



PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL
SCIENCES

PHARMACOGNOSTIC, PRELIMINARY PHYTOCHEMICAL
STUDIES AND ANTICANCEROUS POTENTIAL OF *TRIGONELLA*
FOENUM-GRÆCUM

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ABSTRACT

The present study deals with the pharmacognostic, preliminary phytochemical studies and anticancer properties of seeds of *Trigonella foenum-graecum*. The present paper highlights the macroscopic characters of seeds, physico-chemical evaluation, preliminary phytochemical studies and anticancer properties of the seeds. These observations would be of immense value in the botanical identification and standardization of the drug in crude form and would help distinguish the drug from its other species. Phytochemical standardization parameters such as moisture content, total ash, water soluble and acid insoluble ash, alcohol soluble and water soluble extractives were determined. Preliminary identification of phytoconstituents was performed. HPLC study of the alcoholic extract obtained from the seeds was carried out and seven compounds were separated. A comprehensive overview of the pharmacognostic, phytochemical analysis of the seed extract and the literature survey carried out for the anticancer properties of fenugreek seeds.

Keywords: Medicinal plant, *Trigonella foenum-graecum*, Fenugreek, Physico-chemical studies, Phytochemical Studies.

INTRODUCTION

For the last few decades, phytochemistry (study of plants) has been making rapid progress and herbal products are becoming popular. There has been dramatic rise in the sale of herbal products like *Allium sativum*, *Hypericum perforatum*, *Spirulina*, *Echinacea angustifolia*, *Ginkgo biloba* and *Silybum marianum*. Herbal medicine has produced number of distinguished researchers and due to its accessibility to traditions it is still practiced even by lay practitioners. Ayurveda, the ancient healing system of India,

flourished in the Vedic era in India. According to historical facts, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were written around 1000 B.C. The Ayurvedic Materia Medica includes 600 medicinal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are integral part of Ayurvedic formulations. The formulations incorporate single herb or more than two herbs (polyherbal formulations).

Fenugreek, *Trigonella foenum-graecum* L. is an annual crop from the family Leguminosae. The seed of this plant grown in South Asia, has been known to have health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases. 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 ^[1], 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 ^[1-4]. India is the leading fenugreek producing country in the world ^[3]. Fenugreek has been used for centuries in folk medicine to heal ailments ranging from indigestion to baldness. Fenugreek is regarded as the oldest known medicinal plant in recorded history ^[5]. In parts of Asia, the young plants are used as "pot herbs" and the seed as a spice or herbal medicine. *Trigonella foenum-graecum* is also employed as an herbal medicine in many parts of the world. Its leaves are used for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects. It is assumed to have a stimulating effect on the digestive process. Fenugreek seeds, which are described in the Greek and Latin Pharmacopoeias, are said to have anti-diabetic activity and hypocholesterolaemic effects. In addition, fenugreek has been reported to possess a curative gastric anti-ulcer action, anti-bacterial, anti-helminthic, anti-fertility effects and anti-nociceptive effects. Fenugreek seed contains both saponin and galactomannan polysaccharides which could be of use as natural antibacterial compounds.

In spite of numerous medicinal uses attributed to this plant, there is very less pharmacognostical report on the microscopical and other physicochemical standards required for the quality control of the crude drugs. Hence the present investigation includes macroscopical evaluation, determination of physicochemical constants,

preliminary phytochemical screening and a review on anticancer properties of *Trigonella foenum-graecum*.

MATERIALS AND METHODS

Plant Material

Seeds of *Trigonella foenum-graecum* were collected from the local nursery and its identification was confirmed by NISCAIR and a specimen has been deposited of the seeds vide voucher number: NISCAIR/RHMD/Consult/2008-09/1123/154.

Pharmacognostic Studies

The macroscopy of the seeds were studied by comparing their macroscopical characters mentioned in the literature. Size was measured using a graduated ruler in millimetres which was used for the measurement of the length, width and thickness of seed samples. Since, the seeds are quite small in size they are measured by aligning 10 of them on a sheet of calibrated paper, with 1mm spacing between lines, and dividing the result by 10. The colour was examined by exposing the untreated seed sample under diffuse daylight, and the colour of the seed sample was studied. For analysing the surface characteristics, texture and fracture characteristics the untreated seed sample were examined using a magnifying lens (6X to 10X), seed surface was touched to determine the texture whether soft or hard; bend or ruptured and to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough granular, etc. The odour was analysed by placing a small portion of the crushed seed sample (25g) in a 100ml beaker and then pouring a small quantity of boiling water onto the crushed seed sample. Determined the strength of the odour (none, weak, distinct, strong) and then the odour sensation (aromatic, fruity, musty, mouldy, rancid, etc.) ^[6].

Physicochemical parameters

Different physicochemical parameters such as moisture content and total solids content, ash values and extractive values of the crude drug powder was carried out using reported methods by subjecting the seed powder to various determinations. The fluorescence and general behaviour of the powder of seeds under visible and UV lights (254 and 365 nm) were carried out ^[6].

Preliminary phytochemical screening

20 g of the air-dried powdered plant material was extracted successively using solvents like petroleum ether, methanol, benzene, chloroform and water in a Soxhlet extractor. The extracts were then subjected to various qualitative tests using reported methods to determine the presence of various phytoconstituents. Ethanolic extracts were also subjected to Thin Layer Chromatography in order to separate and detect the presence of different phytoconstituents.

HPLC

Ethanolic extract was prepared from the powder of seeds using Soxhlet apparatus and it was subjected to HPLC studies.

Pharmacological Studies on Anticancer Properties

Fenugreek (*Trigonella foenum-graecum*) is an annual crop from the family Fabaceae, extensively cultivated as a food crop in India, the Mediterranean region, Yemen and North Africa. Fenugreek seeds are well known for their pungent aromatic properties. As a spice, they are a component of many curry preparations and are often used to flavour food and stimulate appetite. Chronic oral administration of ethanolic fenugreek extract (10mg/day per 300g body weight) significantly increases food intake and the motivation to eat in rats, which might be related to the aromatic properties of the seeds. Fenugreek seeds are used in India as a condiment, in Egypt as a supplement to wheat and maize flour for bread making, and in Yemen it is one of the main constituents of the normal daily diet of the general population. Fenugreek leaves are widely consumed in India as a green, leafy vegetable, and are a rich source of calcium, iron, B-carotene and other vitamins.

Active Principles

The nutrient composition of fenugreek seeds consists of approximately 30% protein, 26% starch, 13% natural detergent fibre, 4% gum, 6% lipids and 11% ash. It is also rich in calcium, iron, β -carotene and other vitamins ^[7,8]. In comparison to other legumes, fenugreek seeds contain higher proportions of minerals including Ca, P, Fe, Zn and Mn. Trigonelline is an important alkaloid component of the seeds ^[9], which also contain some aromatic constituents such as n-alkenes, sesquiterpenes and nonalactone.

They have been found also to be rich in saponins, including diosgenin, gitogenin and tigogenin ^[10].

In general, fenugreek contains three important chemical constituents with medicinal value; i.e. steroidal sapogenins; galactomannans and isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products.

Cyclophosphamide (CP) is a commonly used anti-cancer drug which causes toxicity by its reactive metabolites such as acrolein and phosphoramidate mustard. During the study of modulation of toxicity caused by concomitant exposure to CP and L-buthionine-SR-sulfoximine (BSO) by fenugreek (*Trigonella foenum-graecum*); the extract was evaluated by measuring lipid peroxidation (LPO) and anti-oxidants in urinary bladder in mice. Fenugreek, a common dietary and medicinal herb, showed protective effect not only on LPO but also on the enzymatic anti-oxidants. CP-treated animals exhibited a significant decrease in the activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP) and catalase (CAT) when compared to the controls. Level of reduced glutathione (GSH) was also reduced with an increase in LPO in CP-treated animals. BSO treatment depicted an additive toxic effect in CP-treated animals. Pre-treatment of herbal extract restored activities of all the enzymes and thus showed an overall protective effect on additive effect of CP and BSO. Restoration of GSH by extract treatment may play an important role in reversing CP-induced apoptosis and free radical mediated LPO in urinary bladder ^[11]. The ethanolic extract of *Trigonella foenum-graecum*, was also tested for anti-tumour activity in A-549 male lung carcinoma, MCF-7 female breast cancer and HT-29 colon adenocarcinoma cell lines ^[12].

RESULTS

Macroscopy

Seeds vary from rectangular to rounded in outline with a deep groove between the radical and cotyledons, the length is 3.5–6mm and the width 2.5–4mm, light greyish, brown, olive green or cinnamon coloured, with a pronounced radicle that is half the length of the cotyledons and a characteristic bitter pungent smell.



Figure 1: Macroscopy of seeds of *Trigonella foenum-graecum*.

Physicochemical parameters

Different physicochemical parameters for the purpose of standardization such as total solids, moisture content, ash value (total ash, water soluble ash, acid insoluble ash) and extractive values (water soluble and ethanol soluble) for seeds of *Trigonella foenum-graecum* were determined and given in Table 1.

Fluorescence analysis

The powder of seeds were subjected to fluorescence analysis as per the standard procedure and shown in Table 2.

Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical analysis to determine the presence of various phytoconstituents and results are tabulated in Table 3.

TABLE 1: PROXIMATE ANALYSIS OF SEEDS OF
TRIGONELLA FOENUM-GRAECUM

Sr. No	Determination	Percentage (w/w)
1.	Moisture content	1.50
2.	Foreign Matter	1.00
3.	Total ash value	3.92
4.	Acid-insoluble ash value	0.44
5.	Water-soluble ash value	3.48
6.	Alcohol soluble extractive value	14.50
7.	Water soluble extractive value	35.00

TABLE 2: FLUORESCENCE ANALYSIS OF *TRIGONELLA FOENUM-GRACUM* SEED POWDER

Powdered drug	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder as such	Light yellowish brown	Brown	Blackish brown
Powder + 1M NaOH	Yellowish brown	Dark yellowish brown	Dark brown
Powder +1% Picric acid	Yellowish brown	Brown	Black
Powder + Acetic acid	Brown	Dark brown	Blackish brown
Powder + 1M HCl	Brownish yellow	Brown	Dark brown
Powder + Dil HNO ₃	Brownish yellow	Brown	Dark brown
Powder + 5% Iodine	Yellowish brown	Dark brown	Black
Powder + 5% FeCl ₃	Yellowish brown	Brown	Black
Powder + HNO ₃ + 25% NH ₃	Yellowish brown	Yellow	Black
Powder + Methanol	Yellowish brown	Dark brown	Blackish brown
Powder + 50% HNO ₃	Yellowish brown	Brown	Dark brown
Powder + 1M H ₂ SO ₄	Brown	Dark brown	Blackish brown
Powder + Dil. NH ₃	Yellowish brown	Light brown	Brown
Powder + Conc. HNO ₃	Yellowish brown	Brown	Dark brown
Powder +10% Potassium dichromate Solution	Deep yellow	Yellowish brown	Black
Powder + 25% Liquid NH ₃	Yellowish brown	Brown	Blackish brown

TABLE 3: THE RESULTS OF CHEMICAL TESTS PERFORMED WITH THE EXTRACTS OBTAINED BY SUCCESSIVE SOLVENT EXTRACTION OF SEEDS OF *TRIGONELLA FOENUM-GRACUM*

No:	Constituents	Petroleum Ether Extract	Methanol Extract	Chloroform Extract	Water Extract
1	Steroids	-	-	*	-
2	Alkaloids	*	-	-	-
3	Flavonoids	*	+	*	-
4	Saponins	-	+	-	+
5	Tannins	*	-	-	-

*: NOT DONE +: POSITIVE -: NEGATIVE

HPLC analysis of Ethanolic Extract

Ethanolic extract prepared by Soxhlet Apparatus was subjected to HPLC for the separation and identification of constituents present in the oil. Eleven compounds were separated at different retention time. Out of the eleven compounds separated compounds 4, 7, 8 and 10 separated at retention time 9.88, 11.08, 11.45 and 15.72 respectively were found to be identical with HPLC graphs of standard saponins.

TABLE 4: RETENTION TIME (RT) AND % AREA OF ISOLATED COMPOUNDS

Compound	Retention Time	% Area
1.	2.14	7.94
2.	3.58	6.37
3.	9.36	1.85
4.	9.80	15.80
5.	10.35	4.12
6.	10.45	15.37
7.	11.08	11.35
8.	11.45	14.82
9.	11.97	16.96
10.	15.72	3.84
11.	28.23	1.58

Anticancer Activity

Fenugreek seeds showed potential protective activity against 7, 12-dimethylbenz (a) anthracene (DMBA)-induced breast cancer in rats at 200mg/kg body weight. Fenugreek seeds extract significantly inhibited the DMBA-induced mammary hyperplasia and decreased its incidence. Epidemiological studies also implicate apoptosis as a mechanism that might mediate the Fenugreek's antibreast cancer protective effects.

The ethanolic extract of *Trigonella foenum-graecum*, with an ED50 less than 10µg/ml in the brine shrimp cytotoxicity assay, was also observed to possess anti-tumour activity in A-549 male lung carcinoma, MCF-7 female breast cancer and HT-29 colon adenocarcinoma cell lines. The extract gave negative results in the mutagenicity test. The

present study establishes that *Trigonella foenum-graecum* has appreciable anti-cancer activity. Flavonoids seem to be most likely candidates eliciting anti-tumorigenic effect.

CONCLUSION

Trigonella foenum-graecum (fenugreek) is an important culinary and therapeutic plant in many cultures. Fenugreek seeds have been widely studied for their reputed anti-diabetic, hypocholesterolaemic, antifertility and hypolipidemic effects. Properties of fenugreek that have been reported but which have received less attention include anti-cancer, antibacterial, antihelmintic, anti-cholinergic and anti inflammatory effects. The present research was focused on the seeds of *Trigonella foenum-graecum* extracts (using different extracts), for the pharmacognostic studies such as fluorescence analysis, ash value, extractive value, loss by drying etc. This comparative and multidisciplinary approach to the study of *Trigonella foenum-graecum* does help in understanding its identification taxonomical determination, and medicinal importance in depth. The adulterants in drugs obtain from *Trigonella foenum-graecum* can be identified by this investigation. Adulterants if any can be easily identified using these parameters. In conclusion, the pharmacognostic investigations on physicochemical characteristics and fluorescence analysis shows that authentic botanical of this crude drug prevents adulteration, substitution and has a crucial role in standardization of crude drugs. This study also indicates that the fenugreek seeds can be an effective treatment for the cancerous conditions and that the indigenous medicinal plants can be used successfully as an alternative treatment in the management of deadly disease such as cancer.

REFERENCES

1. Petropoulos, G. A. 2002. Fenugreek -The genus *Trigonella*, Pp. 1-127. 1st ed. Taylor and Francis, London and New York.
2. Alberta Agriculture, Food and Rural Development (AAFRD). 1998. Fenugreek, agri-fax. Agdex. 147/20-5.
3. Edison, S. 1995. Spices-Research support to productivity. In N. Ravi (ed.) The Hindu Survey of Indian Agriculture, Kasturi and Sons Ltd., National Press, Madras. Pp.101-105.

4. Fazli, F.R.Y. and Hardman, R. 1968. The spice fenugreek (*Trigonella foenum-graecum* L.). Its commercial varieties of seed as a source of diosgenin. Trop. Sci. 10:66-78.
5. Lust, J.B. 1986. The herb book. Bantam Books Inc. New York. Pp. 1-55
6. WHO, General guidelines for methodologies on research and evaluation of traditional medicine. 2000; HO/EDM/TRM/2000. I. Geneva P. 74.
7. Gupta, K., Kumar, N. and Dahiya D.S. 1998a. Changes in Structural carbohydrates and minerals in developing fenugreek leaves. Int J Tropical Agri 16: 221-227.
8. Gupta, U., Rati, E.R. and Joseph, R. 1998b. Nutritional quality of lactic acid fermented bitter gourd and fenugreek leaves. Int J Food Sci and Nutr 49: 101-108.
9. Shani, J., Goldschmid, A., Ahronson, Z. and Sulman, F.G. 1974. Hypoglycemic effect of *Trigonella foenum-graecum* and *Lupinus termis* seeds and their major alkaloids in alloxan diabetic and normal rats. Arch Int Pharmacodyn Ther 210:27-36.
10. Dawidar, A.M., Saleh, A. A. and Elmofei, S.L. 1973. Steroids sapogenin constituent of fenugreek seeds. Planta Medica 24: 367-370.
11. Bhatia, A.L., K. Manda, S. Patni, A.L. Sharma. 2006. Prophylactic Action of Linseed (*Linum usitatissimum*) Oil Against Cyclophosphamide-Induced Oxidative Stress in Mice Brain - J Med Food, 9 (2): 261–264. Mary Ann Liebert, Inc. and Korean Society of Food Science and Nutrition
12. Alkofahi, A., Batshoun, R., Owais, W. and Najib, N. 1996. Biological activity of some Jordanian medicinal plant extracts. Fitoterapia LXVII, 435–42.

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