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Standardization and quality control parameters of aerial parts (Leaves and Stem) of *Trigonella foenum- graecum* L.-An important medicinal plant

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ABSTRACT

Trigonella foenum- graecum L., commonly known as fenugreek, is a traditional Indian medicinal plant of the Fabaceae family (sub-family: Papilionaceae). Fenugreek has been commonly used as a traditional food and medicine. The plant has been found to possess pharmacological activities such as anti-diabetic and anti-oxidant. The present investigation was therefore undertaken to determine the requisite pharmacognostic standards for the aerial parts (leaves and stem) of T. foenum- graecum L., Pharmacognostic studies including examination of macroscopic and microscopic characters and powder analysis were carried out for aerial plant parts. The physicochemical properties such as moisture content, total ash value, acid insoluble ash value, water soluble ash value and extractive values of aerial parts were also carried out. The presence of anomocytic stomata, simple trichomes, spiral and pitted thickening of vessels are the characteristic features observed in powder microscopy. Aqueous extractive value was found to be more than alcoholic. The preliminary phytochemical analysis indicated presence of tannins, alkaloids, saponins and flavonoids. HPTLC fingerprint profile has been also developed for methanolic extract of Trigonella foenum- graecum; as the chemical fingerprint obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. The results of the study could be useful in setting some diagnostic indices for the correct identification, standardization and preparation of monograph of the plant.

Key words: *Trigonella foenum- graecum* L., Pharmacognosy, phytochemical analysis, HPTLC fingerprint profile

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INTRODUCTION

Herbal medicine has been practiced worldwide and is now recognized by WHO as an essential building block for primary healthcare [1]. Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in the light of modern scientific knowledge [2]. *Trigonella foenum-graecum* L. leaves have great medicinal values in the indigenous system of medicine. It is used for medicinal purpose both, internally as well as externally. It is mostly cultivated in India, Pakistan, Egypt and Middle East countries. Its seeds are being used as spice and leaf as vegetable [3]. Due to its strong flavour and aroma, fenugreek is one of such plants whose leaves and seeds are widely consumed in Indian subcontinents as well in other oriental countries as a spice in food preparations and as an ingredient in traditional medicine ([4-5].

Trigonella foenum- graecum L. is a plant with traditional medicinal use in diabetes. Beneficial effects have been demonstrated in diabetic animals and in both insulin-dependent and non-insulin-dependent diabetic subjects [6]. The hypoglycaemic and antihyperglycemic effects of fenugreek seed and aqueous extracts of the leaf have previously

been reported in experimentally induced diabetic rats [7-8]. This plant has proved to contain many medicinal values such as anti-diabetic activity in animal models and proved to be beneficial in human subjects with non-insulin dependent diabetes and have anti-oxidant potential [9] and hypocholoesterolemic activity of its seeds has been observed in animal and human subjects ([10-11]. It is also having valuable components like galactomannan [12]. This is not widely exploited. The proteins from this plant are observed to be antigenic in nature in some cases [13] and presence of valuable steroids like saponins are also reported in its seeds [14].

No reports are available on the pharmacognostic and phytochemical analysis of the aerial parts (leaves and stem) of *Trigonella foenum- graecum* L. Therefore, the present study deals with the macroscopy, microscopy, physico-chemical investigation, preliminary phytochemical analysis and HPTLC finger print profile of aerial parts (leaves and stem) of *Trigonella foenum- graecum* L. All the parameters were studied according to WHO and Pharmacopoeial guidelines.

EXPERIMENTAL SECTION

Fresh *Trigonella foenum- graecum* L. plants were collected from local vegetable market Kalyan, M.S., India. Herbarium of *Trigonella foenum- graecum* L. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. From the collected plant material, aerial plant parts (stem and leaves) were separated and made free from any foreign matter, washed thoroughly under running tap water, blotted dry and cut into small pieces. The aerial plant parts were dried in preset oven at $40 \pm 2^{\circ}C$ for about one week, ground into powder and used for further analysis.

Physicochemical constants such as total ash, acid insoluble ash and water soluble ash; water and alcohol soluble extractive values were calculated according to the methods described in Pharmacopoeia of India [15]. Preliminary phytochemical analysis of extracts (Petroleum ether, Chloroform, Ethanol, Acetone and Water) of aerial parts was performed as described by Khandelwal [16]. Fluorescence analysis was conducted using methods of Kokoski [17] and Chase and Pratt [18].

A qualitative densitometric HPTLC analysis was performed for methanolic extract to develop characteristic fingerprint profile, which may be used for quality evaluation and standardization of the drug. 10 µl of extract was spotted on pre-coated silica gel G60 F_{254} HPTLC plates (Merck) with the help of CAMAG Linomat V applicator. The plate was developed in twin trough chamber (20 cm x 10 cm) pre-saturated with mobile phase (Toluene: Ethyl acetate: Methanol: Glacial Acetic Acid in the ratio 7.5:1.5:0.8:02). The plate was derivatized using Methanolic Sulphuric acid and scanned using CAMAG TLC Scanner 3 and visualized using CAMAG TLC Visulaizer, Documentation and Evaluation system.

RESULTS AND DISCUSSION

Macroscopy

Trigonella foenum-graceum is an annual herb which is 50-70 cm in height (Plate 1a). the fresh leaves and stem of the plant were spread on a clean dry plastic sheet to observe different features using hand magnifying lens and ruler (where required) and the observations were recorded.

Macroscopic characters of leaf

Colour: Adaxial surface dark green in colour, abaxial surface light green in colour.
Texture: Smooth
Extra features: Pubescent, trifoliate, stipules triangular, leaflets obovate to oblong, 10-30 mm long, 5-15 mm wide.

Macroscopic characters of stem

Colour: Light Green
Texture: Smooth
Extra features: Slender and pubscent

Organoleptic characters of powder

Organoleptic evaluation can be done by means of organs of sense. This evaluation provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample [19]. Organoleptic characters of powder are depicted in Table 1.

Microscopic characters

Numerous free hand sections (transverse section) of fresh leaf and stem were taken, stained with safranin and mounted on a grease free slide and observed under compound microscope [20].

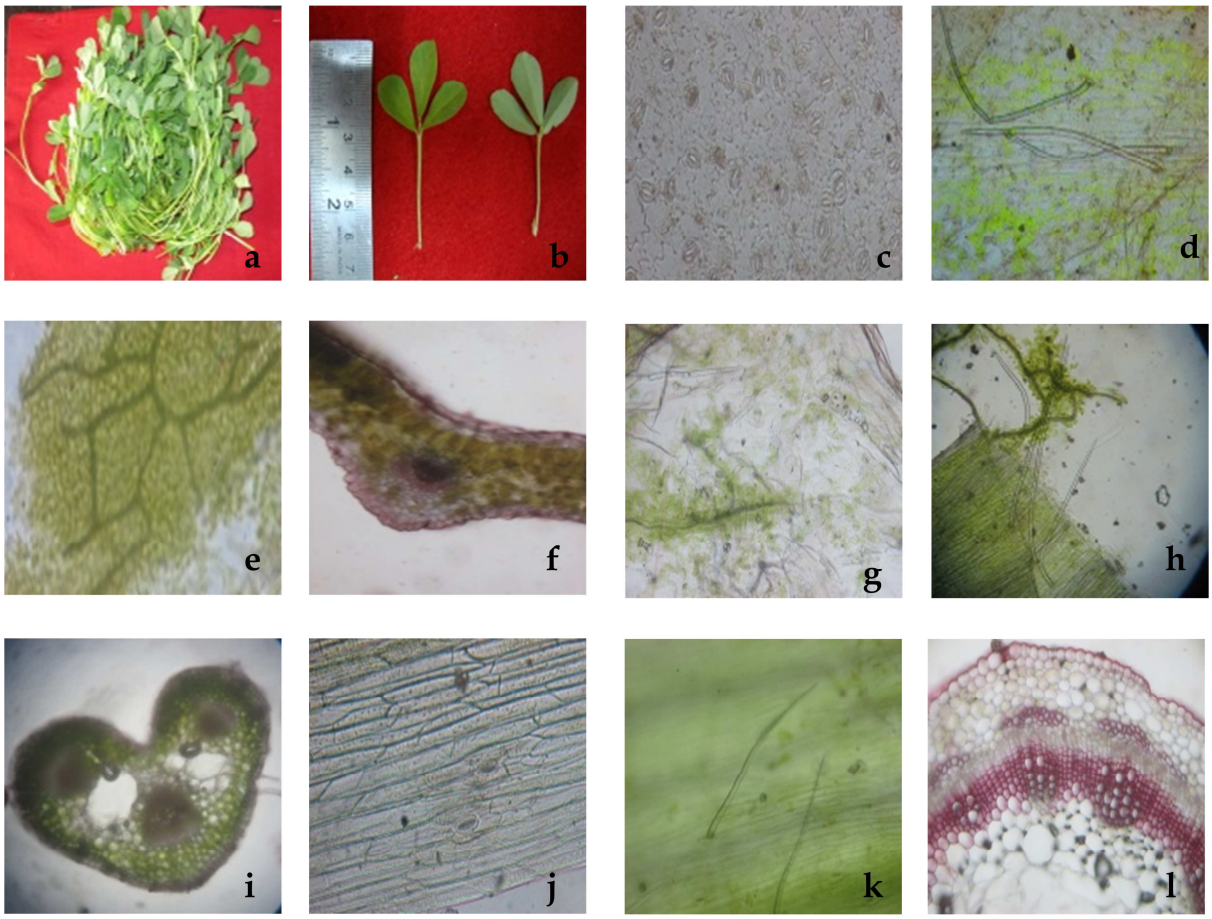

Plate 1: Macroscopy and microscopy of *Trigonella foenum-graecum* L. of leaf, petiole and stem a – Aerial parts, b – Leaves, Leaf: c – Stomata in surface view, d – Trichomes, e – Leaf in surface view showing vein islet, f – T. S. of leaf, Petiole: g – Stomata, h – Trichomes, i – T. S. of Petiole, Stem: j – Stomata, k – Trichomes, l – T. S. of stem

T. S. of leaf

Surface preparation of leaf shows presence of anomocytic stomata, simple trichomes and vein islets (Plate 1 c, d, e). Transverse section of the leaf (Plate 1 f) of *T. foenum- graecum* is distinctly dorsiventral. The lamina portion showed presence of upper epidermis, mesophyll region and lower epidermis. Upper and lower epidermii showed presence of a single layer of tubular cells covered with thin layer of cuticle. The mesophyll tissue is differentiated into adaxial palisade region and abaxial spongy parenchyma region. Both the epidermii are interrupted by stomata.

In the midrib region upper and lower epidermi are continuous. The ground tissue of midrib shows presence of two layered palisade region and parenchymatous cells without any intercellular space. The vascular strand of midrib is small, simple and collateral. The xylem elements are thick walled, angular and compactly arranged in parallel row. Phloem occurs in a thin arc beneath the xylem. Two to three layers of collenchymatous cells with angular thickening are present below vascular bundles in mid-rib region.

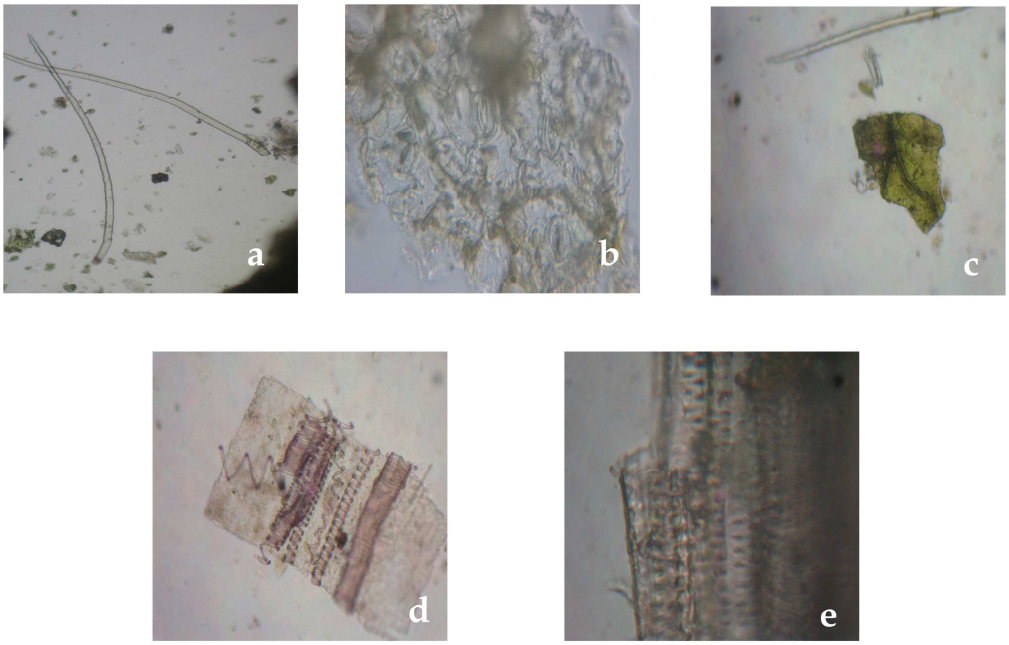

Plate 2: Powder microscopy of *Trigonella foenum-graecum* L. showing a – Trichomes, b – Stomata, c – Fragment of leaf showing vein islet, d – Vessels showing spiral thickening, e – Vessels showing pitted thickening

T. S. of petiole

Surface preparation of petiole shows presence of stomata and simple trichomes (Plate 1g, h). In cross-sectional view (Plate 1i), the petiol is plano-convex in outline. Epidermis consists of single layer of compactly arranged barrel-shaped cells and covered by cuticle. Followed by epidermis, 3-4 layered collenchymatous hypodermis is present. Beneath the hypodermis ground tissue is present made of parenchymatous cells without any intercellular spaces. Two vascular bundles are smaller in size compared to middle vascular bundle. The xylem is found towards the upper side and phloem towards the lower side.

T. S. of stem

Surface preparation shows presence of stomata and simple trichomes (Plate 1j, k). In cross sectional view (Plate 1l), the stem is circular with uneven outline. It consists of outermost layer of epidermis having a single layer of tangentially elongated parenchymatous cells covered with thick cuticle. Epidermis is followed by 4-5 layers of parenchymatous cortex. The endodermis is present below the cortex and separates it from vascular tissues which are present beneath it. The vascular cylinder consists of a narrow zone of phloem and comparatively large zone of xylem tissues. Xylem tissue consists of thick walled radially arranged vessels and fibres. Pith is wide and parenchymatous.

Powder microscopy

Powder microscopy (Plate 2) revealed the presence of simple trichomes, stomata, fragments of leaf showing vein islets, spiral and pitted thickenings of vessel.

Histochemical colour reactions

The Histochemical colour reactions on the leaf and stem were performed for the identification of major cell phytoconstituents. For colour tests transverse sections of fresh leaf and stem were treated with different chemical reagents *viz*. Iodine solution, Dragendorff's reagent, Ferric chloride solution, Sudan III, dil. HCl, Phloroglucinol, Libermann Burchard reagent, Ruthenium red and alcoholic Picric acid. The changes in the histochemical zones were observed under microscope and the results are shown in Table 2.

Physico-chemical analysis

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies

within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs [15]. Therefore, percentage of total ash, acid insoluble ash and water soluble ash were determined.

Table 1: Organoleptic evaluation of *Trigonella foenum-graecum* L. powder

Features	Observation
Colour	Dark green
Odour	Bitter
Taste	Bitter
Texture	Moderately fine

Table 2: Histochemical tests of stem and leaf of *Trigonella foenum-graecum* L

Sr. No.	Test for	Reagents	Colour	Histological Zone	
				Stem Section	Leaf Section
1.	Starch	Iodine	Blue	-	-
2.	Tannins	10% Ferric chloride	Black	-	-
3.	Lignin	Dilute HCl + Phloroglucinol	Pink	Vascular Tissue	Vascular Tissue
4.	Oil globules	Sudan red III	Pink	Lamina	Cortex
5.	Alkaloids	Dragendorff's reagent	Light Orange	Whole Section	Whole Section
6.	Steroids	Libermann Burchard reagent	Green	Lamina	Cortex

Table 3: Physico-chemical analysis of *Trigonella foenum-graecum* L

No.	Parameters	Results (%)
1.	Ash Values	
	a. Total ash content	13.37±0.55
	b. Acid insoluble ash	0.26±0.13
	c. Water soluble ash	7.7±0.57
2.	Extractive Values	
	a. Aqueous	28.85±4.00
	b. Alcoholic	6.96±0.82
3.	Moisture content	90.92±0.35

Table 4: Fluroscence Analysis of *Trigonella foenum-graecum* L. extracts

No.	Extracts	Observation		
		Day Light	UV light	
			254 nm	366nm
1.	Aqueous	Brownish green	ND	Whitish grey colour
2.	Ethanolic	Dark green	ND	Orange red fluorescence
3.	Methanolic	Dark green	ND	Orange red fluorescence
4.	Petroleum ether	Dark green	ND	Orange red fluorescence

Key: ND – Not Detected

Table 5: Fluroscence Analysis of *Trigonella foenum-graecum* L. Powder

Sr. No.	Fluorescence tests	Observations		
		Daylight	U. V. 254nm	U. V. 366nm
1.	Powder as such	Green	Grey	Brown
2.	Powder + nitrocellulose	Green	Grey	Brown
3.	Powder + 1N NaOH in methanol	Dark Green	Brown	Yellow Brown
4.	Powder + 1N HCl	Green	Black	Dark Red
5.	Powder + 1N HCl + nitrocellulose in amyl acetate	Green	Red	Fluroscence Red
6.	Powder + 1N NaOH in water	Green	Brown	Yellow Brown
8.	Powder + H_2SO_4 (1:1)	Green	Black	Dark Brown
9.	Powder + 1% Picric acid	Green	Black	Black
10.	Powder + acetic acid	Green	Dark Red	Fluorescence red
11.	Powder + 5% Iodine	Green	Brown	Fluorescence red
12.	Powder + 5% $FeCl_3$	Green	Black	Black
13.	Powder + 25% NH_3 + HNO_3	Green	Grey	Brown
14.	Powder + Methanol	Dark green	Black	Orange red
15.	Powder + conc. HNO_3	Brown	Brown	Grey
16.	Powder + 10% Potassium dichromate solution	Green	Black	Black
17.	Powder + 50% KOH	Green	Grey	Brown

Table 6: Preliminary Phytochemical Analysis of *Trigonella foenum-graecum* L. extracts

No.	Tests	PE	CE	EE	AE	WE
1.	Tannins	ND	ND	ND	+	+
2.	Alkaloids	+	ND	ND	+	ND
3.	Saponins	ND	ND	ND	ND	+
4.	Amino acids	ND	+	ND	+	+
5.	Carbohydrates	ND	ND	ND	ND	+
6.	Fats & fixed oils	+	+	+	+	+
7.	Glycosides	+	ND	+	+	+
8.	Protein	ND	ND	+	ND	+
9.	Starch	ND	ND	ND	ND	+
10.	Flavonoids	ND	+	ND	+	ND
11.	Essential oil	ND	ND	ND	+	ND

Key: *PE: Petroleum ether extract; CE: Chloroform Extract; EE: Ethanol Extract; AE: Acetone Extract; WE: Water Extract; +: Present; ND: Not Detected.*

The extraction of any crude drug with a particular solvent yields an extract containing different phyto-constituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction [16]. The variation in extractable matter in various solvents is suggestive of the fact the formation of the bioactive principle of the medicinal plant is influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides, as also reported by Sharma *et al.,* [21].

Loss on drying is the loss of mass expressed as percent w/w [22]. Results of physico-chemical analyses are tabulated in Table 3.

Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Many phytocompounds fluoresce when suitably illuminated. The fluorescence characters of powdered drug play a vital role in the determination of quality and purity of the drug material.

The powdered drug exhibit characteristic fluorescence in the presence of different chemical reagents under ultraviolet light. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [23-24]. The change in the colour of the powder and various extracts under UV radiation with reference to day light was observed. Results of fluorescence analysis are tabulated in Table No. 4 and 5.

Phytochemical analysis

The phytochemical compounds are known to play an important role to identify the bioactivity of medicinal plants. From the present study presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds [19]. The preliminary phytochemical screening of powder was carried out using various solvents *viz.* petroleum ether, chloroform, ethyl alcohol, acetone and water. These extracts were subjected to various qualitative chemical analyses. The result obtained showed the presence of proteins, starch, amino acids, fats and fixed oils, glycosides, tannins, alkaloids and flavonoids. The results of phytochemical analysis are tabulated in Table 6.

HPTLC fingerprint profile

HPTLC technique has gained much popularity for standardization of the herbal drugs and formulations in the last decades due to analysis of several samples at a time using very small quantity of marker compound as well as solvent system [25]. Characteristic HPTLC finger printing of a particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. HPTLC fingerprint profile of methanolic extract showed distinct band pattern before and after spraying with derivatizing reagent

Methanolic sulphuric acid (plate 3).

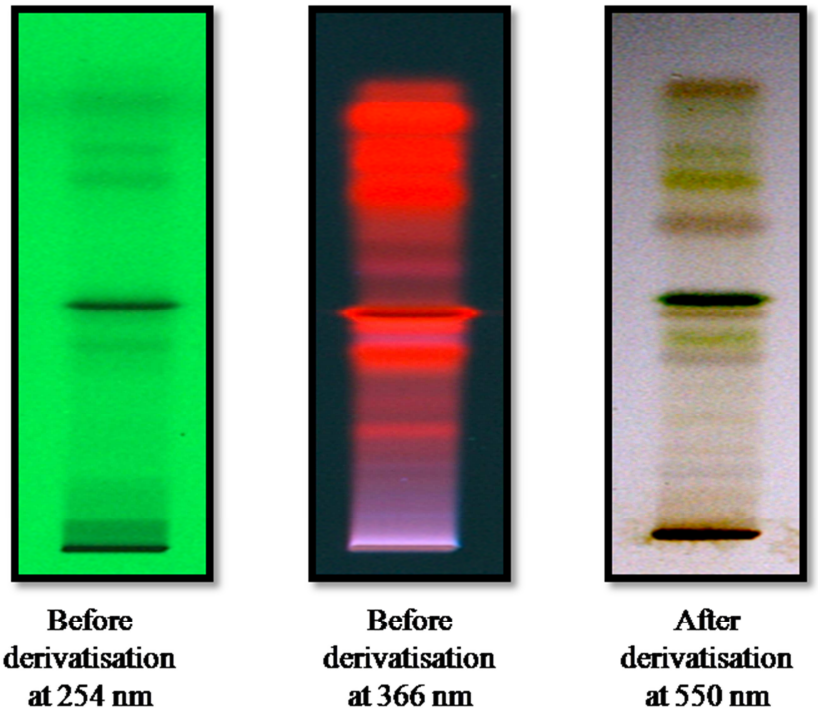

Plate 3: HPTLC fingerprint profile of *Trigonella foenum-graecum* L

CONCLUSION

Herbal drugs are derived from heterogeneous sources leading to variations. This makes the standardization of herbal medicines all the more important as erroneous results can cause variations in pharmacological and phytochemical studies. The pharmacognostic characters reported in this paper could be used as a diagnostic tool for the standardization of *Trigonella foenum-graecum*. Presence of adulterants can be easily identified using these parameters. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the plant. Phytochemical evaluation revealed the presence of various secondary plant metabolites which have been claimed to be responsible for various pharmacological activities. In addition to the above parameters, chromatographic (HPTLC) finger print profile will be helpful in the identification and quality control of *Trigonella foenum-graecum* L. and ensure its therapeutic efficacy. This information generated can also be useful for preparation of monograph of the plant, which could be incorporated in Indian Herbal Pharmacopoeia.

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