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Pharmacognostical and Phytophysicochemical investigations of *Trigonella foenum – graecum* Linn

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.), plant is distributed throughout the world and which belongs to the family Fabacecae. The fenugreek seed contains active constituents such as Carbohydrates, Proteins, Alkaloids, Flavonoids, Free amino acids, Saponins, Glycosides, Vitamins, Minerals, Mucilage, Fixed Oils & Volatile Oils etc. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic effects, Anti-inflammatory effects. Fenugreek has potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry like in steroidal hormones. This review gives view mainly on the Morphological evaluation, Microscopic evaluation, Physicochemical evaluation, Fluorescence Analysis and Phytochemical evaluation.

Keywords: *Trigonella Foenum-groecum* L., Seeds, Fabacecae, Saponins.

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Introduction

Herbal drug technology is used for converting botanicals materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing and biological activity. The phytochemical profile is of special significance since it has a direct bearing on the activity of the herbal drugs[1].

With these objectives in consideration, the raw materials were evaluated on the basis of pharmacognostical, physicochemical and phytochemical parameters.

Fenugreek (*Trigonella Foenum-groecum* Linn) is an annual herb indigenous to the countries bordering on the eastern shores of the Mediterranean 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. The name of the genus, Trigonella, is derived from the old Greek name, denoting 'three-angled', probably refering to the triangular shape of the flowers. The first recorded use of fenugreek is described on an ancient Egyptian papyrus dated to 1500 B.C. Fenugreek seed is commonly used in cooking . Fenugreek has strong flavor and aroma. The plants leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental countries as a spice in food preparations, and as an ingredient in traditional medicine. A wide range of uses were found for fenugreek in ancient times. Medicinally it was used for the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Traditional Chinese herbalists used it for kidney problems and conditions affecting the male reproductive tract. Fenugreek was, and remains, a food and a spice commonly eaten in many parts of the world.

The chemical components of fenugreek seeds include a large carbohydrate fraction (mucilaginous fiber, galactomannan); 20-30% proteins high in tryptophan and lysine; pyridine-type alkaloids; flavonoids ; free amino acids (4-hydroxyisoleucine, arginine, lysine, histidine); saponins; glycosides; vitamins, minerals, (28%) mucilage, (22 %) proteids, 5 % of a stronger-smelling, bitter fixed oil. volatile oils. Bitterness is mainly due to the oil, steroidal saponins and alkaloids.

Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis. The seeds also contain the saponin fenugrin B. Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids (eg, trigonelline, gentianine, carpaine). A large proportion of the trigonelline is degraded to nicotinic acid and related pyridines during roasting. These degradation products are, in part, responsible for the flavor of the seed. The seeds also yield as much as 8% of a fixed, foul-smelling oil. Three minor steroidal sapogenins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin.

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. The pungent aroma of fenugreek may be smelt on the skin and in under-arm perspiration. Fenugreek also has the reputation as a lymphatic cleansing herb. Fenugreek is a practical herb for all mucus conditions of the body, particularly the lungs, by helping to clear congestion. It is a powerful antioxidant and it acts as a mucus solvent and throat cleanser, which also eases the urge to cough. Even drinking the water that seeds have soaked in and been rinsed with, helps to soften and dissolve, accumulated and hardened masses of cellular debris. Use fenugreek for head colds, influenza catarrh, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, hay fever and sinusitis.

Fenugreek has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel. The slightly bitter properties of the seed are beneficial for digestion. Fenugreek has a powerful demulcent action, as it is rich in mucilage and it can soothe irritated or inflamed tissue. 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 is often used in many teas and other products that help balance women's hormones and / or enlarge the breasts. Remedy to Ease Child Birth for Pregnant Women. Fenugreek stimulates uterine contractions and can be helpful to induce childbirth. However, pregnant women should only use Fenugreek for inducing labor after consulting with their doctor.

Fenugreek seeds contain hormone precursors that increase milk supply. Some scientists believe it is possible because breasts are modified sweat glands, and fenugreek stimulates sweat production. It has been found that fenugreek can increase a nursing mother's milk supply within 24 to 72 hours after first taking the herb[2].

Botany

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Trigonella*

Species: *foenum-graecum* Linn

Vernacular names

Eng. – Fenugreek

Guj., Hind., Mar., Punj. – Methi

Kan. – Menthe, Mente.

Mal. – Uluva

Tam. – Mendium, Ventaiyam

Tel. – Mentulu.

Part's used – Seeds & Leaves.

Figure 1: Seeds of *Trigonella foenum-graecum* Linn,

Experimental

1. Procurement and Authentication of drugs

The plant used in this study consists of seeds of *Trigonella foenum-graecum* Linn, was purchased from plant drug supplier Sanjivani Aushadhalay, Ghatkopar local market, Mumbai.

Seeds of *Trigonella foenum-graecum* Linn was identified and authenticated by Dr. (Mrs.) S. S. Rahangdale, Assist. Professor in Botany, Hon'ble B. J. College of Arts, Commerce & Science, Ale.

Material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder. Coarse powder (60#) of dried plants was stored in air-tight containers for their pharmacognostical, physicochemical and phytochemical evaluation.

2. Preparation of Extracts

The dried coarse powder 50 gm. of *Trigonella foenum-graecum* Linn was continuously extracted with methanol as solvent using Soxhlet apparatus for 48 h. After complete extraction, the methanolic extract was concentrated under reduced pressure at 40ºC in a vacuum dryer to obtain dried extract and stored in desiccators[3-4].

3. Morphological Evaluation

Morphological evaluation refers to evaluation of drug, by colour, odour, taste, size, shape and special features like touch, texture etc. seeds of *Trigonella foenum-graecum* Linn was compared with the references for its morphological description.

4. Microscopic evaluation

Free hand sections of the seeds of *Trigonella foenum-graecum* Linn was taken and stained with phloroglucinol and conc. hydrochloric acid to confirm its lignifications.

Seeds powder of *Trigonella foenum-graecum* Linn was observed under high magnification.

5. Physicochemical evaluation

Crude powdered drug of seeds was used for the determination of various physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, extractive values, moisture content and foreign matter[5-7].

6. Fluorescence Analysis

The fluorescence behavior of the seeds powder in the visible light and ultraviolet light were carried out by soaking the powder in different reagent solutions and viewing under the light of required wavelength in a UV Chamber[8-10].

7. Phytochemical Evaluation

The various chemical tests were performed on the drug extract to determine the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phyto-sterols, fixed oils, fats, phenolic compounds and tannins.

A. Detection of Alkaloids

1. Mayer's test

The methanolic extract will be treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream colored precipitate indicates the presence of alkaloids.

2. Wagner's test

The methanolic extract will be treated with solution of iodine in potassium iodide (Wagner's reagent) and the formation of brown precipitate indicates the presence of alkaloids.

3. Hager's test

The methanolic extract will be treated with saturated solution of picric acid (Hager's reagent) and the formation of yellow precipitate indicates the presence of alkaloids.

4. Dragendorff's test

The methanolic extract will be treated with potassium bismuth iodide (Dragendorff's reagent) and the formation of reddish brown precipitate indicates the presence of alkaloids.

B. Detection of Carbohydrates

1. Molish's test

The methanolic extract will be treated with 2 drops of alcoholic solution of α-naphthol, slowly added 1ml concentrated sulphuric acid along the sides of test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

2. Fehling's test

The methanolic extract will be boiled on water bath and added 1ml of Fehling solution A and B. A red precipitate indicates the presence of carbohydrates.

3. Barfoed's test

The methanolic extract will be treated with copper acetate in glacial acetic acid (Barfoed's reagent) and heated on a boiling water bath for 2 min. Red precipitate indicates presence of carbohydrates.

4. Benedict's test

The methanolic extract will be treated with benedict's reagent. This mixture was heated on a boiling water bath for 2 min. A characteristic coloured precipitate indicates the presence of carbohydrates.

C. Detection of Glycosides

1. Borntrager's test

The methanolic extract will be treated with chloroform and 10 % ammonia solution and the formation of pink colour indicates the presence of glycosides.

2. Legal's test

The methanolic extract will be treated with pyridine, sodium nitroprusside and 10 % sodium hydroxide and the formation of pink colour indicates the presence of glycosides.

D. Detection of Saponins

The methanolic extract will be treated with distilled water and shake for 15 min. The formation of foam indicates the presence of saponins.

E. Detection of Proteins and amino acids

1. Millon's test

The methanolic extract will be treated with millon's reagent and formation of white precipitate indicates the presence of proteins.

2. Biuret test

The methanolic extract will be treated with 2 % copper sulphate, ethanol and excess of potassium hydroxide pellets and the formation of pink color indicates the presence of proteins.

3. Ninhydrin test

The methanolic extract will be treated with ninhydrin reagent in acetone and the formation of purple colour indicates the presence of amino acids.

F. Detection of Phytosterols

1. Libermann-Burchard's test

The methanolic extract will be dissolved in acetic anhydride. Then added one or two drops of concentrated sulphuric acid slowly along sides of the test tube. Color changes shows the presence of phytosterols.

G. Detection of Fixed Oils and Fats

1. Spot test

A small quantity of methanolic extract will be pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

2. Saponification test

The methanolic extract will be treated with 0.5 N alcoholic potassium hydroxide solution and phenolphthalein. This mixture is heated on water bath for 2 h. Formation of soap indicates the presence of fixed oils and fats.

H. Detection of Tannins

Small quantities of alcohol and aqueous extract was diluted separately in water and tested for presence of phenolic compounds and tannins.

1. Ferric Chloride test

To the test solutions, a few drops of 5 % ferric chloride solution will be added. Formation of a blue-black or green-black color indicates the presence of phenolic compounds and tannins.

2. Gelatin test

To the test solutions a few drops of 1 % gelatin solution in 10% sodium hydroxide will be added. The formation of white precipitate indicates presence of tannins.

I .Detection of Phenolic compounds

1. Lead acetate test

To the test solutions, a few drops of 10% lead acetate solution will be added. Formation of bulky white precipitate indicates the presence of phenolic compounds.

2. Alkaline reagent test

Test solutions, will be treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

3. Aqueous bromine test

To the test solutions, a few drops of aqueous bromine solution will be added. Formation of a yellow precipitate indicates the presence of tannins.

J. Detection of Gums and Mucilages

The extracts was dissolved in distilled water then added absolute alcohol with constant stirring and formation of white precipitate indicates the presences of gums and mucilage's.

K. Detection of Volatile Oils

In a volatile oil estimation apparatus, 50 gm. of powdered materials (crude drugs) was taken and subjected to hydro-distillation. The distillate is collected in graduated tube of the assembly, where in the aqueous portion automatically separated out from the volatile oils.

8. pH of Extacts

pH was determined by shaking 2.5gm. Of powdered extract in a 25 ml of freshly prepared and cooled distilled water in a 25 ml volumetric flask for 5 minute and the pH determined after filtration using a digital pH meter.

9. Optimization of TLC Solvent System

Different solvent systems were tried for developing a TLC System for the methanolic extract seeds of *Trigonella foenum-graecum* Linn solvent systems were tried identification of constituents in the extracts based on the literature survey and the one showing maximum separation was selected as mobile phase for study.

Thin Layer Chromatography

A stock solutions of methanolic extract seeds of *Trigonella foenum-graecum* Linn was used. For the stationary phase, precoated K5 silica plates were used. 5 µl solution was applied as a band of 5 mm by 2 mm on the same plate. The spots were allowed to dry before developing. The level of the solvent in the developing tank was adjusted to a level 2 to 3 mm below the line of origin on the plate. The plate was considered developed when the distance between point of origin and the distance travelled by the solvent front was not less than ¾ of the length of the plate and no further than 5mm below the top of the plate. The plate was dried and examined the plate under UV light. The Relative front, Rf value for a given spot were calculated [11-12].

HPTLC fingerprinting of extracts

The extract was filtered through 0.45µm filter and HPTLC was performed under the conditions optimized for the seeds of *Trigonella foenum-graecum* Linn extract.

HPTLC Conditions:

Table 1: HPTLC Conditions of *Trigonella foenum-graecum* Linn extract

Standard	*S. T. f. g.* extract
Solvent system	Toluene : Ethyl acetate : Formic acid (6.5 : 1.9 : 0.1 v/v).
Layer	Silica Gel GF 254
Application	Camag100µLsample syringe
Chamber Condition	Twin trough glass
Saturation time and Temperature	20 mins (25±2ºC)
Development distance	80 mm
Migration time	20 mins
Detection	430 nm
Development Mode	Ascending and one dimensional
Slit dimensions	6 mm×0.45 Micro
Scanning Speed	20 mm/s

HPTLC fingerprinting of *Trigonella foenum-graecum* Linn extract by Conditioning method

After scanning at 366 nm, the plates were dipped in Anisaldehyde sulphuric acid reagent for 5 sec and then kept at 110ºC for 20 min for Conditioning. After cooling the plates were scanned at 430 nm to record their fingerprints.

Results and Discussion

1. Extraction

The extracts were subjected to physical examination (Colour, Consistency). Given in Table 2 and Figure 2.

Table 2: Extraction of *Trigonella foenum-graecum* Linn

Sr. No.	Extracts	Colour	Consistency
1.	*Trigonella foenum-graecum* Linn seeds	Yellowish Red	Oily

Figure 2: Extract of *Trigonella foenum-graecum* Linn seeds

2. Morphological Evaluation

***Trigonella foenum-graecum*Linn**

The morphological studies revealed the information about *Trigonella foenum-graecum* Linn seeds are vary from rectangular to rounded in outline with a deep groove between the radical and cotyledons. The length is 3.5 - 6 mm and width 2.5 - 4 mm (Figure 3). The organoleptic evaluation of the *Trigonella foenum-graecum* Linn seeds powder revealed that powder was yellowish brown in colour with characteristic odour and bitter taste (Figure 4)

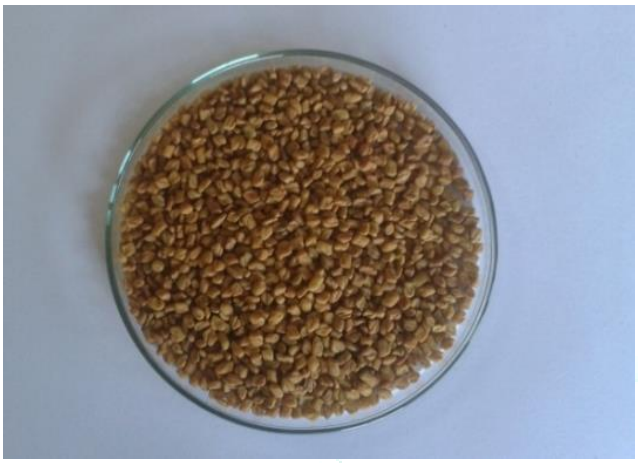


Figure 3: *Trigonella foenum-graecum* Linn seeds

Figure 4: Seeds powder of *Trigonella foenum-graecum* Linn

3. Microscopic Evaluation

Microscopic evaluation allows more detailed examination of a drug and it can be used to identify the drug by their know histological characters.

C. *Trigonella foenum-graecum* Linn

Figure 5 reveals the transverse section of the seeds shows the outermost layer of the testa consist of single row of highly thick walled, cylindrical palisade like lignified cells. Through which a light line, consist of layer sub-epidermis. A row of column cells, with distinct intercellular spaces near their top ends. Their walls get stellately thickened, followed by a zone of narrow, tangentially elongated, compact, thin walled cells of parenchyma. Remaining endosperm cells become large sized of various shapes and filled with mucilage.

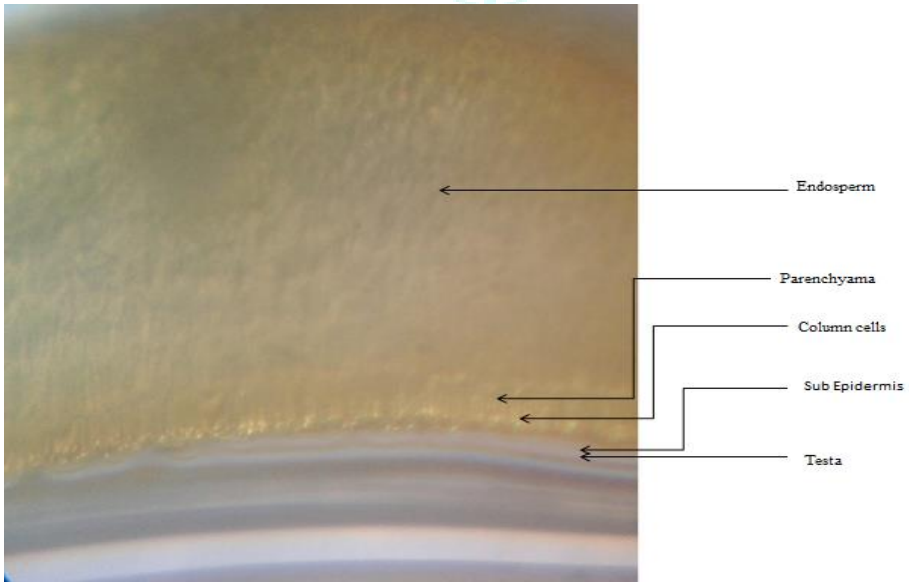
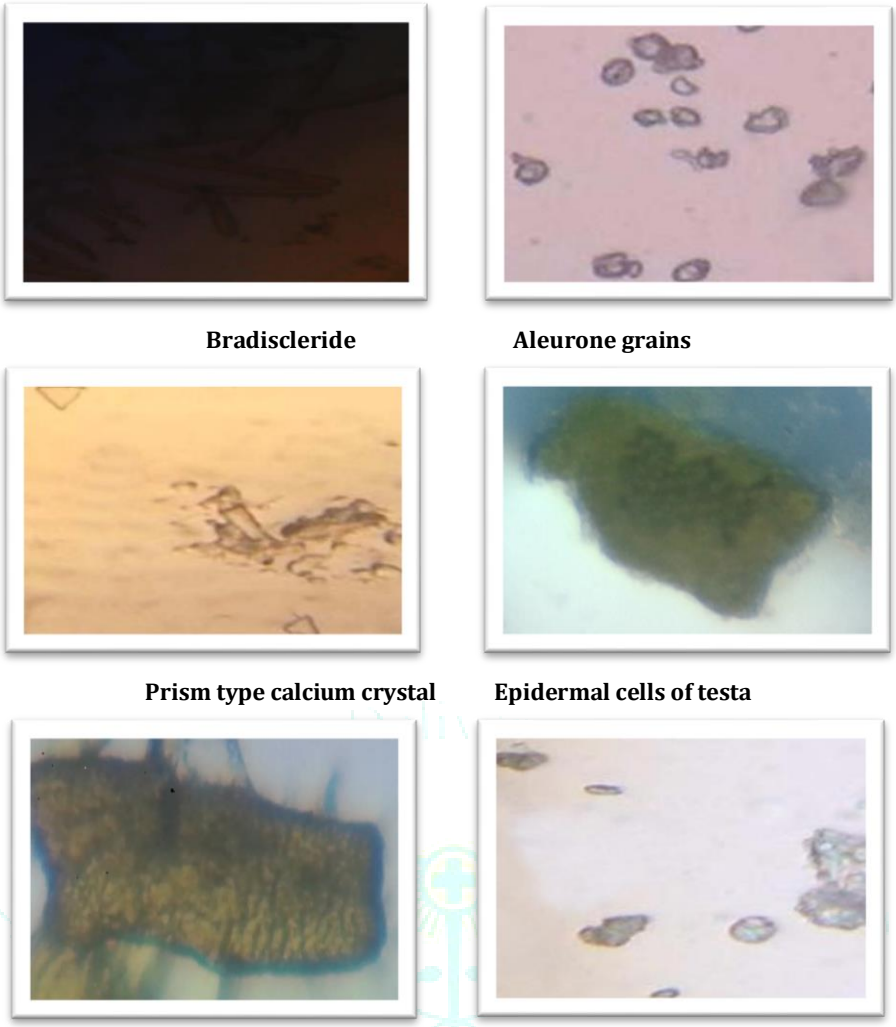


Figure 5: T. S. of *Trigonella foenum-graecum* Linn seed

Microscopic study of *Trigonella foenum-graecum* Linn seed powder showed different characters such as bradiscleride, aleurone grains, prism calcium crystal, epidermal cells of testa, Parenchymatous cells of cotyledonsand oil glands.



Bradiscleride **Aleurone grains**

Prism type calcium crystal **Epidermal cells of testa**

Parenchymatous cells of cotyledons **Oil glands**

Figure 6: Powder Characteristics of *Trigonella foenum-graecum* Linn seed

4. Physicochemical Evaluation

Evaluation of crude drug helps in the identification of a drug and establishes standards for the quality and purity of drugs. Pharmacopoeial specification for the plant materials should be developed to enable the quality control chemists to verify and approve the materials. The physical constant evaluation of drug is an important parameter in detecting adulteration or improper handling of drugs.

Ash values are used to determine quality and purity of crude drug. It indicates presence of varies impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Acid insoluble ash measures the amount of silica present, especially sand. Water soluble ash is the water soluble portion of the total ash. Less amount of these three parameters indicate that the inorganic matter and silica were less in Crude drug.

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. The maximum extractive value of seeds of *Trigonella foenum-graecum* Linn was found in methanol.

The percentage of active chemical constituents in crude drug is mentioned on air dried basis. Moisture content of drug should be minimized in order to prevent decomposition of crude drug. (i.e. Chemical change or microbial contamination) or minimal level to discourage the growth of bacteria, yeast or fungi during storage.

The parts of organ or organs other than those named in the definition and description of the drug are defined as foreign organic matter. The maximum limit for the foreign organic matter is defined in the monograph of crude drugs. If it exceeds the limit, deterioration in quality of the drug takes place.

Physicochemical characterization of powder of seeds of *Trigonella foenum-graecum* Linn are shown in Table 3.

Table 3: Physicochemical Parameters of *Trigonella foenum-graecum* Linn seeds

S.N.	Physicochemical Parameters	*T. f-g*
1.	Ash Values	
	Total ash	3.6%w/w
	Acid insoluble ash	0.4%w/w
	Water soluble ash	3.2%w/w
2.	Extractive Values	
	Methanol soluble extractive	11.80%w/w
	Water soluble extractive	31.40%w/w
3.	Moisture Content	1.5%w/w
4.	Foreign organic matter	0.9 %w/w

5. Fluorescence Analysis

Powders under UV and visible light when treated with different reagents emitted various colour radiations which help in identifying the drug in powder form. Ultraviolet analysis of *Trigonella foenum-graecum* Linn seeds are shown in Table 4.

Table 4: Ultraviolet analysis of *Trigonella foenum-graecum* Linn seeds

Sr. No.	Treatment	Visible light	UV (254nm)	UV (366nm)
1.	Drug Powder	Yellow	Cream Yellow	Dark Yellow
2.	Drug + NaoH	Yellow	Greenish Yellow	Brown
3.	Drug + HNO_3	Yellowish Brown	Brown	Dark Brown
4.	Drug +H_2SO_4	Dark Yellow	Light green	Dark brownish
5.	Drug +Methanol	Yellow	Yellowish Green	Green

6. Phytochemical Evaluation

Phytochemical Evaluation is to detect various phytoconstituents present in crude drug extract and helps in establishing profile of given extract for its chemical composition.

Table 5: Qualitative Phytochemical Tests of *Trigonella foenum-graecum* Linn seeds

S. N.	Tests	*T. f-g*
1.	**Tests for Alkaloids**	
	Mayer's test	+
	Wagner's test	+
	Hager's tests	+
	Dragendorff's test	+
2.	**Tests for Carbohydrates**	
	Molish's test	+
	Fehling test	+
	Barfoed's test	+
	Benedict's test	+
3.	**Tests for Glycosides**	
	Borntrager's test	+
	Legal's test	+
4.	**Test for Saponins**	
	Test solution+20ml distilled H_20	+
5.	**Tests for Proteins & amino acids**	
	Millon's test	+
	Biuret test	+
	Ninhydrin test	+
6.	**Test for Phytosterol**	
	Libermann-Burchard's test	+
7.	**Tests for Fixed oils & fats**	
	Spot test	_
	Saponification test	_
8.	**Tests for Tannins**	
	Ferric chloride test	+
	Gelatin test	+
	Aqueous bromine test	+
9.	**Tests for Flavonoids**	
	Lead acetate	+
	Alkaline reagent test	+
10.	**Test for Gums & Mucilages**	
	Ext. + dis.H_20 +abs. alc. + stirring	+
11.	**Test for Volatile oil**	
	50gm. of powder subjected to hydro-distillation	_

+ ve indicates positive result - ve indicates negative result

***Trigonella foenum-graecum* Linn**

Preliminary phytochemical screening of methanolic extract of *Trigonella foenum-graecum* Linn seeds revealed presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Proteins & amino acids Phytosterols, Tannins, Flavonoids, Gums & Mucilages and absence of Fixed oils & fats, Volatile oil.

Phytochemical constituents detected in crude extracts of *Trigonella foenum-graecum* Linn seeds are shown in table 5.

7. pH of Extacts

pH of *Trigonella foenum-graecum* Linn seeds extract in distilled water mentioned in the table 6.

Table 6: pH of Extacts

Sr. No.	Extract	pH
1.	*Trigonella foenum-graecum*Linnseeds	7.5

8. Optimization of TLC Solvent System

Best solvent system for *Trigonella foenum-graecum* Linn –

For methanolic extract –Toluene: Ethyl acetate: Formic acid (6.5: 1.9: 0.1)

The TLC studies of methanolic extract show best separation using Toluene: Ethyl acetate: Formic acid (6.5: 1.9: 0.1) as a mobile phase and derivatizing agent i. e. Anisaldehyde sulphuric acid.

Figure 7: White Remission Derivatized Image of Methanolic *Trigonella foenum-graecum* Linn seeds extract

Conclusion

This study presents a set of diagnostic characters of *Trigonella foenum-graecum* Linn seeds that will help to identify the drug in whole form. Morphology and microscopy is one of the simplest and cheapest methods to start with for

establishing the correct identity of the source materials. Physiochemical and qualitative chemical analysis of seeds confirms the quality and purity of plant and its identification. The information collected was useful for further pharmacological and therapeutically evaluation along with the standardization of plant material.

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