

In Vitro Assessment of Antidiabetic Activity of Ethanolic Seed Extract of *Abutilon crispum*

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Abstract: Diabetes mellitus is a chronic metabolic disorder that requires effective and safer therapeutic approaches. The present study investigated the in vitro antidiabetic activity of the ethanolic seed extract of *Abutilon crispum* by evaluating its α -amylase inhibitory potential. Mature seeds were collected, shade-dried, powdered, and extracted with ethanol using the maceration technique. The prepared extract was tested at different concentrations, with metformin serving as the reference drug for comparison. The extract demonstrated a dose-dependent inhibition of α -amylase activity, with an IC_{50} value of 66.21 μ g/mL, indicating promising antidiabetic activity. The observed enzyme inhibitory effect may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, alkaloids, tannins, and saponins, which are known to influence carbohydrate-digesting enzymes. These findings suggest that the ethanolic seed extract of *Abutilon crispum* could serve as a potential natural source for the development of antidiabetic agents. However, further in vivo investigations, phytochemical characterization, and toxicity studies are necessary to establish its safety, efficacy, and therapeutic potential.

Keywords: *Abutilon crispum*, Ethanolic Seed Extract, α -Amylase Inhibitory Assay, Antidiabetic, IC_{50} .

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I. INTRODUCTION

Herbal medicine is the oldest type of healthcare known to mankind. Herbs have been employed by every society throughout history. It was an essential component of the evolution of modern civilisation [1]. The plant and its parts are an essential source of medicines, clothing, shelter, and nourishment [2]. The majority of the use of plants for therapeutic purposes seems to have developed through observation and trial-and-error application in animals. Over time, each tribe expanded its knowledge base to include the medical properties of the herbs in their area [3]. Nowadays, a lot of pharmaceuticals are derived from herbs. Herbal treatments are used to cure a wide range of illnesses, including common colds and cancer. Herbal medicine expertise was passed down verbally from one generation to the next [4].

Natural products possess unique chemical structures that often exhibit significant pharmacological properties [5]. Bioactive constituents such as flavonoids, phenolic compounds, alkaloids, tannins, glycosides, and saponins have attracted considerable attention because of their antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities [6]. The growing demand for safer and more effective therapeutic alternatives has encouraged extensive research on plant-based medicines [7]. According to the World Health

Organization (WHO), a substantial proportion of the global population relies on traditional medicinal systems for primary healthcare, emphasizing the continuing importance of medicinal plants in healthcare [8].

Diabetes mellitus is a dangerous disease characterised by insufficient regulation of sugar, polymers of amino acids, and lipid metabolism due to insulin resistance, a relative or complete lack of insulin synthesis, or both at one or more sites in the complex hormone action pathways [9]. It is an inherited or acquired inability to transfer sugar from the bloodstream into cells. Blood glucose levels rise when the body's cells are unable to absorb enough glucose from the blood due to insufficient insulin. This disease, known as hyperglycemia, can damage key organs like the kidney, liver, eyes, nerves, heart, and blood vessels. Abnormally high blood glucose levels are a hallmark of the clinically and genetically diverse group of disorders known as diabetes mellitus. [10]. Insulin resistance, inadequate insulin synthesis, or both may contribute to hyperglycemia. Protein, lipid, and carbohydrate metabolism are often disrupted. The pancreas, small intestine, muscles, and liver are all involved in the metabolism of glucose. Any problem with any of these organs can lead to a deficiency in glucose metabolism and the development of diabetes. Diabetes is characterised by polyuria, polyphagia, and polydipsia. [11].

➤ Plant Profile

Abutilon crispum (Linn) belongs to the family Malvaceae and is a medicinal plant traditionally employed in various parts of South India for the treatment of ailments such as asthma, cough, ulcers, piles, jaundice, and diabetes. Different parts of the plant are reported to possess therapeutic properties due to the presence of biologically active phytochemicals. However, scientific evidence supporting the antidiabetic potential of its seeds remains limited. Therefore, the present study was undertaken to evaluate the in vitro antidiabetic activity of the ethanolic seed extract of *Abutilon crispum* using the α -amylase inhibition assay, thereby providing scientific support for its traditional medicinal use [12, 13].

• Scientific Classification of *Abutilon crispum*



Fig 1 *Abutilon crispum* Plant

- Kingdom: Plantae
- Clade: Tracheophytes
- Clade: Angiosperms
- Clade: Eudicots
- Clade: Rosids
- Order: Malvales
- Family: Malvaceae
- Genus: *Herissantia*
- Species: *H. crispa*
- Binomial name: *Herissantia crispa* (L)
- Brizicky synonyms: *Abutilon crispum*, *Gayoides crispum*.

II. MATERIALS AND METHODS

➤ Identification, Collection and Authentication of the Selected Plant

Fresh *Abutilon crispum* plants were collected from Edappadi, Tamil Nadu, India. The collected plant material was botanically authenticated by Dr. P. Radha, Research Officer (Botany), Siddha Medicinal Plants Garden, Mettur Dam, Tamil Nadu. The authenticated specimen was assigned Authentication No. H300126259C for future reference.

➤ Procurement of Plant Materials

The collected seeds were thoroughly cleaned, shade-dried at room temperature until a constant weight was

obtained, and then pulverized using a laboratory grinder. The powdered material was sieved to obtain a uniform particle size and stored in an airtight container until extraction.

➤ Preparation of Extract

The dried seed powder of *Abutilon crispum* was extracted with ethanol using the maceration method. The powdered material was immersed in ethanol for an appropriate extraction period with occasional stirring to ensure maximum extraction of phytoconstituents.

➤ Evaluate the Antidiabetic Potential: In-Vitro α -Amylase Inhibition Assay

• Principle

The α -amylase inhibition assay was employed to determine the antidiabetic potential of the ethanolic seed extract. During the assay, α -amylase hydrolyses starch into reducing sugars. These sugars react with dinitrosalicylic acid (DNS) reagent to produce a coloured complex, and the intensity of the colour is measured spectrophotometrically. A reduction in absorbance indicates inhibition of α -amylase activity by the test extract.

• Materials Required

α -amylase solution was purchased from Sigma, USA. Sodium phosphate buffer, DMSO, starch, DNS reagent, sodium, potassium tartrate, and NaOH.

• Procedure

Different concentrations (500, 250, 100, 50 and 10 μ g/ml) of the test sample (AC) were remixed with 200 μ l of α -amylase solution (1.0 U/ml in phosphate buffer pH 6.9), and incubated at 25°C for 30 min. After pre-incubation, 400 μ l of 0.25 % starch solution in the phosphate buffer (pH 6.9) was added to each tube to start the reaction. The reaction was carried out at 37°C for 5 min and terminated by the addition of 1.0 mL of the DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium-potassium tartrate in 0.4 M NaOH). After 10 minutes over a boiling water bath, the test tubes were allowed to cool to room temperature. After diluting the reaction mixture with 10 mL of pure water, absorbance (A) was measured at 540 NM. Control incubations representing 100% enzyme activity were conducted similarly by replacing extracts with buffers. For blank incubation (to allow for absorbance produced by the extracts), enzyme solution was replaced by buffer solution, and absorbance was recorded [14,15].

• Calculation

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - (A_{\text{test}} - A_{\text{background}})}{A_{\text{control}}} \times 100$$

Where A_{control} , A_{test} , and $A_{\text{background}}$ represent the absorbance of 100% enzyme activity, the test sample with the enzyme and the test sample without the enzyme, respectively.

III. RESULTS AND DISCUSSION

➤ Collection, Authentication, and Extraction of *Abutilon crispum* (L.) Extract

The plant material was carefully collected from its natural habitat in Edappadi, Tamil Nadu, ensuring that the collected specimens were healthy, undamaged and free from disease. Dr P. Radha, a skilled taxonomist connected to the Siddha Medicinal Plants Garden, Central Council for Research in Siddha (CCRS), Ministry of Ayush, Tamil Nadu, thoroughly verified the plant's botanical identity. By guaranteeing future traceability and reproducibility, the deposition of a voucher specimen (H300126259C) improves the study's scientific validity. After being verified, the roots were shade-dried for 10 to 15 days at room temperature (25 to 30°C) to stop the breakdown of thermolabile components, which is an essential step in preserving the chemical integrity of plant material.

To enhance the surface area for solvent interaction, the seeds were ground into a coarse powder after drying. 92.5 g of this powder was soaked in one litre of ethanol to complete the maceration process. To conserve delicate materials that could break down under high temperatures or mechanical stress, maceration was purposefully chosen over more aggressive methods. Ethanol was selected as a solvent due to its ability to extract a range of phytoconstituents while being safe for human use and compatible with ancient medical systems like Siddha and Ayurveda. Following extraction, the mixture was filtered through Whatman No. 1 filter paper, concentrating the filtrate to create a stable extract suitable for storage and further pharmacological study. During the maceration process, the intermittent shaking promoted the solubility of active compounds into the solvent and assisted in maintaining dynamic equilibrium.

The evaluated yield of 12.16% signifies an efficient extraction process and reflects the abundance of extractable metabolites in *Abutilon crispum* seed. The presence of ethanol-soluble phytochemicals in *Abutilon crispum* seeds, such as flavonoids, alkaloids, tannins, phenolics, glycosides, and saponins—compounds typically associated with a variety of pharmacological actions—is indicated by this high extractive value. It guarantees that a comparatively small amount of raw material can yield enough extract for standardisation research, bioassays, and possible formulation development. Furthermore, in the larger perspective, a high extractive value lowers the environmental impact and resource requirement for increasing output.

This discovery supports the traditional usage of *Abutilon crispum* in ethnomedicine and shows that it is a promising source of bioactive chemicals. The outcomes confirm the extraction protocol's methodological rigour and offer a solid basis for further analyses, such as chromatographic profiling, biological activity testing (such as anti-inflammatory and analgesic assays), and phytochemical screening (to identify major constituents like flavonoids, saponins, sterols, etc.). Furthermore, the effectiveness of this extraction process contributes to the standardisation of *Abutilon crispum* as a phytotherapeutic agent, which is required for its integration into evidence-based alternative medicine. [16].

➤ In Vitro Antidiabetic Activity:

• In Vitro α -Amylase Inhibition Assay

The assay measures the ability of the extract to inhibit the enzymatic hydrolysis of starch, quantified by the reduction in optical density at 540nm. Metformin was used as a standard. Both the standard and the test extract exhibited α -amylase inhibition that was dependent on concentration. The percentage inhibition was calculated with respect to the control absorbance.

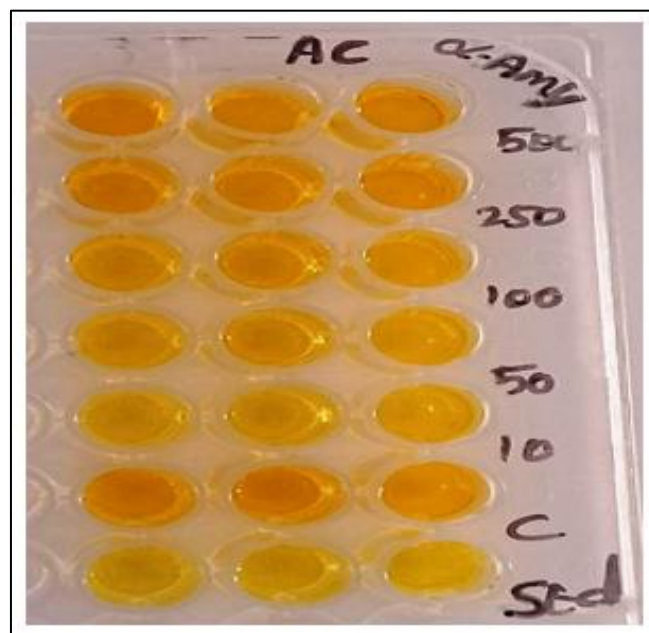


Fig 2 α -Amylase Inhibitory Activity Assay Showing Standard and Sample at Different Concentrations

➤ Results

Table 1 In Vitro α -Amylase Inhibition Activity of Standard (Metformin) and Sample (*Abutilon crispum*)

Concentration(μ g/mL)	OD at 540nm	Percentage inhibition (%)
Control	2.336	0.0000
Sample Code – <i>Abutilon Crispum</i>		
10 μ g/ml	1.653	29.2095
50 μ g/ml	1.439	38.3704
100 μ g/ml	1.240	46.8893
250 μ g/ml	1.131	51.5696
500 μ g/ml	1.067	54.2951
Standard-Metformin (1mg/ml)	0.287	95.9047

- IC₅₀ Value: Sample – *Abutilon crispum* - 66.21µg/mL(Calculated Using ED 50 PLUS V1.0 Software)

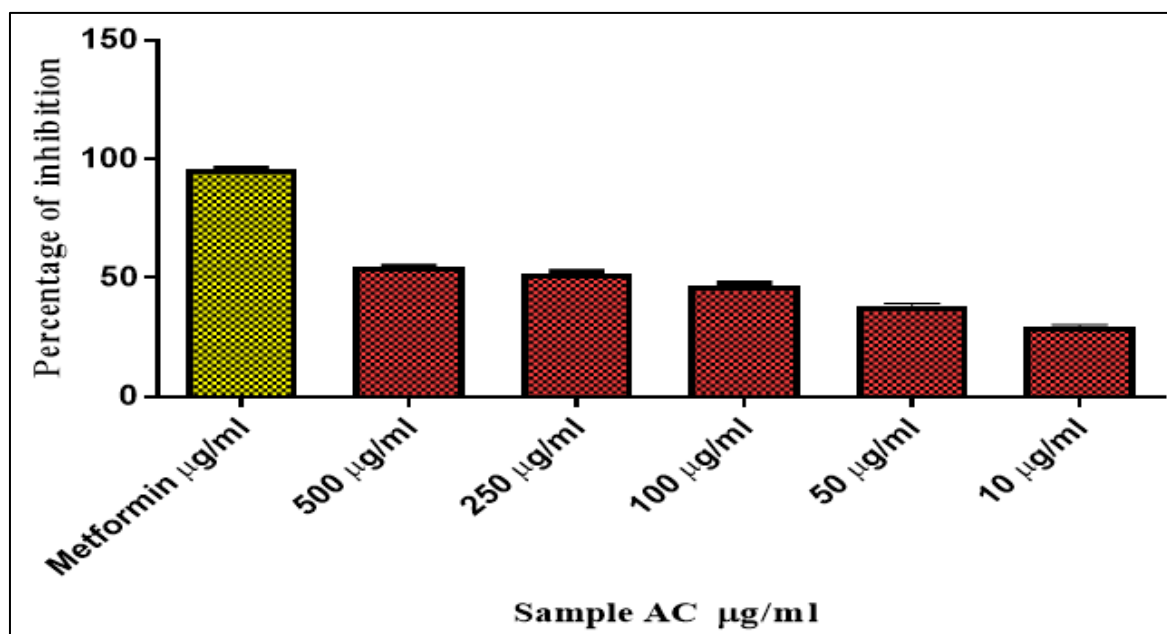


Fig 3 α -Amylase Inhibitory Assay of Ethanolic Seed Extract of *Abutilon Crispum*

➤ Discussion

The test extract of *Abutilon crispum* showed a concentration-dependent inhibition of α -amylase. At the highest tested concentration (500µg/ml), the extract achieved an inhibition of 54.29%, indicating moderate enzyme suppression compared to the standard drug metformin, which reached 95.90% inhibition at a concentration(1mg/ml).

The IC₅₀ value of the ethanolic extract of *Abutilon crispum* was found to be 66.21 µg/mL, indicating strong α -amylase inhibitory activity. The concentration-dependent rise in enzyme inhibition points to the existence of bioactive phytoconstituents that can interact with the α -amylase enzyme's catalytic region. The relatively low IC₅₀ value demonstrates the potent inhibitory effect of the extract, highlighting its potential as a natural antidiabetic agent for controlling postprandial blood glucose levels.

This inhibitory effect may be attributed to phytochemicals that are frequently found in *Abutilon* species, such as flavonoids, alkaloids, phenolics, and tannins. These substances are known to obstruct the enzymes that break down carbohydrates through the following mechanisms:

- Binding to the active site of the enzyme.
- Causing conformational changes that lower the effectiveness of catalysis.
- Preventing the formation of substrate complexes by the enzyme [17, 18].

Crucially, plant-based α -amylase inhibitors, such as those found in *Abutilon crispum*, may have therapeutic benefits over synthetic ones. The extracts offer a safer option with less gastrointestinal distress, which is frequently linked to synthetic inhibitors, and their strength is relatively higher. Additionally, the increased activity seen here lays the

groundwork for additional research, such as structure-activity relationship (SAR) studies to better understand the inhibitory mechanism and bioassay-guided fractionation to identify the most active components.

IV. SUMMARY AND CONCLUSION

Medicinal plants are widely explored as safer alternatives to synthetic drugs due to their antioxidant potential. Although *Abutilon crispum* has long been used as a medicinal plant, science is still confirming its low pharmacological value.

The current study focuses on assessing the ethanolic seed extract of *Abutilon crispum's* antidiabetic efficacy in vitro. Flavonoids, phenolics, tannins, and alkaloids were among the phytoconstituents that were extracted from the seeds using ethanol. Enzyme inhibition assays, such as the α -amylase inhibition assay, were used to assess antidiabetic efficacy.

The findings showed that the ethanolic seed extract significantly reduced the risk of diabetes by blocking the enzymes that break down carbohydrates in a concentration-dependent way. These results validate *Abutilon crispum's* historic usage in diabetic treatment.

The ethanolic seed extract of *Abutilon crispum* has promising antidiabetic action, according to the current in vitro investigation. The indicated pharmacological effects may be attributed to the presence of bioactive phytochemicals, particularly flavonoids and phenolic compounds.

The observed antidiabetic activity, demonstrated through enzyme inhibition assays, suggests its role in controlling postprandial hyperglycemia. Overall, *Abutilon*

crispum can be considered a potential natural source for the development of antidiabetic agents. To establish its therapeutic efficacy and safety, more in vivo research, toxicity assessment, and active chemical isolation are required.

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