ORIGINAL ARTICLE

Comparative study of *in vitro* antidiabetic activity with ethyl acetate, aqueous extracts containing seeds of *Trigonella foenum-graecum*

G. Shanthini Nachiar, S. Shyam Sudarsan, M. Abinesh Babu, V. Agilan, A. Anbu Mani, V. Sandeep

Department of Pharmacy, Sathyabama Institute of Science and Technology, Tamil Nadu, Chennai, India

Abstract

Aims: The aims of this study were to compare the *in vitro* antidiabetic activity with ethyl acetate and aqueous extract containing seeds of *Trigonella foenum-graecum*. **Materials and Methods:** The seeds are dried, powdered, and extracted with the solvents among this ethyl acetate and aqueous extract of fenugreek seeds which were chosen. Phytochemical screening shows the presence of flavonoids, phenol, glycosides, and steroids. *In vitro* antidiabetic potential was prepared by an aggregate of 500 µL of test samples and standard drug metformin (100–1000 µg/mL) was added to 500 µL of 0.20 mM, and phosphate buffer (pH6.9) containing α-amylase (0.5 mg/mL) and was incubated at 25°C for 10 min. After these, 500 µL of a 0.02 M sodium phosphate buffer (pH6.9) was added to each tube, were incubated at 25°C for 10 min. The response was stopped with 1.0 mL of 3, 5 dinitrosalicylic acid color reagent. The test tubes were also incubated in a scorching water bath for 5 min, cooled to room temperature. The response recorded by adding 10 mL of distilled water and absorbance was measured at 540 nm. **Results and Discussion:** There was an increased inhibition by *T. foenum-graecum* seed extracts in a dose-dependent manner. As the concentration of the inhibitor increased, the amylase enzyme activity was decreased. It was concluded that the ethyl acetate extract showed better inhibitory activity compared with the standard and aqueous extract of *T. foenum-graecum* seeds. Inhibition was found to be dose-dependent by standard drug metformin which is a commercially available alpha-amylase inhibitor for the treatment of diabetes. **Conclusion:** The present study entitled the "Relative study of *in vitro* antidiabetic activity with ethyl acetate, waterless excerpt containing seeds of *T. foenum-graecum*" focuses on a factory that is generally available throughout India and traditionally used in the treatment of diabetics.

Key words: Anti-diabetic activity, investigation medicinal plant, phytochemical, pharmacognostic, *Trigonella foenum-graecum*

INTRODUCTION

The seeds of fenugreek seeds are the most important and useful part of fenugreek factory. These seeds are golden-unheroic in color, small in size, and hard and have a four-faced gravestone-like structure. Fenugreek seed is 3–6 mm long, 2–5 mm wide, and 2 mm thick in figure. Raw fenugreek seeds have maple flavor and bitter taste but by the process of riding, their bitterness can be reduced and flavor can be enhanced. Fenugreek seeds are used as spices. The whole seed or its ground greasepaint is used in pickles, vegetable dishes, and spice greasepaint. Dried seeds are used as seasonings. Fenugreek seeds are sticky, stringy, sticky, and sticky in nature. Biologically, its seeds are endospermic in nature. *Trigonella foenum-graecum* is one of the oldest medicinal shops, forming in India and Northern Africa. It is an periodic factory, which grows to an average height of two bases. The leaves and seeds, which develop in long capsules, are used to prepare excerpts or maquillages for medicinal use.

Address for correspondence:
G.Shanthini Nachiar, Department of Pharmacy,
Sathyabama Institute of Science and Technology,
Tamil Nadu, Chennai, India. Mobile: +91-8838571850.
E-mail: shanthini31011980@gmail.com

Received: 22-04-2023
Revised: 18-06-2023
Accepted: 28-06-2023

In India, fenugreek is generally consumed as a seasoning and used medicinally as a lactation goad. There are multitudinous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. Its seeds are being used as spice and splint as a vegetable.

Aim and Scope of the Work

This exploration work exhaustively summarizes the part of fenugreek seeds in health operation with a special emphasis on modulating colorful physical and biochemical conditioning as well as in studies.

Grounded on the ethnomedical information and studies available, the present exploration work has been framed to carry out the following studies on the leaves of *T. foenum-graecum*.

Phytochemical Evaluation

- Preliminary phytochemical screening
- Estimation of total flavonoids content.[1-10]

Pharmacognostical Evaluation

- Macroscopy
- Anatomical section of leave, stem, and seed
- Quantitative microscopy of seeds.

Pharmacological Evaluation[17-21]

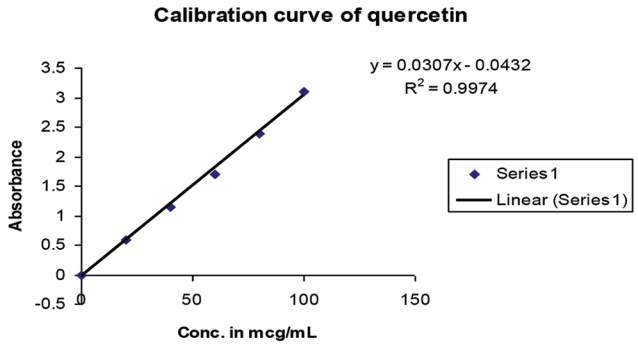
- *In vitro* antidiabetic activity method
- Non-enzymatic glycosylation of hemoglobin assay.

Experimental Work

Preliminary phytochemical screening with the seeds of T. foenum-graecum

Estimation of total flavonoids determination

Procedure



I mL of ethanolic extract at concentrations 50 ug/mL and 100 µg/mL were taken and 0.1 mL of aluminum chloride solution, 0.1 mL of potassium acetate solution, and 28 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min, the absorbance was measured at 415 nm. A calibration curve was constructed by plotting absorbance reading of quercetin at different concentrations. The sample without aluminium chloride was used as a blank.

The total flavonoid content in the extract was expressed as milligrams of quercetin equivalent per gram of extract and the results were tabulated.[10-16]

Pharmacognostical evaluation

Macroscopy

The aerial part of the plant is moderately branched and weak. The stem is erect, branched, green, smooth, and herbaceous. Leaves are petiolate, alternate, compound, trifoliate, fragrant, and stipulate; seeds oblong flattened, or irregularly rhomboidal, hard, a narrow depression dividing radicle pocket from cotyledons is seen, yellowish-brown in color with a characteristic spicy odor and spicy mucilaginous, bitter taste.

S. No	Test	Ethyl acetate	N-butanol	Petroleum ether	Ethanol
1.	Test for flavonoids				
	a. Shinoda test	+	+	+	+
2.	Test for alkaloids				
	a. Dragendroff's reagent	+	+	+	+
	b. Wagner's reagent	+	+	+	+
	c. Mayer's reagent	+	–	+	–
3.	Test for phenol				
	a. $FeCl_3$ test	–	–	–	+
4.	Test for TANNIS				
	a. Lead acetate test	+	–	+	–
5.	Test for carbohydrates				
	a. Fehling's test	–	–	–	–
6.	Test for volatile oils	–	–	–	–

(+) indicates positive reaction, (–) indicates negative reaction

S. No.	Conc. of quercetin in µg/mL	Absorbance at 415 nm	Conc. of ethyl acetate extract in µg/mL	Absorbance at 415 nm	Amt of total flavonoid content in terms mg quercetin equivalent/g of extract
1	20	0.589±0.01	50	0.0383±0.001	467.21±0.351
2	40	1.151±0.04	100	0.0896±0.006	486.36±0.06
3	60	1.710±0.09	150		
4	80	2.390±0.03	-		
5	100	3.112±0.03	-		
				Average	24.08

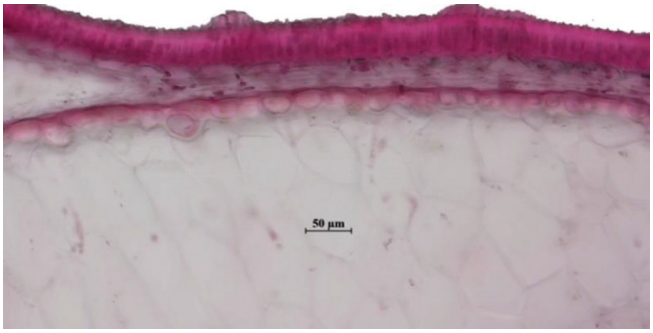
Twig of *Trigonella foenum-graecum* L. stem and leaves

TS of *Trigonella foenum-graecum* stem

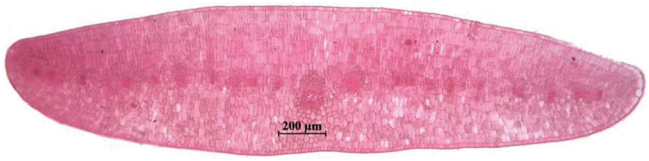
TS of *Trigonella foenum-graecum* petiole

TS of *Trigonella foenum-graecum* midrib

TS of *Trigonella foenum-graecum* lamina



TS of *Trigonella foenum-graecum* seed



TS of *Trigonella foenum-graecum* cotyledon

TS of *Trigonella foenum-graecum* radicle

Quantitative microscopy of T. foenum-graecum leaf

The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded.

The leaf is amphistomatic with anomocytic stomata; the epidermal cells were wavy.

Parameters	Upper epidermis (/mm²)	Lower epidermis (/mm²)
Epidermal number	100–110	110–120
Stomatal number	40–45	45–55
Stomatal index	28–31	30–31
Palisade ratio	25–30	

Pharmacological evaluation

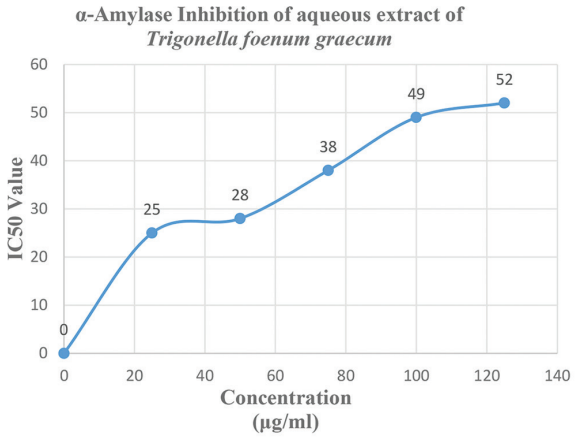
In vitro antidiabetic Potential of *Trigonella foenum-graecum,* alpha-amylase Inhibitory activity.

Procedure

An aggregate of 500 µL of test samples and standard medicine (100–1000 µg/mL) was added to 500 µL of 0.20 mM phosphate buffer (pH6.9) containing α- amylase (0.5 mg/mL) result and were incubated at 25°C for 10 min. After these, 500 µL of a 1 bounce result in 0.02 M sodium phosphate buffer (pH6.9) was added to each tube. The response fusions were also incubated at 25°C for 10 min. The response was stopped with 1.0 mL of 3, 5 dinitro salicylic acid color reagent. The test tubes were also incubated in a scorching water bath for 5 min, cooled to room temperature. The response admixture was also adulterated after adding 10 mL distilled water and absorbance was measured at 540 nm. Control represents 100 enzyme exertion and was conducted in analogous way by replacing excerpt with vehicle.

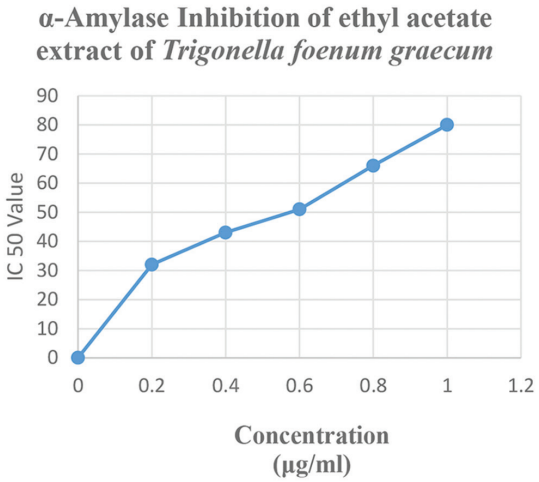
α-Amylase Inhibition of aqueous extract of *Trigonella foenum-graecum*

Concentration (µo/mL)	IC_{50} Value 50% inhibitory concentration
0	0
25	25
50	28
75	38
100	49
125	52



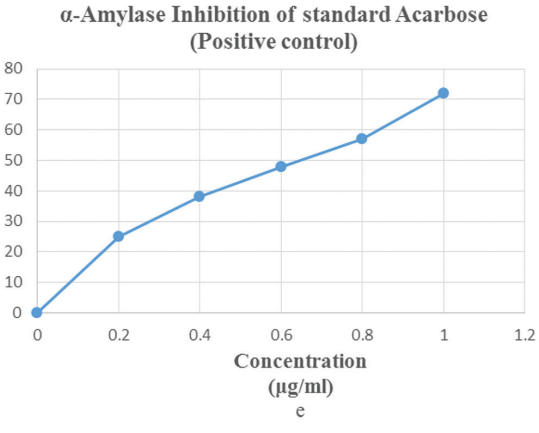
Concentration (µo/mL)	IC50 Value $_{50}$% inhibitory concentration
0	0
25	32
50	43
75	51
100	66
125	80

α-Amylase Inhibition of ethyl acetate extract of *Trigonella foenum-graecum*



α-Amylase Inhibition of standard Drug Metformin (Positive control)

Concentration (µo/mL)	IC_{50} Value 50%inhibitory concentration
0	0
25	25
50	38
75	48
100	57
125	72



There was an increased inhibition by *T. foenum-graecum* seeds extracts in dose dependent manner. As the concentration of the inhibitor increased, the amylase enzyme activity was decreased.

It was concluded that the ethyl acetate extract showed better inhibitory activity compared with the standard and aqueous extract of *T. foenum-graecum* seeds.

Inhibition was found to be dose dependent by standard drug metformin which is a commercially available alpha-amylase inhibitor for the treatment of diabetes.

RESULTS AND DISCUSSION

The preliminary phytochemical screening procedure of the aqueous and *T. foenum-graecum* extract of fenugreek seeds showed the presence of carbohydrates, alkaloids, and flavonoids, whereas phenol, tannins, and volatile oil were absent.

The linear regression equation was found to be y=0.0307x−0.0432 while the correlation was found to be 0.9974. The amount of flavonoid content presents in the extract in terms mg quercetin equivalent/g of extract was found to be 24.08 using the above linear regression equation.

TS is oblong circular with uniseriate epidermis covered by a thin cuticle; a single layered collenchymatous hypodermis is present; cortical region is made up of 5 to 6 layers of thin-walled parenchyma cells; collateral vascular bundles are arranged as a ring; discontinuous pericyclic fibers are seen surrounding the vascular region; a few prismatic crystals are seen randomly distributed in the pericyclic region; and centrally placed thin walled parenchymatous pith is present.

Cotyledon consists of the upper and lower epidermis; 4 – 5 layers of palisade cells are seen below the upper epidermis; on the dorsal side 7 – 10 layers of spongy, parenchyma cells are seen which are filled with oil globules and aleurone grains; small vascular bundles are present in the mesophyll region.

Radicle TS is circular in outline; the single-layered epidermis is present covered by cuticle followed by broad cuticle made up of thin-walled parenchyma cells filled with aleurone grains; vascular region is present in the center.

Phytochemical investigation reveals the presence of flavonoids, and role of flavonoid in diabetic, the results reveals that alpha-amylase inhibition activity was high with 125 mg/mL of ethyl acetate *Trigonella foenum*, compared with aqueous extract of 125 mg/mL.

T. foenum exhibits toward reduction in glucose level, which plays a key role equivalent to synthetic molecule in curing the diabetic conditions.

In future, the study would be conducted in animals (*in vivo* studies) to prove the betterment of fenugreek seeds toward reducing the glucose level in patients suffering with diabetics without any adverse side effects.

Organic drugs are first born in India to cure various diseases. Among these life-threatening diseases such as diabetes, some herbal plants are used traditionally in olden days to cure diabetes. Patients suffering from diabetes may affect with skin allergy, cornea encephalopathy, etc.

SUMMARY AND CONCLUSION

The present study entitled the "Relative Study Of *in vitro* AntiDiabetic Activity with Ethyl Acetate, Waterless Excerpt Containing Seeds of *T. foenum-graecum*" focuses on a factory which is generally available throughout India and traditionally used in treatment of colorful diabetics. Studies on the seeds of *T. foenum-graecum* are still lacking. Hence to exploit this, present study with Trigonella seeds proved to be effective in curing diabetic conditions. The chapter on literature review deals with the information regarding the pharmacognostical, phytochemical, and pharmacological evaluation of the seeds of *T. foenum-graecum*. (The antidiabetic exertion of excerpt of seeds of *T. foenum-graecum* was carried out by) nascence amylase inhibition assay system. The excerpt showed better nascence-glucosidase inhibition property the excerpt parade significant inhibition of glycosylation as compared with the standard medicine metformin. Decreases the conformation of the glucose-hemoglobin complex and therefore quantum of free hemoglobin increases it act by inhibiting glucose-haemoglobin complex. The excerpt showed lesser effectiveness in adding the glucose uptake by incentive cells as compared to standard medicine metformin. Type 2 diabetes is characterized by the insufficiency of insulin causing increased quantum of glucose in blood. After the treatment of the incentive cells with these splint excerpts, the glucose uptake was set up to increase in a cure-dependent manner nascence amylase impediments bind to the nascence bond of polysaccharide and help break down of polysaccharide in mono and disaccharide. The result showed an excerpt of *T. foenum-graecum* significant exertion as compared to a standard medicine. The *in vitro* assays of the present study indicated that the excerpt of *T. foenum-graecum* possesses good antidiabetic exertion.

REFERENCES

1. Mosihuzzaman M, Iqbal C. Safety, efficacy, standardization, and documentation of herbal medicine. Int Union Pure Appl Chem 2008;80:2195-230.
2. Aggarwal A. Critical issues in quality control of herbal products. Pharma Times 2005;37:9.
3. Ayurvedic Pharmacopoeia. Ministry of Health and Family Welfare. New Delhi: Government of India; 1996. p. 1-43.

4. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehradun: International Book Distributors; 1999.
5. The Ayurvedic Pharmacopoeia of India, Part. 1st ed., Vol. 2. India: Government of India Ministry of health Education; 1996. p. 107-8.
6. Petropoulos GA. Fenugreek Trigonella. 1st ed. London, New York: Taylor and Francis; 2002. p. 1-127.
7. Alberta Agriculture, Food and Rural Development (AAFRD), Fenugreek, Agrifax, Agdex. Vol. 5. Taylor and Francis: Agdex; 1998. p. 147.
8. Edison S. Spices productivity. In: Ravi N, editor. The Hindu Survey of Indian Agriculture. Madras: Kasturi and Sons Ltd.; 1995. p. 101-5.
9. Fazli FR, Hardman R. The spice fenugreek (*Trigonella foenumgraecum*). Its commercial varieties of seed as a source of diosgenin. Trop Sci 1968;10:66.
10. Mukherjee PK. Evaluation of Indian traditional medicines. J Drug Inform 1996;35:631.
11. Mukherjee PK. Quality Control Herbal Drugs: An Approach to Evaluation of Botanicals. New Delhi: Horizons; 2002. p. 800.
12. Anonymous. Quality Control Methods for Medicinal Plant Materials. Geneva: World Health Organization, Phar; 1992. p. 559.
13. Kokate CK, Purohit AP, Gokhale SB. A Text Book of Pharmacognosy. Vol. 31. Pune: Sathya Publishers; 2005. p. 130
14. Anonymous, WHO. Publication Data, Quality Control Methods for Medicinal Plant Materials. Typeset in Hong Kong, England. Geneva: Anonymous, WHO; 1998.
15. Indian Pharmacopoeia. Ministry of Health and Family Welfare. New Delhi: Government of India; 2007.
16. Escot N. Atoms summar. In: Fenugreek. Berlin, Germany: Springer; 1994/1995. p. 7-12.
17. Passano P. The Many uses of Methi. New Delhi: M/S,AC Specialist publishers pvt.,Ltd.; 1995. p. 31-4.
18. Yoshikawa M, Murakami T, Komatsu H, Murakami N, Yamahara J, Matsuda H, *et al*. Medicinal Foodstuffs: IV. Fenugreek seeds (1): Structures of trigoneosides Ia, Ib, IIb, IIIa and IIIb new furostanol saponins from the seeds of Indian *Trigonella foenum-graecum* L. Chem Pharmacol Bull 1997;45:81-7.
19. Abdo MS, Al-Kafawi AA. Experimental studies on the effect of *Trigonella foenum-graecum*. Planta Med 1969;17:14-8.
20. Xue W, Lei J, Li X, Zhang R. *Trigonella foenum-graecum* seed extract protects kidney function and morphology in diabetic rats via its antioxidant activity. Nutr Res 2011;31:555-62.
21. Gupta R, Nair S. Antioxidant flavonoids in common Indian diet. South Asian J Prev Cardiol 1999;3:83-94.
22. Broca C, Gross R, Petit P, Sauvaire Y, Manteghetti M, Tournier M, *et al*. 4-Hydroxyisoleucine: Experimental evidence of its insulinotropic and antidiabetic properties. Am J Physiol 1999;277:617-23.

Source of Support: Nil. **Conflicts of Interest:** None declared.