Estimation and Phytochemical screening from different plant parts of *Trigonella foenum graceum* (L.)

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ABSTRACT: *Metabolites such as carbohydrates, organic and amino acids, vitamins, hormones, flavonoids, phenolics, are essential for plant growth, development, stress adaptation, and defense. Besides the importance for the plant itself, such metabolites determine the nutritional quality of food, color, taste, smell, anti-oxidative,anti-carcinogenic, anti-hypertension, anti-inflammatory, antimicrobial, immune stimulating ,and cholesterol lowering properties. Plants have been an important source of medicine for thousands of years. Recently, the world health organization estimated that upto80%of people still rely mainly on traditional remedies such as herbs for the medicines. In the present study various plant parts of Fenugreek were evaluated separately for the presence of primary metabolites. Trigonella foenum graceum L. belongs to family Fabaceae, and commonly known as methi, is a common herb. It is employed as an indigenous medicine for variety of ailments including Jaundice. The plant also has heapto protective activity and it is used in Pancreas stimulant, digestive stimulant, carminative, Anti-carcinogenic,anti oxidant Neuroprotective etc. Phytochemical screening and quantification of primary metabolites in different plant parts i.e. leaf, seeds and stem of mature as well as its cotyledonary stage revealed the presence of, proteins, lipids, chlorophyll, ash content, moisture content and crude fiber. It showed higher concentration of lipid in Kasoori methi (dried plant) and lower in green seeds, concentration of protein was observed higher in brown seeds and lower in green seeds whereas chlorophyll content was found higher in mature plant leaf and crude fiber content was found higher in Kasoori methi(dried plant).*

Key Words: *Trigonella foenum graecum, Primary metabolites, medicinal plant.*

Introduction:

Fenugreek (Trigonella Foenum-graceum) is a plant from the family of Leguminase that grows annually and is widely cultivated in Mediterranean countries and Asia. The dried seeds have been traditionally used in India, China, Egypt and in some parts of Europe for their beneficial health effects such as, galactogouge, antibacterial, anti-inflammatory, insulinotropic, and rejuvenating effects (1). Pleasantly bitter and slightly sweet fenugreek seeds which are available in whole and ground forms are used as a source of flavoring for foods including curry powders, spice blends and teas. The seed have horny and relatively large layer of white and semi-transparent endosperm encircling central hard, yellow embryo (2).Wonderful functional and medicinal values of fenugreek are attributed to its chemical composition (20-25% proteins, 45-50% dietary fiber, 20-25% mucilaginous soluble fiber, 6-8% fixed fatty acids.

Fenugreek seeds have traditionally and commonly been used to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis, to ease labor pains and menstruation pain, and as an appetite stimulant. Fenugreek has also been used as an external poultice to control inflammation and dandruff. Modern medicine is beginning to provide confirmation of many of the traditional medicinal applications of fenugreek seeds (3,4). To the best of our knowledge, no previous studies have been reported for the mature &cotyledonary stage plant, dried plant and two different types of seeds of fenugreek.

Materials and Methods

Quantitative estimation of Primary metabolities:

Stem, leaf (both mature and immature plant), dried fenugreek (Kasoori Methi) and Seeds (both green and brown) parts of the plant were evaluated the evaluation of the nutritional value and organic content of the plant materials. It was achieved by the measure of percentage proximate composition which includes the quantification of the amount of lipid, moisture, fiber and ash by the method of Raghuramalu et.al.

To estimate the total level of sugar, protein, lipids &chlorophyll content were extracted from different plant parts in vivo. The protein was estimated by Lowry et.al ,Chlorophyll was estimated by Arnon

et.al,Carbohydrates were estimated by Loomis et.al. They were hydrolysed into simple sugars by dilute in hot acidic medium. Lipids were measured by the method of Jayaraman.

[1] Ash Content:

For determination of ash content, 5 g of powdered seed material weighed and taken in porcelain crucible and heated first over a low flame till all the material was completely charged, followed by heating in a muffle furnace for about 3-5 hrs at 600 °C. Then the sample was cooled in a desiccators and weighed. It was again heated in muffle furnace for 1 hour, cooled and weighed. This was repeated consequently till the weight of sample became constant (Ash became white or grayish white). The loss in weight of the sample gives the ash content calculated using the formula as follows.

% Ash = Weight of ashed sample / Weight of sample taken x 100.

[2] Moisture Content:

For determination of moisture content 1 g powdered seed material is kept in pre-weighed watch glass/moisture box and dried at 100 – 105 °C over night in an oven. The sample with watch glass is cooled at room temperature in a desiccators and final weight is taken after achieving constant weight. The weight loss in sample regarded as moisture content. The moisture content was calculated using the formula as follows.

% Moisture = (Total weight – Final weight) / Weight of the sample x 100

[3] Crude Fibre :.

For determination of Crude fibre, 2 g of moisture and fat free seed material were treated with 200ml of 1.25% H_2SO_4 with 30 min boiling. After filtration and washing, the residue was treated with 1.25% NaOH with 30 min boiling, then filtered, washed with hot distilled water. The residue was dried overnight at 80-100 °C and weighed (W1). It was then ignited and the ash weighed (W2). Loss in the weight gives the weight of crude fiber calculated using the formula.

% Crude fibre = [100 – (Moisture + Fat)] x [W1-W2] / Weight of moisture and fat free sample

% Crude fat = (W2 - W1) / Fresh sample weight x 100.

[4] Lipid Content:

Crude fat were determined by extracting 10 g of moisture free seed material with petroleum ether in a soxhlet extractor for 10-16 hours. This petroleum ether extract that contained crude fat, was taken in a pre-weighed beaker (W1) and petroleum ether was evaporated. The weight of beaker along with the residual extract after evaporation (Crude fat, W2) was taken and crude fat content of the sample was calculated using the formula.

[5] Protein:

Chemicals:

Reagent A: 2.0% sodium carbonate in 0.1N sodium hydroxide.

Reagent B: 0.5% copper sulphate (CuSO4.5H2O) in1% sodium potassium tartarate.

Reagent C: - 50% of reagent A. was mixed with at 1.0 ml of reagent B. This was prepared fresh, before use.

Reagent D: - Folin-ciocalteau reagent.

Protein solution 1: Protein standard solution was made by dissolving. 50 mg of BSA (Bovine Serum Albumin) in 0.1N NaOH and final volume was made upto 50 ml.

Working standard: 10 ml of this stock solution was diluted to 100 ml of distilled water. A series of volumes range of 0.1 of 1.0 ml of this standard gave a concentration range from 10 □ g200 □ g and procedure was followed as for that sample.

[6] Chlorophyll:

Extraction and Quantification Chlorophyll content was observed by Arnon's (1949) method using 80% acetone and the absorbance was read at 663 nm and 645 nm using spectrophotometer. The value of chlorophyll content was calculated by the following formula.

V= Final volume of chlorophyll extract in 80% acetone

W =Fresh weight of tissue extract

Material and Method

80% acetone (pre-chilled) , Plant material (1 gm)

Procedure

1 gm plant material was macerated in 80% acetone and centrifuged, thrice, at 5000rpm for 5 min. The superanatnt was pooled and final volume was made to 4ml. OD was taken at 645 and 663 nm against 80% acetone as blank. OD was taken at 645 and 663 nm against 80% acetone as blank.

Estimation Results are presented as the average of three replicates and the chlorophyll content was expressed as mg/g dw fresh weight .

Chlorophyll contain is mg/gm dry weight is follows:
Chlorophyll a(mg/g)=12.3×O.D. at 663nm- 0.86×O.D. 645 ×v/a×1000×w.
Chlorophyll b(mg/g)=19.3×a 645nm – 3.6 a 663 nm×v/a×1000×w.
Total chlorophyll= a+b (mg/gm).
V = final volume of chlorophyll extracted 80% acetone.
W= dry weight of plant material.
a=the length of light path in the cell (v=1 cm).
A =abs at 663 nm and 645 nm.

Discussion:
The moisture content of the fenugreek seeds were comparatively similar to those reported by Mullaicharametal.,, (2013); Krishanetal.,,(2013); Snehlata et al.,,(2012); and Naidu et al.,, (2011). Moisture of fresh processed foods gives an indication of its freshness and shelf life and thus high moisture content increase microbial spoilage, deterioration and short shelf life (Tressieretal.,,1980) [10,11,12,13,14].
The moisture content of fenugreek seeds was found to be 4.00%,this result is lower than that reported by Mounir et al. (1978) who found a value of 9.3%, and higher than the values reported by both Abdel Aal et al.(1985) and P. Udayasekhara and Sharma (1987),who reported 3.4% and 2.4%,respectively.The variations in moisture content reported by various investigators could be attributed to the differences in the environmental conditions, the time of harvesting and the storage conditions as stated by Sulieman (1995) [15,16,17,18,19].
In the present research work the crude fiber content of fenugreek seeds was 6.50%.This value is lower than the range9.3%–11.97% and the value 10.4% reported by Haram (1991) and Shankaracharya and Nalarjan (1972), respectively. In contrast, the value found in the present study was nearly similar to that reported by Nour and Magboul (1986) who reported a value of 6.7% crude fiber content in Sudanese fenugreek seeds [20,21,22].
According to (Ullah Khan F, A Ullah ,S Rehman, S Naz and N Naureen Rana)the crude fiber content found to be 6.28% [23].
Crude fat determines the free fatty lipids of a product and is used as basis for determining processing temperature as well as autoxidation which can lead to rancidity. The result of the crude fat obtained from this study compares favorably with the works of Singh et al.,(2015);and Naidu,(2011) [24,25].
Low fat content in foods enhance storage life due to reduced chance of lipid per oxidation, however, it may not be a good source of fat soluble vitamins nor contribute much to energy content. T.foenum graceum seed is good source of fat content is 6.53% (Ullah Khan F, A Ullah, S Rehman, S Naz and N Naureen Rana) [23].
The fat content of fenugreek seeds was found to be 4.00%. This result was lower than that reported by Abdel Hamid et al. (1984), Abdel Aal et al. (1985) and Sulieman (1995) who reported 7%, 7.6% and 8.04%,respectively [16,19,26].
Ash is the substance that remains after burning an organic substance; it contains almost all macro-as well as micronutrients except organic carbon and nitrogen. The ash content of the fenugreek seeds was found to be 3.20%. This value was in a close agreement with that reported by Shankaracharya and Nalarjan (1972) which was 3.15%.On the other hand, the ash content obtained in the present work was lower than that recorded by Abdel Hamid et al. (1984) for the Egyptian fenugreek seed which was 7.6%. The variation in the ash content could be due to the variety of fenugreek or the soil conditions (Sulieman1995) [19,20,26].
The ash content was also similar to results obtained from previous studies (Mullaicharam et al.,,2013).Determination of ash content in food samples has no nutritional significance perse, but the value for a shisa useful check in summing up the proximate composition of food and a measure of its mineral content [10].
The fenugreek seeds protein content was 28.55%. This value was higher than reported by Wunschendroff (1919) and Shankaracharya and Nalarjan (1972) who found a value of 27% and24.7% ,respectively [20].
The protein content of the fenugreek seeds was significantly lower than those reported by Tori (2011);Mullaicharametal.,,(2013):,where they reported higher proportion of protein ranging from 20to30 % as well as the presence of the amino acid 4 hydroxy isoleucine. These differences may be due to climatic condition, temperature, type of vegetation, rainfall or type of cultivation practice of the plant [10].
In these study Fenugreek seed contained about 46.25% carbohydrates. This value was higher than that reported by Abdel Aal and Rahma (1986) and Haram (1991), who reported total carbohydrates in fenugreek seeds was 40.6%, and 44%, respectively ,and much lower than that reported by Yousif et al. (1973) who determined 60.01% carbohydrates in fenugreek seeds [16,22,30].

Conclusion

The analysis of proximate composition gives basic chemical composition of both stages of plant parts. These components are essential for assessment of nutritive quality of food being analyzed. Primary metabolites of different plant parts were evaluated quantitatively to estimate chlorophyll, starch content, total protein and total lipid content. Proximate analysis of Crude fibre, Ash, Moisture was also done by described protocols .In order to study further, this plant species was also evaluated for the presence of different bioactive secondary metabolites through different plant parts i.e. leaves, stem of mature and cotyledonary stage of plant and dried plant(Kasoori methi) .

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Result:

The various plant parts (under present study) exhibit variation in total content of various metabolites shown in Tables.

Table:1
Proximate Analysis of Ash , moisture, Lipid and crude fibre:

Names	Ash content	Moisture content	Lipid content	Crudefiber content
Brown seeds	83%	9.64%	1%	0.85%
Green seeds	84.93%	9.80%	0.50%	4.65%
Mature plant leaf	96.40%	10.23%	2.90%	10%
Immature plant leaf	89%	11%	0.80%	2.75%
Mature plant Stem	93.40%	9.64%	1.90%	12.55%
Immature plant Stem	86.70%	8.74%	0.20%	0.85%
Kasoori methi	91%	13.30%	6%	17.65%

Graph:1: Comparsion of Proximate Analysis of Ash , moisture, Lipid and crude fibre:

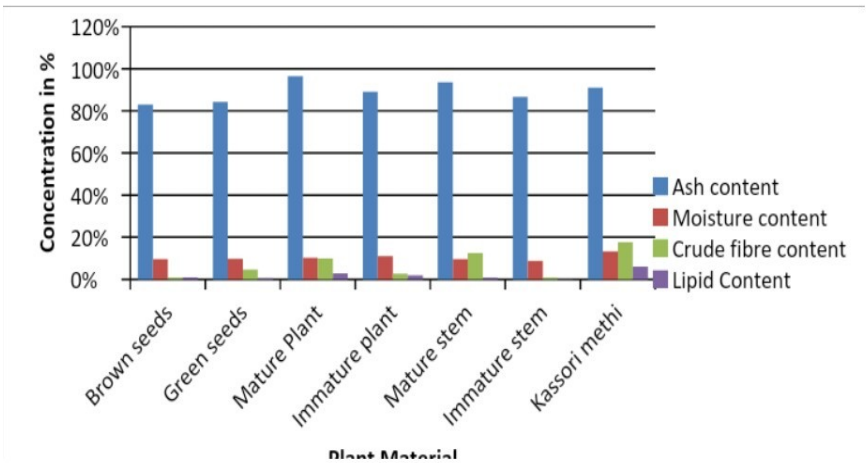

Table:2
Protein Content:

Name	Protein mg/gm. d.wt.
Brown seeds	0.1
Green seeds	0.19
Mature Plant Leaf	0.33
Immature Plant Leaf	0.45
Mature Plant Stem	0.19
Immature Plant Stem	0.34
Kasoori Methi	0.58

Graph:2 : Protein Content

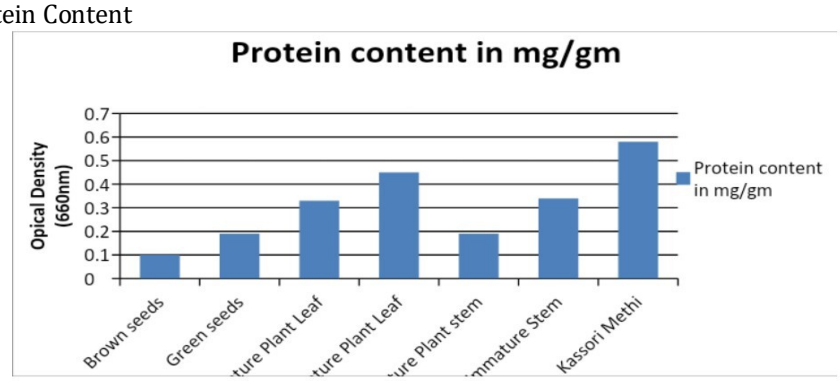

Table:3
Cholorophyll Content:

NAMES	Total Chlorophyll Content
Brown seeds	0.9873 gram
Green seeds	4.4073gram
Mature Plant Leaf	4.74096gram
Mature Plant stem	1.8249gram
Immature plant Leaf	0.76732 gram
Immature Plant stem	0.05324 gram
Kasoori methi	1.33524 gram