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Pharmacognostic evaluation of *trigonella foenum-graecum linn* leaves

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Abstract
Trigonella Foenum-graecum Linn., is an annual herb of bean family, reaching 30-60 cm and largely cultivated in India, Egypt and Morocco. It is commonly known as 'Fenugreek', which belongs to the family *Fabaceae*. The present study attempts to evaluate Pharmacognostic studies including examination of macroscopic and microscopic character and powder analysis of Fenugreek leaves. The detailed Pharmacognostic studies have given a clear idea regarding the different cell characters and various constants. The physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, loss on drying and extractive values were also determined. These Physicochemical parameters have given standard numerical values for comparison and detection of adulterants. The results of this study will possibly prove useful for establishing pharmacognostic standards for the identification, purity and quality of drug.

Keywords: *trigonella foenum-graecum linn*., leaves, pharmacognostic studies, physicochemical parameters

1. Introduction
In recent times, there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is always supposed to be the safe. The advantage of natural drugs is their easy availability, economic and less or no side effects but the disadvantage is that they are the victims of adulteration. The more effective the natural drug, more is its demand and the chances of non-availability increases. To meet the growing demand, the natural drug is easily adulterated with low grade material. Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basically deals with standardization, authentication and study of natural drugs. Pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also. This is again necessary because once the plant is dried and made into powder form, it loses its morphological identity and becomes easily prone to adulteration. Such studies will help in authentication of the plants and ensure reproducible quality of herbal products which will lead to safety and efficacy of natural products.
Fenugreek, *Trigonella Foenum-graecum Linn*., is an annual herb of bean family, reaching 30-60 cm and largely cultivated in India, Egypt and Morocco. The name fenugreek comes from *foenum-graecum*, meaning 'Greek hay', as the plant was traditionally used to scent inferior hay and the name of the *Trigonella* is derived from the old Greek name, denoting 'three angled', probably referring to the triangular shape of flowers. Fenugreek has strong flavor and aroma. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriented countries as a spice in food preparations and as a ingredient in traditional medicine. Medicinally it was used for the treatment of wound abscesses, arthritis, bronchitis, ulcer and digestive problems. The plant grows to height of about 3 feet. *Trigonella Foenum-graecum Linn* has long stalked leaves up to 5cm long stipules triangular; lanceolate leaflets about 2.5 cm long. The root mass of fingery structure. Flowers are white and pale yellow. The plant radiates spicy odor which persist on the hands after touching. Fenugreek is best grown as a annual crop from seeds by the line sowing method. The leaves contain 7 saponins, known as *graecunins*. These compounds are glycosides of diosgenin. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1% and carbohydrate 6% & the vitamins (calcium, iron, phosphorous, carotene, thiamine, riboflavin, niacin and vitamin c).
Fabaceae: A large family that comprises the peas, beans and related herbaceous or woody plants with pea like flowers and a legume as fruit and that is now usually included in the family Leguminosae.

Fig 1: Trigonella Foenum-graecum leaves

2. Plant Profile
It is a biennial plant, but is usually grown as an annual. Modern varieties typically grow to a height of 15 to 45 cm (6 to 18 in). The leaves are yellowish to bluish green and grow alternately in a flattened, fan-shaped swathe. They are

fleshy, hollow, and cylindrical, with one flattened side. They are at their broadest about a quarter of the way up, beyond which they taper towards a blunt tip. The base of each leaf is a flattened, usually white sheath that grows out of a basal disc. From the underside of the disc, a bundle of fibrous roots extends for a short way into the soil. As the onion matures, food reserves begin to accumulate in the leaf bases and the bulb of the onion swells.

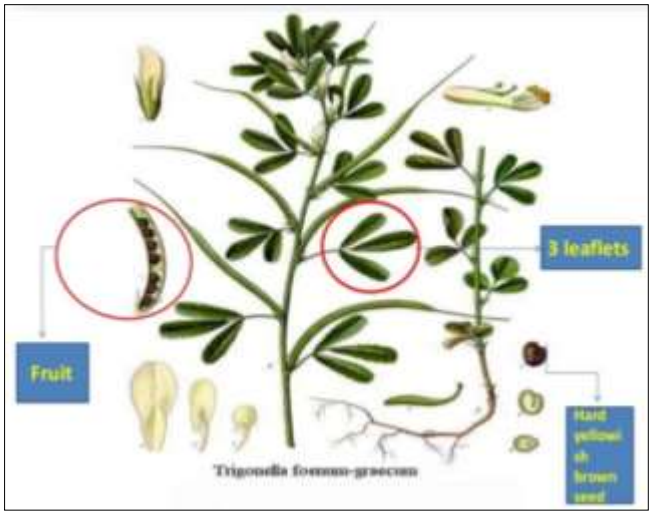


Fig 2: Trigonella Foenum-graecum plant (Fabaceae)

2.1. Chemical Constituents

The alkaloid trigonelline, trigocoumarin, trimethyl coumarin and nicotinic acid are present in stem. About 28% mucilage; 5% of a stronger-smelling, bitter fixed oil, 22% proteins; a volatile oil; two alkaloids, Trigonelline and Choline, and a yellow colouring substance are present in stem. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1%, and carbohydrate 6%. Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis. These seeds are aromatic, bitter, carminative, antibacterial and may be eaten raw or cooked.

2.2. Traditional Uses

- Traditional Chinese herbalists used plant for kidney problems and conditions affecting the male reproductive tract. The seeds also function as a preservative and are added to pickles, chutneys and other similar products. In modern food practice, the seeds or the extract are used in bakery products, frozen dairy products, meat products, relish, condiments, candy, gravy sauces, gelatin puddings and in alcoholic and non-alcoholic beverages.
- In China, the fenugreek seeds are used as a pessary to treat cervical cancer. In the Middle East and the Balkans, the aerial parts of plant are a folk remedy for abdominal cramps associated with both menstrual pain and diarrhea or gastroenteritis. Fenugreek has a beneficial action on cleansing the blood. As a diaphoretic it is able to bring on a sweat and to help detox the body. This takes place through the pores of the skin.
- Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel, it absorb toxic material and eliminate it. The healing and soothing action creates a protective coating, like a lubricant, over inflamed areas. The slightly bitter properties of the seed are beneficial for digestion.
- The Fenugreek herb has been known to help reduce fever when taken with lemon and honey, since it nourishes the body during an illness. Some health food stores also sell herbal Fenugreek teas, which can be used instead of the green tea.
- Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. It has been found that fenugreek can increase a nursing mother's milk supply within 24 to 72 hours after first taking the herb.
- Medicinally, Fenugreek seeds and leaves are anti-inflammatory, antitumor, carminative, expectorant and uterine tonic, and useful in burning sensation.

2.3. Taxonomical Classification

Table 1

Kingdom	**Plantae**
Division	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Trigonella L.
Species	Trigonella Foenum-graecum L., (T. Foenum-graecum), - Sickle fruit Fenugreek

2.4. Synonyms

Table 2

Botanical name	***Trigonella Foenum-graecum***
English name	Fenugreek, Sickle cell Fenugreek, Greek hay
Hindi name	Methi
Sanskrit name	Methika
Telugu name	Menthulu
Tamil name	Vendhayam

2.5. Pharmacognostic evaluation of *Trigonella Foenum-graecum* leaf

Table 3

Colour	Dark green
Odour	Bitter
Taste	Bitter

3. Material and Methods

3.1. Collection, Identification and Authentication of Plant Material

The fresh Leaves of *Trigonella Foenum-graecum* was collected from local region of Nanded i.e. from local market and authenticated by Dr. Shrirang S. Bodke, Head, Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded.

3.2. Processing of Plant Material

Shade drying of the leaves up to complete removal of moisture was done. (Took around 15 days). Dried leaves were powdered by hand crushing and sieved through sieve number 30.

Fig 3: Leaves in powder form

3.3. Pharmacognostic evaluation of plant material

3.3.1. Leaf Macroscopy

Morphological characters of plant like shape of leaves, apex, base, margin etc were examined properly. The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste.

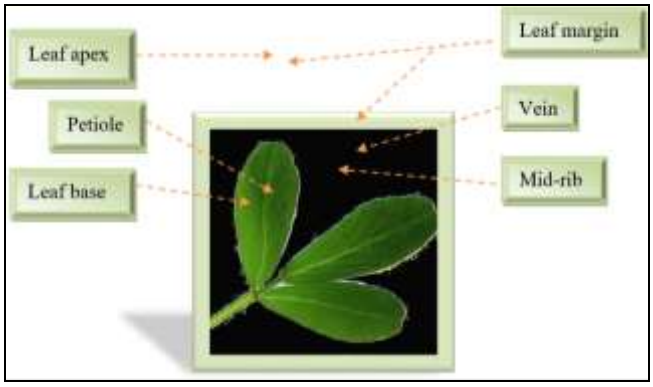
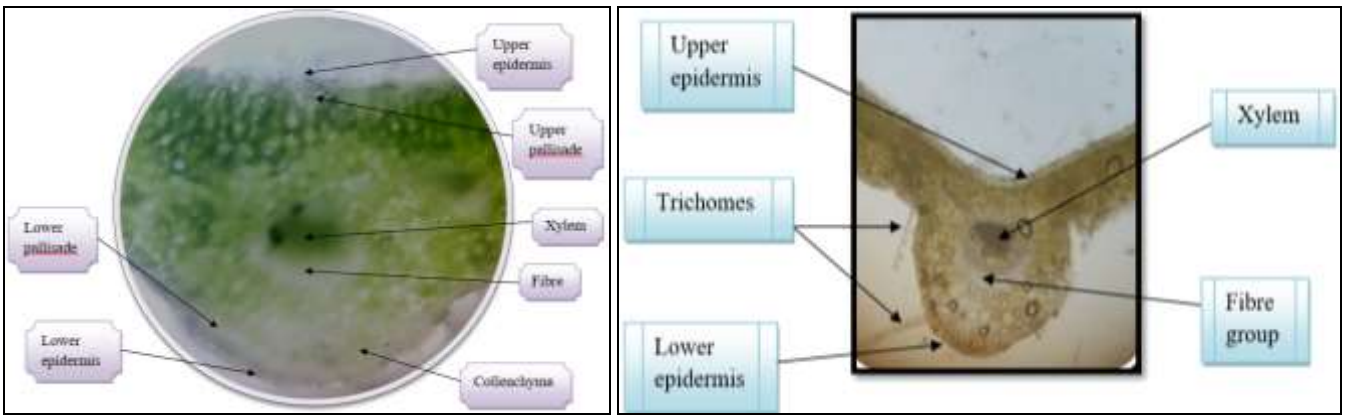


Fig 4: Leaf of *Trigonella Foenum-graecum*

3.3.2. Leaf Microscopy

The outer epidermal membranous layer (in fragments) were cleared in chloral hydrate, mounted with glycerine and observed under a compound microscope. The presence/absence of the following was observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution), epidermis (upper and lower), hypodermis, spongy parenchyma, stomata number, stomatal index, vein islet and veinlet termination number were determined by using fresh leaves of the plant. Xylem elements and ground tissue were also observed under microscope. The transverse sections of the fresh leaves through the lamina and the midrib were also cleared, mounted and observed.



(A) **(B)**

Fig 5: (A) and (B): T.S. of *Trigonella Foenum-graecum* leaf

3.3.3. Microscopical evaluation of powdered drug

Some amount of powdered drug stained with phloroglucinol and HCL (1:1). It gives pink colour to fibers and vascular bundles which are observed under microscope with 40X lens. When it was stained with dil. HCL calcium oxalate crystals were observed. Under microscope fibrous layer of anther with reticulated cell was observed. Shows fragments of straight walled, polygonal, thin walled epidermal cells, simple pitted cells, vessels with spiral thickening, anisocytic stomata, Unicellular, bent or curved trichomes, a few prismatic crystals of calcium oxalate.

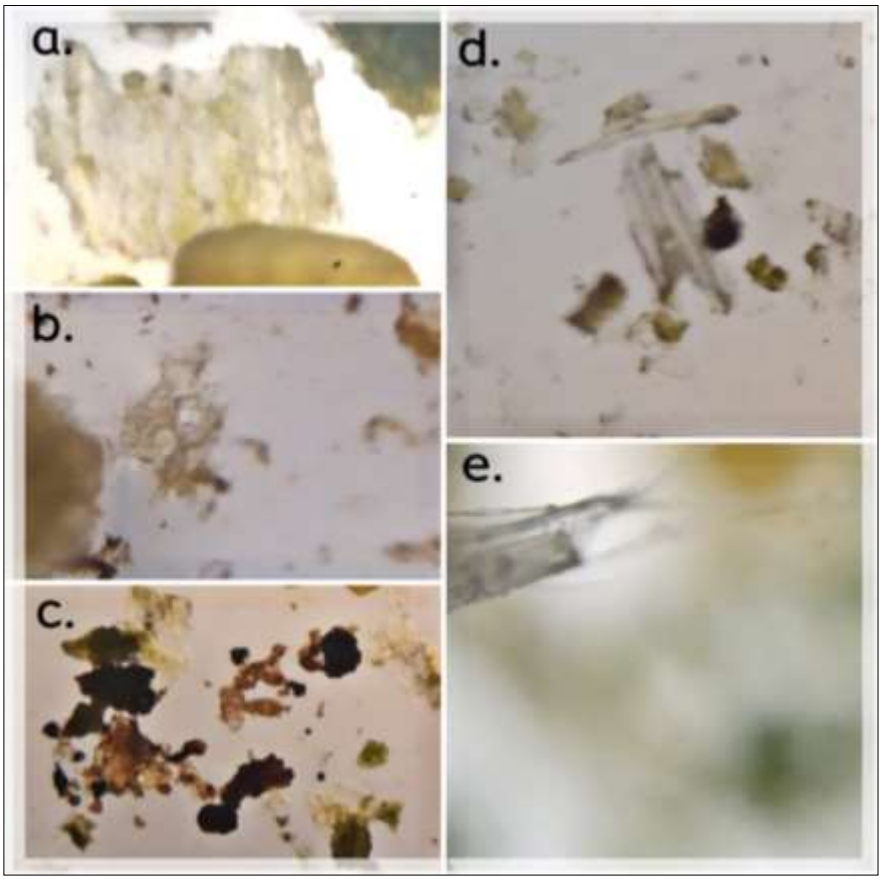


Fig 6: Powder characteristics of *Trigonella Foenum-graecum* leaf

(Parts include: a) Lignified Fibre; b) Epidermis in Surface View ; c) Calcium Oxalate Crystals; d) Vascular Bundle; e) Covering Trichome)

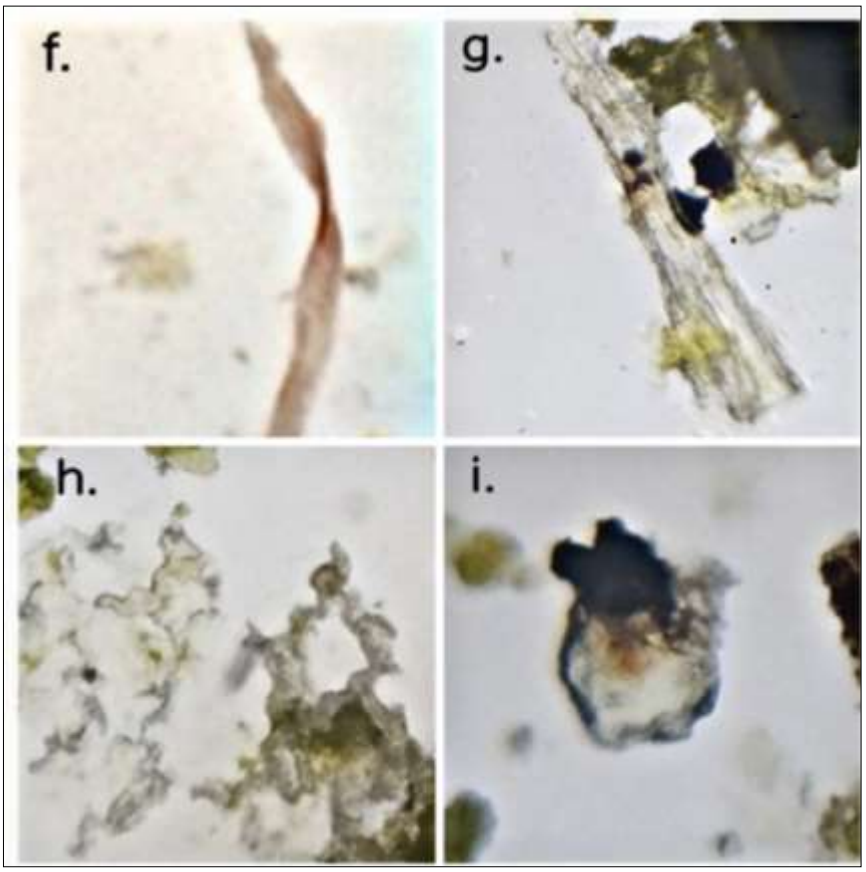


Fig 7: Powder characteristics of *Trigonella Foenum-graecum* leaf

(Parts include: f) Cotton fibre ; g) Portion of Fibre group with Crystal sheath ; h) Polyhedral sharp angle type starch granules ; i) unknown shape of crystal)

3.4. Physical Evaluation

The dried leaves of plant was used for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content(LOD).

3.4.1. Determination of ash values of crude drug:

a. Total ash value

Procedure

Weigh and ignite flat, thin, porcelain dish or a tarred silica crucible. Weigh about 2g of the powdered drug into dish/crucible. Support the dish on pipe-clay triangle placed on ring of retort stand. Heat with a burner, using a flame about 2cm high and supporting the dish about 7cm above the flame, heat till vapours almost cease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off. Cool in dessicator. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug (Khandelwal K., 2010).

Fig 8: Ash of plant

b. Determination of Acid–Insoluble ash value:

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further using 25ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into 100 ml beaker. Place mere gauze over a Bunsen burner and boil for five minutes. Filter through an 'Ash less' filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter-paper and residue together into crucible; heat gently until vapors cease to be evolved and then more strongly until all carbon has been removed. Cool in a dessicator. Weigh the residue and calculate acid-insoluble ash of the crude drug with reference to the air dried sample of the crude drug (Khandelwal K., 2010).

c. Determination of Water soluble ash value:

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further-Using 25ml of water wash the ash from the dish used for total ash into 100 ml beaker. Place mere gauze over a Bunsen burner and boil for five minutes. Filter through an 'ash less' filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter-paper and residue together into crucible; heat gently until vapors cease to be evolved and then more strongly until all carbon has been removed. Cool in a dessicator. Weigh the residue and calculate water soluble ash of the crude drug with reference to the air dried sample of the crude drug (Khandelwal K., 2010).

3.4.2. Determination of Loss on Drying (LOD)

Procedure:

Weigh about 2g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100°C or 105°C, until two consecutive weighing do not differ by more than 0.5mg. Cool in a dessicator and weigh. The loss in weight is usually recorded as moisture. (Khandelwal K., 2010)

3.4.3 Extractive value determination

a. Determination of water soluble extractive Values

5 gm of air dried drug was macerated with 100ml of distilled water in a closed flask for 24 hours; it was frequently shake during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly without loss of water, dried in tarred flat -bottomed shallow dish, dried at 105^0C and weighed. The percentage of water soluble extractive value was calculated with reference to air dried drug. (The Indian Pharmacopoeia, 1996)

b. Determination of Alcohol-Soluble Extractive Values

5 gm of air dried drug was macerated with 100ml of ethanol of the specified strength in a closed flask for 24 hours; it was frequently shake during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly without loss of ethanol, dried in tarred flat-bottomed shallow dish, dried at 105^0C and weighed. The percentage of alcohol-soluble extractive value was calculated with reference to air dried drug.

Fig 9: Extractive value of different solvents

3. Results

3.1 Determination of total ash content

Table 4: Determination of Total ash content of *Trigonella Foenum-graecum* leaves powder

Weight of empty crucible (g)	17.45 (g)
Weight of crucible + air dried material (g)	19.45 (g)
Weight of crucible + ash (g)	17.65 (g)
Wt. of Total ash (g)	0.2
Total ash value (% w/w)	10%

The total ash content of *Trigonella Foenum-graecum* leaves powder was found to be 10 %

4.2. Determination of acid insoluble ash value

Table 5: Determination of acid insoluble ash value of *Trigonella Foenum-graecum* leaves powder

Weight of crucible (g)	17.45(g)
Weight of crucible + acid soluble ash (g)	17.46(g)
Total acid insoluble ash (g)	0.01(g)
Acid insoluble ash (% w/w)	0.5%

The acid insoluble ash value of *Trigonella Foenum-graecum* leaves powder was found to be 0.5%.

4.3. Determination of water soluble ash value

Table 6: Determination of water soluble ash value of *Trigonella Foenum-graecum* leaves powder

Weight of crucible (g)	17.45
Weight of crucible + water insoluble ash (g)	17.48
Total water soluble ash (g)	0.03
Water soluble ash (% w/w)	1.5%

The water soluble ash value was found to be 1.5%.

4.4. Determination of Loss on Drying:

Table 7: Determination of Loss on Drying of *Trigonella Foenum-graecum* leaves powder

Weight of empty crucible (g)	17,420
Weight of crucible (g)+ air dried material (g)	19,420
Weight after drying of crucible + dried material	19,100
Weight of material after drying (g)	0.016
Loss on drying (% w/w)	1.67%

The percentage of loss on drying was found to be 1.67%

4.5. Determination of Extractive values

Table8: Determination of Extractive Values

Sr. No.	Solvent	Extractive value (%w/w)
1	Ethanol soluble	7.5%
2	Methanol soluble	13%
3	Ethyl acetate soluble	1.5%
4	Acetone soluble	1.5%
5	Chloroform soluble	0.5%

5. Conclusion

Standardisation is essential measure for quality, purity and sample identification. Leaf macroscopy, microscopy and powder analysis of leaf along with physicochemical parameters are one of the simplest and cheapest methods to start with for establishing the correct identity of the source material. Physicochemical analysis of fenugreek leaves confirm the quality and purity of plant and its identification. These studies will serve as standard reference for identification and distinguishing powder of leaves of *Trigonella Foenum-graecum Linn.* from its substituents and adulterants. Taking into consideration the fact that since more than 7500 species are being used in healthcare, Standardisation of drugs thus becomes a stupendous task. The present work is a small contribution in this direction.

6. Acknowledgement

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