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PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF *TRIGONELLA FOENUM GRAECUM* SEEDS EXTRACT

INTRODUCTION:

Fenugreek (*Trigonella foenum-graecum*) is an annual leguminous bean, and belongs to Fabaceae family, Its seeds and green leaves used as food possess medicinalapplications, and is an old practice of human history, it has been used for diverse medicinal benefits that include wound healing, aid in digestion, treatment of sinus and lung congestion, inflammation and infection, mitigation, hair treatment, breast enhancement and aphrodisiac effects.

Fenugreek is consumed in various parts of the world in different forms and has beenregarded as a treatment for many ailments known to man. Recent advances in nutraceutical and phytochemical research stimulated a renewed interest in fenugreek to be used as a functional food.

Medicinally, the fenugreek seeds are the most important and useful part of fenugreekplant. These seeds are golden-yellow in color, small, hard and have four-faced stone like structure. The biological and pharmacological actions of fenugreek seeds are mostly attributed to the variety of its bioactive chemical constituents that serve as raw materials for the manufacture of various hormonal and therapeutic drugs.

MATERIALS AND METHOD:

The purest chemical materials are used to analyzing the main phytochemical, nutrient and estimate some of the active group composition of fenugreek seeds study.

A. **Collection of plant material**: The seeds of *Trigonella foenum greacum* were obtained from local market and authenticated by Dr. Sahar Idris, Asst Prof, NRI Institute of Pharmaceutical Sciences, Bhopal.

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

C. **Physico-Chemical Analysis:** The powdered plant material of was subjected to standard procedure for the determination of various physicochemical parameters.

D. **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 makinguse of water-soluble ash and acid insoluble ash.

E. **Total ash value:** Accurately about 3 grams of air-dried powder was weighed ina tared silica crucible and incinerated at a temperature not exceeding 4500C 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 oftotal ash with reference to the air-dried drug was calculated.

F. **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 acidinsoluble ash was calculated with reference to the air-dried drug.

G. **Water soluble ash**: The ash obtained in total ash was boiled for 5 minutes with25 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. Theweight 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.

H. **Determination of moisture content (Loss on drying)**: About 10 g of drug (without preliminary drying) after accurately weighing was placed in a evaporating dish and kept in oven at 1050 C for 5 hours and weigh. The percentage loss on drying with reference to the air-dried drug was calculated.

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.

Phytochemical Screening: 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:

i. **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.

ii. **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.

iii. **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 colour, showing the presence of glycosides.

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 colour ppt
- Hager's reagent - Yellow colour ppt

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 colour.
- 10% lead acetate solution - White precipitate

Test for flavonoids:

i. **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.

ii. **Shinoda's test:** small quantities of the sample were dissolved in alcohol, to this piece of magnesium followed by concentrated hydrochloric acid drop wise added and heated. Appearance of magenta colour shows the presence of flavonoids.

RESULT AND DISCUSSIONS:

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 was screened for the presence of various medicinally active constituents. The results of the phytochemical screening of fenugreek seed extract were present in Table.

Preliminary phytochemical screening was useful in prediction of nature of drugs and 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.

S. No.	Test	Petroleum ether	Chloroform	Ethanol	Water
1.	Alkaloids	-ve	-ve	+ve	-ve
2.	Flavonoids	-ve	-ve	+ve	-ve
3.	Steroids	+ve	-ve	+ve	+ve
4.	Tannins	-ve	-ve	+ve	+ve

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