

Preliminary Phytochemical and Pharmacognostic Studies on a Well Known Medicinal Plant *Trigonella foenum-graecum*

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Research Article

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Abstract

Fenugreek is commonly used as a spice in cooking and in small quantities is categorized as "Generally Recognized as Safe (GRAS)" by the U.S. Food and Drug Administration. Fenugreek is a member of the Leguminosae (Fabaceae) family and is commonly cultivated in India, Egypt, the Middle East and North Africa. The seeds of the plant have been used as a traditional remedy for numerous conditions including gastrointestinal disorders, gout, wound healing and inflammation, hyperlipidemia and diabetes. Bioactive compounds isolated from fenugreek seeds include saponins (ie: fenugreekine, diosgenin), alkaloids (i.e.: trigonelline, gentianine, carpaine), amino acids, some of which act as insulin secretagogues (i.e.: 4-hydroxyisoleucine, arginine), coumarins, mucilaginous fibers (galactomannan), nicotinic acid and other vitamins and minerals.

Keywords: *Trigonella Foenum-Graecum Fenugreek*; Phytochemical; Pharmacognostic; Medicinal Plant

Introduction

Trigonella foenum-graecum Linn. Commonly known as Fenugreek, also called Greek Hay, is an erect annual herb (Figure 1). Ayurveda recognizes the use of 'seeds' for a pathological condition called "Madhumeha" (Diabetes) or "Honey urine", which was first described by Sushruta around 1000 BC. It is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use.



Figure 1: Habit.

Applications of fenugreek were documented in ancient Egypt, where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread making. In ancient Rome, fenugreek was purportedly used to aid labor and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and edema of the legs. In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. The possible hypoglycemic and anti hyperlipidemic properties of oral fenugreek seed powder have been suggested by the results of preliminary animal and human trials. Its actions are partly mediated by stimulation of insulin signaling pathway. Constituents responsible for biological actions of fenugreek have been studied. While 4- hydroxyisoleucine is shown to potentiate insulin secretion in a glucose dependent manner, saponins of fenugreek are reported to inhibit the absorption of glucose in the intestine.

The effect of Oral supplements of fenugreek (*Trigonella foenum graecum*) seeds (Fen) in Zucker obese rats (Ob), an animal model of obesity and related disorders, such as dyslipidemia and hepatic steatosis was carried out by Raju, et al. [1]. A study was conducted by Mahdavi, et al. [2] to evaluate the underlying mechanisms, including the role of nitric oxide (NO) and cyclooxygenase pathways, in diabetic rats. Male Wistar rats were divided into control, extract-treated control, diabetic, and extract-treated diabetic groups. Diosgenin suppresses proliferation, inhibits invasion and suppresses osteoclastogenesis through inhibition of NF-kappaB-regulated gene expression and enhances apoptosis induced by cytokines and chemotherapeutic agents [3]. Anti-inflammatory and antipyretic effects of the *Trigonella foenum graecum* (TFG) leaves extract, an Iranian medicinal plant, were examined by Ahmadiani, et al. [4]. For anti-inflammatory activity, the formalin-induced edema model was used. Hyperthermia was induced by intraperitoneal injection of 20% (w/v) aqueous suspension of brewer's yeast. Effective concentrations of TFG extract in the above mentioned experiments did not inhibit COX enzymes in EIA tests. In conclusion, the results indicate that the blocking of spinal purinoceptors may contribute in the analgesic effect of TFG leaves extract [5]. The seeds of have been reported to have antidiabetic and hypocholesterolaemic properties in both animal models and humans. The antidiabetic activity of the seeds of *Trigonella foenum graecum* (fenugreek) has been attributed largely to its saponin and high fibre content, and is probably not related to its major alkaloid

trigonelline. Its regular consumption may therefore be beneficial in the management of diabetes and the prevention of atherosclerosis and coronary heart disease. In a comparative study by Pipelzadeh, et al. [6] between Fenugreek (*Trigonella foenum graecum*) with that of lovastatin the effectiveness in restoration of endothelial function in the aorta taken from aged N-Mari rats. However, improvement in endothelial function was significantly increased in all treatment groups. The histological findings showed significant reduction in thickness and lipid deposits in the aorta in all treatment groups. The improvement in the epithelial function was correlated with LDL-cholesterol lowering and partly with the reduction in the thickness of the aortic intimal layer. This study demonstrated that fenugreek is as effective as lovastatin in reducing the features associated with atherosclerosis. The hypolipidaemic effect of fenugreek was associated with the defatted fraction of the seed powder, and among the extracts analysed, the crude saponin extract was the most effective fraction with respect to the fenugreek seeds. The improvement of the plasma lipid profile by fenugreek treatment further supports the use of fenugreek seeds as a hypolipidaemic agent in the improvement of lipid disorders [7].

In view of the available literature and scientific evidence stated above Fenugreek may have applications in the areas of many pharmacological conditions? In this context it is very important to standardize this plant using pharmacognostic studies, hence this present effort.

Materials and Methods

Voucher specimen: The plant material Rhizome of *Trigonella foenum graecum* was collected from the wild and Identity was confirmed with the voucher specimen using Gamble, et al. [8].

Physico Chemical and Fluorescence Studies

Organoleptic Characters: The present investigation comprises studies on both physical and sensory characteristics such as color, sensation, taste, oily stain and mucilage of the species under study.

Determination of Total Ash: Two grams of powdered drug was incinerated in a sintered silica crucible by gradually increasing heat up to 450°C until the drug is free from carbon and then cooled. This ash kept in a dessicator for 15-20 min. and weighed using Anamed Electronic balance, India and noted down the readings [9].

$$\text{Ash \%} = \frac{B - C}{A} \times 100$$

Where, weight of empty crucible=C, weight of plant material=A, weight of crucible + ash = B, weight of ash = B-C.

Determination of Acid Soluble Ash: Total ash obtained was boiled for 15 min. in 25 ml of 25% hydrochloric acid and filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in a dessicator for 15 min. The residue was weighed in Anamed Electronic balance and the acid soluble ash was calculated using the formula.

Determination of Extractive Values: Hundred gram of powdered plant material of both plants understudy were extracted with ethanol (95%) and water using Soxhlet extractor. Thus obtained extracts were allowed to dry to room temperature. After complete evaporation, weight, nature and colour of the extracts were recorded [10].

$$\% \text{ Extractive value} = \frac{\text{Weight of the residue obtained}}{\text{Weight of plant material taken}} \times 100$$

Fluorescence Studies [11]: The fluorescent study of the seed powder of *Trigonella foenum graecum* was treated with chemicals such as benzene, chloroform, acetic acid, ethanol, water, concentrated H₂SO₄ and concentrated HCl. The powdered materials gave different color reactions

with different chemicals and fluorescent colors of treated and untreated drugs were observed under visible and UV light and the observations were noted (Table 3).

Preliminary Phytochemical Tests: The qualitative phytochemical tests were carried out for phenols, flavonoids, steroids, triterenes, diterpenes, lactones, tannins, lignins, saponins, alkaloids following the methods of Gibbs, et al. [12-14].

Physico-chemical values such as the percentage of total ash, acid-insoluble ash, water-soluble ash, and water and alcohol-soluble extractives were calculated as per the Indian Pharmacopoeia.

TLC fingerprinting profile carried as per Stahl E, et al. [15]. For the Anatomical studies, transverse sections (TS) were prepared and stained [16]. A standard, Limit for total microbial count provided by WHO Guidelines [17] was followed and also Indian herbal pharmacopoeia.

Histological Studies [18]: For the purpose of studying the micro scopical characters free hand sections were used. These sections were washed in tap water and stained with saffranine for further observations and photographed using Magnus microscope.

Results and Discussions

Sl. No	Sample	Color	Texture	Odor	Taste
1	Fenugreek (seed)	Yellow	Coarse	Pungent	Bitter

Table 1: Organoleptic characters.

Sample Name	Total Ash Content (%)	Acid Insoluble Ash (%)	ASE (%)	WSE (%)
Results	3.2592	0.66	3.00	12.9
Limits(API)	NMT 4	NMT 0.5	NLT 5	NA

API-Ayurvedic Pharmacopoeia of India NMT-Not more than NLT- Not Less than.

Table 2: Physicochemical parameters.

Sl. No	Solvent	Visible	UV
1.	Ethanol	Yellow	Black
2.	Methanol	Yellow	Green
3.	Toluene	Milkish yellow	Transparent
4.	HNO ₃	Brownish red	Dark yellow
5.	H ₂ SO ₄	Orangish red	Dark yellow
6.	HCl	Light orange	Greenish yellow
7.	Water	Milky yellow	Yellowish green

Table 3: Powder analysis of Fenugreek after treating.

Phytochemicals	Test	Observation	Results
Phenols	Phenol test	Intense color	+
Flavonoids	Shinoda test	Yellowish brown color	+
Steroids	Salkowski test	Red color	-
Triterpenes	Salkowski test	Yellow color	-
Saponins	Foam test	Foam	-
Alkaloids	Meyer's test	Creamy white precipitate	+
Lignans	Labat test	Olive green color	-

Table 4: Preliminary Phytochemical analysis of Fenugreek.

TLC profile of Trigonella

Solvent system: 1ve Toluene: Ethyl acetate (8:2)

Extracted in Methanol.

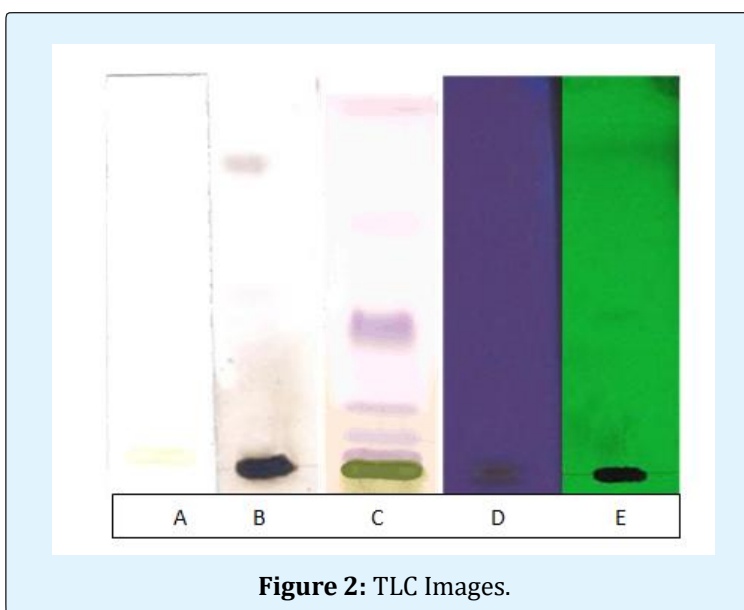


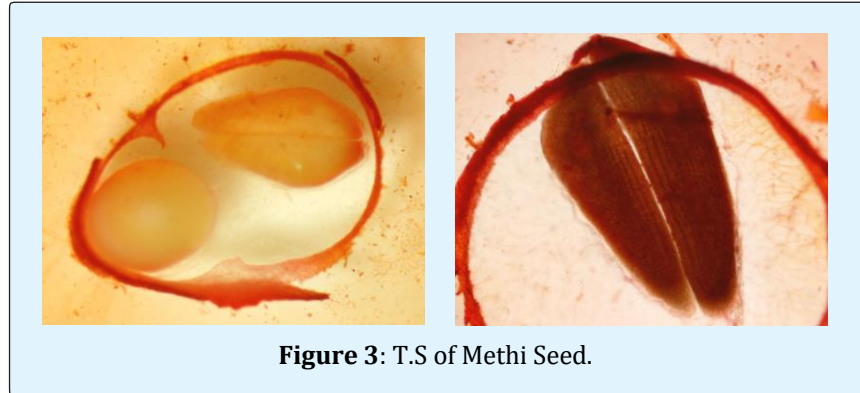
Figure 2: TLC Images.

A. Under Visible Light								
Rf Values	-	-	-	-	-	-	-	-
B. Sprayed with 10% H2SO4								
Rf Values	0.82	-	-	-	-	-	-	-
C. Sprayed with Anisaldehyde								
Rf Values	0.04	0.09	0.17	0.38	0.67	0.97	-	-
D. Under LongUV (366nm)								
Rf Values	0.41	-	-	-	-	-	-	-
E. Under Short UV (254 nm)								
Rf Values	-	-	-	-	-	-	-	-

Table 5: TLC Finger Printing Profile.

Anatomy of Trigonella seed

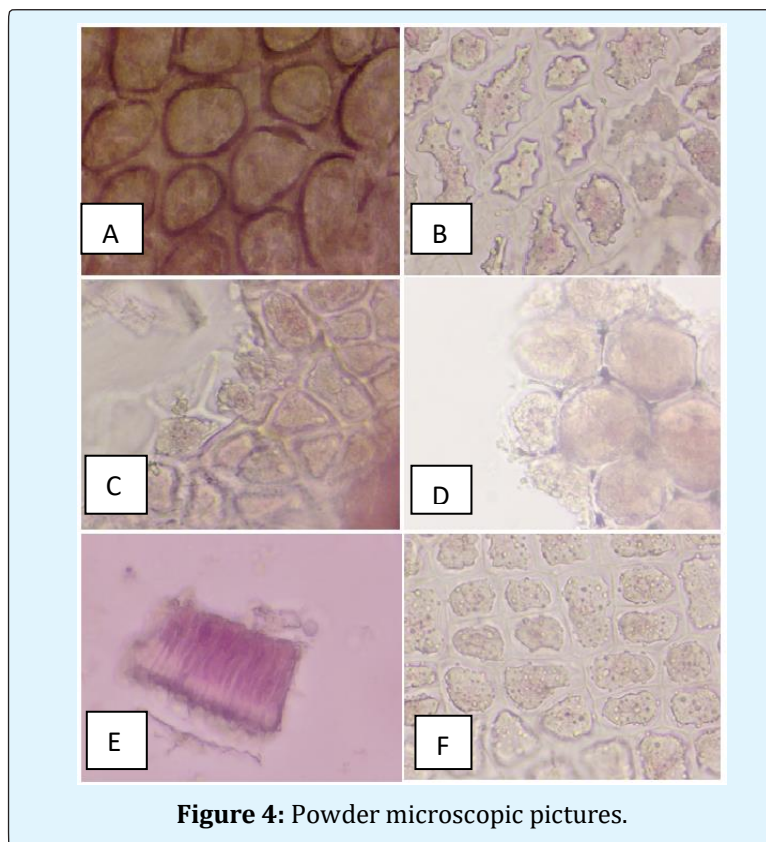
Histology Characters



- Thick layered of thick walled columnar palisade covered extremely with thick cuticle cells flats at base, mostly pointed but a few flattened at apex, supported internally wide bearer cells having radial rib like thickenings followed by tangentially elongated cells, thin walled parenchymatous
- Endodermis consists of thin layer of thick walled containing of thick walled cells containing aleuron grains.
- Several layers of a thin-walled mucilaginous cell, varying size, long axis radially elongated in outer region and tangentially elongated in inner region.
- Cotyledon consists of 3-4 layers of palisade cells varying in size with axis and few layers of rudimentary spongy tissue and vascular tissue situated in mesophyll containing aleuron grains and oil globules.

Powder Microscopy

Powder microscopic characters are very important in Ayurveda for the proper identification and authentication of plant materials during drug standardization [19-22].



Powder characteristics of Fenugreek A-Hypodermis of testa in surface view, from below. B-Outer most layer of the endosperm in surface view, C-Epidermis of the testa in surface view, from below, D-Epidermis and parenchymatous cell of the cotyledons in sectional view, E-Cuticle, epidermis and hypodermis of the testa in sectional

view, F-Epidermis of the testa in surface view, from above [23,24].

Microbial Studies

The microbial limit tests reveal that the TABC and TYMC are well within the prescribed limit as per IHP and E Coli and Salmonella are absent.

Raw herb Sample	Total Aerobic Bacterial Count (TABC) Cfu/gm	Total Yeast And Mould Count (TYMC) (Cfu/gm)
<i>Trigonella</i>	45 x 10 ⁵	23 x 10 ³

Table 6: Microbiological Limits.

Conclusion

The Plant understudy Fenugreek is most commonly used for culinary purposes and has got many therapeutic properties. The Organoleptic characters (Table 1), physicochemical parameters and Microbial limit tests revealed that there is no harm or side effect if consumed (Table 2 & 6). The fluorescent studies help in identifying and authenticating the sample based on its color when treated with different chemicals (Table 3). The secondary metabolites viz Phenols, Flavonoids and alkaloids are present and the efficacy of this plant may be attributed to this group of compounds (Table 4). The TLC results reveal the finger print profile of the plant part (Table 5). The Histology and microscopic studies will help in standardizing and authenticating the drug Fenugreek seed.

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