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Research Article

PHARMACOGNOSTIC EVALUATION OF *TRIGONELLA FOENUM GRACEUM* L. LEAF AND STEM

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ABSTRACT

Evaluation of *Trigonella foenum graceum* leaf showed anisocytic stomata with stomatal index of 39.82 and a long trichome of 1205 – 2451µm in epidermal peeling. The maceration studies revealed presence of fibers,tracheids with scalariform thickening. Alkaloids, protein, starch, lignin and mucilage were present in histochemical studies. Aqueous, petroleum ether and dichloromethane extracts of leaf and stem powder showed the presence of alkaloids in all the extracts . Bradysclerides , Macrosclerids , Tracheid with scalariform thickening,resin and calcium oxalate crystals were present in the powder analysis . Under 365nmUV, the stem and leaf powder treated with different reagents showed characteristic blue green fluorescent in Nitric acid and Ammonia and in sodium hydroxide and methanol. Physical parameters such as Total ash content, water soluble, acid insoluble and moisture content was also determined.

Keywords: Fenugreek, Sclerides, Anisocytic stomata, Trichome, Alkaloid, Powder analysis.

INTRODUCTION

Plants are always an exemplary source of drugs; in fact many of the currently available drugs were derived either directly or indirectly from them. In developing countries 80% of population are dependent on traditional medicine for primary health care. India is a country of vast biodiversity and traditional knowledge for using herbal medicines to cure many ailments in various cultures and tribes (Bhowmik et al 2010). *Trigonella foenum-graecum* commonly known as methi belongs to the family *Leguminosae* is used as food and for medicinal purposes (Chadha 1976). Fenugreek is traditionally used in India, especially in the Ayurveda and Unani systems (Grover 2002;Srinivasan 2006). *Trigonella foenum graecum* is one of the oldest medicinal plants, originating in India and Northern Africa. It is an annual plant, which grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. Its seeds are being used as spice and leaf as vegetable (Udayasekhara et al 1996).

Various medicinal properties like anticholestrolemic, anti-inflammatory, antitumour, cardio tonic, carminative, demulcent, diuretic, emollient, expectorant, febrifuge, galactogogue, hypoglycemic, hypotensive and laxative have been attributed to this plant in the traditional system of Indian medicine. It is a good source of many essential elements such as iron, phosphorus and sulfur (Phillips and Foy 1990). *T. foenum graecum* extract is also reported to have immunomodulatory effects in mice (Bin-Hafeez et al 2003). There are several reports concerning the antinociceptive, anti-inflammatory and anti-pyretic effects of the plant. In Indian traditional system of medicine, seeds of this plant are widely used as anthelmentic, astringent, cure leprosy, emetic, anti-inflammatory, anti-arthritis, emmenagogues and also useful in heart disorders (Kirtikar 1993).

Earlier workers have reported that seeds possess, antidiabetic and wound healing activities (Chauhan et al 2010; Shah and Seth 2010).The key obstacles which has hindered the acceptance of the alternative medicines in the developing countries is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines, hence it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. Hence this work attempts to bring out the pharmacognostic features of leaves and stem of *Trigonella foenum graecum.*

MATERIALS AND METHOD

Microscopic characters

For microscopic studies, the leaves were cut and removed from the plant and fixed in FAA. After 24 hours of fixation, the epidermal peel and transverse sections of leaf were taken by free hand. The section were stained in safrain (1%) and mounted in glycerol .

Quantitative microscopic

The total number of stomata was calculated by stomatal index =.No. of stomata X 100/ total no. of epidermal cells. The type of the stomata, vein islet and vein termination was recorded in the epidermal peeling. Quantitative microscopy was studied as per the procedure given by (Wallis 1958;Lala 1981).

Organoleptic characters and Anatomical study

Organoleptic characterization of dried leaf powder was carried out. The texture, smell, colour and taste were observed. Free hand section of Leaf and Stem of *Trigonella foenum greacum* were taken, stained with Safrain and mounted in glycerol and observed under light microscope and photographed at 40x.

Maceration

The stems of *Trigonella foenum graecum* were cut into small piece, boiled in water and the cooled material was repeatedly boiled to expel air and repeated for 3-5 times untill the pieces settled down. Treated pieces of the plants was soaked in jeffery's fluid (equal volume of 10% of nitric acid and 10 % chromic acid) for 24 hours at 30-40ºC, decanted washed and then stored in 50% alcohol. Pieces of macerated stem was treated with aqueous safrainin overnight, dehydrated through alcohol series (50%,60%,70%,80%,90%,100%) for five minutes and passed through alcohol: xylol (1:1 ration) series for five minutes. Then each material was macerated and observed.

Histochemical test

The plant section treated with various reagent such as Wagners reagent (Potassium iodide and Iodine) for detection of alkaloid, Toluidine blue 0 for lignin, Ferric chloride in IN Hydrochloric acid for Tannin, Sulphuric acid for detection of calcium oxalate Crystals and Methylene blue for phenols.

Phytochemical screening

The leaves and stem were washed thoroughly, blotted dry and completely dried. The dried leaves were extracted with aqueous, petroleum ether and Dichloromethane. The extracts were used for the following phytochemical tests. Chemical tests for various

extracts were also carried out according to the standard procedures described by (Harborne 1998; Kokate 1986).

Powder analysis

The dried leaves and stems were powdered and sieved to obtain coarse powder. The power thus obtained was placed on to a clean slide and observed under microscope.

Fluorescence analysis

The dry powder was placed on a slide and treating with several drops of specified reagent like Hydrochloric acid, Sodium hydroxide, Nitric acid, Sulphuric acid, Ferric chloride, Iodine Acetic acid, HNO3+ Ammonia, Methanol, NaOH+ Methanol. The slides were observed under UV 265 nm and 365 nm and the emitted fluorescence was observed that helps in identifying the drug in powdered sample. Flourescence analysis has been carried out according to the method of (Kokoshi et al 1958).

Physical parameters

Determination of total ash, acid Insoluble ash, water soluble ash and moisture content was done according to Indian Pharmacopoeia (Indian Pharmacopoeia 1985;1996).

RESULTS AND DISCUSSION

Quantitative microscopy

The total no of stomata in the epidermal peelings were counted. *Trigonella foenum graecum* showed 39.82% of stomatal index, No of stomata-92, vein islet no 19-30/ sqmm, vein termination no 16-22-/sqmm (Table-1).The stomata was observed to be anisocytic with 3 subsidary cells surrounding the guard cell (Fig-1).

Organoleptic and Anatomical character

Trigonella foenum graecum dried leaves were Dull Green, brittle, aromatic, bitter, and astingent.

Stem: Transverse section of stem showed single layered epidermis covered with thin cuticle 3-5 layers of parenchymatous cortical cells are present. A Single layer of endodermis was present. Vascular bundles consisted of xylem and phloem. The xylem consisted of protoxylem and metaxylem.. Vascular bundle was endarch. Small parenchymatous pith was present.

Leaf: Anatomical section of *Trigonella fonum graecum* Showed upper epidermis and lower epidermis. Mesophyll was differentiated into palisade and spongy parenchyma. Palisade parenchyma cells were arranged in three layer. Spongy parenchyma cell were arranged in 6 rows and contained many intercellular spaces. Trichomes were present. A Central vascular bundle was present. Single layer of endodermis consisted of xylem and phloem beneath it..

Maceration

The macerated stem of the plant showed various structures. In *Trigonella foenum graecum* xylem fibers, tracheids with scalariform thickening were commonly observed. (Fig-1).

Histochemical studies

The histochemical studies showed the presence, alkaloids, protein, starch, lignin, mucilage and phenol; tannin and lipids were absent.(Fig-1).

Phytochemical screening

The preliminary analysis of leaf and stem aqueous, petroleum ether and dichloromethane extract showed the presence of Alkaloids, flavanoids, tannin, saponin, resin and steroid was recorded (Table-2). Alkaloids was present in all the three extract, while tannin was present only in dichloromethane extract .Absence of phenol in the leaf and stem extracts was reported by (Summayya et al 2012). It was reported that phenol are abundantly present in seeds only. Aqueous extracts showed presence of saponins. Presence of high ascorbic acid and total phenol was reported by (Singh et al 2010). Phenolic acids like caffic acid, ferculic acid, vanillic acid and flavanoid are responsible for the antioxidant activity.Protein and carbohydrate content was reported to increase in mature leaf and stem. In present study presence of protein and carbohydrates was not observed in aqueous extract.

Powder analysis

Resins, Bradyscleride, Macroscleride , Tracheids with scalariform thickening, calcium oxalate crystal, anisocytic stomata with subsidiary cells, epidermal cell were present. (Fig-1)

Fluorescence analysis

Under white light, leaf powder with Hydrochloric acid, sodium hydroxide, nitric acid, sulphuric acid, ferric chloride, Iodine, Acetic acid, HNO_3+Ammonia, methanol, NaOH + Methanol showed brownish green, green, yellowish red, blackish brown, black, reddish brown respectively. Under UV light 265nm it exhibited yellowish with NaOH, reddish with NaOH+Methanol. Fluorescence was observed in 365nm and it appeared bluish green with HNO3+ ammonia, and NaOH+ Methanol (Table-3).

Qualitative analysis

Analysis of physical parameters of leaf and stem powder ,the moisture content was 5.3%, water insoluble ash 10.9% ,acid insoluble ash 0.61% and total ash 22.77%.(Table-4). It was reported that analysis of seed powder revealed moisture content was 1.5% whereas acid insoluble ash was 0.44% and the soluble extract 35 %w/w (Summayya et al 2012).

Herbal drugs play an important role in health care programs especially in developing countries. Pharmacognistic studies play a vital role in standardization and purification of herbal drugs. Since there is a great demand for herbal drugs there is need to standardize the plant material. The present pharmacognistic studies on *Trigonella foenum graecum* will be of great importance in maintaining quality of these herbal drugs.

Table 2: Phytochemical analysis of *Trigonella foenum graecum* extracts

Test	Aqueous Extract	Petroleum ether Extract	Dichloromethane Extract
Wagners (Alkaloids)	+	+	+
Tannin ($Fecl_2$)	-	-	+
Flavonoids	-	+	+
Anthroquinone	-	-	-
Saponins	+	-	+
Phenol	-	-	-
Terpenoids	-	-	-
Gum	-	-	-
Resins	+	-	-
Phlobalanins	-	-	-
Steroids	-	+	+
Glycosides	-	-	-
Protein	-	-	-
Carbohydrate	-	-	-

Table 1: Quantitative Microscopy of *Trigonella foenum graecum*

Quantitative Parameters	No.
No. of Stomata	92 / Sq.
Stomatal Index	39.82 %
Vein islet	19 – 30
Vein Termination	16 – 22
Trichome length	1205 – 2451 µm

Table 3: Fluorescence Analysis of *Trigonella foenum graecum* powder

Reagent	White light	UV Light	
		265nm	365nm
$Fecl_2$	Black	Black	Black
Iodine	Reddish Brown	Black	Black
NaOH	Green	Yellowish Red	Pale Green
Hcl	Brownish Green	Brownish Green	Pale Green
Acetic Acid	Brownish Green	Brownish Green	Pale Green
HNO_3 +Ammonia	Brownish Green	Brownish Green	Bluish Green Fluorescence
Methanol	Green	Green	Pale Green
H_2SO_4	Blackish Brown	Blackish Brown	Dark Green
NaoH + Methanol	Green	Reddish	Bluish Green Fluorescence
Nitric Acid	Yellowish Red	Yellowish Red	Pale Green

Table 4: Qualitiative Analysis of *Trigonella foenum graecum* powder

Physical parameters	Percentage
Moisture	5.3%
Water soluble ash	10.9%
Acid insoluble ash	0.61%
Total ash	22.77%

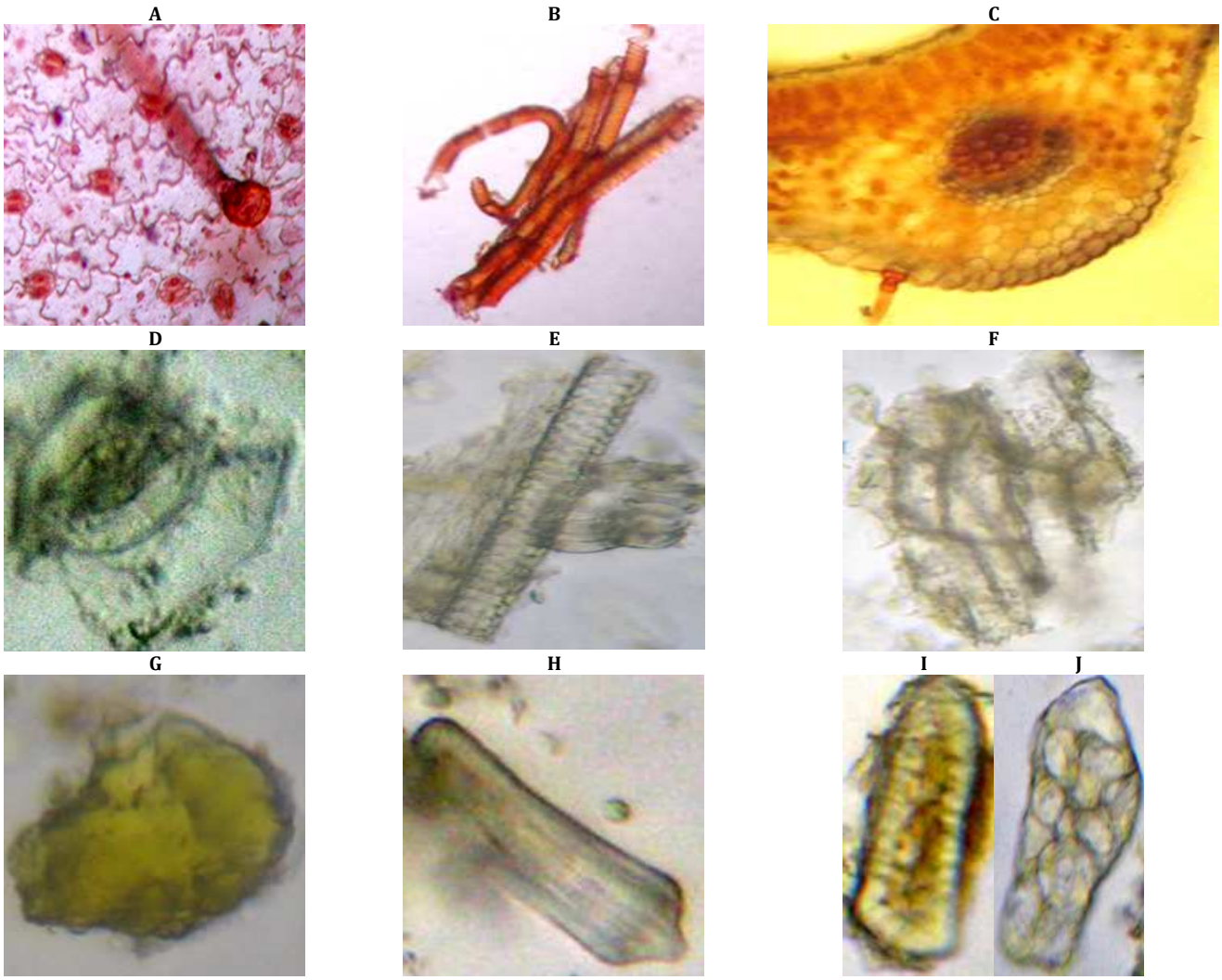

Fig. 1: Pharmacognostic evaluation of *Trigonella foenum graceum* showing

A- Epidermal peeling of leaf showing Anisocytic stomata with trichome; **B**- Tracheid with sclariform thickening; **C**- Histochemical test showing the presence of alkaloids; **D-J** Powder analysis showing stomata, tracheid with sclariform thickening, epidermal cells , resin, calcium oxalate crystal, Bradyscleride and Macroscleride respectively.

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