.55±6.52	260.15±2.14$^{###}$	105.80±6.72***	115.70±6.82***	90.41±5.08***
8	86.17±4.22	261.45±2.10$^{###}$	110.42±5.13***	120.62±5.03***	110.74±3.42***
10	87.02±7.73	261.83±4.58$^{###}$	117.77±3.07***	125.87±3.05***	115.35±4.10***
12	89.60±4.34	263.10±5.14$^{###}$	120.86±4.24***	130.66±4.14***	120.06±8.44***

The values are given as Mean ±SD with n = 6. After doing a two-way ANOVA, the Tukey-Kramer multiple comparison test was run. ###p<0.001 in comparison with normal control; ##p< 0.01 in comparison with normal control; #p<0.01 in comparison with normal control; ***p < 0.001 in comparison with disease control; **p < 0.01 in comparison with disease control; *p <0.05 in comparison with disease control; nsp >0.05 in comparison with disease control.

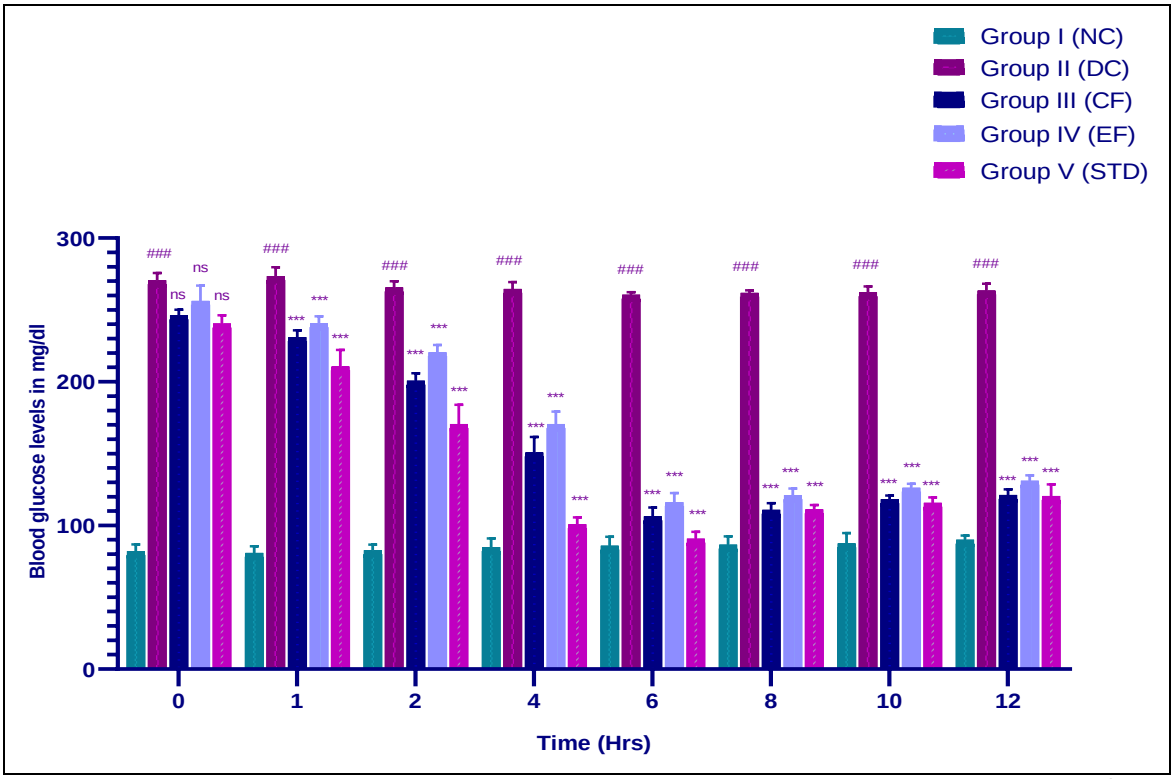

Figure 7: Graphical representation of *In-vivo* antidiabetic study in Rats. Values are expressed as Mean ±SD; n =6. Two-way ANOVA was performed followed by Tukey–Kramer multiple comparisons test: $^{###}$p<0.001 in comparison with normal control; $^{##}$p< 0.01 in comparison with normal control; $^{#}$p<0.01 in comparison with normal

control; ***p < 0.001 in comparison with disease control; **p < 0.01 in comparison with disease control; *p <0.05 in comparison with disease control; ^{ns}p >0.05 in comparison with disease control.

3.6. GLUT4 Gene Expression

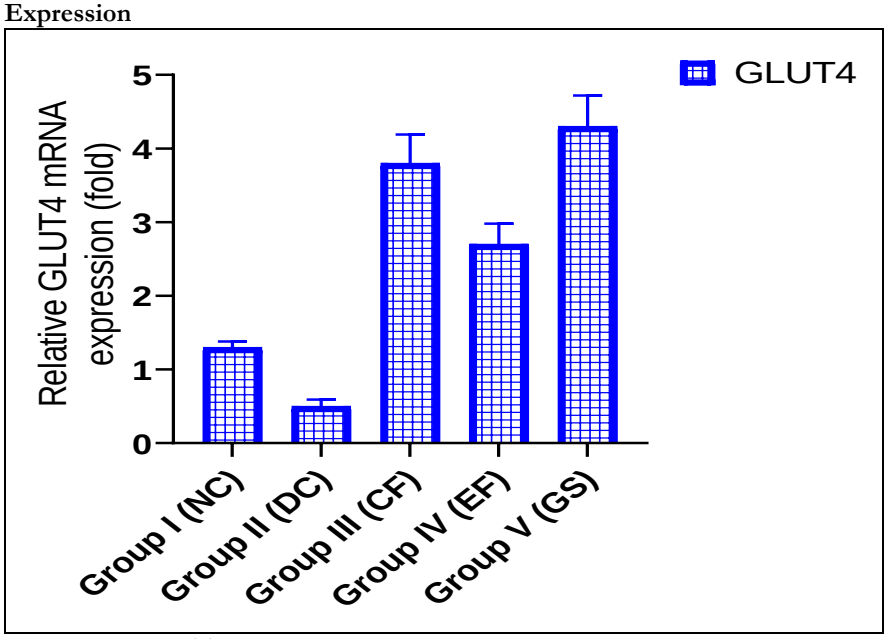

Figure 8: The expression of GLUT4 in blood samples of rats treated with different treatments: Group I Normal Control, Group II Diabetic Control, Group III Chloroform Fraction (200 mg/kg), Group IV Ethanol fraction (200 mg/kg), and Group V Glibenclamide Standard.

3.7. PPAR.γ Gene Expression

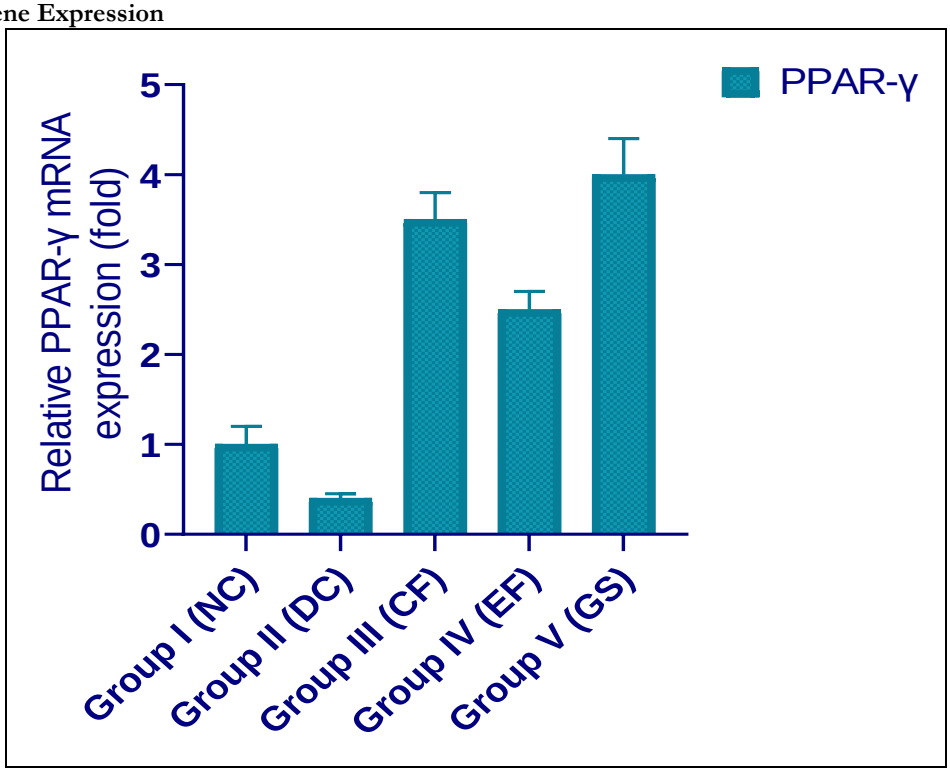

Figure 9: The expression of PPAR.γ in blood samples of rats treated with different treatments: Group I Normal Control, Group II Diabetic Control, Group III Chloroform Fraction (200 mg/kg), Group IV Ethanol fraction (200 mg/kg), and Group V Glibenclamide Standard.

4. Discussion

The total phenolic content (TPC) and total flavonoid content (TFC) of Tamarindus indica seed fractions were quantified to evaluate their potential contribution to the observed antidiabetic effects. As shown in Table 2, the chloroform fraction (CHTI) exhibited a significantly higher TPC (128.67 ± 5.90 mg GAE/g E.) compared to the ethanolic fraction (ETTI) (89.22 ± 3.78 mg GAE/g E.). This indicates that the chloroform fraction is richer in phenolic compounds, which are known for their potent antioxidant properties and potential to modulate carbohydrate metabolism. Similarly, the TFC was found to be 66.32 ± 2.32 mg QE/g E. for the chloroform fraction and 78.3 ± 3.41 mg QE/g E. for the ethanolic

fraction, suggesting a notable presence of flavonoids in both fractions. The higher flavonoid content in the ethanolic fraction underscores its potential role in enhancing insulin sensitivity and glucose uptake.

The differences in TPC and TFC between the chloroform and ethanolic fractions of Tamarindus indica seeds highlight the varied phytochemical profiles and their possible synergistic effects on antidiabetic activity. Phenolic and flavonoid compounds have been extensively studied for their ability to scavenge free radicals, inhibit α-glucosidase and α-amylase enzymes, and enhance glucose transporter (GLUT4) expression, thereby contributing to better glycemic control. These findings align with previous research that associates high phenolic and flavonoid contents with significant antidiabetic effects, supporting the potential use of Tamarindus indica seed extracts as a natural therapeutic agent for diabetes management. The comprehensive phytochemical profiling and subsequent bioactivity studies provide a robust foundation for further exploration of these fractions in preclinical and clinical settings (Table 2).

The HR-LCMS analysis of the chloroform fraction of Tamarindus indica seeds revealed a diverse array of bioactive compounds, as illustrated in Figure 1 and detailed in Table 3. Among the identified compounds, apigenin and epicatechin stood out due to their well-documented antidiabetic properties. Apigenin (R.T. 2.619) and epicatechin (R.T. 3.042) are flavonoids known for their antioxidant, anti-inflammatory, and insulin-enhancing activities. Apigenin has been reported to enhance GLUT4 translocation, which facilitates glucose uptake in cells, thus improving glycemic control. Similarly, epicatechin has been shown to improve insulin secretion and sensitivity, further supporting its role in diabetes management. Other notable flavonoids identified include catechin (R.T. 2.988) and naringenin (R.T. 11.001), both of which exhibit significant antidiabetic and antioxidant effects. Naringenin, in particular, acts as an insulin mimetic, inhibits α-glucosidase, and activates AMP-activated protein kinase (AMPK), which are crucial mechanisms in regulating blood glucose levels. The chloroform fraction contained several compounds that contribute to metabolic regulation and anti-inflammatory activities, such as eicosanoic acid (R.T. 3.120), medicagenic acid (R.T. 3.758), and tetradecanedioic acid (R.T. 5.945). Medicagenic acid, a saponin, is particularly noteworthy for its multifaceted biological activities, including antidiabetic and antioxidant effects. The presence of palmitoyl glucuronide (R.T. 10.038) and taxifolin (R.T. 10.977) further underscores the therapeutic potential of this fraction, given their roles in metabolic regulation and anti-inflammatory responses. These findings align with previous studies highlighting the benefits of such compounds in diabetes management, suggesting that the chloroform fraction of Tamarindus indica seeds could be a valuable source of natural antidiabetic agents.

The HR-LCMS analysis of the ethanolic fraction of Tamarindus indica seeds, as shown in Figure 2 and summarized in Table 4, also revealed a range of bioactive compounds with potential antidiabetic and antioxidant properties. The ethanolic fraction contained notable compounds such as E-15-heptadecenal (R.T. 2.997) and eriodyctiol (R.T. 5.500). E-15-heptadecenal, an aldehyde, exhibits significant anti-inflammatory and antimicrobial activities, which could be beneficial in managing inflammation associated with diabetes. Eriodictyol, a flavonoid, is known for its antidiabetic and antioxidant effects, enhancing its potential as a therapeutic agent. The identification of β-sitosterol (R.T. 7.420), a compound with known antidiabetic properties, further supports the efficacy of the ethanolic fraction in glucose regulation. The presence of various ester derivatives such as 10-octadecenoic acid, methyl ester (R.T. 2.997), and ethyl (E,Z)-decadienoate (R.T. 9.910) in the ethanolic fraction indicates their role in metabolic regulation and anti-inflammatory responses. These compounds are known to impact lipid metabolism, which is crucial in the context of diabetes management. The diverse phytochemical profile of the ethanolic fraction highlights the potential synergistic effects of these compounds in exerting antidiabetic effects. Collectively, the HR-LCMS analysis of both chloroform and ethanolic fractions of Tamarindus indica seeds provides a comprehensive understanding of their phytochemical composition and underscores their potential as natural sources of antidiabetic agents (Figure 2, Table 4).

The molecular docking studies of twenty-three compounds derived from the HR-LCMS analysis of the chloroform (CHTI) and ethanolic (ETTI) fractions of Tamarindus indica seeds against key proteins involved in diabetes mellitus, GLUT4 and PPARgamma, revealed significant insights into their potential antidiabetic mechanisms. The binding affinities and detailed interactions of these compounds are presented in Table 5 and visualized in Figures 3 to 7. Among the compounds docked, β-sitosterol exhibited the highest binding affinity against GLUT4 with a docking score of -11.7, indicating a strong interaction potential. This was followed closely by apigenin and naringenin, with docking scores of -10.6 and -9.8, respectively, suggesting their significant role in enhancing glucose uptake through GLUT4 translocation (Table 5). The detailed 3D and 2D interaction maps (Figures 3 and 4) show that apigenin forms conventional hydrogen bonds and pi interactions with key amino acid residues of GLUT4, which could facilitate its antidiabetic action.

PPARgamma, naringenin showed the highest binding affinity with a docking score of -11.7, followed by epicatechin and β-sitosterol, both with scores of -10.3. The strong binding affinities suggest that these compounds could effectively modulate PPARgamma activity, which plays a crucial role in lipid metabolism and glucose homeostasis (Table 5). The interaction details in Figures 4 and 5 highlight that naringenin and eriodictyol form multiple pi and alkyl interactions with PPARgamma, which may enhance their stability and effectiveness in the protein's active site. These interactions, such as the pi-sigma and pi-alkyl bonds formed by naringenin (Table 6), underscore the potential of these compounds to act as potent agonists of PPARgamma, thereby improving insulin sensitivity and exerting anti-inflammatory effects. The docking results indicate that the compounds identified from Tamarindus indica seed fractions, particularly β-sitosterol, apigenin, naringenin, and eriodictyol, have promising antidiabetic properties through their interactions with GLUT4 and

PPARgamma. These findings provide a molecular basis for the observed hypoglycemic effects of Tamarindus indica seeds and support their potential use in diabetes management.

The *in-vivo* antidiabetic activity study of Tamarindus indica seed fractions was evaluated by measuring the blood glucose levels in diabetic rats at various time intervals, as presented in Table 7 and graphically represented in Figure 7. The results indicate a significant reduction in blood glucose levels in the treatment groups compared to the diabetic control (Group II). At the 0-hour mark, the blood glucose levels in Group II (DC) were 270.28 ± 5.34 mg/dL, which remained consistently high throughout the study. In contrast, Group III (treated with the chloroform fraction) and Group IV (treated with the ethanol fraction) showed a progressive decrease in blood glucose levels, with the most pronounced effects observed at the 6-hour mark, where the levels dropped to 105.80 ± 6.72 mg/dL and 115.70 ± 6.82 mg/dL, respectively. These reductions were statistically significant when compared to the diabetic control group, highlighting the hypoglycemic potential of both fractions. The marketed antidiabetic drug (Group V) exhibited a rapid and sustained reduction in blood glucose levels, reaching 90.41 ± 5.08 mg/dL at the 6-hour mark, which serves as a benchmark for evaluating the efficacy of the Tamarindus indica seed fractions. The chloroform fraction demonstrated a comparable hypoglycemic effect, particularly in the early hours post-administration, while the ethanol fraction showed a slightly less pronounced but still significant reduction in glucose levels. By the 12-hour mark, the blood glucose levels in the chloroform and ethanol fraction-treated groups were 120.86 ± 4.24 mg/dL and 130.66 ± 4.14 mg/dL, respectively, indicating a sustained hypoglycemic effect throughout the study period.

The *in-vivo* study confirms the antidiabetic efficacy of Tamarindus indica seed fractions, with both chloroform and ethanol extracts significantly lowering blood glucose levels in diabetic rats. These findings suggest that the bioactive compounds identified in the HR-LCMS analysis contribute to the observed hypoglycemic effects, supporting their potential use as natural therapeutic agents for diabetes management. Further studies are warranted to elucidate the mechanisms of action and to optimize the dosage and formulation for clinical applications.

The gene expression analysis of GLUT4 and PPAR-γ provides further insights into the molecular mechanisms underlying the antidiabetic effects of Tamarindus indica seed fractions. As shown in Figure 8, the expression levels of GLUT4 in blood samples from rats treated with the chloroform fraction (CF), ethanol fraction (EF), and glibenclamide (GS) were significantly elevated compared to the diabetic control (DC) group. The diabetic control group exhibited a markedly reduced GLUT4 protein level, with only a 0.5-fold increase. In contrast, the chloroform fraction (200 mg/kg) enhanced GLUT4 expression by 3.8-fold ($p < 0.05$), indicating a substantial upregulation compared to the diabetic control. The ethanol fraction also improved GLUT4 levels but was slightly less potent than the chloroform fraction, though the difference was not statistically significant. Notably, glibenclamide treatment resulted in a higher GLUT4 protein expression than both the chloroform and ethanol fractions, demonstrating its strong efficacy in enhancing GLUT4 translocation. These findings suggest that the bioactive compounds in Tamarindus indica seeds can significantly increase GLUT4 expression, which is crucial for glucose uptake and insulin sensitivity.

The expression of PPAR-γ in the blood samples, illustrated in Figure 9, showed significant differences across the treatment groups compared to the diabetic control group. The diabetic control rats had a reduced PPAR-γ protein level, with only a 0.4-fold increase. The chloroform fraction treatment resulted in a 3.5-fold increase in PPAR-γ expression ($p < 0.05$), highlighting its potential in modulating lipid metabolism and glucose homeostasis. The ethanol fraction was also effective, though less potent than the chloroform fraction, in enhancing PPAR-γ protein levels. Glibenclamide treatment exhibited the highest increase in PPAR-γ expression, with a 4.0-fold enhancement, surpassing both fractions of Tamarindus indica. The elevated PPAR-γ levels in the treatment groups indicate that the bioactive compounds in the seed fractions can activate PPAR-γ pathways, contributing to improved insulin sensitivity and anti-inflammatory effects. These results underscore the therapeutic potential of Tamarindus indica seed fractions in regulating key proteins involved in diabetes management and support their further investigation as natural antidiabetic agents.

5. Conclusion

The comprehensive study on *Tamarindus indica* seed fractions reveals significant antidiabetic potential through various mechanisms. Phytochemical profiling using HR-LCMS identified numerous bioactive compounds with known hypoglycemic and antioxidant properties. Molecular docking studies demonstrated strong binding affinities of these compounds with key diabetic proteins, GLUT4 and PPAR-γ, indicating their potential to enhance glucose uptake and improve insulin sensitivity. In vivo studies confirmed the hypoglycemic effects of the seed fractions, significantly reducing blood glucose levels in diabetic rats. Furthermore, gene expression analysis showed substantial upregulation of GLUT4 and PPAR-γ proteins, reinforcing the therapeutic promise of Tamarindus indica seeds in diabetes management. These findings collectively suggest that Tamarindus indica seed fractions are potent natural agents for antidiabetic therapy, warranting further exploration and development.

Abbreviations

DM: Diabetes mellitus; HR-LCMS: High-resolution liquid chromatography-mass spectrometry; GLUT4: Glucose transporter type 4; PPAR-γ: Peroxisome proliferator-activated receptor gamma; CHTI: Chloroform fraction of Tamarindus indica; ETTI: Ethanolic fraction of Tamarindus indica; GAE: Gallic acid equivalent; QE: Quercetin equivalent; STZ: Streptozotocin; RT-qPCR: Real-time quantitative reverse transcription-polymerase chain reaction; GS: Glibenclamide standard; NC: Normal control; DC: Diabetic control; SD: Standard deviation; ANOVA: Analysis of variance.

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Author Contribution

All Authors Contributed Equally.

Competing Interests

The authors declare that they have no competing interests.

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