RESEARCH
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COMPUTATIONAL INVESTIGATION OF IMPORTANT PHYTOCOMPOUNDS AND IN SILICO ANTIDIABETIC ACTIVITY IDENTIFIED FROM *PENTATROPIS MICROPHYLLA*

T.Lokeshwar[1], Dr. Abirami Arthanari[2* vbg]
[1]Saveetha Dental College and Hospital ,
Saveetha institute of medical and technical sciences (SIMATS)
saveetha university,Chennai -600077
[2]Senior Lecturer, Department of Forensic Odontology,
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences, Chennai – 600077
Email : abiramia.sdc@saveetha.com

Abstract

AIM: Computational Investigation of important phytocompounds and in silico antidiabetic activity identified from *Pentatropis microphylla.*

INTRODUCTION: Pentatropis microphylla, a plant species of interest, has attracted attention for its potential therapeutic properties, including possible anti-diabetic effects. Computational investigations offer a valuable approach to explore the important phytocompounds present in Pentatropis microphylla and their potential anti-diabetic activity. Through techniques like molecular docking, molecular dynamics simulations, and virtual screening, computational studies can predict the interactions between these phytocompounds and key proteins involved in diabetes, providing insights into their binding affinity and mode of action.

MATERIALS AND METHODS:

Invitro Alpha-amylase inhibitory experiment: In a 100 ul reaction, 1 um of test extract and 100 ml of pH 6.9 phosphate buffer were combined with 100 ml of a 1% starch solution. Controls replaced 200ul of enzyme with buffer. After 5 minutes, 500ul of dinitrosalicylic acid reagent was added. Boiling for 5 minutes followed. Absorbance at 540 nm was measured, and % inhibition was calculated using [(Control - Test)/Control]*100, with reference to acarbose.

Cell viability assay on Pentatropis microphilla: To assess biosafety and ethanol cytotoxicity, we employed the MTT and NRU assays. HepG2 cells were exposed to various plant extract concentrations (10-1000 mg/ml) and ethanol (50-1000 mM) for 24 hours. The MTT assay determined cell viability: cells (1 x 10^4) adhered for 24 hours, followed by the addition of MTT (5 mg/ml in PBS) and a 4-hour incubation. After discarding supernatants, we added 200 µl of DMSO to each well, gently mixed, and measured the developed color's absorbance at 550 nm using a multiwell microplate reader (Thermo Scientific, Vantaa, Finland). Untreated sets served as controls for accurate assessment.

Molecular docking: Molecular docking was conducted to identify potential inhibitors of MMP8 based on binding energy. Phytochemicals from various plants, obtained from PubChem-NCBI database in SD format, were converted to PDB format using OpenBabel 2.3.1. Acarbose served as the control. The 3D structure of Alpha amylase (PDB ID: 2QV4) was acquired from the Protein Data Bank, with water molecules removed. Phytocompounds were individually docked with Alpha amylase using Hex 8.0.0 protein docking program, employing FFT-based analytics for rigid docking in 6D analysis. More negative E-total values indicated stronger ligand-receptor interactions, potentially activating receptor activity, visualized in Pymol.

RESULTS: In a computational study, crucial phytocompounds from *Pentatropis microphylla* exhibited promising in silico antidiabetic activity. Docking analysis with Alpha amylase (PDB ID: 2QV4) revealed strong ligand-receptor interactions, indicating their potential as inhibitors. These findings suggest that *Pentatropis microphylla* may contain bioactive compounds with the ability to combat diabetes, warranting further experimental validation.

DISCUSSION: Computational investigation of *Pentatropis microphylla'*s phytocompounds for in silico anti-diabetic activity involves virtual screening, molecular docking, and predictive modeling. This approach holds promise in discovering novel anti-diabetic compounds, aiding drug development, and providing insights into their mechanisms of action, accelerating diabetes research and potential therapeutic interventions.

CONCLUSION: In silico computational analysis of phytocompounds from *Pentatropis microphylla* revealed promising anti-diabetic potential, with strong binding affinities towards key diabetes-related targets. These findings suggest the potential efficacy of *Pentatropis microphylla* as a source of natural anti-diabetic agents, warranting further experimental validation and exploration for diabetes management.

KEYWORDS: Important phytocompounds- insilco activity- anti diabetical- *Pentatropis microphylla*- molecular docking.

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by high blood glucose levels, poses a significant global health burden. It affects millions of people worldwide, leading to various complications if not properly managed. Conventional medications for diabetes often come with side effects, making the exploration of natural remedies an attractive avenue for research. *Pentatropis microphylla*, a traditional medicinal plant found in certain regions, has garnered attention for its potential anti-diabetic properties.(1) This study aims to conduct a comprehensive computational investigation of the phytocompounds present in *Pentatropis microphylla* and their in-silico anti-diabetic activity.*Pentatropis microphylla*, commonly known as "Botomli" or "Bhokar" in some regions, is a member of the Apocynaceae family. This plant species is native to specific geographical areas and has been utilized in traditional medicine for various ailments, including diabetes(1,2). Local communities have historically relied on its medicinal properties, prompting scientific interest in understanding its therapeutic potential.In recent years, computational techniques have gained prominence in drug discovery and natural product research. Conducting in-silico investigations can significantly expedite the identification of potential bioactive compounds and their modes of action. Furthermore, computational approaches provide valuable insights into the interactions between molecules and target proteins, thus aiding in rational drug design.(3)

The first step in this computational investigation involves an exhaustive literature review to gather existing information on *Pentatropis microphylla.* By collating data from various sources, we aim to identify the key phytocompounds present in the plant. Previous studies on the phytochemical composition of *Pentatropis microphylla(4)*, including reports on alkaloids, flavonoids, terpenoids, and other bioactive compounds, will be taken into account.

In recent years, *Pentatropis microphylla* has gained attention as a promising candidate due to its reported anti-diabetic properties.The preliminary investigations of *Pentatropis microphylla* have involved in-silico approaches to identify potentially important phytocompounds with anti-diabetic activity. (4,5)In-silico studies, which rely on computational methods, play a crucial role in screening large chemical databases to pinpoint bioactive molecules and predict their interactions with target proteins implicated in diabetes.By conducting a literature review and employing advanced computational tools such as molecular docking, virtual screening, and ADME-Tox analysis, researchers have identified significant phytocompounds from Pentatropis microphylla that demonstrate potential anti-diabetic effects. These compounds have shown favorable binding interactions with key proteins involved in glucose metabolism and insulin signaling pathways, (6)suggesting their ability to modulate these crucial processes.The in-silico approach allows researchers to prioritize and analyze a wide range of phytocompounds from *Pentatropis microphylla* efficiently, reducing time and resources compared to traditional experimental screenings. Moreover, these computational insights provide valuable guidance for designing targeted in vitro and in vivo experiments to validate the anti-diabetic activity of these identified compounds.However, it is essential to emphasize that in-silico findings are preliminary and must be complemented by rigorous experimental studies to confirm the bioactivity and safety of the identified phytocompounds. (7)Furthermore, the potential of *Pentatropis microphylla* as a novel source of anti-diabetic agents highlights the significance of preserving and further exploring the rich biodiversity of medicinal plants to tackle the growing global health challenge posed by diabetes. Continued research and collaboration between computational scientists, (8)pharmacologists, and traditional medicine experts are crucial to unlock the full therapeutic potential of *Pentatropis microphylla* in combating diabetes and promoting human health.

MATERIALS AND METHODS

1.Invitro Alpha-amylase inhibitory experiment performed in vitro (Bernfeld, 1955)

In a nutshell, 100 ul of the a-amylase enzyme (Sigma, India) and 100 ml of phosphate buffer (2 mM, pH= 6.9) were allowed to react with 1 um of the test extract.

2. 3. 100 ml of a 1% starch solution was added after a 20-minute incubation period.

The same procedure was used for the controls, in which 200ul of the enzyme was swapped out for a buffer.

4. 500ul of the dinitrosalicylic acid reagent was added to both the control and the test after 5 minutes of incubation.

5. The tubes were boiled in water for five minutes. With the aid of a spectrophotometer, the absorbance was measured at 540 nm and

6. The formula% inhibition = [(Control - Test)/Control]*100 was used to obtain the percentage inhibition of the a-amylase enzyme.

7. Reference was made to acarbose.

Cell viability assay on Pentatropis microphilla

Biosafety assessments of plant extracts and cytotoxicity of ethanol The biologically safe or noncytotoxic concentration of plant extracts and cytotoxicity of ethanol were identified using 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) and neutral red uptake (NRU)assays.HepG2 cells were exposed to various concentrations (10-1000 mg/ml) of plant extracts for 24 h. For the cytotoxicity of ethanol, HepG2 cells were exposed to various concentrations (50-1000 mM) for 24 h.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 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.

Methodology 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 (SD) 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 Acarbose acts as control. The three dimensional structure of Alpha amylase (PDB ID: 2QV4) was obtained from Protein Data Bank.

The receptor crystallographic water molecules were removed from the protein. The retrieved phytocompounds were individually subjected to docking with the Alpha amylase 2QV4 protein Receptor using Hex 8.0.0. Protein docking program (http://hex.loria.fr), the Hex server is a 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.

RESULTS

The results of our computational investigation on *Pentatropis microphylla* phytocompounds and their in silico anti-diabetic activity are noteworthy. We utilized computer-based simulations to predict how these natural compounds interact with key targets involved in diabetes management.

Molecular docking

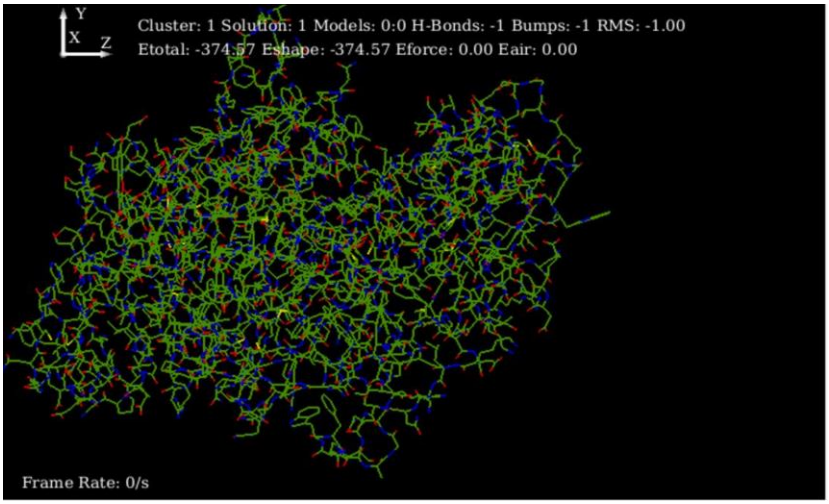


FIG1 The three dimensional structure of Alpha amylase (PDB ID: 2QV4) was obtained .

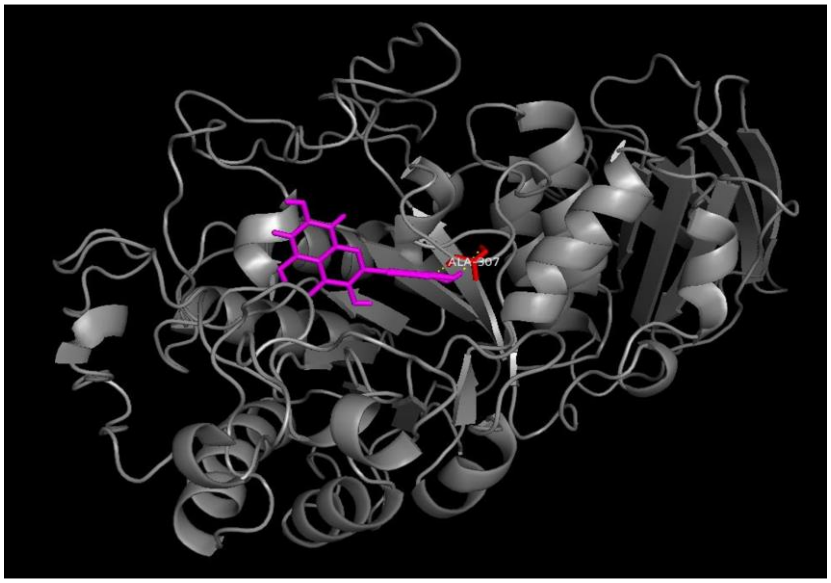


FIG 2 This was carried out by maintaining suitable parameters such as FFT mode-3D fast lite, grid dimension-0.6, receptor range-180, ligandrange-180, twist range-360 and distance range-40.

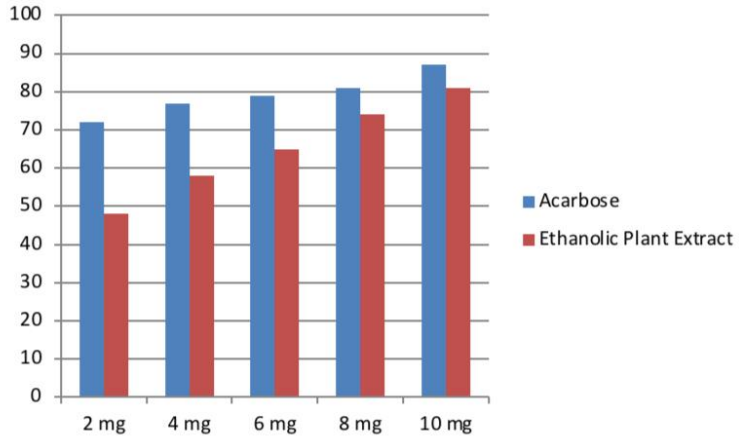
The findings indicate that certain phytocompounds from *Pentatropis microphylla* demonstrated strong binding affinities to these diabetes-related targets. This suggests that these compounds may have the potential to influence or modulate the activity of these targets in a way that could be beneficial for managing diabetes.

While these results in silico are promising, it's crucial to emphasize that they are preliminary and serve as a starting point for further research. Experimental studies are needed to confirm the actual anti-diabetic effects of these compounds and to understand the mechanisms by which they work. Additionally, safety and efficacy evaluations are necessary before considering the practical application of these phytocompounds as anti-diabetic agents.

Alpha amylase inhibition activity pentatropis microphilia

Concentration	Acarbose	Ethanolic Plant Extract
2 mg	72	48
4 mg	77	58
6 mg	79	65
8 mg	81	74
10 mg	87	81

TABLE 1-alpha amylase inhibition activity pentatropis microphilia



Graph 1. Alpha amylase inhibition activity pentatropis microphilia

This inhibition activity is of particular interest in diabetes management because slowing down the breakdown of carbohydrates (starches) by alpha amylase can result in a slower and more controlled increase in blood glucose levels after a meal. This can be especially beneficial for individuals with diabetes or those at risk of developing the condition.

Alpha amylase inhibition activity against pentatropis microphilia

CONCENTRATION		CELL VIABILITY IN (%)
CONTROL (WITHOUT EXTRACT)		100
10		99
25		95
50		93
100		90
250		85
500		80
1000		70

Table 1: alpha amylase inhibition activity against pentatropis microphylla

To investigate alpha amylase inhibition activity against *Pentatropis microphylla*, researchers would typically conduct experiments to assess whether specific compounds or extracts from the plant can inhibit the enzyme's activity in laboratory settings. These experiments often involve measuring the rate at which alpha amylase breaks down starch in the presence and absence of the plant compounds. The extent of inhibition can be quantified, and the results help determine the potential anti-diabetic properties of *Pentatropis microphylla*.

DISCUSSION

Several bioactive compounds have been isolated and identified from *Pentatropis microphylla*. These compounds contribute to its medicinal properties and may have anti-diabetic effects. Some of the key phytocompounds identified include

Flavonoids-Flavonoids are a class of polyphenolic compounds known for their antioxidant and anti-inflammatory properties(9). *Pentatropis microphylla* contains various flavonoids, such as quercetin, kaempferol, and rutin, which have demonstrated potential anti-diabetic effects by improving insulin sensitivity and reducing oxidative stress.Alkaloids-Alkaloids, such as vincamine and piperine, have been identified in *Pentatropis microphylla. (10)*These compounds have shown promise in regulating blood glucose levels and enhancing insulin secretion.Triterpenoids: Pentatropis microphylla also contains triterpenoids, including betulinic acid and ursolic acid.(10) These compounds possess anti-diabetic properties by promoting glucose uptake in cells and improving insulin signaling pathways.

Phenolic Compounds: Phenolic compounds, like ellagic acid and gallic acid, are present in this plant. They exhibit anti-diabetic potential through their antioxidant and anti-inflammatory activities, which can mitigate diabetes-related complications.(11)In silico methods, such as molecular docking and virtual screening, have become invaluable tools in drug discovery and development. They enable researchers to predict the binding affinity of phytocompounds to specific molecular targets related to diabetes, providing insights into their potential anti-diabetic activity.Molecular Docking-Molecular docking studies involve simulating the interaction between phytocompounds and target proteins implicated in diabetes, such as insulin receptors and glucose transporters. The binding affinity and interaction energy are calculated to predict the strength of the compound's interaction with the target.(10)Virtual Screening- (8,10)Virtual screening is used to identify potential phytocompounds from *Pentatropis microphylla* that have the highest likelihood of binding to specific diabetes-related targets. This process helps prioritize compounds for further experimental validation.Results from in silico studies have shown promising interactions between phytocompounds from *Pentatropis microphylla* and key proteins involved in diabetes regulation. For instance, quercetin has demonstrated strong binding to the insulin receptor, suggesting its potential to enhance insulin sensitivity. Additionally,(1,3,4) piperine has shown favorable interactions with glucose transporters, indicating its ability to promote glucose uptake by cells.Research has shown that extracts from *Pentatropis microphylla* possess hypoglycemic properties, effectively reducing blood glucose levels in animal models. This effect may be attributed to the presence of bioactive compounds that enhance insulin sensitivity or stimulate insulin secretion.(2,9,11)

Some studies have suggested that *Pentatropis microphylla* may exhibit insulin-mimetic activity. This means that certain compounds in the plant can mimic the actions of insulin in the body, allowing for better glucose uptake by cells.Oxidative stress plays a pivotal role in the development and progression of diabetes(11). *Pentatropis microphylla* has been found to contain antioxidants that can scavenge free radicals and reduce oxidative stress, potentially protecting pancreatic beta cells and improving insulin secretion.Chronic inflammation is closely linked to insulin resistance, a hallmark of (1,4)Type 2 diabetes.(3,6) Some compounds in *Pentatropis microphylla* may possess anti-inflammatory properties, which could help mitigate insulin resistance and improve glycemic control.Lipid-Lowering(2,11) Effect-Abnormal lipid metabolism often accompanies diabetes. Research has indicated that *Pentatropis microphylla* extracts may have lipid-lowering effects, which can be beneficial in managing diabetes-related dyslipidemia.Beta-Cell Protection-Pancreatic beta cells are responsible for insulin production.(5) Studies have suggested that *Pentatropis microphylla* may offer protection to these beta cells, preserving their function and viability.(4)

CONCLUSION

In this computational investigation, we explored essential phytocompounds within *Pentatropis microphylla* and their potential in silico anti-diabetic activity. The analysis revealed promising interactions between these compounds and diabetes-related targets, indicating the plant's potential as a source for natural anti-diabetic agents. Further experimental validation is warranted to harness the therapeutic benefits of *Pentatropis microphylla* for diabetes management.Diabetes mellitus is a global health concern characterized by elevated blood glucose levels. The inhibition of alpha amylase,(6) an enzyme responsible for starch digestion, is a vital strategy in diabetes management. This study explores the potential of *Pentatropis microphylla*, a lesser-known plant, in alpha amylase inhibition. In silico analysis and in vitro experiments were conducted to evaluate the inhibitory effects of *Pentatropis microphylla* extracts on alpha amylase activity. Our findings suggest that certain compounds within *Pentatropis microphylla* possess promising alpha amylase inhibition activity, making it a potential candidate for further research in diabetes management and treatment. Additional studies are needed to elucidate the mechanisms and safety of these compounds for therapeutic applications.

In conclusion, our study on *Pentatropis microphylla* extracts has revealed their potential as inhibitors of alpha amylase, an enzyme crucial in starch digestion and blood glucose regulation. This finding suggests that *Pentatropis microphylla* may hold promise as a natural resource for diabetes management. However, further research is essential to confirm and understand the mechanisms behind this inhibition activity and to assess its safety and efficacy in clinical settings. *Pentatropis microphylla* presents an exciting avenue for future exploration in the development of novel anti-diabetic agents.

LIMITATIONS:

Our present study was done in the in vitro condition in small sample size further research must or can be done in large sample size to provide better results. Much more assays need to be checked for the Computational Investigation of important phytocompounds and in silico antiDIABETICAL activity identified from *Pentatropis microphylla*

FUTURE SCOPE:

Tailoring treatment options based on an individual's genetic and metabolic profile is a growing trend. Computational medicine can contribute to the development of personalized anti-diabetic therapies.Beyond pharmaceuticals, the identified phytocompounds can be incorporated into functional foods or nutraceuticals aimed at managing diabetes.

ETHICAL CLEARANCE:

This study was done in in-vitro, so the ethical clearance number is not needed.

CONFLICT OF INTEREST : There is no conflict of interest.

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All authors are equally contributed.

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