RESEARCH
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ALPHA GLUCOSIDASE INHIBITION ACTIVITY OF MIMOSA PUDICA LINN

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Abstract

Introduction: Diabetes is a metabolic disorder of high blood sugar levels which leads to various chronic health-related complications. The digestive enzymes *α*-amylase and *α*-glucosidase play a major role in the hydrolysis of starch to glucose; hence, inhibiting these enzymes is considered an important strategy for the treatment of diabetes. Medicinal plants such as *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica* are commonly used in traditional remedies due to their numerous health benefits.

Aim and Objective: To evaluate the alpha glucosidase inhibition activity of *Mimosa pudica* and to find the alpha glucosidase inhibition activity of *Mimosa pudica.*

Materials and Methodology: Cell viability assay and molecular docking were analyzed on *Mimosa pudica Linn.* Biologically safe or noncytotoxic concentration of plant extracts and cytotoxicity of ethanol were identified using MTT and NRU assays. Molecular docking was also performed.

Results: The results were expressed as percentage inhibition, which was calculated using the formula,
Inhibitory activity (%) = (1 − As/Ac) ×100. The ethanolic plant extract was compared with acarbose. Various concentrations such as 2 mg, 4 mg, 6 mg, 8 mg and 10 mg were considered.
When comparing the standard with the ethanolic plant extract, significant similarities were observed

Conclusion: From this study it can be concluded that *Mimosa pudica* plays a role in alpha glucosidase inhibition and can be useful in controlling diabetes.

Keywords: Mimosa pudica, alpha glucosidase inhibition, molecular docking, cell viability, MTT assay, NRU assay.

INTRODUCTION:

Diabetes is a common metabolic disease characterized by abnormally high plasma glucose levels, leading to major complications, such as diabetic neuropathy, retinopathy, and cardiovascular diseases(1). It has also been used for centuries in traditional medicines to cure different diseases like fever, diabetes, constipation, jaundice, ulcers, biliousness, and dyspepsia(2).Alpha-glucosidase inhibitors are important therapeutic agents used in the management of diabetes mellitus. They work by slowing down the digestion of carbohydrates in the small intestine, which leads to a reduction in postprandial glucose levels. This mechanism helps in controlling blood glucose spikes after meals and improves overall glycemic control.

Mimosa pudica Linn, commonly known as the "Sensitive Plant" or "Touch-Me-Not," is a medicinal plant that has been used in traditional medicine for various health conditions, including diabetes. It is known for its diverse bioactive compounds, such as alkaloids, flavonoids, and tannins, which contribute to its pharmacological properties.

Alpha-glucosidase is an enzyme that plays a role in breaking down carbohydrates into simple sugars in the digestive tract. Inhibition of alpha-glucosidase can help slow the absorption of glucose from the digestive system, which is beneficial for individuals with diabetes to better control their blood sugar levels.(1,2)

The in vitro assay will involve incubating the enzyme with the Mimosa pudica Linn extract and a substrate, followed by measuring the release of glucose using a colorimetric method. Acarbose, a known alpha-glucosidase inhibitor, will be used as a positive control for comparison.Understanding the alpha-glucosidase inhibition activity of Mimosa pudica Linn extract can provide valuable insights into its potential as a natural anti-diabetic agent(3). If the extract shows significant inhibition, it could serve as a basis for further research to isolate and identify the specific bioactive compounds responsible for the inhibitory activity.(4)

The use of natural products like Mimosa pudica Linn in diabetes management offers several advantages, including fewer side effects compared to synthetic drugs. (3,5)Furthermore, harnessing the therapeutic potential of traditional medicinal plants can contribute to the development of more accessible and affordable treatments for diabetes, particularly in resource-limited settings.(1)

This study aims to investigate the alpha-glucosidase inhibition activity of Mimosa pudica Linn extract. The plant material will be collected, dried, and extracted using appropriate solvents(5). The resulting extract will be evaluated for its inhibitory effects on alpha-glucosidase enzyme activity(6).

MATERIALS AND METHODOLOGY:

This research has been conducted under the department of forensic odontology at Saveetha Dental College. Duration of this study is 5 months

Materials:

Plant Materials from Mimosa pudica: The plant material from Mimosa pudica was gathered, including leaf and stem extracts. After drying, the plant material was ground into a fine powder in preparation for extraction.

Solvents: Common solvents employed in the extraction of the plant extract included ethanol.

Alpha-Glucosidase Enzyme: The in vitro experiment used purified alpha-glucosidase enzyme. This enzyme is available for purchase. The alpha-glucosidase inhibition assay uses p-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate.

Alpha-glucosidase inhibitor, or acarbose, is a positive control or standard that is used to compare the activity.

Centrifuges, spectrophotometers, microplate readers, and other tools required for sample extraction and analysis are included in the category of laboratory equipment.

Methodology:

Plant Extraction:

The dried and ground Mimosa pudica plant material is typically macerated or extracted with a suitable solvent (i.e Ethanol).The extraction may be performed using different techniques, such as maceration, sonication, or Soxhlet extraction. The extract is then concentrated and dried to obtain a crude extract.

Preparation of Enzyme and Substrate:

The alpha-glucosidase enzyme is prepared by dissolving it in a buffer solution(phosphate buffer). The substrate pNPG is prepared by dissolving it in a buffer solution as well.

Alpha-Glucosidase Inhibition Assay:

A series of test solutions are prepared, including the crude extract of *Mimosa pudica*, positive control (acarbose), and negative control (buffer).The enzyme and substrate are mixed with the test solutions, and the reaction is initiated.The reaction progress is monitored by measuring the absorbance at a specific wavelength over time using a spectrophotometer or microplate reader.The inhibition of alpha-glucosidase by the *Mimosa pudica* extract is determined by comparing its effects on the rate of substrate hydrolysis to that of the positive and negative controls.

Data Analysis:

The percentage inhibition of alpha-glucosidase by the *Mimosa pudica* extract is calculated based on the absorbance data.Graphs may be generated to visualize the dose-response relationship, and IC50 values (concentration required for 50% inhibition) was then calculated.

Statistical analysis:

Cell viability assay on *Mimosa pudica Linn:*

Evaluations of the cytotoxicity of ethanol and the biosafety of plant extracts The cytotoxicity of ethanol and the biologically safe or noncytotoxic concentration of plant extracts were determined by neutral red uptake (NRU) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tests. Plant extracts at different concentrations (10–1000 mg/ml) were applied to HepG2 cells for a full day. To test ethanol's cytotoxicity, HepG2 cells were exposed to a range of doses (50–1000 mM) for a full day. MTT assay Percentage cell viability was assessed using MTT assay as described (Siddiqui et al., 2008).

Briefly, cells (1 104) were allowed to adhere for 24 h in a CO2 incubator at 37C in 96-well culture plates. After the respective exposure, MTT (5 mg/ml of stock in phosphate-buffered saline; PBS) was added (10 ml/ well in 100 ml of cell suspension) and the plates were incubated for 4 h. Then the supernatants were discarded and 200 ml of DMSO were added to each well and

mixed gently. The developed color was read at 550 nm using a multiwell microplate reader (Thermo Scientific, Vantaa, Finland). Untreated sets were also run under identical conditions and served as control.

Screening of docked molecules was performed based on highest binding energy. Docking of target enzyme-substrate was performed to determine the binding energy of interaction and analysis of the docking result was carried out for identification of potential inhibitor. phytochemicals were selected and plants were retrieved from extensive literature survey for ligand preparation act against MMP8. Their respective two-dimensional chemical structures in structured data format (SDF) were retrieved from PubChem-NCBI database and SDF format was converted into Protein data bank (PDB) format through OpenBabel 2.3.1 version. The chemical structure of Glucosidase acts as control. The three dimensional structure of Glucosidase(PDB ID:3L4Y) was obtained from Protein Data Bank. The receptor crystallographic water molecules were removed from the protein. The retrieved phytocompounds were individually used using Hex 8.0.0. Protein docking program (http://hex.loria.fr), the Hex server is the first Fourier Transform (FFT) based analytics. In this method, rigid docking is undertaken taking into consideration different orientations through 6D analysis.

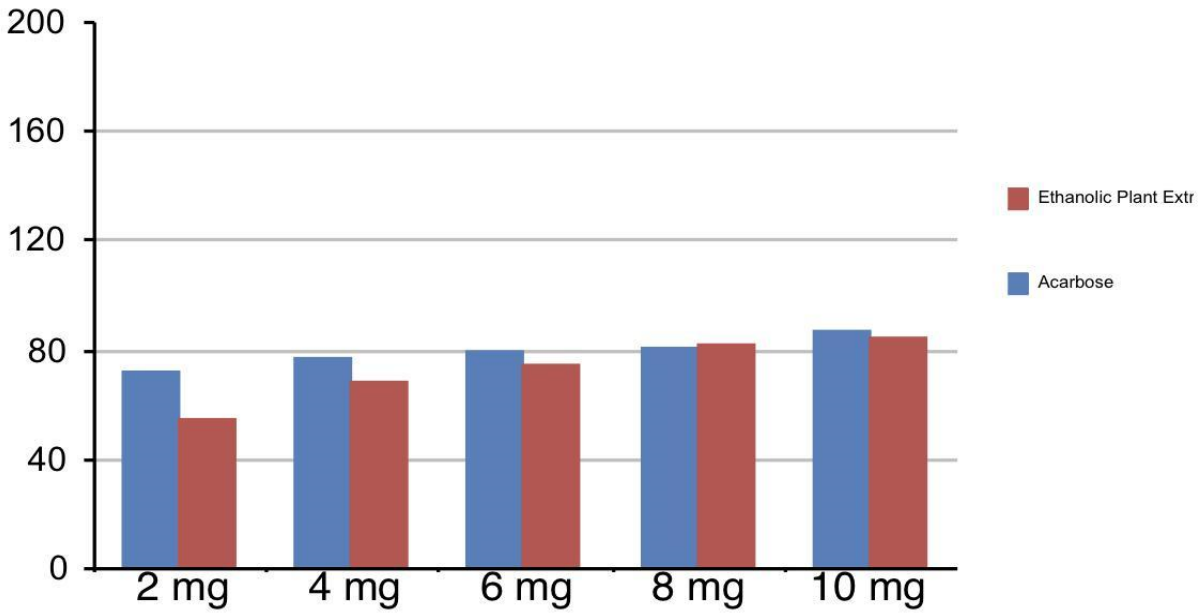
The HEX program carries out a complete search over all six rigid-body degrees of freedom by rotating and translating the expansion coefficients. This was carried out by maintaining suitable parameters such as FFT mode-3D fast lite, grid dimension-0.6, receptor range-180, ligand range-180, twist range-360 and distance range-40. Docked complexes of protein and ligand interaction were visualized in Pymol.In the Hex Docking server 8.0 versions, more negative E-total value implied that there exists a strong interaction between ligand and receptor and that leads to activation of receptor activity.

Methodology of Molecular Docking:

Screening of docked molecules was performed based on highest binding energy. Docking of target enzyme-substrate was performed to determine the binding energy of interaction and analysis of the docking result was carried out for identification of potential inhibitor. phytochemicals were selected and plants were retrieved from extensive literature survey for ligand preparation act against MMP8. Their respective two-dimensional chemical structures in structured data format (SDF) were retrieved from PubChem-NCBI database and SDF format was converted into Protein data bank (PDB) format through OpenBabel 2.3.1 version. The chemical structure of Glucosidase acts as control. The three dimensional structure of Glucosidase(PDB ID:3L4Y) was obtained from Protein Data Bank. The receptor crystallographic water molecules were removed from the protein.The retrieved phytocompounds were individually used using Hex 8.0.0. Protein docking program (http://hex.loria.fr), the Hex server is the first Fourier Transform (FFT) based analytics. In this method, rigid docking is undertaken taking into consideration different orientations through 6D analysis. The HEX program carries out a complete search over all six rigid-body degrees of freedom by rotating and translating the expansion coefficients. This was carried out by maintaining suitable parameters such as FFT mode-3D fast lite, grid dimension-0.6, receptor range-180, ligand range-180, twist range-360 and distance range-40. Docked complexes of protein and ligand interaction were visualized in Pymol.In the Hex Docking server 8.0 versions, more negative E-total value implied that there exists a strong interaction between ligand and receptor and that leads to activation of receptor activity.

concentration	acarbose	Ethanolic plant extract
2mg	72	55
4mg	77	68
6mg	79	74
8mg	81	82
10mg	87	85

Table 1: shows the values of both ethanolic plant extract and the standard. There is a general trend toward an increase in the observed outcome as the acarbose concentration rises. Similar to this, there is a general trend for the measured outcome to increase with concentration in the case of the ethanolic plant extract. This implies that there may be a dose-dependent effect of the plant extract, with greater amounts having a more noticeable effect. As the quantities of acarbose and the ethanolic plant extract rise, so does the observed result. This suggests that both drugs have dose-dependent effects.



Graph 1: shows the inhibition activity. The % of inhibition is shown with the increase in concentration (2 mg, 4 mg, 6mg, 8 mg and 10 mg) in mean standard and extract inhibition. The above graph represents the above reference chart that addresses the relationship between the % of inhibition with increase in concentration of mean. The red colour denotes the ethanolic plant extract while the blue colour denotes the standard. SD:Standard deviation, acarbose

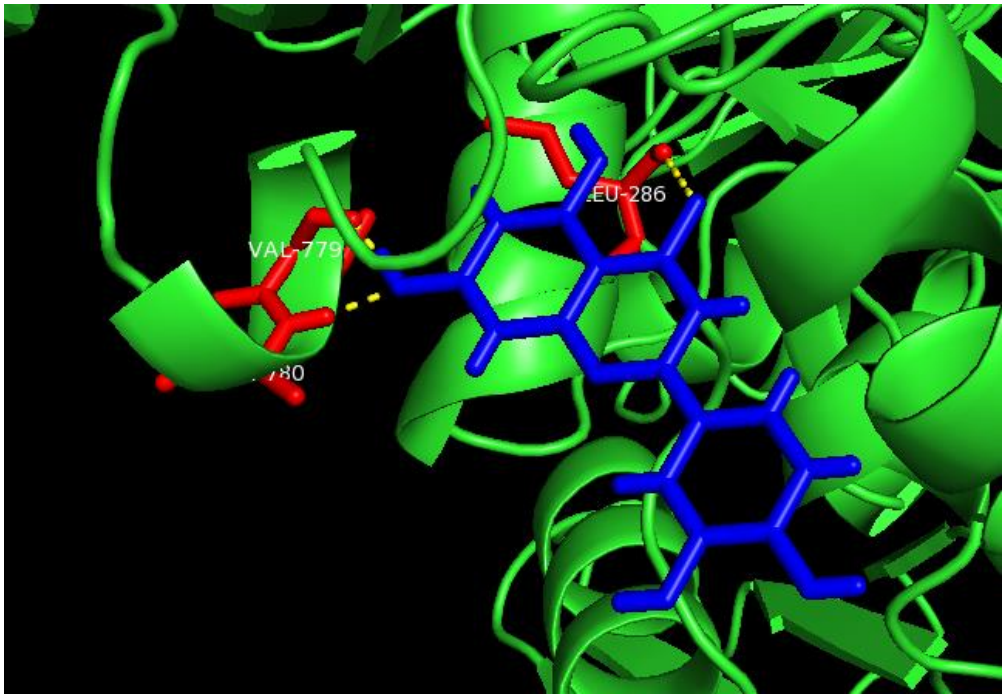


Fig 1: shows the molecular docking of *Mimosa pudica* plant. The chemical structures of the ligands (compounds from Mimosa pudica) and the receptor proteins are prepared for computational analysis. This may involve obtaining the three-dimensional structures of the molecules from databases or predicting them using computational methods.

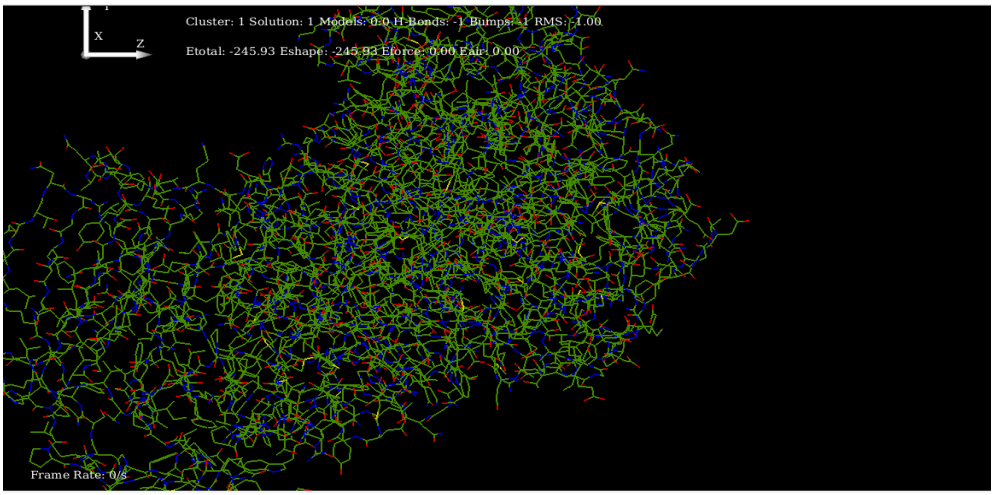


Fig 2: shows the complete cluster of the inhibitor. This indicates that the inhibitors being studied are derived from or related to compounds found in Mimosa pudica. These inhibitors could be usually natural compounds extracted from the plant or synthetic analogs designed to mimic the activity of natural compounds.

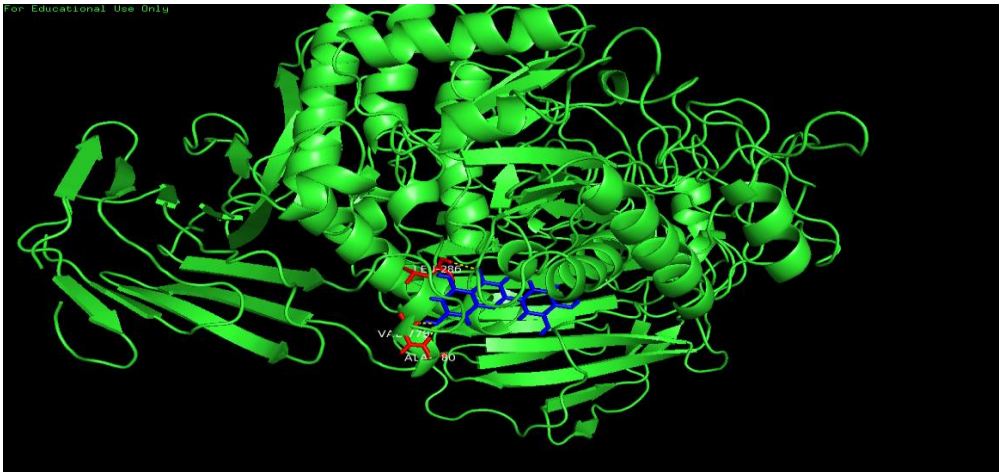


Fig 3: shows the selected region of the inhibitor.This indicates that the inhibitors being studied are derived from or related to compounds found in Mimosa pudica. These inhibitors could be usually natural compounds extracted from the plant or synthetic analogs designed to mimic the activity of natural compounds.

Concentration	Cell viability
control (without extract)	**100**
10	**100**
25	**98**
50	**93**
100	**90**
250	**83**
500	**80**
1000	**77**

Table 2: shows the values of cell viability. At a concentration of 1000, the cell viability drops to 77%, indicating a significant decrease in cell viability compared to the control. The results suggest that the extract has a concentration-dependent effect on cell viability, with higher concentrations showing a greater decrease in viability

RESULTS:
As a percentage of inhibition, the data were presented, and this was computed using the following formula: inhibitory activity (%) = (1 − As/Ac) ×100. Acarbose and the ethanolic plant extract were contrasted. Different dosages, including 2 mg, 4 mg, 6 mg, 8 mg, and 10 mg, were taken into consideration.
There were notable correlations between the ethanolic plant extract and the standard. The protein bound to the ligand is depicted in Figures 1 and 3. And the alpha glucose-inhibiting element, luteolin, is depicted in Fig 3.Plant extract displayed a value of 55 and acarbose a value of 72 at 2 mg. Acarbose displayed a value of 77 at 4 mg, while the plant extract displayed a value of 68. Acarbose had a value of 79 at 6 mg, while the plant extract displayed 74. At 8mg acarbose showed a value of 81 and plant extract showed 82. At 10 mg acarbose showed a value of 87 and plant extract showed 85.

DISCUSSION:
The alpha-glucosidase inhibition activity of Mimosa pudica suggests its potential as a natural remedy for diabetes management. Inhibition of alpha-glucosidase can slow the digestion and absorption of carbohydrates, leading to reduced post-meal blood sugar spikes. According to the present research it can be seen that there are potential alpha glucosidase inhibition properties that can be seen in *Mimosa pudica* plant extract. Drug resistance is still a major challenge that needs to be addressed with efficient methods, despite the fact that the pharmacological therapy for type 2 diabetes mellitus has improved to some level during the past 10 years.
According to research conducted by Lan PT et al; *Mimosa pudica* leaves were extracted with 80% of ethanol. Bioactive compounds were isolated using a column chromatographic technique and elucidated the structure based on the nuclear magnetic resonance and electrospray ionization mass spectrometry spectral data. The α- glucosidase and PTP1B inhibitory activities of the isolated compounds were evaluated using p nitrophenyl phosphate and p-nitrophenyl-α-D-glucopyranoside as a substrate, respectively. Molecular docking and molecular dynamics are used to study the interaction between isolated compounds and proteins. Lipinski's rule of five was used to evaluate the drug-like properties of isolated compounds. Predict pharmacokinetic parameters were evaluated using the pkCSM tool. My research aimed at evaluating cell viability molecular docking.
Another study conducted by Shreshtha et al; showed that the inhibitory activity against α-amylase and α-glucosidase was performed using CNPG3 and PNPG, respectively. Antioxidant activity was estimated using DPPH free radical scavenging assay. The well diffusion method was used for the antibacterial. Using folin- ciocalteu's reagent, the total phenolic content was determined. The total flavonoid content was determined using the aluminum trichloride method. In addition, molecular docking was performed using autodock vina.
Uday Hossain et al examined the most recent modifications in the category of bioactive -glucosidase inhibitors. This review gives a general overview of the -glucosidase inhibitory potentials and effectiveness of controlling postprandial hyperglycemia of various bioactive substances, including flavonoids, phenolic compounds, polysaccharide, betulinic acid, tannins, anthocyanins, steroids, polyol, polyphenols, galangin, procyanidins, hydroxyl--sanshool, erythritol.

CONCLUSION:
According to this study, Mimosa pudica has alpha glucosidase inhibition properties and after conducting further in-vivo studies can be used to treat patients with diabetes. Inhibiting or reducing the synthesis of glucose in the small intestine is one of the methods used to monitor blood glucose levels in people with type II diabetes mellitus. Inhibitors of glucosidase prevent the breakdown of carbs, improving glycemic management. Therefore, natural products with a wide range of structural variations are still a great choice to look for such inhibitors, which encourages researchers to investigate biologically active substances from the highly diverse plants. Several drawbacks persist despite the field's amazing advancements over the past few decades and the widespread use of docking techniques. In particular, protein flexibility is a significant obstacle in current protein ligand docking attempts that has to be more effectively taken into consideration in order to fully grasp the principles that govern ligand binding in proteins.

CONFLICT OF INTEREST:
The authors hereby declare that there is no conflict of interest in this study.

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3. Saveetha University

ETHICAL CLEARANCE:
Since this is an In-vitro study ethical clearance number is not required.

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