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Investigation of Physicochemical and Phytochemical Parameters of Different Extracts of *Trigonella foenum-graecum*

C.Girish[1], Y. Narsimhareddy*[2].

[1] S.V.U.College of Pharmaceutical Sciences, S.V.University, Tirupati - 517502.A.P, India.
[2]Department of pharmacology, University College of pharmaceutical sciences, Kakatiya University, Warangal - 506009, A.P, India.

Abstract: *Trigonella foenum-graecum* has been used for several diseases. *Trigonella foenum-graecum* generally known as "Fennel", and is belongs to family Papilionaceae. The present study comprises physico-chemical and phytochemical evaluation of different extracts of *Trigonella foenum-graecum* by using different standard methods. The physico chemical evaluation was carried out by the determination of ash values, extractive values and moisture content. Whereas phytochemical evaluation was carried out to estimate the presence of carbohydrates, glycosides, flavonoids, tannins, phytosterols and phenolic compounds in different extracts of *Trigonella foenum-graecum.* Results revealed the presence of carbohydrates, proteins, alkaloids, saponons, tannins, phytosterols, flavonoids, glycosides, fats and phenolic compounds. The present study will helpful in determining the quality and purity of a crude drug and laying down pharmacopoieal standards for *Trigonella foenum-graecum.*
Key words : *Trigonella foenum-graecum*, Fennel, Phytochemical Evaluation, glucosides and Flavonoids.

Introduction:

*Trigonella foenum-graecum*belonging to family Papilionaceae is commonly known as Fennel which is an aromatic annual plant, and is cultivated in India (1). The plant grows upto35 to 65 cm tall.The shape of the seed isrhomboidial shape and having 3 to 5 mm length with a thickness of 2 mm. The seeds are having yellowish brown colour and is giving a characteristic spicy odour with mucilaginous bitter taste.

The *Trigonella foenum-graecum* suppress blood glucose levels ([2]). It is also has antiplasmodic activity ([3]).The extracts of the seeds are also has hypolipidemic activity [4]. The seed extracts are more effective against E.coli, Salmomellatyphi and Staphylococus aureus[5]. The seed extract is also having anthelmintic activity [6]. The whole plant is also having analgesic and antiinflammatory activity [7]. The plant is also having antioxidant [8].Previously reported chemical constituents of *Trigonella foenum-graecum*are trigonelline, neurin, quercetin, rutin, fenugrin B and fenugreekine [9].The present objective of the study is to determine the physiochemical parameters and the phytochemical screening of the different extracts of *Trigonella foenum-graecum.*

Experimental Methods:

I. Collection of plant material

The seeds of *Trigonella foenum-graecum* were collected from local market of Tirupati. They were identified and verified taxonomically and authenticated in the Department of Botany, S.V.University, Tirupati. The seeds were coarsely powdered by using a rotary grinderand the powder is stored in airtight plastic containers. This powder wasused for all phytochemical analysis.

II. Preparation of extracts

The collected plant material was washed and dried at room temperature for 15-20 days and was subjected for size reduction. The prepared powder was used for extract preparation. The plant material (100 g) was extracted with Soxhlet apparatus by using 400 ml petroleum ether for about 48h. After defatting, the marc was dried in hot air oven at50°C and it is packed in Soxhlet apparatus for further extraction with 400 ml of 95% ethanol until it does not shows the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for 7days with frequent shaking. Solvents were removed from the extracts with the help of rotary vacuum evaporator.

III. Physicochemical evaluations

1. Moisture content

Weighed quantity of the shade dried powder of *Trigonella foenum-graecum*(3 g) was taken in a tared glass bottle and initial weight was taken. The powder was heated at 105°C in an oven and is weighed. This procedure was repeated untill the constant weight was obtained. The moisture content of the sample was calculated in the percentage with reference to shade dried plant powder by using formula [17].

$$\% \text{ Moisture content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

2. Ash values [18]

a) Determination of total ash

An accurately weighed quantity of the shade dried powder of *Trigonella foenum-graecum*(2 g) was incinerated in a crucible at a temperature of 450°C in a muffle furnace until carbon free ash was obtained. Then it was cooled and weighed. The percentage of total ash was calculated with reference to the shade dried powder by using the following formula.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of the crude drug taken}} \times 100$$

b) Determination of acid insoluble ash

The ash obtained was boiled with 25 ml of 2M HCl for 5min and it was filtered using an ash less filter paper. Insoluble matter retains on the filter paper and it was washed with hot wate rand filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

c) Determination of water soluble ash

The ash above obtained, was boiled for 5min with 25 ml of distilled water, cooled and the insoluble matter was collected on an ash less filter paper. Paper was washed with hot water and ignited for 15min at a temperature not exceeding 450°C in a muffle furnace. The difference in weight of ash and weight of water insoluble matter gave the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the shade dried plant powder by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Weight of total ash-Weight of water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

3. Extractive values [19]

Extractive values of shade-dried powder of *Trigonella foenum-graecum* were determined using following methods.

a) Determination of alcohol soluble extractive

An accurately weighed quantity of the shade dried powder of *Trigonella foenum-graecum*(5 g) was macerated with 100 ml of alcohol (Ethanol) in a closed flask for 24h, with occasional shaking during the first 6 h. It was then allowed to stand for 18 h and it was filtered rapidly to prevent any loss during evaporation. Evaporate 25 ml of the filtrate in a porcelain dish and dried at 105°C and weighed. The percentage of alcohol (Ethanol) soluble extractive wasc alculated with reference to the shade dried plant powder.

b) Determination of water soluble extractive

Weighed quantity of the shade dried powder of *Trigonella foenum-graecum*(5 g) was macerated with 100 ml of water in a closed flask with occasional shaking for the first6 hrs and allowed to stand for 18 hrs. Then it was filtered taking precaution against loss of water. Evaporate25 ml of filtrate in a tared flat bottom shallow dish dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the shade dried plant powder.

c) Determination of petroleum ether soluble extractive

Weighed quantity of the shade dried powder of *Trigonella foenum-graecum*(5 g) was macerated with 100 ml petroleum ether in a closed flask for 24 h, with occasional shaking for the first 6 hrs and allowed to stand for 18 hrs. Then it was filtered rapidly taking precaution against loss of petroleum ether due to its volatility. Evaporate 25ml of filtrate in a porcelain dish and dried at105°C and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to the shade dried plant powder.

IV. Phytochemical Evaluation

The freshly prepared petroleum ether, methanolic andaqueous extracts of *Trigonella foenum-graecum* were qualitatively analyzed for the presence of major phytochemical constituents using the following standard procedures.

1. Detection of Carbohydrates [20]

100 mg of extracts were dissolved in water (10 ml)and filtered. The prepared filtrate was used to test the presence of proteins and amino acids.

(a) Molisch's Test

To the 1 ml of filtrate add 2 drops of Molisch's reagentin a test tube and add 2 ml of concentrated sulphuric acid carefully along the sides of the test tube. Formation of violet color at the interface indicates the presence of carbohydrates.

(b) Fehling's Test

To the 1 ml of filtrate, 4 ml of Fehling's reagent (2ml Fehling A and 2 ml Fehling B solutions) were added in atest tube and heated for 10 minutes in a water bath. Formation of red precipitate indicates the presence ofreducing sugar.

(c) Barfoed's Test

The 1 ml of Barfoed's reagent is heated with 5 drops of filtrate in a test tube on boiling water bath. Formation of abrick-red precipitate within five minutes indicates the presence of monosaccharides. Disaccharides generally don't give any reaction even for ten minutes.

2. Detection of Proteins and Amino acid [21]

100 mg of extracts were dissolved in 10 ml of water and it was filtered. The filtrate was used to test the presence ofproteins and amino acids.

(a) Millon's Test

2 ml of filtrate was treated with 2 ml of Millon's reagent in a test tube and it was heated in a water bath for 5min, cooled and few drops of NaNO2 solution were added. Formation of white precipitate which turns to red upon heating indicates the presence of proteins and amino acids.

(b) Ninhydrin Test

To the 2 ml of filtrate, 2-3 drops of Ninhydrin reagent were added in a test tube and boiled for 2 min. Formation of blue colour indicates the presence of amino acids.

(c) Biuret Test

To the 2 ml of filtrate, add 2 ml of 10% sodium hydroxide solution in a test tube and heated for 10 min,to the above solution add a drop of 7% of copper sulphate. Formation of violet colour confirms the presenceof proteins.

3. Detection of Glycosides [22]

0.5 g of extract was hydrolyzed with 20 ml of dilute HCL (0.1N) and filtered. The filtrate obtained was usedto test the presence of glycosides.

(a) Legal Test

To 1 ml of filtrate add sodium nitropruside (3 ml) inpyridine and methanolic alkali (KOH) in a testtube. Appearance of blue colour in the alkaline layer indicates thepresence of glycosides.

(b) Keller-killiani Test

1 ml of filtrate was shaken with 1 ml of glacial acetic acid which contains traces of ferric chloride. Add 1 ml of concentrated H_2SO_4along the sides of the test tubes. Formation of blue colour in acetic acid layer and red colour at the junction of the two liquids indicates the presence of glycosides

(c) Modified Borntrager Test

To the 1ml of filtrate add 2 ml of 1% ferric chloride solution in a test tube and heated for ten min in boiling water bath. The mixture was cooled and then shaken with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Appearance of pink colour in the ammonical layer indicates the presence of glycosides.

4. Detection of Alkaloids [23]

0.5 g. of extract was taken and it was dissolved in 10 ml of dilute HCL (0.1N) and filtered. The filtrate was used to test the presence of alkaloids.

(a) Dragendorff's Test

To the 2 ml of filtrate add2-3 drops of Dragendorff's reagent. Appearance of reddish brown colored precipitate indicates the presence of alkaloids.

(b) Hager's Test

To the 2 ml of filtrate add Hager's reagent. Formation of yellow colored precipitate indicates the presence of alkaloids.

(c) Mayer's Test

To the 2 ml of filtrate, 2-3 drops of Mayer's reagent was added, formation of cream colored precipitate indicates the presence of alkaloids.

(d) Wagner's Test

To the 1 ml of the extract, add 2 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

5. Detection of Flavonoids [24]

(a) Shinoda Test

To the 100 mg of extractadd few fragments of magnesiummetal in a test tube. To the test tube add 3 to 4 drops of concentrated HCL. Formation of magenta colour or light pink colour indicates the presence of flavonoids.

(b) Alkaline Reagent Test

To the 100 mg of extract add few drops of NaOH solution in a test tube. Intense yellow colour is formed. After adding few drops of dilute hydrochloric acid, the yellow colour becomes colourless which indicates the presence of flavonoids.

(c) Fluroscence test

To the 100 mg of extract add 0.3 ml boric acid solution (3 %w/v) and then add 1 ml oxalic acid solution (10 %w/v) and evaporated to dryness. The residue obtained was dissolved in10 ml of ether. Under UV light the ethereal layer shows greenish fluorescence which indicates presence of flavanoids.

6. Detection of Phenolic Compounds and Tannins [25]

100 mg of extract mixed with one ml of distilled water and it was boiled and filtered. The filtrate was used for the following test.

(a) Ferric Chloride Test

Take 2 ml of filtrate in a test tube and add two ml of 1%ferric chloride solution. Formation of bluish to black colourindicates the presence of phenolic nucleus.

(b) Lead Acetate Test

To the two ml of filtrate add few drops of lead acetate solution in a test tube. Appearance of yellowish precipitate indicates the presence of tannins.

7. Detection of Fats and Oils [26]

Oily Spot Test

One drop of extract was placed on filter paper and the solvent was allowed to evaporate. Appearance of oily stain on the filter paper indicates the presence of fixed oil.

8. Detection of Saponins [27]

Foam Test

To 1 ml of extract, 20 ml of distilled water was added and shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

9. Detection of Phytosterols [28]

To 0.5 g of extract add10 ml of chloroform and filtered. The filtrate was used to test the presence ofphytosterols and triterpenoids.

(a) Libermann's Test

To the 2 ml of filtrate in hot alcohol addfew drops ofacetic anhydride. Formation of brown precipitate indicates the presence of sterols.

(b) Salkowski Test

To the 2 ml of extract, few drops of concentrated sulfuric acid were added, shaken and then allowed to stand. Appearance of red colour in lower layer indicates the presence of sterols.

Results and Discussion:

*Trigonella foenum-graecum*was subjected to systematic physicochemical and phytochemical screening by extracting with different organic solvents of increasing polarity to determine the soluble constituents in a given amount of plant material. The present work is helpful in determining the quality and purity of a crude drug. In this study the parameters used for the evaluation of *Trigonella foenum-graecum* were moisture content, extractive values by different solvents (includes petroleum ether, methanol and water), ash values (total ash, water soluble and acid insoluble ash) (Table 1). On incineration, drugs leave an ash which consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The determination of ash value is useful for detecting exhausted drugs, low-gradeproducts and excess of sandy matter which is especially applicable to powdered drugs.

Phytochemical analysis was performed on the petroleum ether, methanol and aqueous extracts of *Trigonella foenum-graecum*. Petroleum ether extract was foundto contain proteins and aminoacids, tannins, saponons, fats and oils. Methanolic extractcontains carbohydrates, proteins and aminoacids, glycosides, alkaloids, flavonoids, phenolic compounds andtannins. Aqueous extract contains carbohydrates, proteins and aminoacids, glycosides, alkaloids, flavonoids, phenolic compounds, phytosterols and tannins(Table 2).

Table. 1: Physico-chemical investigation of *Trigonella foenum- graecum*

S.No.	Quality parameters		Results
1	**Moisture content**		4.6
2	**Ash value**		
	A	Total ash value	6.6
	B	Acid insoluble ash value	1.2
	C	Water soluble ash value	2.1
3	**Extractive values**		
	A	Petroleum ether soluble extract	5.9
	B	Methanol soluble extract	6.1
	C	Aqueous soluble extract	7.3

Table. 2: Phyto-chemical investigation of *Trigonella foenum-graecum*

S.No	Tests	Petroleum ether extract	Methanolic extract	Aqueous extract
1	Carbohydrates	-	+	+
2	Proteins and aminoacids	+	+	+
3	Glycosides	-	+	+
4	Alkaloids	-	+	-
5	Flavonoids	-	+	+
6	Phenolic compounds	-	+	+
7	Tannins	+	+	+
8	Saponins	+	-	-
9	Phytosterols	-	-	+
10	Fats and oils	+	-	-

Conclusion:

Herbal remedies serve as the vital means of therapeutic medical treatment. The people are turning to use herbal plants and phyto-chemicals in health care. India has one of the oldest cultural traditions of use of itsherbal flora since from vedic period. Ayurveda, Unani, Siddha and other different traditional systems of medicine are the oldest systems of medicine and utilize huge number of medicinal plants. Phytochemical screening, biological screening of randomly collected plants and phytochemical examination of plants have proved to be helpful in discovering new drugs.

Trigonella foenum-graecum, generally known as fennel ,is very important medicinal plant belonging to family Papilionaceae. The present study concluded that the plant *Trigonella foenum-graecum*contains variety of phytoconstituents. The physicochemical evaluation of *Trigonella foenum-graecum*revealed that the standardquality and purity of drug. Phytochemical studies on the extracts of *Trigonella foenum-graecum* showed presence of phytosterols, carbohydrates, glycosides, flavonoids, proteins and amino acids, tannins & phenolic compounds. This information may be further used forisolation of various compounds from *Trigonella foenum-graecum* for treatment of diseases in human beings.

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