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**Research Article**

# PHARMACOGNOSTIC STUDY OF *TRIGONELLA FOENUM GRAECUM*

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**ABSTRACT**

The present research article reveals the pharmacognostic study of *Trigonella foenum graecum*. Standardization in medicinal plants is complicated by the complex chemical makeup of plants and the difficulty in obtaining the pure materials needed to compare and measure the amounts of any one particular compound in a plant mixture. In this, study standardization parameters are performed to ensure the quality, safety and efficacy of given herbal drug according to Indian Ayurveda pharmacopoeia.

**Keywords:** Methi, standardization, HPTLC, Heavy metals, evaluation, Physiochemical parameters.

## INTRODUCTION

"***Health for all***" is a dream and a goal which humanity at large shares and strives for. [1] Ayurveda emphasized the relationship between men and plants throughout the development of human culture. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures. [2] Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. Among them, *Trigonella foenum-graecum* (Family *Fabaeace*) is called *methika* in Ayurveda and used as medicine for the treatment of wounds, abscesses, arthritis, bronchitis and digestive disorders etc since oldest time [3]

### Description

Fenugreek is a aromatic, 30-60 cm tall, annual herb, [4.5]. The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Fenugreek is believed to be native to the Mediterranean region [3], but now is grown as a spice in most parts of the world. It is reported as a cultivated crop in parts of Europe, northern Africa, west and south Asia, Argentina, Canada, United States of America (USA) and Australia [6,7,8,9,]. India is the leading fenugreek producing country in the world [8]

## TAXONOMIC CLASSIFICATION

**Kingdom:** Plantae

**Division:** Magnoliophyta

**Class:** Magnoliopsida

**Order:** Fabales

**Family:** Fabaceae

**Genus:** *Trigonella*

**Species:** ***T. foenum-graecum***

**Botanical name: -** ***Trigonella foenum-graecum***

***Common name:*** Methi, Fenugreek

### Material and Methods

**Plant material:** The dried seeds of *Trigonella foenum graecum* used for the present study were received from the

Botanical Research Department of Dabur India Ltd, Sahibabad, U.P. The plant material was identified by Dr. G.P.Kimothi, Taxonomist, Dabur India Ltd, Sahibabad, U.P. A voucher specimen has been retained in the department. As per the information given by supplier, the raw materials were collected from different places such as Madhya Pradesh (TM-1) and Rajasthan (TR-2).

**Standardization Parameters**

**1. Macroscopic characters:**

The morphological studies were carried out for different parameters such as shape, size, color, odor and taste and fracture identification of the fenugreek seed. [10]

**2. Microscopic studies and powder analysis:**

Microscopical examination of the plant drugs is not only essential to the study of adulterants but also is indispensable in the correct identification [11].

a. **Section Cutting**: Healthy and suitable dried seeds of *Trigonella foenum graecum* were taken and soaked overnight in tertiary butyl alcohol. Next day pieces were transfer in ethanol for 2 hours and processed for microtome. The sections were stained with safranin, fast green, phloroglucinol and examined under microscope.

b. **Powder analysis**: The raw materials were powdered and the powder was passed through sieve no. 60 and was examined for its microscopic characters. The powder of the drug was boiled with chloral hydrate to remove the coloring matters, mounted on the glass slides using glycerin, covered with a cover slip and viewed under microscope. The powder was also stained with safranin, fast green, phloroglucinol and hydrochloric acid and examined under microscope. Further iodine water was used to locate the starch.

**Photomicrography:** As demanded by the anatomical details the photomicrography of the sections and the powder at different magnifications was taken with the help of Axio vision camera.

**3. Physicochemical parameters:**

The various physio-chemical values of seed such as ash values, extractive values, moisture content, fluorescence Analysis were determined according to the standard method. [10, 12]

**4. Phytochemical screening:** The Phytochemical evaluation of drug was carried out as per the method described. Previously dried powdered seeds were extracted in a Soxhlet apparatus with petroleum ether, chloroform, methanol, ethanol, acetone and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrate, phenolic, flavonoids, proteins, amino acids, saponins, and resins etc. [12, 13]

**5. MICROBIOLOGICAL STUDIES [14, 15]**

This is one of the major parameters for standardization in Indian herbs. Following are certain limits for number of microorganisms per gram of material as per IP, 2007.

| **Micro-organisms** | **Crude drug for processing** | **Ready for topical use** | **Ready for oral use** |
|---|---|---|---|
| Total aerobic count | $1\times10^7$ | $10^7$ per g or ml | $10^7$ per g or ml*<br>$10^5$ per g or ml** |
| Total fungi | $1\times10^5$ | $10^3$ per g or ml | $10^5$ per g or ml*<br>$10^3$ per g or ml** |
| *Staphylococcus aureus* | Absent | Absent in 1 g or 1 ml | Absent in 1 g or 1 ml |
| *Salmonella sp* | Absent | Absent in 10 g or 10 ml | Absent in 10 g or 10 ml |
| *Escherichia coli* | $1\times10^3$ | Absent in 10 g or 10 ml | $10^2$ per g or ml* Absent in 1 g or 1 ml** |
| *Pseudomonas aeruginosa* | Absent | Absent | Absent |

* Products to which boiling water is added before use

** Products to which boiling water is not added before use

**6. Determination of Heavy Metals (Analysis by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS):** Contamination of medicinal plant materials either accidentally or intentionally with heavy metal such as arsenic, lead, cadmium, copper etc. can be attributed to many causes including environmental pollution and traces of pesticides. These may prove to be very dangerous for human health, even if present in trace amounts [14]. Hence, the evaluation of heavy metals in herbal raw materials, their herbal extracts and marketed formulations being used for the proposed study is of utmost importance. Analysis by the use of advanced instruments like Atomic absorption spectroscopy, inductively coupled plasma optical omission spectroscopy (ICP-OES) and inductively coupled plasma mass spectroscopy (ICP-MS) is being employed now days for their effective and accurate determination.

**7. Chromatographic techniques**

Standardized manufacturing procedures and suitable analytical tools are required to establish the necessary framework for quality control in herbals. Among those tools, separation techniques including High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC) and gas chromatography are the most widely used to establish reference fingerprints of herbs, against which raw materials can be evaluated and finished products, can be assayed [11].

**RESULTS AND DISCUSSION**

**1. Macroscopical evaluation**

**Fig1. Seeds of Trigonella foenum graecum**

**Seeds**: Seed oblong, rhomboidal with deep furrow running obliquely from one side,dividing seed into a larger and smaller part, 0.2-0.5 cm long, 0.15-0.35 cm broad,smooth, very hard; dull yellow; seed becomes mucilaginous when soaked in water;odour, pleasant; taste, bitter. [3]

**2. Microscopical characters**

**a. Transverse section**

Transverse section of seed is present in the Fig: 2.

**b. Seed Powder:** The seeds can be identified by presence of aleurone grains, parenchymatous cells of testa, epidermal cells of testa, parenchymatous cells of cotyledons and radical, hypodermis of testa, outer layer of the endosperm, fibers and oil containing cells presence in the Fig: 3-11.

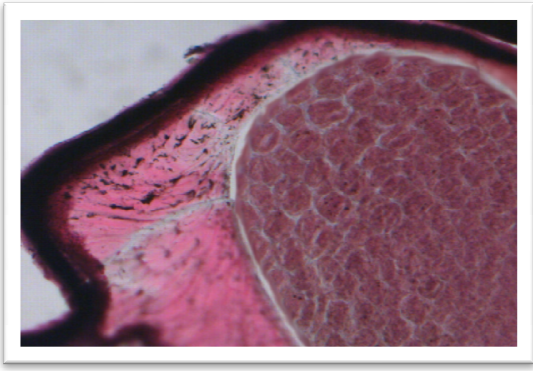


**Fig. 2: T.S. of Seed**

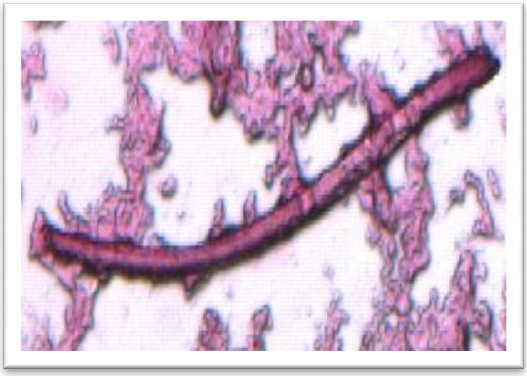


**Fig.3: fibre of seed**

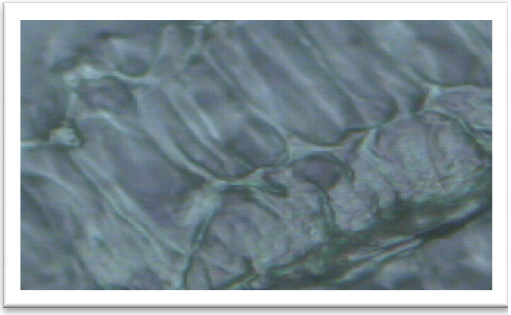


**Fig. 4: Epidermal cell of testa**

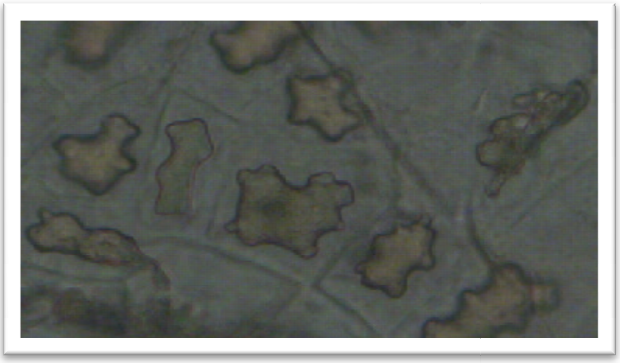


Fig. 5: Outer Layer of Endosperm

Fig. 6: Palisade Cells In Epidermal Cells

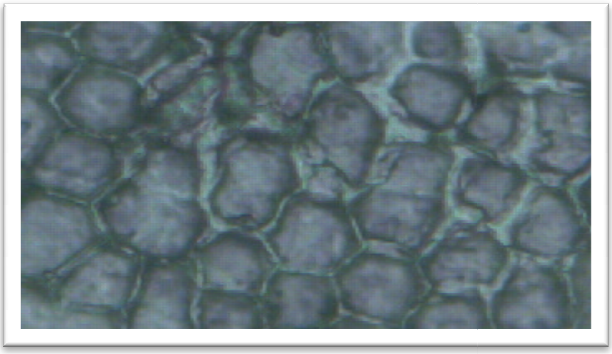


Fig. 7: Epidermal Cells

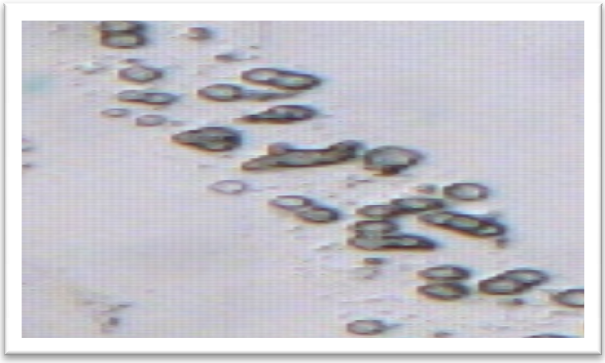


Fig. 8: Aleurone Grains

Fig. 9: Prism Type Crystals

**3. Physio- chemical parameters**

All physio-chemical parameters ash value, extractive value, moisture contents, fluorescence study were performed and the results are present in Table 1 and 2.

**4. Phytochemical screening**

Phytochemical screening was useful for the determination of the presence of significant chemical of constituents. The results are present in Table 3.

**Table 1. Physicochemical studies of raw materials of *Trigonella foenum graecum***

| Sample name | Water soluble extractive value (%w/w) | Alcohol soluble extractive value (%w/w) | Total ash (%w/w) | Acid insoluble ash (% w/w) | Loss on drying (%w/w) | pH of 1% w/v solutions |
|---|---|---|---|---|---|---|
| Limits for crude raw material (API)* | ** | NLT 5 | NMT4 | NMT 0.5 | NMT | ** |
| TM-1 | 30.81 | 9.26 | 3.55 | 0.03 | 8.56 | 6.54 |
| TR-2 | 32.25 | 11.8 | 3.45 | 0.11 | 8.12 | 6.57 |

*Ayurvedic Pharmacopoeia of India (2007);

** Not specified;

**Table 2. Fluorescence analysis of *Trigonella foenum graecum***

| S. no | Reagent | Day light | 254 nm | 366 nm |
|---|---|---|---|---|
| 1. | Drug powder as such | Yellow | Cream yellow | Dark yellow |
| 2. | Drug powder + conc. H2SO4 | Dark yellow | Light green | Brownish dark |
| 3. | Drug powder+ 1m H2SO4 | Brown | Dark brown | Blackish brown |
| 4. | Drug powder+ conc. HCL | Dark yellow | Yellowish green | Dark brown |
| 5. | Drug powder+ 1m HCL | Brownish yellow | Brown | Dark brown |
| 6. | Drug powder+ conc. HNO3 | Dark yellow | Brownish yellow | Black |
| 7. | Drug powder+ conc. HNO3+ 25% NH3 | Yellowish brown | Yellow | Black |
| 8. | Drug powder+ methanol | Yellow | Yellowish green | Green |
| 9. | Drug powder+ chloroform | Yellow | Yellowish green | Light brown |
| 10. | Drug powder+ petroleum ether | Light yellow | Yellow | Yellow |
| 11. | Drug powder+ acetic acid | Brown | Dark brown | Blackish brown |
| 12. | Drug powder+ picric acid (1%) | Yellow | Yellow | Black |
| 13. | Drug powder + sodium hydroxide | Yellow | Greenish yellow | Brown |
| 14. | Drug powder+ sodium hydroxide+ dist. water | Yellow | Yellow | Light brown |
| 15. | Drug powder + 5% iodine | Yellowish brown | Dark brown | Black |
| 16. | Drug powder + 5% FeCL3 | Yellowish brown | Brown | Black |

**Table 3. Phytochemical screening of extracts of *Trigonella foenum graecum***

| Sr. No. | Plant Constituents | Water extract | Acetone extract | Chloroform extract | Petroleum ether extract | Methanol extract | Ethanol extract |
|---|---|---|---|---|---|---|---|
| 1. | ALKALOIDS | + | + | _ | _ | + | + |
| 2. | GLYCOSIDES | + | _ | _ | _ | + | + |
| 3. | CARBOHYDRATES | + | __ | _ | _ | + | + |
| 4. | SAPONINS | + | _ | _ | _ | + | _ |
| 5. | PHENOLICS COMPUNDS & TANNINS | + | _ | _ | + | + | _ |
| 6. | FLAVONOIDS | + | + | _ | _ | + | + |
| 7. | PROTEINS & | + | _ | _ | + | + | * |
| 8. | AMINOACIDS | + | + | _ | + | + | * |
| 9. | STARCH | + | _ | _ | _ | + | + |

**5. Microbial Studies**

To study the presence of bacteria and moulds in the test samples microbiological studies were performed. The results are shown in the **Table 5.**

***6. Heavy Metal Analysis***

To study the presence of heavy metals that come from environmental pollution and use of pesticides etc, heavy metal analysis was performed. All the results were compared with WHO guidelines (1998)

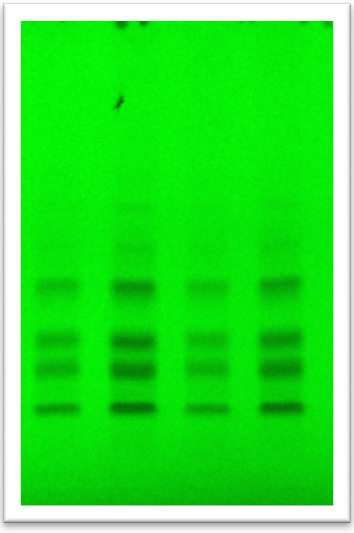
**Table 5. Microbial counts for *Trigonella foenum graecum* samples**

| Sample name | Total bacterial count (cfu/g) | Total yeast and mould count (cfu/g) | *E.Coli* (cfu/g) | *Salmonella typhi* (cfu/g) | *Pseudomonas aeruginosa* (cfu/g) | *Staphylococcus aureus* (cfu/g) | *Enterobacters* (cfu/g) |
|---|---|---|---|---|---|---|---|
| Limits for crude raw material * | $1\times10^7$ | $1\times10^5$ | $1\times10^3$ | Absent | Absent | Absent | $1\times10^4$ |
| TM-1 | $7.0\times10^2$ | 15 | Absent | Absent | Absent | Absent | Absent |
| TR-2 | $3.1\times10^3$ | $1.7\times10^3$ | Absent | Absent | Absent | Absent | Absent |

**Table 6. Heavy metals analysis for *Trigonella foenum graecum* samples**

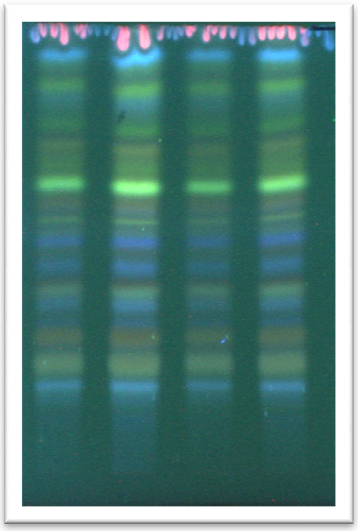
| Sample name | Arsenic (ppm) | Mercury (ppm) | Lead (ppm) | Cadmium (ppm) |
|---|---|---|---|---|
| Limits for raw materials* | NMT 5 | NMT 1 | NMT10 | NMT 0.3 |
| TM-1 | ≤ 1 | ≤0.1 | ≤0.1 | ≤0.1 |
| TR-2 | ≤ 1 | ≤0.1 | ≤0.1 | ≤0.1 |

**7. High Performance Thin Layer Chromatography (HPTLC) Studies**



**(i)**

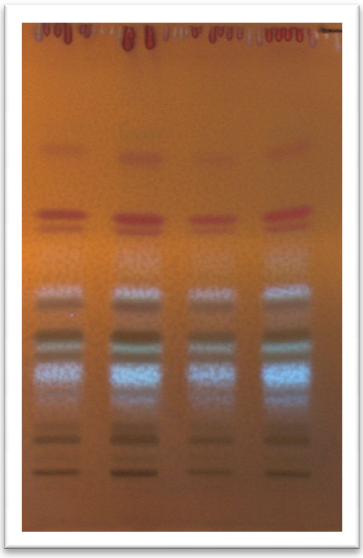
A B C D



**(ii)**

A B C D

(iii)



(iv)

**Plate: (i) At 254nm (ii) At 366nm (iii) After spray with Anisaldehyde Sulphuric acid and (iv) At 366nm after spraying respectively.**

**A & B spots of TM (5 & 10 µl); C &D spots of TR (5 &10µl).**

## CONCLUSION

Methi is a very common name in Indian kitchen used as a spice as well as for its therapeutic properties. Therefore, to ensure the reproducible quality of raw materials, standardization is a very important aspect of manufacture and supply of herbal drugs. It is only in the recent years that the importance of standardization of herbals is realized and efforts are being made to satisfy the regulatory requirements. It is the establishment of the reproducible pharmaceutical quality by comparing an herbal with established references. All evaluation of *Trigonella foenum graecum* seeds was successfully performed.

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