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In Vitro Antidiabetic Activity of Ethanolic Extract from Justicia tranquebariensis: Insights from GC-MS Analysis and Computational Docking

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The research described focuses on exploring the potential antidiabetic properties of Justicia tranquebariensis, a medicinal plant from the Acanthaceae family. The dried and powdered leaves of Justicia tranquebariensis were extracted using ethanol, employing the Soxhlet extraction method. This process is common in phytochemical extraction to obtain bioactive compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on the ethanol extract. GC-MS is a technique used to identify the chemical composition of a substance, in this case, the phytoconstituents present in the plant extract that might contribute to its antidiabetic properties. After identifying the phytoconstituents through GC-MS, in silico docking tests were conducted. Docking is a computational technique used to predict how a small molecule (ligand) interacts with a target protein (receptor).The study found that 2-furancarboxyaldehyde, a compound identified in the GC-MS analysis, effectively docked with the alpha amylase receptor. This suggests a potential mechanism through which the plant extract might exert its antidiabetic effects. The antidiabetic activity of the ethanolic extract of Justicia tranquebariensis was further evaluated using an in vitro method that involved testing its ability to inhibit beta galactosidase enzyme. The results indicated that the ethanolic extract at a concentration of 500 µg/ml exhibited 86.82% inhibition of beta galactosidase. This inhibition level was compared to conventional metformin, which showed 87.95% inhibition. This demonstrates significant enzyme inhibitory activity, supporting the plant's traditional use for diabetes. The study concludes that Justicia tranquebariensis possesses promising antidiabetic properties, as evidenced by its inhibition of beta galactosidase and the docking results with alpha amylase. However, there is a need for further clinical research to validate these findings and to explore the efficacy of the plant extract in managing diabetes in human subjects.

Keywords: Ethanolic extract, Justicia tranquebariensis, Beta galactosidase, and Antidiabetic activity.

1. Introduction

Chronic hyperglycemia is a hallmark of diabetes, a metabolic condition that can cause significant harm to the heart, blood vessels, eyes, kidneys, nerves, and heart over time. Usually affecting adults, type 2 diabetes is the most prevalent kind and is brought on by an insufficient or resistant response to insulin in the body. Countries of all income levels have seen a sharp increase in the prevalence of type 2 diabetes over the last three decades. In type 1 diabetes, sometimes referred to as juvenile diabetes or insulin-dependent diabetes, the pancreas generates little or no insulin on its own. It is a chronic illness. The availability of inexpensive medical care, such as insulin, is essential for the survival of those with diabetes. Stopping the rise in diabetes and obesity by 2025 is a goal that has been widely accepted.

A quarter of a million deaths globally are directly linked to diabetes, and 422 million individuals with the disease live mostly in low- and middle-income nations. During the past few decades, there has been a steady rise in both the number of cases and the prevalence of diabetes. [1]

These days, medication, food therapy, and insulin therapy are among the several diabetic treatments available. Various glucose-lowering medications have anti-diabetic effects through distinct mechanisms. These mechanisms include the following: biguanides and thiazolidinediones increase peripheral glucose absorption, alpha-glucosidase delays intestinal carbohydrate absorption, and biguanides reduce hepatic gluconeogenesis. Sulfonylurea and meglitinides stimulate insulin secretion. Even while diabetes treatment has advanced significantly over the last three decades, patient outcomes are still far from ideal. Some drawbacks of these treatments are side effects, toxicity, and medication resistance, which is a decrease in efficacy. [2]

Justicia transquebariensis has been used traditionally in Ayurveda medicine to cure toxic bites. Juices from leaves are administered to children during small pox therapy and serve as aperients and coolants. On contusions, crushed leaves are administered. To lessen the pain, leaf paste is applied topically to the swelling. To treat toothaches, apply root paste. The antidote for cobra bites is to apply leaf paste physically at the site of the bite, or administer about 20 ml of leaf juice orally. The application of plant extract in the treatment of arthritis and inflammation supports the use of medicinal plants. Historically, the leaves have been used as an expectorant, diuretic, antispasmodic, antiseptic, fever, rheumatism, asthma, pneumonia, tuberculosis, and to reduce swelling. It is used to treat skin disorders, cancer, edema, abscesses, and leprosy. [3]

1.1 Classification [4]

Kingdom :Plantae

Phylum :Streptophyta

Class:Equisetopsida

Subclass:Magnoliidae

Order: Lamiales

Family: Acanthaceae

Genus: Justicia

Species:Justicia tranquebariensis

Fig.1: Justicia tranquebariensis

Plant based medicine play a vital role in the treatment of diabetes. The aim of the research work was to study the invitro antidiabetic properties of Justicia tranquebariensis.

2. Material and Methods

Beta galactosidase, Sodium acetate, o-nitrophenyl b-D-galactopyranoside, Sodium carbonate, Magnesium chloride procured from merck.

2.1 Collection and Authentication

Justicia tranquebariensis was collected from the Thoothukudi district, Tamilnadu. The plant was authenticated by Dr.S.Mutheeswaran, M.Sc., M.Phil, Ph.D, Xavier research foundation, St. Xavier's college, Palayamkottai, Tamilnadu. The herbarium with voucher specimen No is XCH-40474.

2.2 Extraction of Plant material

The Soxhlet apparatus's thimble chamber held the crushed sample in this approach. The bottom flask was heated to evaporate the extraction solvent ethanol, which then condenses in the condenser and drips back. The liquid contents emptied into the bottom flask once more and

the process was repeated until they reached the siphon arm. The extraction technique uses 250 ml of ethanol in a Soxhlet apparatus for 48 hours after about 100 g of dry sample powder was weighed. After evaporating at 70 °C for eight hours, the extract was dried and concentrated.

2.3 GC-MS analysis of ethanolic extract of Justicia tranquebariensis

One of the most reliable methods for determining the identity and profile of secondary metabolites in both plant and non-plant species is gas chromatographic-mass spectrometry (GC-MS). Inorder to determine which bioactive substances in Justicia tranquebariensis ethanolic extract may be in charge of the plant's antidiabetic properties. The GC-MS analysis that is being done was completed. The extract included 35 phytoconstituents, according to the GC-MS data.

2.4 Insilico docking study[5-7]

2.4.1 Preparation of Ligand

The phytocompound named 2-Furancarboxyaldehyde (PubChem CID: 237332) was downloaded from the PubChem website in 3D SDF format and visualized using BIOVIA Discovery Studio software and converted from SDF to PDB format. Preparation of ligand was performed using AutoDock 4.2.6 software. 3D structure of 2-Furancarboxyaldehyde (PubChem CID: 237332) was shown in Figure 2.

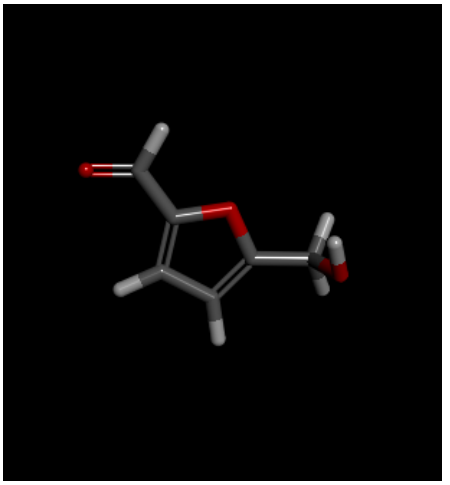


Figure 2: 3D structure of 2-Furancarboxyaldehyde

In 2-Furancarboxyaldehyde Gasteiger charges merged 5 non polar hydrogens and found 4 aromatic carbons and rotatable bonds in 2-Furancarboxyaldehyde was 3. PDB format of 2-Furancarboxyaldehyde was converted to PDBQT format using AutoDock 4.2.6 software.

2.4.2 Preparation of Target protein

The selected target protein was Human pancreatic alpha-amylase (PDB id: 2QV4) which was determined by X-ray diffraction method with the resolution of 1.97 Å and having single chain named A. The PDB format of the target protein was retrieved from RCSB Protein Data Bank

database and visualized and optimized using BIOVIA Discovery Studio software. The optimization of the target protein was performed by deleting the heteroatoms and water molecules. The polar hydrogens was added and active site was determined and attributes of xyz were 2QV4 (13.758000, 58.583058, 22.465406) was noted. Target protein was converted from PDB to PDBQT format using AutoDock 4.2.6 software. The optimized target protein was subjected to the molecular docking study. Figure 3, shows the 3D structure of target protein.

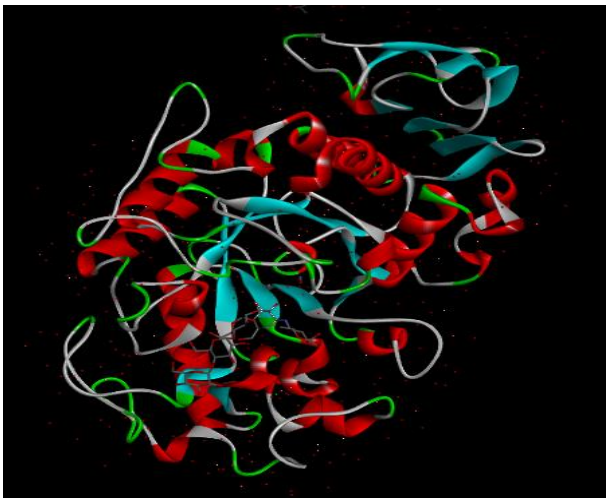


Figure 3: Structure of target protein

2.4.3 Molecular Docking study

The optimized ligand 2-Furancarboxyaldehyde was docked with Human pancreatic alpha-amylase using AutoDock vina software. To perform molecular docking, conf file was prepared and subjected to molecular docking. Figure 4: shows the optimized target protein,

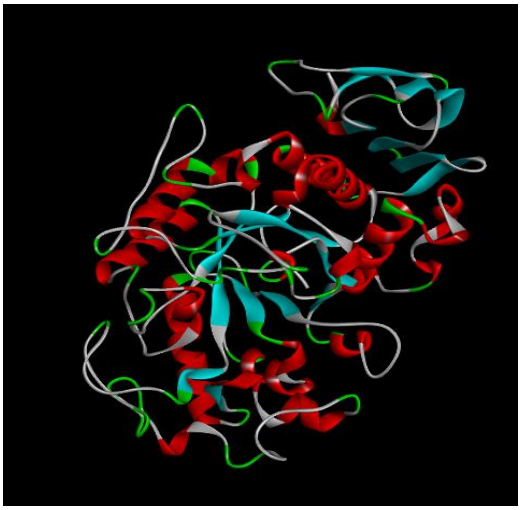


Figure 4: Structure of optimized protein

Lamarckian genetic algorithm was followed with the maximum 2.5 million energy evaluations were subjected for exploration of molecular docking analysis. Molecular Docking was performed using AutoDock vina comments using command prompt. The topmost conformation of the docked complex was interpreted using BIOVIA Discovery Studio

2.5 Invitro antidiabetic activity

2.5.1 Beta Galactosidase activity:

For the inhibition of β-galactosidase activity,a total of 0.5 mL (500, 250, 100, 50 and 10 µg/ml) of the different concentration of (JT) sample was pre-incubated with β-galactosidase in Na-acetate buffer at room temperature for 20 min. Then 0.5 mL of substrate mixture (8.3 mM o-nitrophenyl b-D-galactopyranoside, 1 mM MgCl2, and 0.1 M b-mercaptoethanol in 0.1 M Na-phosphate buffer, pH 7.0) was added to the sample mixture. After the incubation at 30°C for 20 min, the reaction was terminated with 0.5 mL of 0.5 M Na_2CO_3buffer. Release of o-nitrophenol was recorded at 420 nm using a microplate reader (Thermo scientific, USA). Each measurement was performed with three independent biological triplicates using 96 well plates. Inhibition of enzyme activity was determined by using the following formula.[8]

$$\text{Percentage of inhibition (\%)} = \frac{\text{Absorbance of control- Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

3. Results and Discussion

Diabetes mellitus is a condition where the body's tissues are unable to utilize glucose, which causes an increase in the use of proteins and a subsequent decrease in body weight. [9]

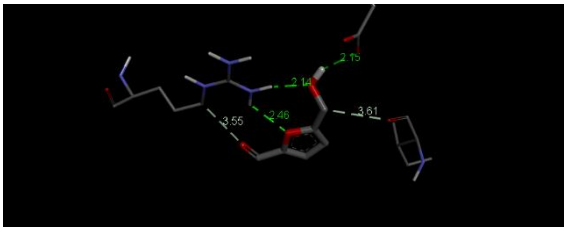
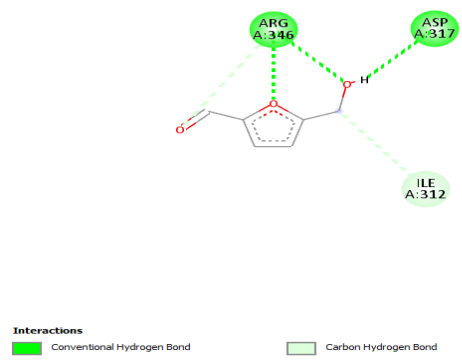
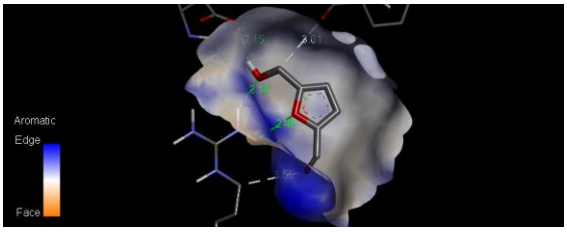
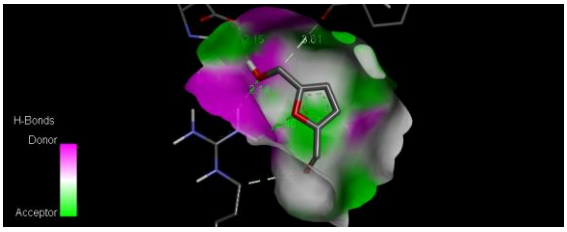
The two most prevalent forms of diabetes mellitus, type 1 and type 2, both result in hyperglycemia. An extremely severe state of insulin shortage leads to the death of pancreatic cells, which is the hallmark of type 1 diabetes mellitus, an autoimmune illness. Conversely, type 2 diabetes mellitus is more widely recognized and impacts 90 to 95% of all people with diabetes. Abnormal insulin secretion and peripheral insulin resistance are its defining characteristics. Even though diabetes is a non-communicable disease, experts caution that the disease is still serious because there will be about 438 million diabetics worldwide by 2030. [10]

The inhibition of α-glucosidase and β-galactosidase has the ability to decrease the release of glucose and monosaccharide from carbohydrates following a meal, hence contributing to blood sugar regulation and the prevention of hyperglycemia. Acarbose and voglibose, two synthetic inhibitors of α-glucosidase, are frequently taken orally as antidiabetic medications for the treatment of type 2 diabetes mellitus. However, both medications may have unfavorable side effects. [11]

The possibility for treating hyperglycemic properties exists in the Justicia species. GC-MS analysis was done to determine which phytoconstituents were present in the compound. 35 chemicals were found in the ethanolic extract of Justicia tranquebariensis according to the results of the GC-MS analysis.

Literature review suggested that the compunds of 2(5H)-Furanone, [12] 1,2-

Cyclopentanedione, [13] 2,5-Heptadecadione and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- [14] and 2-Furancarboxaldehyde, 5-(hydroxymethyl)- [15-16] have possessed the antidiabetic property. Based on the literature review 2-Furancarboxaldehyde was docked with Human pancreatic alpha-amylase (PDB id: 2QV4). The docking study's findings confirm the ligand's predicted anti-diabetic effect.The results were depicted in the figure 5 and table 1.

S.no	Images
3D image	
2D image	
Aromatic	
Hbond	

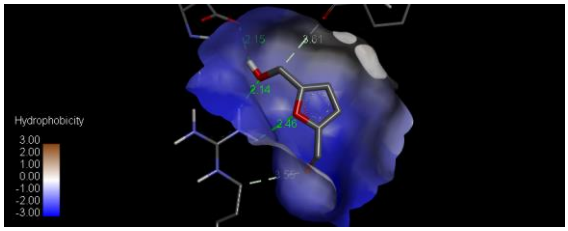
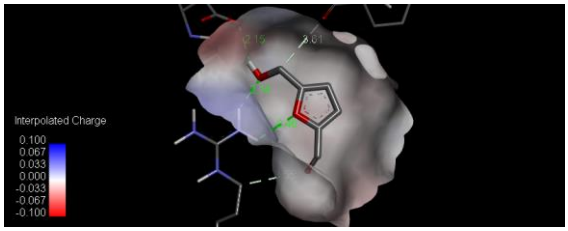
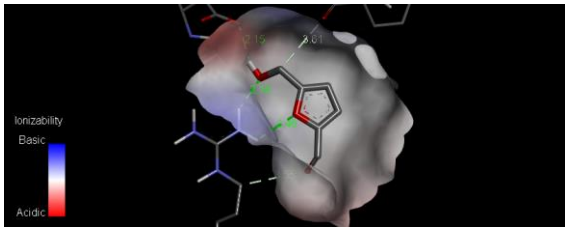
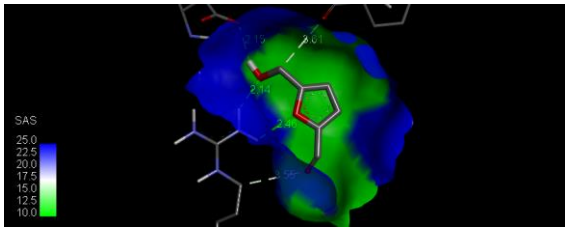
Hydrophobicity	
Charge	
Ionizability	
SAS	

Figure 5: Docked poses of 2-Furancarboxaldehyde with alpha amylase

Table 1: Docking score of 2 Furancarboxyaldehyde

S.No	Binding affinity	Bonds	Amino acid Residues
2QV4_2 Furancarboxyaldehyde	-4.7 Kcal/mole	3 Hydrogen bonds	ARG (A) 346- 2.46 Å ARG (A) 346- 2.14 Å ASP (A) 317- 2.15 Å
		2 Carbon hydrogen bonds	ARG (A) 346- 3.55 Å ILE (A) 312- 3.61 Å

Further it was evaluated by β-galactosidase activity. The percentage inhibition of Justicia tranquebariensis showed the 86.82% compared with metformin 87.95%. It proves the ethanolic extract of Justicia tranquebariensis antidiabetic potential. The results of the findings are shown in the figure 6&7 and table 2.

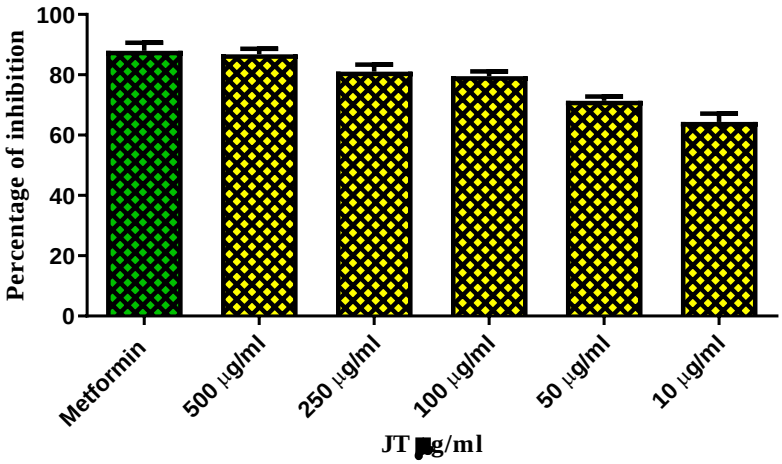


Fig.6: Percentage inhibition of Justicia tranquebariensis

Table 2: Percentage inhibition of enzyme action on Justicia tranquebariensis

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Metformin	86.12288	86.75847	90.99576	87.95904
2.	500 µg/ml	88.2415	84.8517	87.3941	86.8291
3.	250 µg/ml	78.8136	80.7203	83.4746	81.0028
4.	100 µg/ml	77.7542	79.9788	80.8263	79.5198
5.	50 µg/ml	72.5636	71.7161	69.8093	71.363
6.	10 µg/ml	61.6525	64.3008	67.161	64.3715

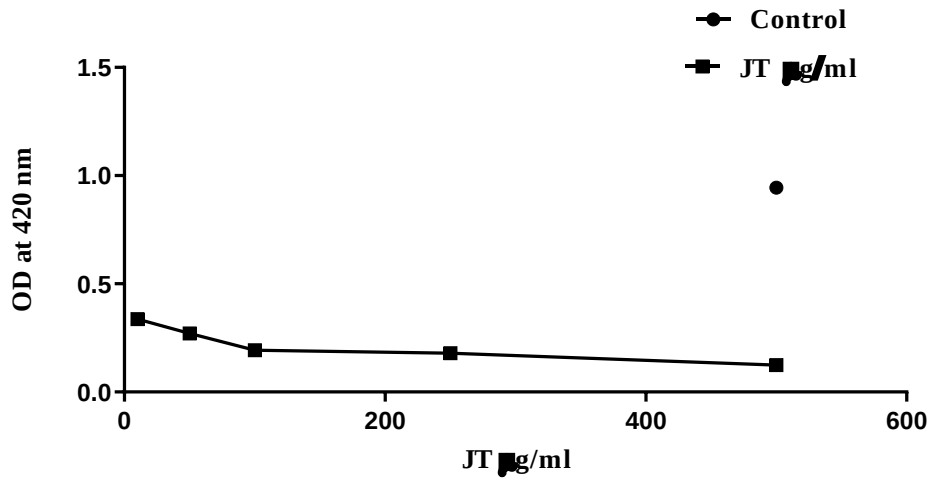


Fig .7 : Absorbance against concentration of ethanolic extract of Justicia tranquebariensis

4. Conclusion

The present study's findings confirm the potential of Justicia tranquebariensis as an antidiabetic agent, marking the first instance where its antidiabetic effects have been scientifically verified. This beneficial activity is likely due to phytoconstituents and secondary metabolites present in the ethanolic extract of the plant. Given these results, Justicia tranquebariensis could potentially serve as a safer alternative to existing traditional medicines currently available on the market. Further clinical investigations are warranted to explore and validate its effectiveness for diabetes management. Additionally, it is crucial to delve into the precise mechanisms underlying its unique characteristics in exerting antidiabetic effects.

In summary, the study underscores the promising role of Justicia tranquebariensis in diabetes treatment, encouraging continued research into its therapeutic potential and mechanisms of action.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data analyzed in this article are available from the author on reasonable request.

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