

# Physicochemical and Phytochemical Screening of Fenugreek (*Trigonella foenum graecum*) Indian Medicinal Plant

*Anwar Iqbal Khan<sup>1\*</sup>, Shailesh Gupta<sup>2</sup>, Girendra Gautam<sup>1</sup>*

1. Pharmacy department, Bhagwant University, Ajmer, Rajasthan.
2. Millennium college of Pharmacy and Science, Madhya Pradesh, Bhopal.

## Abstract

*Trigonella foenum-graecum* L. (Fenugreek) commonly known as methi (in Hindi) has been used as a culinary spice, a flavoring agent and as a medicinal plant from ancient time. The present study comprises physico-chemical and phytochemical evaluation of different extracts of *Trigonella foenum-graecum*. The physico-chemical evaluation was carried out by the determination of ash values, extractive values and moisture content. Whereas phytochemical evaluation was carried out to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in different extracts of *Trigonella foenum-graecum*. Results revealed the presence of carbohydrates, proteins, alkaloids, saponins, tannins, phytosterols, flavonoids, glycosides, fats and phenolic compounds. The present study will be helpful in determining the quality and purity of a crude drug.

Key words: *Trigonella foenum-graecum*, Phytochemical Evaluation, alkaloids and Flavonoids.

## Introduction

*Trigonella foenum-graecum* L. (Fenugreek) commonly known as methi (in Hindi) has been used as a culinary spice, a flavoring agent and as a medicinal plant from ancient time

The seeds of fenugreek Fenugreek seeds are the most important and useful part of fenugreek plant. These seeds are golden-yellow in colour, small in size, hard and have four-faced stone like structure. Fenugreek seed is 3-6 mm long, 2-5 mm wide and 2 mm thick in geometry. Raw fenugreek seeds have maple flavour and bitter taste but by the process of roasting, their bitterness can be reduced and flavour can be enhanced. Fenugreek seeds are used as spices. The whole seed or its ground powder is used in pickles, vegetable dishes and spice powder. Dried seeds are used as condiments. Fenugreek seeds are gummy, fibrous, sticky and gummy in nature. Biologically, its seeds are endospermic in nature.<sup>1-2</sup>

## Material & Methods

### 1.1 Collection of plant material

The seeds of *Trigonella foenum greacum* were obtained from local market and authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

### 1.2 Preparation of plant powder

The seeds of *Trigonella foenum greacum* were pulverized, sieved through 40 mesh to obtain a coarse powder.

### 1.3 Physico-Chemical Analysis

The powdered plant material was subjected to standard procedure for the determination of various physicochemical parameters.

#### Determination of ash values

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

#### Total ash value

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

#### Acid insoluble ash

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

#### Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

#### Determination of moisture content (Loss on drying)

About 10 g of drug (without preliminary drying) after accurately weighing was placed in a tared evaporating dish and kept in oven at 105°C for 5 hours and weighed. The percentage loss on drying with reference to the air dried drug was calculated.<sup>3-4</sup>

#### 1.4 Preparation of extracts

About 250-250 gm of dried powder of *Trigonella foenum greacum* seed was subjected to soxhlation separately . It was first defatted with petroleum ether then exhaustively extracted with ethanol solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.This extract is used for the formulation.<sup>5-6</sup>

#### 1.5 Phytochemical Screening<sup>7-12</sup>:

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts.

##### Tests for carbohydrates and glycosides

###### Molisch's test

Sample was treated with 2-3 drops of 1% alcoholic - naphthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

###### Legal's test

To the sample 1 ml of pyridine and few drops of sodium nitroprusside solutions was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

###### Borntrager's test

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

##### 5.2.3.2 Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff's reagent - Reddish brown ppt
- Wagner;s reagent - Reddish brown ppt
- Mayer's reagent - Cream color ppt
- Hager's reagent - Yellow color ppt

##### 5.2.3.3 Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million's reagent: Appearance of red color shows the presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the presence of Proteins and free amino acids

- Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

#### 5.2.3.4 Test for tannins

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Violet color.
- 10% lead acetate solution - White precipitate

#### 5.2.3.5 Test for flavonoids

##### Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

##### Shinoda's test

Small quantities of the sample was dissolved in alcohol, to this piece of magnesium followed by concentrated hydrochloric acid drop wise added and heated. Appearance of magenta color shows the presence of flavonoids.

#### 5.2.3.6 Tests for fixed oils and fats Spot test

- A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.
- Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolphthalein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

#### 5.2.3.7 Tests for steroids and triterpenoids

##### Libermann-burchard test

Sample was treated with few drops of acetic anhydride, boils and cooled. Then concentrated sulphuric acid was added from the side of test tube, brown ring was formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

##### Salkowski test

Sample was treated with few drop of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

#### 5.2.3.8 Test for mucilages and gums

Small quantities of sample was added separately to 25 ml. of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

#### 5.2.3.9 Test for waxes

To the test solution alcoholic alkali solution was added, the waxes get saponified.

## Results and conclusion

### Extractive value

The dried powder of plant was extracted with solvents i.e., Ethanol solvent for fenugreek seed **extract**. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of various extract was presented in Table 2.

### Phytochemical Screening

The extracts obtained were subjected to preliminary phytochemical screening. The extraction was carried out with water, ethanol, chloroform and petroleum ether the extract were screened for the presence of various medicinally active constituents.

The results of the phytochemical screening of fenugreek seed extract were present in Table-2. Preliminary phytochemical screening was useful in prediction of nature of drugs and also useful for the detection of several constituents present in different polarity solvent. Different types of secondary metabolites such as alkaloids, tannins, terpenoids, carbohydrates, glycosides, protein and mucilage & gum were presented in fenugreek seed extract.

**Table 1. Physico-Chemical Analysis of fenugreek seed**

S.No.	Physicochemical constants	values (%w/w)
1	Percentage of loss on drying	1.89% w/w
2	Percentage of ash content	4.25 % w/w
3	Percentage of acid insoluble ash	0.42 % w/w
4	Percentage of water soluble ash	3.82% w/w
5	Percentage of alcohol soluble extractive value	15.13 %w/v
6	Percentage of water soluble extractive value	17.4% w/v

**Table No. 2: Phytochemistry screening of the fenugreek seed extract**

S.No	Test	Petroleum ether	Chloroform	Alcohol	Water
1.	<b>Alkaloids</b>	- ve	- ve	+ ve	- ve
2.	<b>Flavonoids</b>	- ve	- ve	+ ve	- ve
3.	<b>Steroids</b>	+ ve	+ ve	- ve	- ve
4.	<b>Tannins</b>	+ ve	- ve	+ ve	+ ve
5.	<b>Saponins</b>	- ve	- ve	+ ve	+ ve

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