

IN VITRO ASSESSMENT OF THE ANTIDIABETIC ACTIVITY OF TRIGONELLA FOENUM-GRAECUM SEED EXTRACT BY ALPHA-AMYLASE INHIBITION METHOD

Dr. Malepati Sandhya Rani*, G. Mounica, H. Bharathi, H. Rakshitha, K. Shannu, K. Devi

Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Kothapeta, Proddatur, Kadapa, A.P. Department of Pharmacognosy.

Article Received: 16 January 2026 | Article Revised: 6 February 2026 | Article Accepted: 26 February 2026

*Corresponding Author: Dr. Malepati Sandhya Rani

Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Kothapeta, Proddatur, Kadapa, A.P. Department of Pharmacognosy.

DOI: <https://doi.org/10.5281/zenodo.18831422>

How to cite this Article: Dr. Malepati Sandhya Rani, G. Mounica, H. Bharathi, H. Rakshitha, K. Shannu, K. Devi (2026) IN VITRO ASSESSMENT OF THE ANTIDIABETIC ACTIVITY OF TRIGONELLA FOENUM-GRAECUM SEED EXTRACT BY ALPHA-AMYLASE INHIBITION METHOD. World Journal of Pharmaceutical Science and Research, 5(3), 272-277. <https://doi.org/10.5281/zenodo.18831422>



Copyright © 2026 Dr. Malepati Sandhya Rani | World Journal of Pharmaceutical Science and Research.

This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. The growing global burden of diabetes necessitates the exploration of safer and cost-effective therapeutic alternatives. The present study aimed to evaluate the in vitro antidiabetic activity of Trigonella foenum-graecum seed extract using the alpha-amylase inhibition assay. Methanolic extraction was performed by maceration, followed by qualitative phytochemical screening. The inhibitory potential was assessed at various concentrations and compared with the standard drug acarbose. The extract exhibited significant concentration-dependent inhibition, with 67.88 percent inhibition observed at 60 microgram per milliliter, comparable to acarbose (69.49 percent). The results indicate that fenugreek seeds possess promising antidiabetic potential, supporting their traditional use. Further in vivo and clinical investigations are recommended to validate therapeutic applicability.

KEYWORDS: Trigonella foenum-graecum; Antidiabetic activity; Alpha-amylase inhibition; Herbal medicine; phytochemical screening.

INTRODUCTION

Diabetes mellitus is one of the most prevalent metabolic disorders worldwide, characterized by chronic elevation of blood glucose levels. It arises due to defects in insulin secretion, insulin action, or a combination of both mechanisms.^[1,2] Long-term hyperglycemia is associated with complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases. The increasing incidence of diabetes poses significant health and economic

burdens globally.^[3] Conventional antidiabetic medications, including oral hypoglycemic agents and insulin therapy, are effective but may produce adverse effects such as gastrointestinal disturbances, hypoglycemia, and weight gain.^[4] These limitations have encouraged the exploration of plant-based therapeutic alternatives. Medicinal plants have been used traditionally for the management of diabetes due to their safety, accessibility, and affordability.^[5] *Trigonella foenum-graecum*, commonly known as fenugreek, is widely used in traditional medicine systems for its hypoglycemic properties.^[6,7] The seeds contain bioactive constituents such as alkaloids, flavonoids, saponins, tannins, and fibers, which may contribute to glucose-lowering effects.^[8,9] One of the mechanisms by which plant extracts exert antidiabetic effects is through inhibition of carbohydrate-digesting enzymes such as alpha-amylase.^[10] Inhibition of this enzyme delays carbohydrate digestion and reduces postprandial hyperglycemia.^[11] The present study was designed to evaluate the *in vitro* antidiabetic activity of methanolic extract of *Trigonella foenum-graecum* seeds using alpha-amylase inhibition assay.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fenugreek seeds were obtained from the local market and authenticated by a qualified botanist. The seeds were cleaned, shade dried, and pulverized into coarse powder.

Preparation of Extract

The powdered material was subjected to methanolic extraction by maceration for 72 hours with intermittent shaking. The mixture was filtered and concentrated using a rotary evaporator under reduced pressure. The dried extract was stored in an airtight container for further analysis.

Phytochemical Screening

Preliminary qualitative phytochemical tests were performed to detect alkaloids, flavonoids, saponins, tannins, glycosides, and carbohydrates using standard procedures.

Alpha-Amylase Inhibition Assay

The inhibitory activity was evaluated using the standard alpha-amylase inhibition method. Various concentrations of the extract (10, 20, 40, and 60 microgram per milliliter) were prepared. The reaction mixture contained phosphate buffer, alpha-amylase enzyme, and plant extract. After incubation, starch solution was added and further incubated. The reaction was terminated using dinitrosalicylic acid reagent, and absorbance was measured spectrophotometrically.

Acarbose served as the standard reference drug.

Percentage inhibition was calculated using the formula: Percentage inhibition equals Control absorbance minus Sample absorbance divided by Control absorbance multiplied by 100.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean percentage inhibition.



Figure-1: Filtration and process of extraction.

RESULTS

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract of *Trigonella foenum-graecum* confirmed the presence of alkaloids, flavonoids, tannins, steroids, fixed oils, phenols, and carbohydrates (Table 1).

In Vitro Antidiabetic Activity

The α -amylase inhibitory activity of the extract was evaluated and compared with Acarbose. The extract exhibited concentration-dependent inhibition (10–60 $\mu\text{g/mL}$). Absorbance values decreased from 0.84 to 0.35 with increasing concentration, while percentage inhibition increased from 22.93% to 67.88%. Acarbose showed inhibition ranging from 28.81% to 69.49% across the same concentrations. At higher concentrations (50 and 60 $\mu\text{g/mL}$), the extract demonstrated inhibition comparable to the standard drug.

Table-1: preliminary phytochemical screening of ethanolic extract of *Trigonella foenum graecum*.

S.NO	TEST FOR	ETHANOLIC EXTRACT
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Tannins	Positive
4	Steroids	Positive
5	Fixes oils	Positive
6	Phenols	Positive
7	Carbohydrates	Positive

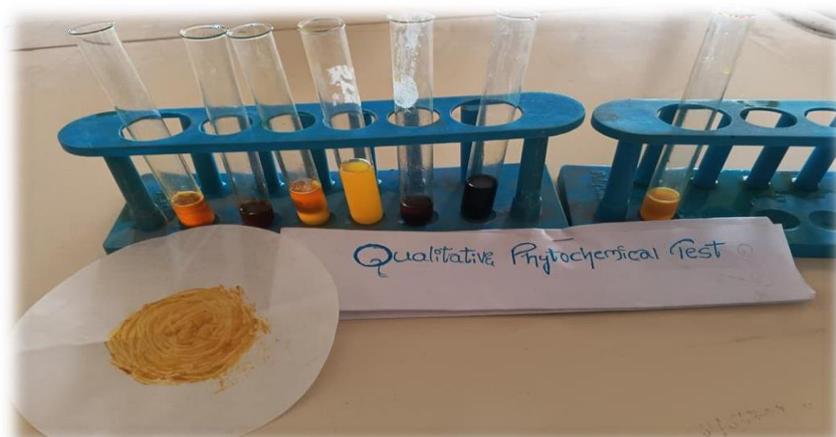


Figure-2: Phytochemical screening of *Trigonella foenum graecum*.

ANTIDIABETIC ACTIVITY

The results have been summarized in Table 2

Standard ACARBOSE series in ml	Sample Solution in ml	Conc. in µg/ml	SAMPLE Absorbance (540nm)	% Inhibition of sample	ACARBOSE Absorbance (540nm)	% Inhibition of Acarbose
1	1	10	0.84	22.93%	0.42	28.81%
2	2	20	0.79	27.52%	0.38	35.59%
3	3	30	0.68	37.61%	0.33	44.06%
4	4	40	0.59	45.87%	0.28	52.54%
5	5	50	0.47	56.88%	0.22	62.71%
6	6	60	0.35	67.88%	0.18	69.49%
Blank	Blank	00	1.09	-	0.59	-

CALCULATION

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample} \times 100}{\text{Absorbance control}}$$

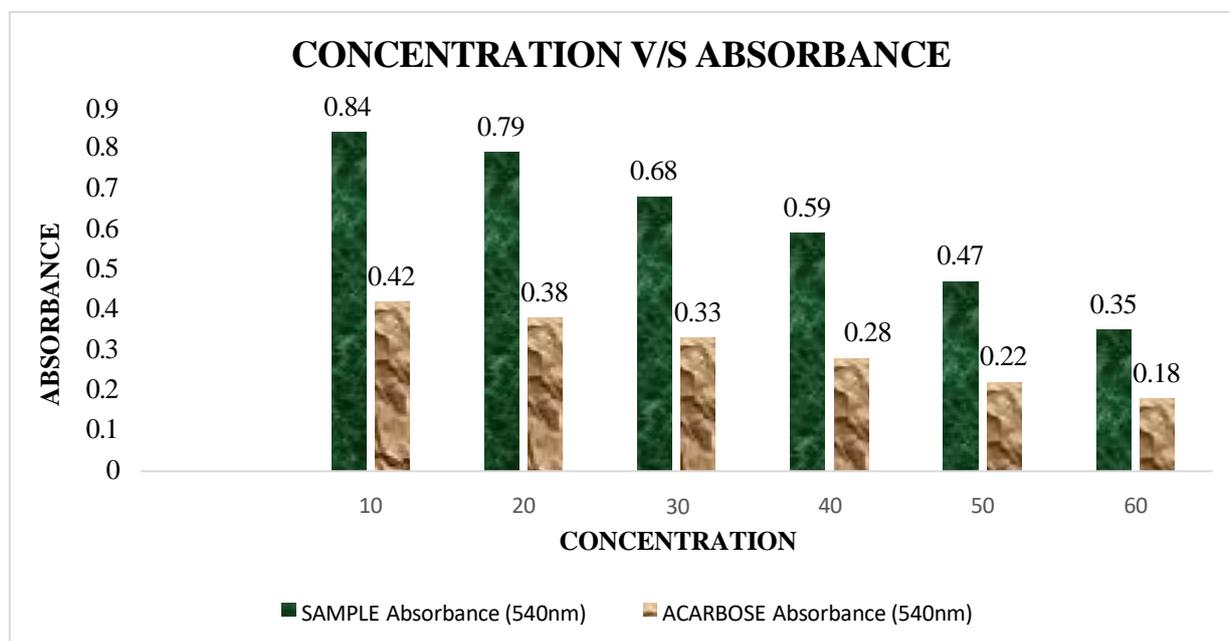


Figure 3: concentration vs absorbance.

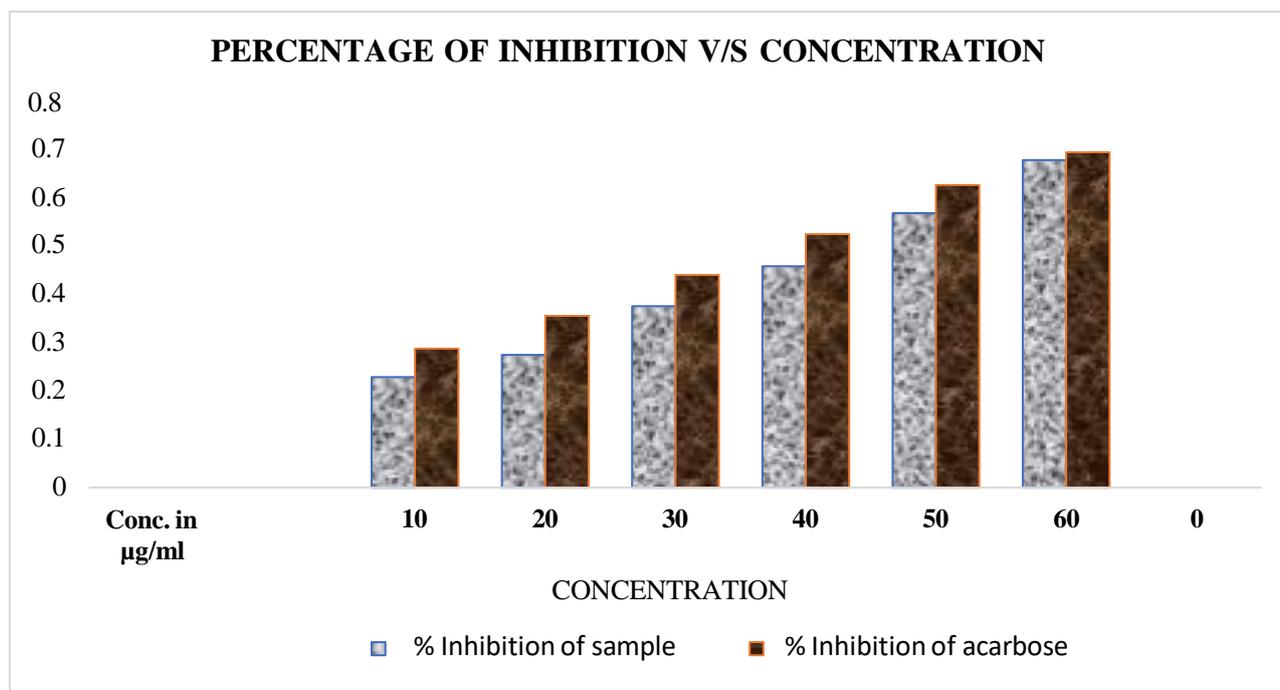


Figure 3: percentage of inhibition vs concentration

DISCUSSION

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the ethanolic extract revealed the presence of alkaloids, flavonoids, tannins, steroids, fixed oils, phenols, and carbohydrates. The presence of flavonoids and phenolic compounds is particularly important, as these compounds are known for their antioxidant and enzyme inhibitory activities. Alkaloids and tannins have also been reported to exhibit hypoglycemic effects.

The presence of these bioactive constituents suggests that the extract may exert antidiabetic activity through multiple mechanisms, including enzyme inhibition and antioxidant action.

In Vitro Antidiabetic Activity

The α -amylase inhibitory activity of the ethanolic extract was evaluated and compared with acarbose.

Absorbance Values

The absorbance values of the extract decreased progressively with increasing concentration, indicating enhanced enzyme inhibition. At 10 micrograms per milliliter, the absorbance was 0.84, which decreased to 0.35 at 60 micrograms per milliliter. Similarly, acarbose showed a decrease from 0.42 to 0.18 across the same concentration range. The inverse relationship between concentration and absorbance confirms the concentration-dependent inhibition of α -amylase.

Percentage Inhibition

The percentage inhibition of the extract increased from 22.93 percent at 10 micrograms per milliliter to 67.88 percent at 60 micrograms per milliliter. Acarbose showed inhibition ranging from 28.81 percent to 69.49 percent. At lower concentrations, acarbose demonstrated higher inhibitory activity compared to the extract. However, at higher concentrations, the extract exhibited inhibition values comparable to the standard drug. This suggests that the

ethanolic extract possesses significant enzyme inhibitory potential.

CONCLUSION

The ethanolic extract of *Trigonella foenum-graecum* demonstrated significant and concentration- dependent α -amylase inhibitory activity. The activity was comparable to acarbose at higher concentrations. The presence of bioactive phytoconstituents supports its potential role as a natural antidiabetic agent. Further in vivo and clinical studies are required to establish its therapeutic efficacy and safety.

ACKNOWLEDGEMENTS

The authors acknowledge Sri Lakshmi Venkateshwara Institute of Pharmaceutical Sciences for providing necessary facilities for conducting this research.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 2014; 37(Supplement 1): S81–S90.
2. Bailey CJ, Day C. Traditional plant medicines as treatments for diabetes. *Diabetes Care*, 1989; 12(8): 553–564.
3. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of alpha-amylase and alpha-glucosidase by aqueous extract of *Morinda lucida*. *BMC Complementary and Alternative Medicine*, 2013; 13: 91.
4. Kumar P, Bhandari U. Common medicinal plants with antidiabetic potential. *Journal of Ethnopharmacology*, 2012; 142(3): 556–572.
5. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pacific Journal of Tropical Biomedicine*, 2012; 2(5): 411–420.
6. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. *Alternative Medicine Review*, 2003; 8(1): 20–27.
7. Madar Z, Abel R, Samish S, Arad J. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *European Journal of Clinical Nutrition*, 1988; 42(1): 51–54.
8. Hannan JMA, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YHA. Soluble dietary fibre fraction of *Trigonella foenum-graecum* improves glucose homeostasis in animal models of type 1 and type 2 diabetes. *British Journal of Nutrition*, 2007; 97(3): 514–521. <https://doi.org/10.1017/S0007114507336744>
9. Gupta A, Gupta R, Lal B. Effect of *Trigonella foenum-graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: A double blind placebo controlled study. *Journal of Association of Physicians of India*, 2001; 49: 1057–1061.
10. Khosla P, Gupta DD, Nagpal RK. Effect of *Trigonella foenum-graecum* (fenugreek) on blood glucose in normal and diabetic rats. *Indian Journal of Physiology and Pharmacology*, 1995; 39(2): 173–174.
11. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology*, 2006; 52(2): 149–153. <https://doi.org/10.3177/jnsv.52.149>