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Shawrif Zaman
Research Trainee, Bio Edge Solutions, Bengaluru, Karnataka, India

Dr. Shruthi SD
CEO & Scientist, Bio Edge Solutions, Bengaluru, Karnataka, India

Corresponding Author:
Shawrif Zaman
Research Trainee, Bio Edge Solutions, Bengaluru, Karnataka, India

In-vitro and *in-silico* study of plant extract isolated from leaves of *Trigonella foenum* (fenugreek) for anti-diabetic activity

Shawrif Zaman and Shruthi SD

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Abstract
Type 2 diabetes is a chronic condition characterized by insulin resistance, influenced by genetics, obesity, and lifestyle factors. Fenugreek, rich in bioactive compounds, has shown promise in diabetes care due to its hypoglycemic and anti-inflammatory properties. Advanced techniques like Gas Chromatography-Mass Spectrometry (GC-MS) aid in detecting bioactive substances, helping refine therapeutic applications.

Fenugreek leaves were processed for phytochemical analysis, revealing compounds such as Squalene, Phytol, and Azelaic acid. In-vitro studies focused on α-amylase inhibition to assess glucose regulation potential, while in-silico docking examined ligand-protein interactions to evaluate therapeutic efficacy. Using PyRx and Discovery Studio, computational studies validated Fenugreek's potential as an antidiabetic agent. ADMET analyses with Swiss ADME and ADMET LAB 2 predicted pharmacokinetics and toxicity, confirming the compounds' drug-likeness and metabolic stability.

These findings highlight Fenugreek's therapeutic value in managing Type 2 diabetes. The combined use of biochemical assays and computational methods underscores its potential to regulate glucose metabolism and mitigate postprandial hyperglycemia, paving the way for further research into its clinical applicability.

Keywords: Fenugreek, alpha-amylase inhibition, diabetes, molecular docking, GC-MS, *Trigonella foenum*, ADMET

Introduction

Type 2 diabetes, a chronic condition, affects millions globally. It is characterized by insulin resistance, where cells fail to respond effectively to insulin, leading to elevated blood sugar levels. Research highlights the role of genetics, obesity, and lifestyle factors in its development. Early menopause has been linked to an increased risk of type 2 diabetes, emphasizing the importance of hormonal changes on metabolic health. Innovative tools are being developed to identify the most effective glucose-lowering drugs, offering personalized treatment options [1].

Recent advancements in diabetes management focus on a comprehensive approach, addressing not only glycaemic control but also cardiovascular risk factors and mental health. Studies reveal that lifestyle modifications, such as regular exercise and a balanced diet, can significantly reduce the risk of developing type 2 diabetes. Additionally, understanding the differences between type 1 and type 2 diabetes is crucial for effective treatment strategies. As research continues to evolve, it brings hope for better prevention and management of this widespread condition [2].

Fenugreek (*Trigonella foenum-graecum* L.), a versatile herb, has been cherished for its medicinal properties for centuries. Its leaves and seeds are rich in bioactive compounds such as flavonoids, alkaloids, saponins, and polyphenols, contributing to their therapeutic potential. Often used in culinary dishes, fenugreek leaves boast anti-inflammatory and antioxidant properties, with methanolic extracts proving effective in topical creams for inflammation and joint pain [3].

Studies highlight fenugreek leaves' diverse health benefits, including hypoglycemic and hypocholesterolemic effects for managing diabetes and cardiovascular diseases. Their antimicrobial and antifungal properties aid in treating infections, while neuroprotective and hepatoprotective effects make them promising for neurodegenerative disorders and liver

diseases. Fenugreek leaves enhance metabolic enzyme activity, regulating blood sugar and cholesterol synthesis, and their rich nutrient profile further boosts overall health. This natural therapeutic agent continues to show promise in scientific research [4].

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique that combines the separation capabilities of gas chromatography with the molecular identification prowess of mass spectrometry. In this process, a sample is first vaporized and separated into its components based on their chemical properties. These components are then analyzed by the mass spectrometer, which identifies them by their unique mass-to-charge ratios. This dual approach allows scientists to unravel complex mixtures with precision, making GC-MS indispensable in fields like environmental analysis, forensic science, and pharmaceutical [5].

The versatility of GC-MS lies in its ability to detect even trace amounts of substances, offering unparalleled sensitivity and specificity. From identifying pollutants in air and water to detecting drugs in forensic investigations, its applications are vast and impactful. For instance, in food safety, GC-MS ensures the detection of harmful contaminants, safeguarding public health. Its role extends to space exploration, where it helps analyze the chemical composition of extraterrestrial samples [6].

In-silico studies have revolutionized the exploration of antidiabetic activity by leveraging computational tools to predict molecular interactions and drug efficacy. These studies often involve molecular docking and dynamic simulations to evaluate the binding affinity of bioactive compounds with key enzymes like α-amylase, α-glucosidase, and DPP-IV, which are crucial in diabetes management [7].

For instance, compounds such as diffractaic acid have shown promising inhibitory effects against DPP-IV, as highlighted in recent research. By simulating biological processes, in-silico methods provide a cost-effective and efficient approach to screening potential antidiabetic agents. This computational strategy accelerates drug discovery, offering insights into the structural and functional properties of therapeutic compounds [8].

Molecular docking is a computational approach that predicts the interaction between protein targets and ligands, forming stable complexes. This technique is widely used in drug discovery to identify potential therapeutic agents. Docking involves determining the optimal orientation of a ligand within the binding site of a protein, which helps estimate binding affinity and interaction strength [9].

Advancements in docking algorithms, such as flexible receptor docking and improved scoring functions, have enhanced the accuracy of predictions. These methods allow for a better understanding of protein-ligand interactions, enabling the design of novel drugs. Recent research highlights the use of molecular dynamics simulations to validate docking results and assess the stability of protein-ligand complexes. Such approaches are crucial for optimizing drug candidates and reducing the time required for experimental validation. Molecular docking continues to play a pivotal role in the development of targeted therapies for various diseases [10].

Methods

Collection of Plant Material

Leaves of Fenugreek were purchased from herbal shop in Bengaluru, India. After collecting the leaves were thoroughly washed with cleaned water. The leaves were then shade-dried in room temperature.

Isolation of Plant Extract

The Fenugreek fresh leaves were cut into small thin pieces by using clean scissor. The leaf pieces (10g) were transferred into a clean glass bottle. In the bottle Methanol (100 ml) were added to submerge the leaves. The Fenugreek leaf pieces were soaked for 3-4 days so that all photochemicals get dissolve into Methanol solution. Further the separated extracts were filtered through Whatman No. 1 filter paper and the methanol filtrate was condensed to dryness using rotary evaporator at 40 °C, yielding approximately 20% of methanol extract [11].

Identification of Phytoconstituents

Gas Chromatography - Mass Spectroscopy [GC-MS]

GC-MS analysis

The chemical composition of the extracts was evaluated using Shimadzu GCMS-QP2010 S instrument with GC-MS solutions software and compounds were separated using Rtx-5, capillary column (0.25 mm, 0.25 µm). Split ratio 1:25 injector temperature was 300 °C; the column temperature was maintained at 60 °C, followed by 10 min at 300 °C. About 1 µl sample was injected to the column by the split mode. GC-MS is performed by an electron ionization system, with ionization of 70 eV. Helium is used as a carrier gas at flow rate of 1 ml/min; Mass scanning range was 40-500 m/z. The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 µL) were injected with syringe into the injector with a split ratio of 30:1. All data were obtained by collecting the full-scan mass spectra within the scan range 40-550 amu. The percentage composition of the crude extract constituents are expressed as percentage by peak area and identification, characterization of chemical compounds in crude extracts were based on GC retention time. The mass spectra obtained was computer matched with standards available in mass spectrum libraries. Interpretation on Mass-Spectrum GC-MC was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library as well as by comparison of the retention time. The name, molecular weight and structure of the compounds present in extracts and formulation was ascertained [12].

In vitro Antidiabetic Activity

Diabetes is a complex disease with various symptoms. It occurs due to improper utilization of glucose obtained by digestion of food in the cells due to failure of secreting hormone by pancreas [13]. Insulin is the hormone that regulates the glucose level in the blood. Due to lack of hormone, the level of glucose raises in blood and lead to the condition called hyperglycemia. The patients with diabetics show other problems like kidney failure, cataract, heart problem, highly prone to infections, skin cancer, etc. due to rise in glucose level. During diabetics, the improper metabolization of glucose leads in to the formation of free radicals which can damage the cells that lead into early aging and cancer [17].

α-amylase assay

Principle

α-amylase activity can be measured by the determination of reducing groups arising from hydrolysis of soluble starch by α-amylase according to the protocol of Rick and Stegbauer (1970). The reduction of 3, 5-dinitrosalicylic acid to nitroaminosalicylic acid produces a color shift which is followed photometrically by changes in the absorbance at

546nm. Inhibition of starch hydrolysis by an α-amylase inhibitor results in a diminished absorbance at 540nm in comparison with the controls [14].

Reagents required

• Phosphate buffer saline - 0.02 mol./L (pH 6.8)
• α-amylase - 0.1 mg/ml of PBS
• Starch solution: 1.0%
• DNS reagent
1. Dinitrosalicylic acid - 1%
2. Sodium sulphite - 0.05%
3. Sodium hydroxide - 1%

Procedure

0.5ml of plant extract of different concentration such as 100, 200, 300, 400 and 500 µg were taken and dissolved with 0.25 ml of α-amylase solution and mixed thoroughly. The sample was incubated at 37 °C for 5 min. Add 0.5 ml of starch solution and incubate for 3 min at 37 °C. Then DNS reagent was added and boiled at 100 °C for 5 min to stop the reaction. The reaction mixture was cooled to room temperature and the absorbance was read at 540 nm in spectrophotometer. 15
Percentage of inhibition was calculated using the equation:

$$\text{Inhibition (\%)} = [(Abs1 - Abs2)/Abs1] \times 100$$

OR

$$\text{\% inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \text{ X } 100$$

In silico docking studies

In silico docking techniques are being used to investigate the complementarity at the molecular level of a ligand and a protein target. As such, docking studies can be used to identify the structural features that are important for binding and for in silico screening efforts in which suitable binding partners can be identified [16].

Softwares used for Insilco docking

PYRX

PYRX is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with an easy-to-use user interface which makes it a valuable tool for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for structure-based drug design.

Discovery Studio

Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Dassault Systemes BIOVIA (formerly Accelrys). The product suite has a strong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 and more

Discovery Studio provides software applications covering the following areas:

Simulations

- Including Molecular Mechanics, Molecular Dynamics, Quantum Mechanics. For molecular mechanics based simulations: Include implicit and explicit-based solvent models and membrane models.

Ligand Design

- Including tools for enumerating molecular libraries and library optimization.

Pharmacophore modeling

- Including creation, validation and virtual screening

Structure-based Design

- Including tools for fragment-based placement and refinement, receptor-ligand docking and pose refinement, de novo design

Macromolecule design and validation

Macromolecule engineering

- Specialist tools for protein-protein docking. Specialist tools for Antibody design and optimization. Specialist tools for membrane bound proteins, including GPCRs

QSAR

- Covering methods such as multiple linear regression, partial least squares, recursive partitioning, Genetic Function approximation and 3D field based QSAR

ADME

- Predictive toxicity

Ligand preparation for docking

Download the ligand file in 3D SDF format from PubChem and open it in PyRx for Auto Docking.

Protein preparation for docking

Protein molecules can be downloaded from Protein Data Bank website (www.rcsb.org). The downloaded protein structure in their ".pdb" format has to be edited to remove the non amino acid residues, such as water molecules, ions, ligands that are in the complex. These can be removed using either PyMol software or WordPad. This has to be done, since, these molecules will interfere with the interaction between the target molecule and protein in AutoDock. Once the ".pdb" file is downloaded, right-click the file and open it with WordPad. Scroll down to the end of the document and delete all the lines that begin with "HETATM" and "CONNECT," these are the atoms that do not belong to amino acids and their interaction with the amino acids, respectively. The last line of the document should contain a like begins with "TER" (e.g.: "TER 8910 SER B 557") this indicates the end of the protein chain. Once this is done, save the document, now it is ready for AutoDock analysis.

ADMET studies

1. Swiss ADME

Swiss ADME, developed by the Swiss Institute of Bioinformatics, is a computational tool used to predict Absorption, Distribution, Metabolism, and Excretion (ADME) properties of chemical compounds. It offers free access to predictive models for evaluating physicochemical descriptors, pharmacokinetics, drug-likeness, and medicinal chemistry aspects of small molecules. In drug discovery, Swiss ADME is critical for selecting suitable compounds by predicting their behaviour within biological systems, reducing reliance on extensive experimental testing. In molecular docking, it ensures that chosen compounds possess favourable ADME properties alongside binding affinity. Swiss ADME minimizes drug development failures due to poor pharmacokinetics, aiding researchers in creating safer and more effective drugs.

Lipinski's rule of 5

Lipinski's "rule of five" is the first qualitative attempt to guide the design of "orally deliverable" compounds and is based on the limits on properties (clog P, molecular weight and number of hydrogen-bond donors and acceptors) beyond which oral activity is predicted to be poor. Therefore, the recommended strategy during pharmaceutical development is to improve the solubility of the lead compound even if the permeability of the molecule is compromised as a result. ADMETLAB 2.0

2. ADMET LAB 3

ADMET LAB 3 is a computational tool that predicts Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of chemical compounds. It plays a key role in drug discovery and molecular docking by assessing pharmacokinetic and toxicological profiles of potential candidates. By predicting compound behaviour in biological systems, it helps researchers identify suitable drugs, saving time and resources otherwise spent on extensive experimental testing. In molecular docking, it ensures selected compounds have favorable ADMET properties alongside strong binding affinities. Its significance lies in reducing drug development failures and enabling the creation of safer and more effective therapies, speeding up progress toward clinical applications.

Results

In this study, one plant extract and the solvent fractions were evaluated for their possible α-amylase inhibitory activities alongside acarbose as a positive control. The result is shown in the chart -

Table 1: Constituents of Fenugreek Leaf Extract

Sl no.	Name	Molecular Formula	CAS	Retention Time	Similarity	Base m/z	Properties
1	Squalene	$C_{30}H_{50}$	111-02-4	17.50	95	410.00	Hydration, Anti-inflammatory, Antioxidant
2	Phytol	$C_{20}H_{40}O$	150-86-7	30.14	93	123.00	Anti Inflammatory, Antioxidant
3	Azelaic acid	$C_9H_{16}O_4$	123-99-9	13.76	98	187.00	Insulin Sensitivity, Antioxidant, Antidiabetic
4	Stearic acid	$C_{18}H_{36}O_2$	57-11-4	16.60	97	129.00	Anti diabetic, Cardiovascular health, Metabolic function
5	2-ethyl-Heptanoic acid	$C_8H_{16}O_2$	106-76-3	13.74	96	129.00	Metabolic function
6	9,12-Octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	60-33-3	22.97	91	67.05	Glucose Metabolism, Cholesterol Regulation
7	9E,11E)-Octadecadienoic acid	$C_{18}H_{32}O_2$	544-71-8	24.32	93	67.05	Metabolic function
8	Quinoline	C_9H_7N	92-22-5	14.50	97	129.00	Antimicrobial, Antioxidant
9	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	80060-76-0	26.02	88	55.05	Antioxidant
10	2,3-dihydroxypropyl ester	$C_5H_{10}O_4$	2277-28-3	28.68	88	67.05	Lipid Metabolism
11	Caryophyllene	$C_{15}H_{24}$	87-44-5	19.63	96	204.00	Anti-inflammatory
12	Linoleic acid ethyl ester (Ethyl Linoleate)	$C_{20}H_{36}O_2$	544-35-4	32.28	89	67.05	Glucose Metabolism, Cardiovascular Health
13	Ricinoleic acid	$C_{18}H_{34}O_3$	141-22-0	34.09	82	98.05	Anti-inflammatory, Metabolic stability
14	1,8,11-Heptadecatriene	$C_{17}H_{30}$	56134-03-3	14.5	87	234.00	Cellular metabolism
15	Elaidic Acid (9-Octadecenoic acid)	$C_{18}H_{34}O_2$	112-79-8	18.5	98	282.00	Antidiabetic, Lipid metabolism
16	9-Tetradecenal	$C_{14}H_{26}O$	1937-62-8	16.5	79	210.00	Biochemical process

The Phytochemical analysis of Fenugreek leaves by using GC-MS indicated presence of different constituents like Squalene, Phytol, Azelaic acid, Stearic acid, 2-ethyl-Heptanoic acid, 9,12 Octadecadienoic acid (Z,Z), 9E,11E)-Octadecadienoic acid, Quinoline, (Z)-18-Octadec-9-enolide, 2,3-dihydroxypropyl ester, Caryophyllene, Linoleic acid ethyl ester (Ethyl Linoleate), Ricinoleic acid, 13-Hexyloxacyclotridec-10-en-2-one; 1,8,11-Heptadecatriene, Elaidic Acid (9-Octadecenoic acid), 9-Tetradecenal (Table 1).

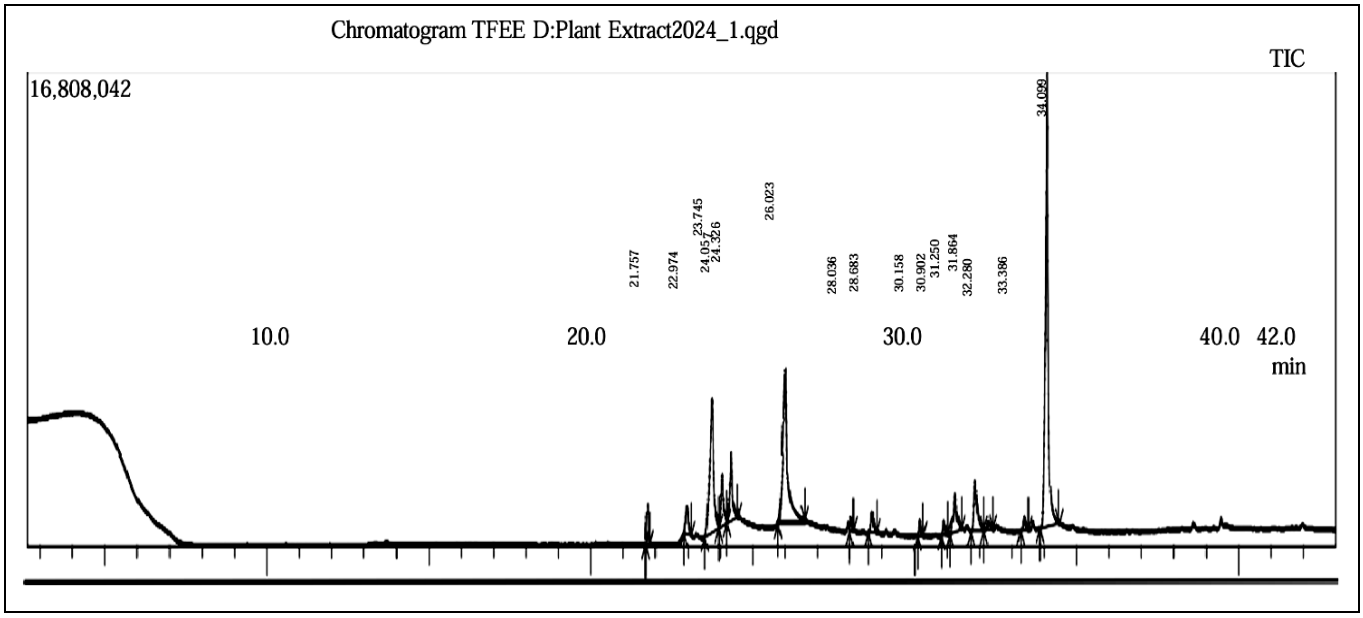
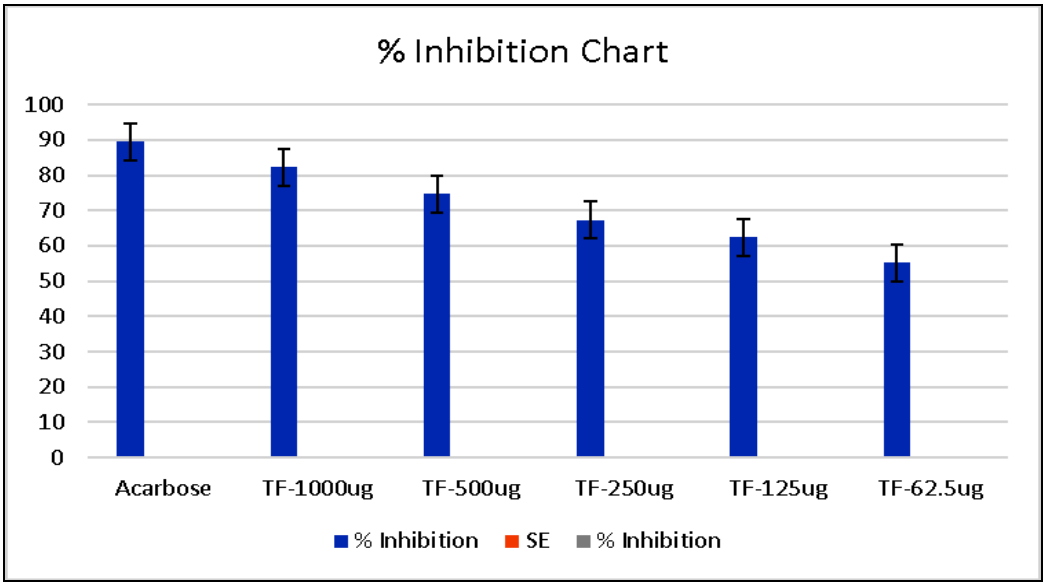


Fig 1: GC-MS Graph of Phytoconstituents of *Trigonella foenum* (Fenugreek) leaves

Table 2: Percentage Inhibition

Groups	% Inhibition	SE	% Inhibition
Acarbose	89.57	0.003	89.57±0.003
TF-1000ug	82.21	0.003	82.21±0.003
TF-500ug	74.72	0.002	74.72±0.002
TF-250ug	67.23	0.003	67.23±0.003
TF-125ug	62.4	0.002	62.4±0.002
TF-62.5ug	55.21	0.004	55.21±0.004



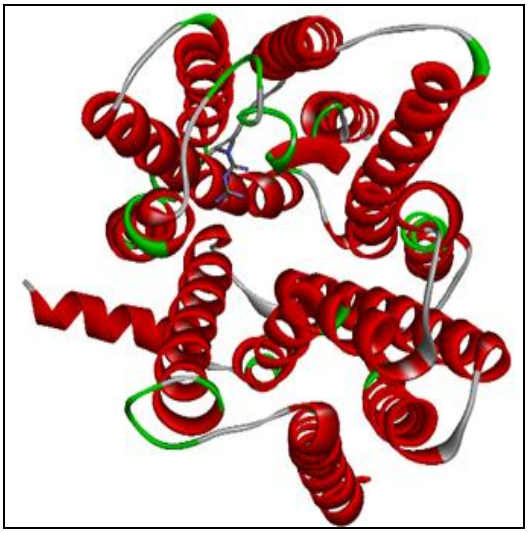
In in-silico study the phytoconstituents were docked with '8z7w protein'. The structure of the protein was downloaded from Protein Data Bank. When this protein was docked with the phytoconstituents as ligands, they formed 'Protein-Ligand Complex'. After Docking different parameters like Binding Affinity, number of Hydrogen Bonds Amino Acids and Van der Waals Bonds were identified and recorded (Table 2). Based upon these parameters few Protein-Ligand Complex of Ricinoleic Acid, Elaidic acid, Isolinoleic Acid, Stearic Acid, and Azelaic Acid were selected for further study (Table 3)

Table 2: Docked All Compound

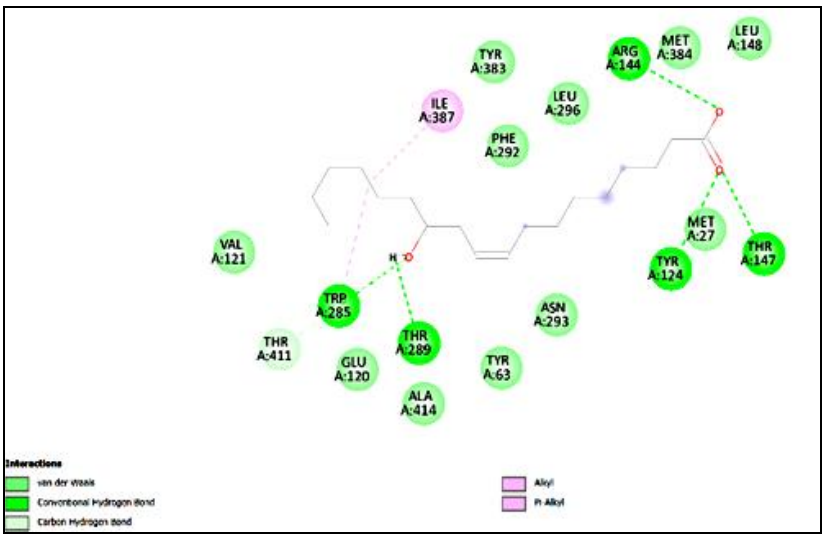
Sl No.	Protein_Ligand Complex	Ligand Name	Binding Affinity	No. of H-bond	Amino Acid	Va der Waals Bond	Amino Acid
1	8z7w_5283469_uff_E=2651.48	(Glyceral monoenolenoleate), 9,12-Octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester	-5.3	5	ASN, TRP, THR, TYR, ARG	10	TYR, THR, TYR, TRP, PHE, MET, LEU, MET, THR, LYS
2	8z7w_13573140_uff_E=54.18	1,8,11-Heptadecatriene	-4.8	0	-	11	THR, TYR, GLU, ASN, LEU, THR, MET, THR, TYR, ARG, PHE
3	8z7w_18648_uff_E=39.01	2-ethyl Heptanoic acid	-5.3	2	THR, TRP	9	ALA, TYR, GLU, PHE, ASN THR, ARG, TYR, ILE
4	8z7w_3931_uff_E=81.58	9,12-Octadecadienoic acid	-5.4	1	LEU (A:415)	13	TYR (A:63), TRP, ASN, MET, THR, TYR, ARG, TYR, VAL, GLU, THR, THR, ALA
5	8z7w_5282796_uff_E=65.28	9E,11E)-Octadecadienoic acid	-5.6	3	TRP, THR, THR	10	TYR, THR, ASN, ALA, ARG, TYR, GLU, MET, LYS, THR
6	8z7w_445639_uff_E=80.35	Elaidic Acid (9-Octadecenoic acid)	-5.3	3	TRP, THR, THR	12	GLU, THR, ASN, TYR, TRP, PHE, MET, LEU, THR, LYS, ALA, MET
7	8z7w_5283368_uff_E=47.31	9-Tetradecenal	-5	1	TYR	10	GLU, TYR, ARG, ILE, MET, THR, ASN, THR, ALA, THR
8	8z7w_6536948_uff_E=167.75	13-Hexyloxacyclotridec-10-en-2-one	-7.2	1	TYR	11	TRP, TYR, THR, ASN, THR, GLU, LEU, MET, PHE, THR, ARG
9	8z7w_6428982_uff_E=175.45	18-Octadec-9-enolide	-7.6	2	TYR, ASN	14	PHE, ILE, ARG, TYR, THR, THR, MET, THR, LEU, LYS, LEU, TYR, MET, TRP
10	8z7w_2266_uff_E=42.88	Azelaic acid	-4.9	3	TRP (A:285), ASN (A:293), TYR (A383)	7	ILE (A:387), THR (A:411), TYR (A:63), GLU (A:120), THR (A:289), THR(A:117), PHE (A:292)
11	8z7w_5281515_uff_E=740.89	Caryophyllene	-6.4	0	-	8	GLU, LYS, MET, THR, LEU, MET, PHE, ASN
12	8z7w_5282184_uff_E=2055.50	Ethyl Linoleate	-5.6	2	THR, LYS	15	GLU, MET, ASN, THR, THR, TYR, LEU, TYR, LEU, ARG, MET, TYR, THR, GLU, VAL
13	8z7w_5280435_uff_E=165.89	Phytol	-5.5	2	ARG, TYR	16	THR, LYS, LEU, THR, MET, LEU, PHE, TYR, ALA, THR, MET, ASN, GLU, THR, TYR, ILE
14	8z7w_643684_uff_E=131.80	Ricinoleic acid	-5.7	5	ARG, TYR, THR, TRP, THR	12	TYR, PHE, LEU, MET, LEU, MET, ASN, TYR, ALA, GLU, THR, VAL
15	8z7w_638072_uff_E=248.91	Squalene	-6.7	0	-	18	TRP, TYR, THR, ASN, TYR, LEU, THR, MET, ARG, TRP, MET, THR, GLN, LYS, ASN, TYR, ARG, GLU, THR

Table 3: Docked Selected Compound

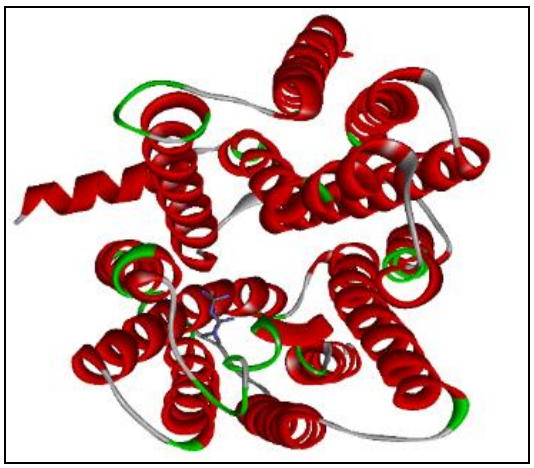
Sl No.	Protein_Ligand Complex	Ligand Name	Binding Affinity	No. of H-bond	Amino Acid	Va der Waals Bond	Amino Acid
1	8z7w_643684_uff_E=131.80	Ricinoleic acid	-5.7	5	ARG, TYR, THR, TRP, THR	12	TYR, PHE, LEU, MET, LEU, MET, ASN, TYR, ALA, GLU, THR, VAL
2	8z7w_445639_uff_E=80.35	Elaidic acid (9-Octadecenoic acid)	-5.3	3	TRP, THR, THR	12	GLU, THR, ASN, TYR, TRP, PHE, MET, LEU, THR, LYS, ALA, MET
3	8z7w_5282796_uff_E=65.28	Isolinoleic Acid (9E,11E-Octadecadienoic acid)	-5.6	3	TRP, THR, THR	10	TYR, THR, ASN, ALA, ARG, TYR, GLU, MET, LYS, THR
4	8z7w_5281_uff_E=4383.69	Stearic acid	-4.9	3	THR (A:411), TRP, THR (A:289)	9	ALA, ARG, MET, THR, LYS, ARG, MET, ASN, LEU
5	8z7w_2266_uff_E=42.88	Azelaic acid	-4.9	3	TRP (A:285), ASN (A:293), TYR (A383)	7	ILE (A:387), THR (A:411), TYR (A:63), GLU (A:120), THR (A:289), THR(A:117), PHE (A:292)



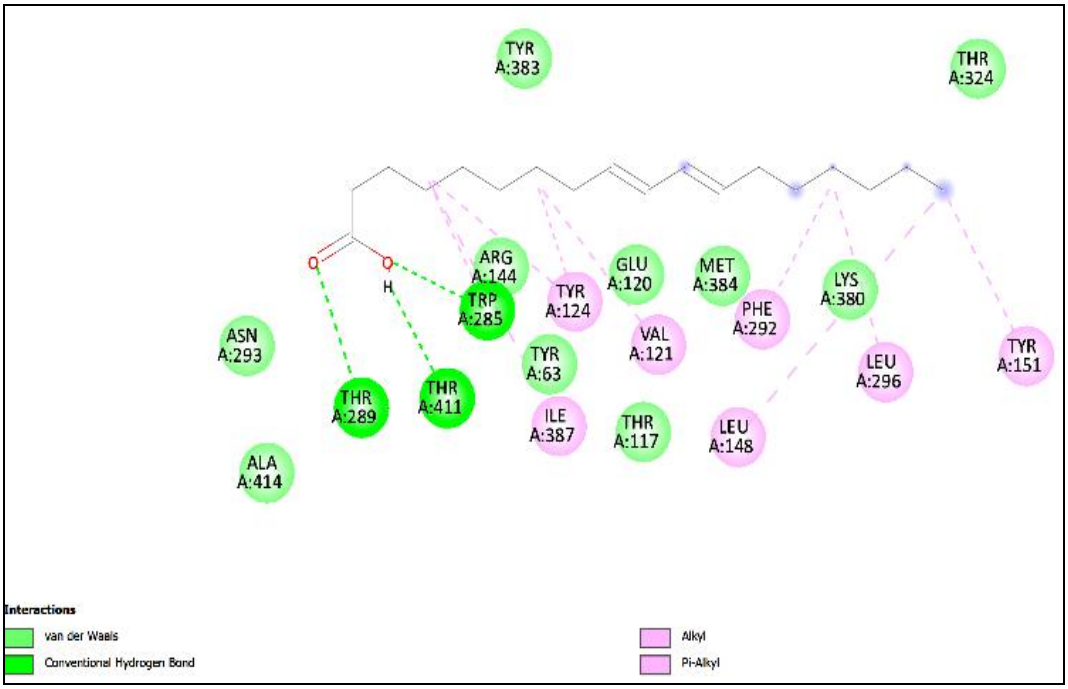
Docking Protein 8z7w



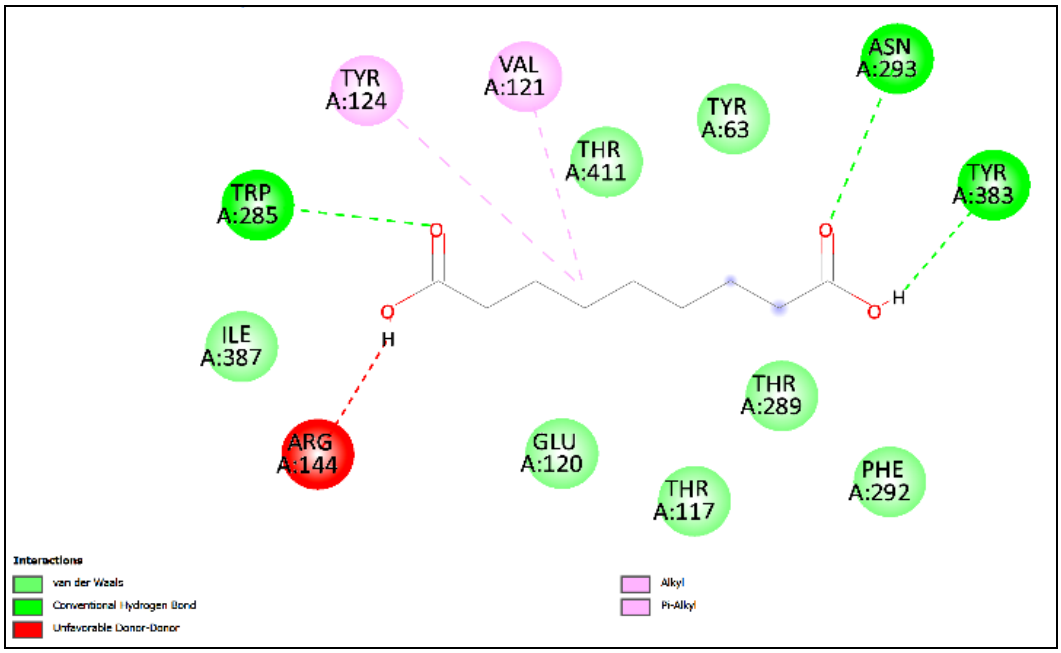
2D Diagram of Protein-Ligand Complex



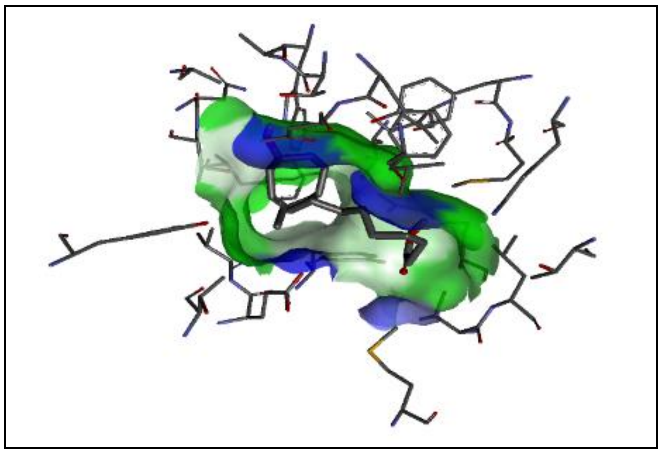
1. Ricinoleic Acid



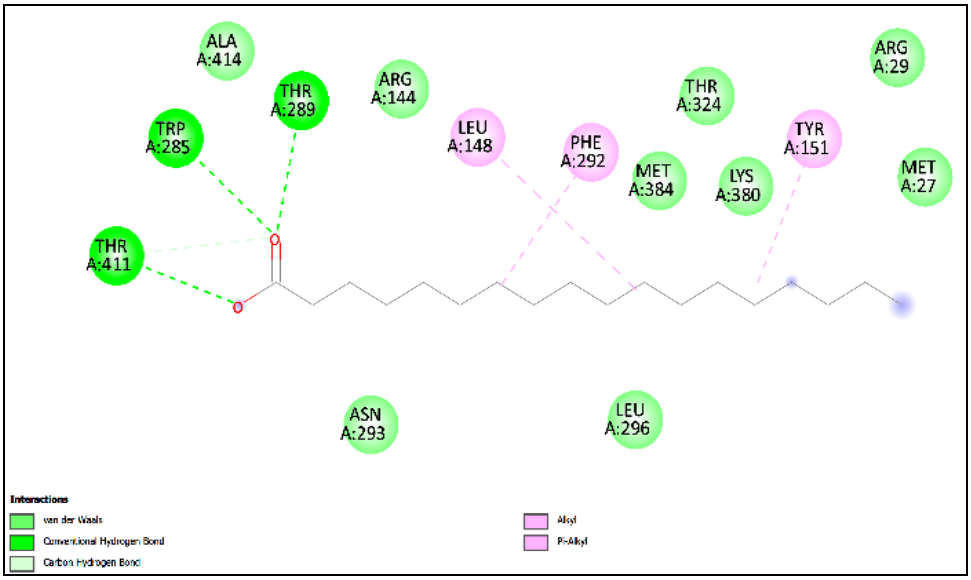
2. Elaidic Acid



3. Isolinoleic Acid

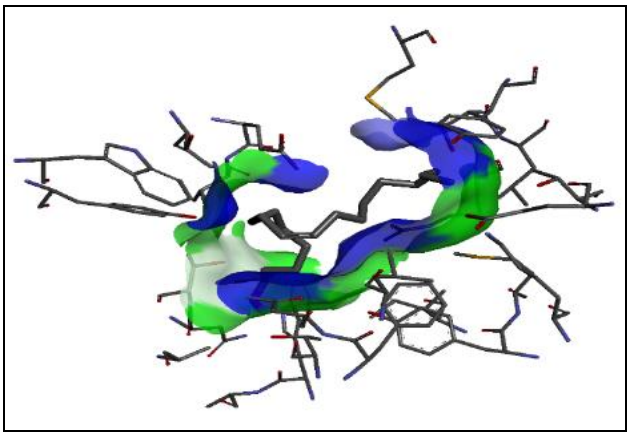


4. Stearic Acid

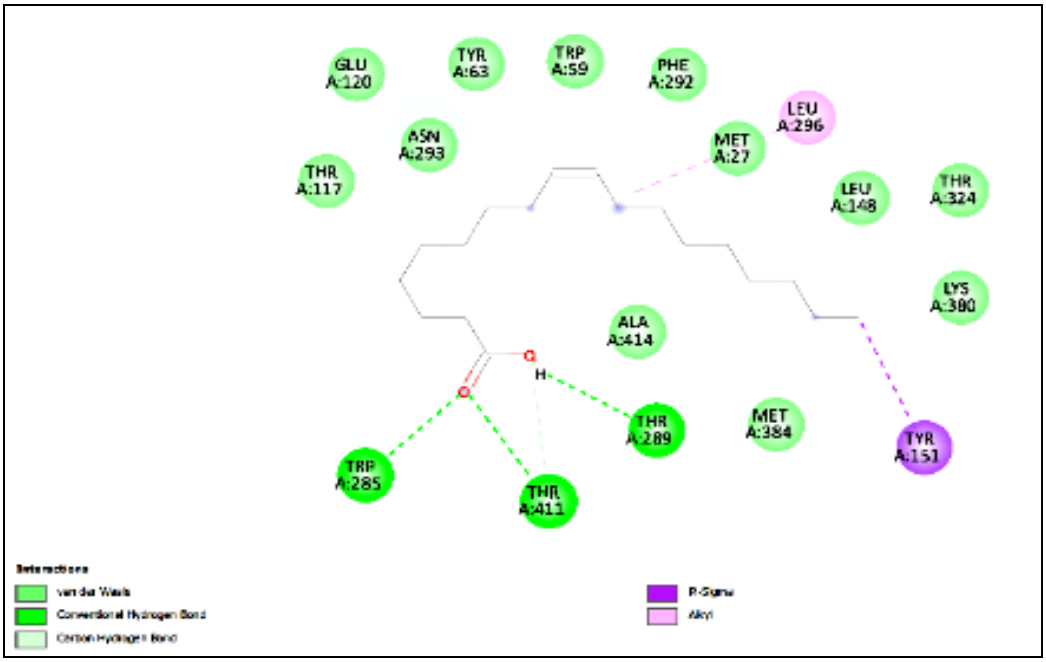


5. Azelaic Acid

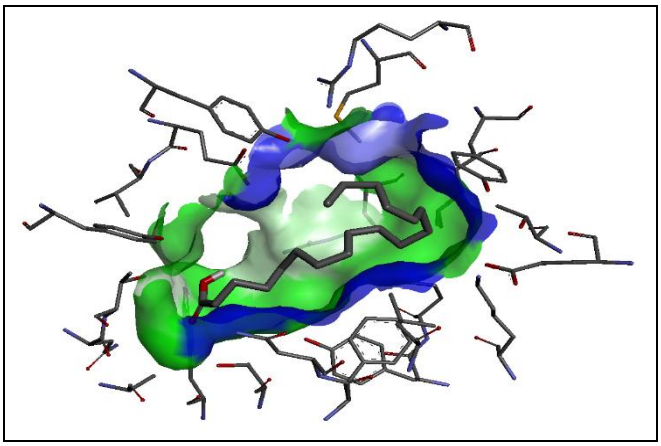
Docking Protein 8z7w
3D Picture Protein-Ligand Complex



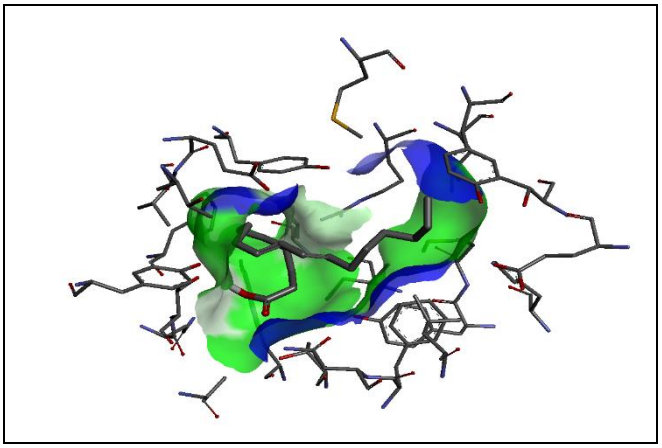
1. Protein-Ricinoleic acid Complex



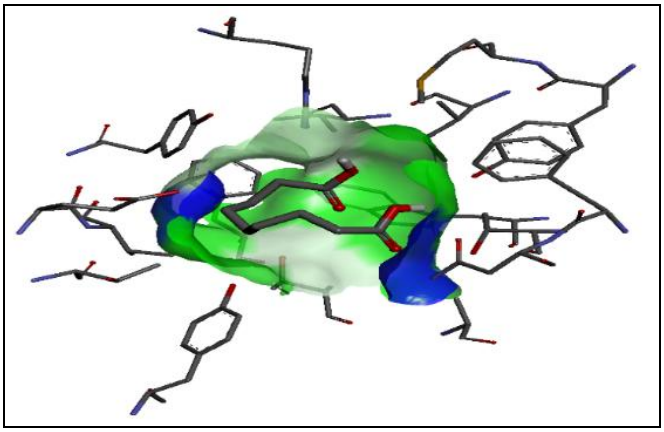
2. Protein-Elaidic Acid Complex



3. Protein-Isolinoleic Acid Complex



4. Protein-Stearic Acid Complex



5. Protein-Azelaic Acid Complex Docking Protein 8z7w

ADMET Study of the phytoconstituents of *Trigonella foenum* (Fenugreek) were done by using study Tools like 'SWISS ADME' and 'ADMET LAB 2'. For the study, SMILES of Ricinoleic acid, Elaidic Acid, Isolinoleic Acid, Stearic Acid and Azelaic Acid were collected from 'PubChem' Website. SWISS ADME analysis of Water Solubility [Log S (ESOL)], Drug likeness - Lipinski and Synthetic Accessibility showed good results (Table 4). ADMET LAB 2 analysis included Golden Triangle, $F_{20\%}$, Volume of distribution, Metabolism, Excretion and Toxicity showed positive results (Table 5).

Table 4: ADME Selected Compound - SWISS ADME

Sl no.	Name	Molecular Formula	Molecular Weight (g/mol)	SMILES	Water Solubility Log S (ESOL)	Druglikeness, Lipinski	Synthetic Accessibility
1	Ricinoleic acid	C18H34O3	298.46	CCCCCC[C@H](C/C=C\CCCCCCCC(=O)O)O	-4.56 Moderately soluble	Yes, 0 Violation	3.73
2	Elaidic Acid (9-Octadecenoic acid)	C18H34O2	282.46	CCCCCCCC/C=C/CCCCCCCC(=O) O	-5.41 Moderately soluble	Yes, 1 Violation	3.07
3	Isolinoleic Acid (9E,11E-Octadecadienoic acid)	C18H32O2	280.45	CCCCCC/C=C/C=C/CCCCCCCC(=O) O	-5.10 Moderately soluble	Yes, 1 Violation	3.35
4	Stearic Acid	C18H36O2	284.48	CCCCCCCCCCCCCCCCCC(=O) O	-5.73 Moderately soluble	Yes, 1 Violation	2.54
5	Azelaic acid	C9H16O4	188.22	C(CCCC(=O)O) CCCC(=O)O	-1.47 Very soluble	Yes, 0 Violation	1.57

Table 5: ADME Selected Compound - ADMET LAB 2

Sl no.	Name	Molecular Formula	SMILES	Golden Triangle	$F_{20\%}$	Volume Distribution	Metabolism	Excretion Clearance	Acute Toxicity
1	Ricinoleic acid	$C_{18}H_{34}O_3$	CCCCCC[C@H](C/C=C\CCCCCCCC(=O) O)O	Accepted	Low	0.5.8 Good	4.13	3.872 Low	0 Alert
2	Elaidic Acid. (9-Octadecenoic acid)	$C_{18}H_{34}O_2$	CCCCCCCC/C=C/CCCCCCCC(=O) O	Accepted	Low	0.858 Good	3.43	2.414 Low	0 Alert
3	Isolinoleic Acid (9E,11E-Octadecadienoic acid)	$C_{18}H_{32}O_2$	CCCCCC/C=C/C=C/CCCCCCCC(=O) O	Accepted	Moderate	0.608 Good	3.34	1.652 Low	0 Alert
4	Stearic Acid	$C_{18}H_{36}O_2$	CCCCCCCCCCCCCCCCCC(=O) O	Accepted	Moderate	0.752 Good	3.81	2.425 Low	0 Alert
5	Azelaic acid	$C_9H_{16}O_4$	C(CCCC(=O)O) CCCC(=O)O	Rejected	High	0.26 Good	2.29	1.727 Low	0 Alert

Discussion

In-vitro study investigates glucose metabolism in Diabetes, focusing on enzymatic activity. Using starch solution as a glucose source, the research simulated enzymatic hydrolysis, highlighting the role of starch breakdown in postprandial glucose regulation and the value of *in-vitro* methods for studying metabolic processes. 20

Alpha-amylase, a key enzyme in starch digestion, was examined alongside its inhibitors. The study showed that alpha-amylase inhibitors effectively reduced glucose production, demonstrating their therapeutic potential in managing postprandial hyperglycemia in Type 2 Diabetes by delaying carbohydrate metabolism. The findings emphasize the importance of in-vitro methods as complementary to computational and clinical approaches in Diabetes management. 21

This *in silico* study investigates glucose metabolism and its regulation using computational modeling. Starch solution was employed as a glucose source to simulate enzymatic hydrolysis during carbohydrate digestion. The computational approach effectively highlights the dynamics of glucose release, demonstrating the potential of in silico methods to predict metabolic responses and enhance therapeutic strategies for Diabetes management. 22

The study focuses on alpha-amylase, an enzyme critical for starch breakdown, and explores the inhibitory effects of alpha-amylase inhibitors. These inhibitors, known to control postprandial hyperglycemia in Type 2 Diabetes, were found to significantly reduce glucose release in computational models. 23

The findings align with experimental studies and emphasize the inhibitors' therapeutic potential for delaying carbohydrate digestion and improving blood sugar regulation. *In silico* methods are shown to be vital for complementing experimental research by accelerating insights into enzymatic processes and identifying effective interventions for Diabetes care. 24

Fenugreek (*Trigonella foenum*-graecum) leaves are rich in phytoconstituents like Ricinoleic acid, Elaidic acid, Stearic acid, and Azelaic acid, which exhibit antidiabetic properties. *In-vitro* studies demonstrate that Azelaic acid effectively reduces blood glucose levels by modulating insulin sensitivity and inhibiting glucose absorption. These findings highlight its potential in managing Type 2 Diabetes by targeting key metabolic pathways. 25

In-silico docking studies further reveal Azelaic acid's strong binding affinity to enzymes involved in glucose metabolism, such as alpha-amylase. By inhibiting alpha-amylase activity, Azelaic acid delays carbohydrate digestion, reducing postprandial hyperglycemia. Computational models validate its role in improving glycemic control and mitigating Diabetes-related complications.

The combined in-vitro and in-silico approaches underscore the therapeutic potential of fenugreek's phytoconstituents, particularly Azelaic acid, in Diabetes management. These methods provide valuable insights into molecular interactions and pave the way for developing effective antidiabetic treatments.

Conclusion

In conclusion, the *in-vitro* and *in-silico* docking studies strongly indicate the positive antidiabetic potential of fenugreek leaves. Through a combination of biochemical assays and computational modeling, these studies highlight the efficacy of fenugreek's phytoconstituents, particularly Azelaic acid, in regulating glucose metabolism and mitigating postprandial hyperglycemia. The inhibition of key enzymes, such as alpha-amylase, underscores its role in controlling blood sugar levels and managing Type 2 Diabetes effectively. These findings reinforce the therapeutic value of fenugreek leaves as a natural antidiabetic agent and provide a solid foundation for further research into their clinical applicability in Diabetes management.

Conflict of Interest

Authors declare that there is no conflict of interest.

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