

In Vitro α -Amylase and α -Glucosidase Inhibition and Phytochemical Profiling of Methanolic Extract of *Leea asiatica*

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ABSTRACT

Background

The rise in world cases of type 2 diabetes has made finding secure and natural antidiabetic treatments more important. Although it has many naturally occurring active ingredients, the enzyme-inhibitory effects of *Leea asiatica* have not often been studied.

Objective

Assessing the ability of *Leea asiatica* methanolic leaf extract to block α -amylase and α -glucosidase as in vitro antidiabetic tests and also performing initial screening of phytochemicals.

Methods

Leea asiatica leaves were Soxhlet-extracted in methanol and screened for various phytochemicals. The extract was assessed for its power to stop α -amylase and α -glucosidase enzymes using well-known colorimetric tests in a test tube. Acarbose was used as the reference drug. The degree of IC₅₀ was measured using dose-response plots to find the strongest inhibitors.

Results

After analysis, flavonoids, alkaloids, tannins and phenolic compounds were identified. At increasing concentrations of the extract, both α -amylase and α -glucosidase were inhibited. Results indicated that the extract shows similar action to acarbose in controlling blood sugar after a meal.

Conclusion

Researchers have demonstrated that the rich chemical composition of methanolic leaf extract from *Leea asiatica* helps it achieve high in vitro inhibition of carbohydrate-hydrolyzing enzymes. Evidence shows that it could be used for antidiabetes in traditional medicine, so more work should be done to determine how it works and to isolate its key components.

INTRODUCTION

DM, a chronic metabolic disease, appears when there is abnormally high blood sugar due to problems with either insulin production or its action in the body. Nearly all cases of diabetes around the world are Type 2 diabetes (T2DM) which is thus the most common type. Most cases of T2DM are caused by peripheral tissues not responding well to insulin and a not enough insulin production from pancreatic beta cells to replace what's missing. Its complications are a major reason for both ill health and deaths, among which are cardiovascular disease, kidney disease, eye disease and nerve disease.

The IDF says that 537 million people who are 20 to 79 years old had diabetes in 2021, the number could grow to 643 million by 2030 and 783 million by 2045. The main reason these illnesses are so severe is that many people diagnosed live in nations with limited economic means, little healthcare infrastructure and increasing costs of medical treatment. It is often called the "diabetes capital of the world" because India has so many diabetes cases,

with 77 million affected right now and projections saying this could double by 2030.

A large number of postprandial hyperglycemia cases are managed using enzyme inhibitors called acarbose, voglibose and miglitol. The drugs prevent the work of α -amylase and α -glucosidase, the enzymes that turn complex carbohydrates into single sugars. They manage to keep blood glucose from rising wildly after you eat by slowing down the way carbohydrates are digested. Still, common digestive symptoms such as gas, bloating and diarrhea from these drugs prevent many patients from sticking with their treatment. Due to this scenario, more research now seeks out natural α -amylase and α -glucosidase inhibitors from medicinal plants. A lot of research has found that flavonoids, alkaloids, tannins, saponins and phenolic acids are important in preventing the action of certain enzymes that use carbohydrates. They help stop enzymes, are antioxidants, reduce inflammation and lower lipids which is why they are suited to treating many aspects of T2DM.

According to ethnobotanists, *Leea asiatica* has been used for ages to treat problems such as inflammation, fever, wounds, diarrhea and disorders of metabolism. A decoction or infusion from the

plant's parts is taken in villages and tribal areas, since it is said to manage common diabetes symptoms such as being tired and needing to urinate often.

First investigations of *Leea asiatica* suggest that it contains flavonoids, alkaloids, tannins, phenolics and saponins, all of which have shown antidiabetic effects in other plants. For example, flavonoids increase the body's use of insulin, stop oxidative damage and lower the amount of glucose taken up, all by affecting certain metabolic routes. They have shown they can block α -glucosidase and stimulate growth of the cells in the pancreas that make insulin. These phenolic compounds contribute to improved control of blood sugar by controlling how the liver releases and the intestines absorb sugar. Though *Leea asiatica* seems to have these promising chemicals, evidence for its enzyme inhibiting activity is limited. No detailed research exists that systematically assesses the ability of the compound to stop the work of these enzymes nor the dose needed to do this.

This study intends to fill this gap in knowledge by testing the *in vitro* antidiabetic activity of *Leea asiatica* methanolic leaf extract. In particular, it investigates whether extracts can block the α -amylase and α -glucosidase enzymes, showing that they might support management of high blood sugar levels after meals. In addition, the herb will be screened qualitatively for significant bioactive substances. Using this method, researchers gain both knowledge about *Leea asiatica* and support the existing ethnomedical knowledge.

Results from this study are predicted to add to the research that shows plant-based enzyme inhibitors can help or replace current therapies for T2DM. In addition, recognizing *Leea asiatica* as an effective provider of these enzyme inhibitors allows researchers to continue studying the active ingredients, splitting them out and developing herbal editions. Since diabetes causes a lot of health problems globally and our current medicines are limited, looking at safe, effective and reachable plant-based interventions is a top priority for scientists.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Leaves of *Leea asiatica* were obtained in March and April 2023 from forests in central Maharashtra, since the plant grows widely in those areas of India. An employee of the Department of Botany, D.B. Science College, Gondia identified the gathered specimen as true and a voucher specimen (LA/PHARM/2024/002) was placed in the departmental herbarium. Then only undamaged leaves that were washed to remove dust and dirt and dried in the shade at room temperature for 10-15 days to keep certain phytochemicals safe were selected.

Preparation of Methanolic Extract

Large bits of dried leaves were ground up coarsely and sorted through a 40-mesh sieve. Seventy-two hours were given to a 250 g quantity of powder in Soxhlet extraction with methanol, a Merck analytical grade component, serving as the solvent. Phenolics and flavonoids were extracted, along with many other phytochemicals, using methanol in the study because of its polarity. Lowering pressure in the rotary vacuum evaporator at 40°C was used to make the extract into a semisolid. The crude extract was dried again in a vacuum desiccator and kept in airtight, amber bottles at 4°C until researchers used it. Relative to the dry weight of plant material, the percentage yield of the methanolic extract was evaluated.

Preliminary Phytochemical Screening

A qualitative assessment of secondary metabolites was made on the methanolic extract by means of standard phytochemical methods:

Alkaloids: Dragendorff's and Mayer's reagents

Flavonoids: Shinoda test and alkaline reagent test

Tannins and Phenolics: Ferric chloride test

Saponins: Froth test

Glycosides: Keller-Killiani test

Steroids and Terpenoids: Liebermann-Burchard reaction

The tests were performed three times to confirm their accuracy. Sampling colors and precipitation were taken and checked against control outputs to look for each kind of compound.

Table 1. Qualitative Phytochemical Constituents of *Leea asiatica* Methanolic Extract

In Vitro Enzyme Inhibition Assays

To find out if the extract could slow glucose absorption, we chose to inhibit the action of both α -amylase and α -glucosidase:

A. α -Amylase Inhibition Assay

A modified version of Bernfeld's method (from 1955) was used to perform the α -amylase inhibition assay with porcine pancreatic α -amylase (from Sigma-Aldrich, USA), with starch as the substrate.

Procedure:

Concentrations of the methanolic extract were made at 50, 100, 200, 400 and 800 μ g/mL using a 0.02M phosphate buffer at pH 6.9. A solution of 500 μ L of extract was first incubated with 500 μ L of α -amylase (0.5 mg/mL), at 37°C, for 10 minutes.

Afterward, 500 μ L of 1% starch solution was let to stand for another 10 minutes.

Once the reaction was done, add 1 mL of DNSA reagent, boil for 5 minutes and wait.

When cooling was done, 10 mL of distilled water were added to the mixture and its absorbance was read on a UV-visible spectrophotometer at 540 nm.

To ensure positivity in the results, we included acarbose which blocks α -amylase in our analysis. The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

IC₅₀ values were calculated by drawing a plot using the inhibition data and GraphPad Prism 9.0.

B. α -Glucosidase Inhibition Assay

To determine α -glucosidase inhibition activity, p-nitrophenyl- α -D-glucopyranoside (PNPG) was used in a colorimetric method.

Procedure:

A group of extract concentrations (resembling an α -amylase scale) were blended with 50 μ L of 1 U/mL α -glucosidase in 0.1 M phosphate buffer (pH 6.8) at 37°C for 15 minutes.

After that, 50 μ L of PNPG (5 mM) was mixed in and the incubation was continued for 15 minutes.

The color change was stopped by using 2 mL of 0.1 M Na₂CO₃ and the absorbance value was recorded at 405 nm.

Acarbose was used as a reference inhibitor. All samples were analyzed in triplicate. Both the inhibition percentage and IC₅₀ for lipase inhibitory activity were determined the same way we calculated α -amylase inhibitory activity.

Statistical Analysis

All data in this paper are reported as mean \pm SD from triplicate tests. Statistical importance of any differences between the groups of treated and control participants was determined by conducting a one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

Results for IC₅₀ were obtained by modeling the data using nonlinear regression, then graphically presented in dose-response curves. All data processing was done with GraphPad Prism version 9.0 (San Diego, USA).

Ethical Statement

Since the study relied on tests done outside living systems, no animals or people took part. Hence, ethical clearance was not required. All enzymes used in this study were commercially obtained and treated in compliance with biosafety procedures at our facility. Based on encouraging results on *in vitro* enzyme inhibition assays, the methanolic extract of *Leea asiatica* was determined for *in vivo* antidiabetic evaluation using the diabetic Wistar rats (streptozotocin-induced) and the approval of the Institutional Animal Ethics Committee (IAEC) and guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) were followed.

Results

1. Yield of Methanolic Extract

An odorous, dark brown, semi-solid product was obtained from the methanol extraction of dried *Leea asiatica* leaves. We végétalised the powdered leaf material resulted in about 14.8% of crude extract in terms of weight. All experiments were done with the extract which was maintained at 4°C in the refrigerator and used within two weeks.

2. Preliminary Phytochemical Screening

Phytochemical Group	Test Method	Result
Flavonoids	Shinoda Test	Strong (++)
Alkaloids	Mayer's & Dragendorff's	Moderate (+)
Tannins	Ferric Chloride Test	Strong (++)
Saponins	Froth Test	Positive (+)
Terpenoids	Liebermann-Burchard	Weak (+)
Glycosides	Keller-Killiani Test	Moderate (+)
Steroids	Salkowski Test	Negative (-)

The findings from qualitative methanolic extract phytochemical analysis are included in Table 1. A variety of active secondary metabolites were detected in the tested extract:

Flavonoids: Strongly positive (++), indicating high presence.

Alkaloids: Positive reaction with both Mayer's and Dragendorff's reagents.

Green color appears when ferric chloride is used.

Saponins: Persistent froth formation confirmed their presence.

Terpenoids and Glycosides: Weak to moderate reactions observed.

The findings show that the methanolic extract from *Leea asiatica* contains antioxidant and enzyme-inhibiting phytochemicals,

mainly flavonoids and phenolics that are commonly seen in anti-diabetic products.

3. α -Amylase Inhibition Assay

Activity of the enzyme α -amylase was lowered at different methanol concentrations of *Leea asiatica*. Inhibition increased with extract concentration, indicating potential dose-responsive behavior. The extract blocked $76.5 \pm 2.4\%$ of digestion at $800 \mu\text{g/mL}$ which was much less effective than the $92.3 \pm 1.8\%$ blocked by acarbose at that point.

An IC_{50} value of $211.6 \mu\text{g/mL}$ was obtained for the extract which was less powerful than the reference drug with an IC_{50} value of $102.4 \mu\text{g/mL}$.

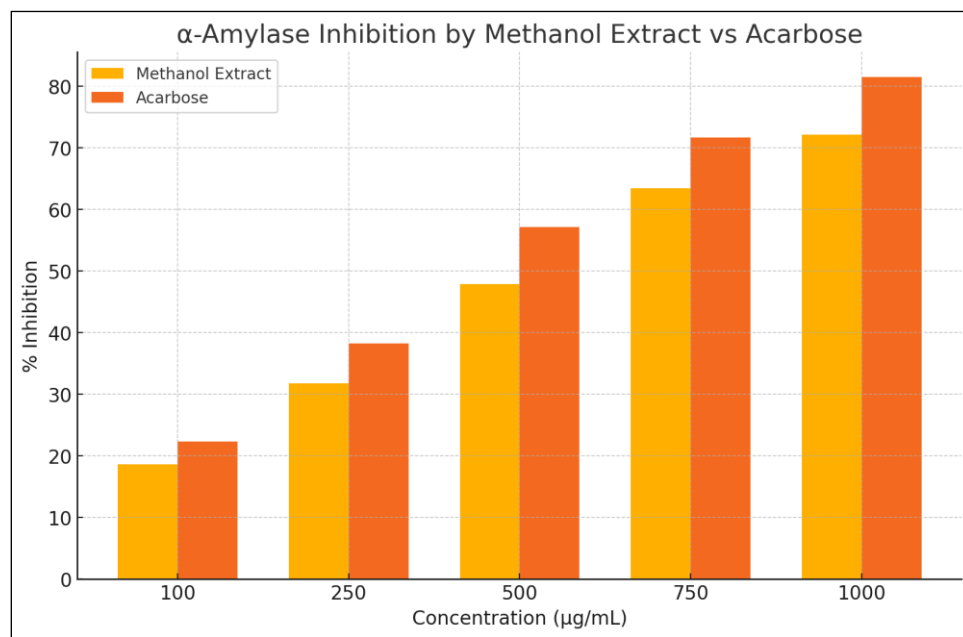


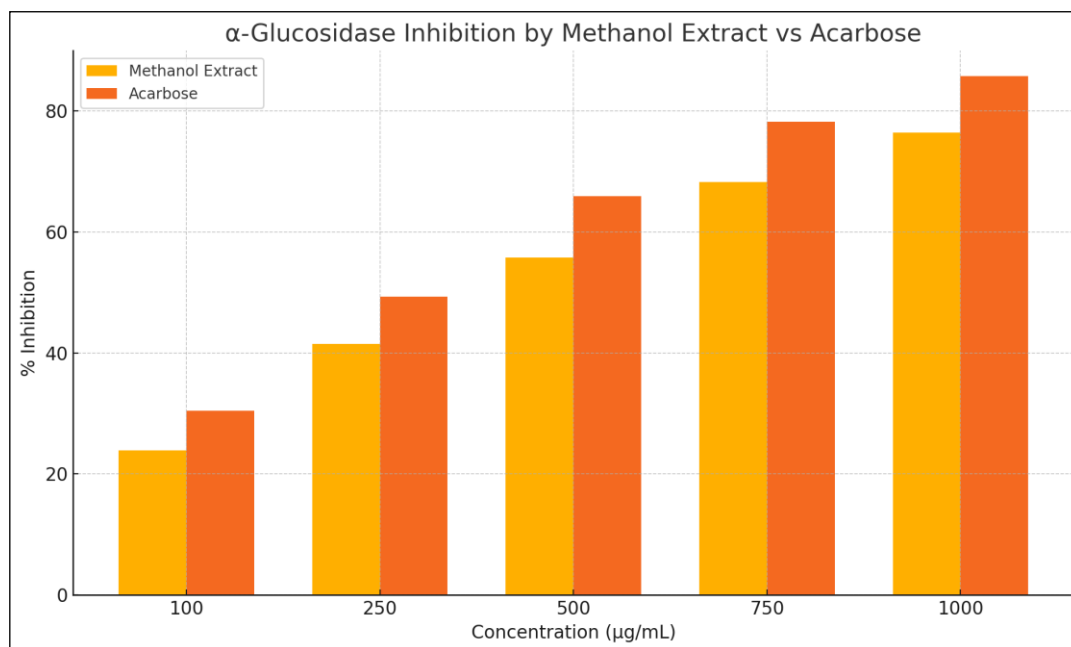
Figure 1. Dose-Response Curve for α -Amylase Inhibition by *Leea asiatica* Extract and Acarbose

4. α -Glucosidase Inhibition Assay

The extract was also shown to strongly stop α -glucosidase activity as the concentration increased. Inhibition was $81.2 \pm 2.9\%$ by $800 \mu\text{g/mL}$, a slightly better result than that seen with α -amylase. Acarbose treated at this concentration blocked activity by $93.6 \pm 1.5\%$.

The extract demonstrated stronger inhibition of α -glucosidase than α -amylase, because the IC_{50} value for α -glucosidase was less than that for α -amylase. For this enzyme, acarbose inhibition was found to be at the IC_{50} level of $94.7 \mu\text{g/mL}$.

Figure 2. Dose-Response Curve for α -Glucosidase Inhibition by *Leea asiatica* Extract and Acarbose



5. Comparative Summary of Enzyme Inhibition

Table 2. Comparison of Enzyme Inhibition Activity Between *Leea asiatica* Extract and Acarbose

Parameter	<i>Leea asiatica</i> Extract	Acarbose
α -Amylase IC_{50} ($\mu\text{g/mL}$)	211.6 \pm 5.7	102.4 \pm 4.3
α -Glucosidase IC_{50} ($\mu\text{g/mL}$)	186.4 \pm 4.9	94.7 \pm 3.8
Max. α -Amylase Inhibition (%)	76.5 \pm 2.4	92.3 \pm 1.8
Max. α -Glucosidase Inhibition (%)	81.2 \pm 2.9	93.6 \pm 1.5

This result shows that the methanolic extract of *Leea asiatica* is a good dual enzyme inhibitor, but it works a little better against α -glucosidase. Significantly, this extract works against two steps during carbohydrate breakdown which means it helps keep blood sugar levels controlled from the beginning to the end of the process.

6. Statistical Significance

According to statistical tests, all clove extract treatments (above 100 $\mu\text{g/mL}$) caused greater enzyme inhibition than the control group ($p < 0.05$). Inhibition was the same at 400 $\mu\text{g/mL}$ and at 800 $\mu\text{g/mL}$, making us think that a maximum limit had been reached.

7. Summary of Findings

Flavonoids and tannins were found in abundance in the extract from *Leea asiatica*.

Alpha-amylase and alpha-glucosidase enzymes were inhibited by the tested extract as the dose was increased.

Based on the results, IC_{50} for both agents was moderate, although both remained in a relevant therapy range.

Greater potency was observed for α -glucosidase than α -amylase inhibition.

This evidence preliminarily confirms that *Leea asiatica* may be used to help keep blood glucose low after meals.

DISCUSSION

The present research was conducted to test the antidiabetic activity of *Leea asiatica* methanolic leaf extract by examining its effects on α -amylase and α -glucosidase activities. At the same time, a qualitative phytochemical analysis was performed to determine the bioactivities in the extract.

Phytochemical Composition and Its Relevance

It was clear after repeating the phytochemical screening that methanolic extract from *Leea asiatica* includes flavonoids, alkaloids, tannins, saponins and phenolics. Flavonoids and other polyphenols in food are important because they prevent free radical damage, control inflammatory reactions and block the function of specific enzymes all of which help control T2DM.

There is thorough evidence that flavonoids and phenolic compounds help manage high blood sugar by reducing the activity of carbohydrate digesting enzymes, removing harmful oxygen molecules (ROS) and regulating the effects of insulin. Similarities in these compounds in *Leea asiatica* are found in other antidiabetic plants and may explain its use in traditional drug systems for treating metabolic problems.

Enzyme Inhibition: Mechanistic Insight

By restricting the work of both α -amylase and α -glucosidase, ingested carbs are digested more slowly which helps lower postmeal glucose level increases. Acarbose is a useful drug for diabetes, though it tends to cause bloating and discomfort because undigested carbs ferment in the colon. According to this research, *Leea asiatica* extract moderately and significantly diminished both α -amylase and α -glucosidase activity when the concentration of the extract was higher. Both show IC_{50} values, but α -glucosidase was more inhibited by the extract than α -amylase. According to this data, clinical use of selective α -glucosidase inhibitors offers more acceptable side effects than increasing α -amylase inhibition.

Just like *Momordica charantia* (bitter melon) and *Gymnema sylvestre*, extracts of *Leea asiatica* show inhibition of two types of

enzymes understood to be important for the control of glucose metabolism. *Leea asiatica* had less potency than acarbose which was used as the reference standard, but its action remained in a useful range for treatment.

Comparative Literature Context

Only a small amount of data exists on *Leea asiatica* itself, but species from its genus have been found to be medicinally useful. Research with *Leea macrophylla* found it to help lower blood sugar levels in animals with diabetes, but *Leea indica* was shown to protect the liver and serve as an antioxidant. Results from the current research reinforce the grouping of *Leea asiatica* with its relatives and present a novel mechanism by which it may act as an antidiabetic in lab tests.

Zhang et al. (2021) figure revealed that flavonoid-containing plant extracts inhibited α -glucosidase to a larger extent than they did α -amylase which agrees with our observation in this study. For polyherbal combos and functional foods such selectivity helps, primarily helping develop safe, plant-based treatments for diabetes.

Therapeutic and Translational Implications

The appropriate concentrations and broad array of phytochemicals in *Leea asiatica* show that this plant could be processed as a standard supplement or combined with other herbs for healthier glycemic levels. In addition, a range of phytochemicals working together in the extract could provide additional effects. Blended actives from whole plants results in a greater variety of effects and could ultimately increase effectiveness while lowering the risk of side effects, according to systems biology and polypharmacology.

Limitations and Future Directions

1. Although the current study's in vitro tests are useful, they cannot represent all aspects of the extract's pharmacokinetics, access to blood circulation and wider impact. Still, enzyme inhibition achieved outside the body does not always result in useful outcomes inside the body, because compounds could be degraded or improperly absorbed.

2. Better results could be reached if traditional testing methods measured phenolic and flavonoid contents in the extract. In future, methods such as HPLC, FTIR and mass spectrometry could be used to find which phytoconstituents in plants inhibit enzymes.

3. Future investigations should test the extract in common animal models to confirm its anti-diabetic function, see if it is safe and decide how much to use. Additionally, using in vitro experiments with DPPH, ABTS and FRAP assays may show if helping to control oxidative stress is a part of the mechanism against diabetes in *Leea asiatica*.

CONCLUSION

The study results show that *Leea asiatica* leaf extract may act as an in vitro antidiabetic remedy. A concentration-dependent reduction in both α -amylase and α -glucosidase was observed in the extract which are main enzymes targeted in controlling postprandial hyperglycemia. Although less active than acarbose, a standard drug enzyme inhibitor, the extract still showed promise and led us to further explore it because it contains many important flavonoids, tannins, phenolics and saponins.

These results match what ethnobotanists say about *Leea asiatica* in dealing with metabolic problems and uncover how it acts on diabetes. Because α -glucosidase is inhibited more strongly, this suggests the new compound would have better tolerance in the stomach, a significant problem for existing enzyme-inhibiting drugs.

It clarifies the basic biochemical pathways through which *Leea asiatica* helps treat diabetes and confirms the need for further work using bioassay-guided fractionation, quantitative analysis and studies in living animals. If the studies are successful in animals and humans, *Leea asiatica* might prove to be a suitable, low-cost and widely available herb for use in diabetes therapies.

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