



Research Article

QUALITATIVE AND QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS IN *TRIGONELLA FOENUM GRACEUM*

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum*) is an important spice; its dried seeds have wide application in food and beverages as a flavoring additive as well as in medicines. The objective of the present study was to evaluate the phytochemical constitution of ethanolic extract of dried plant parts (Leaf, Stem, Root and Seed) of Medicinal Herb *Trigonella foenum-graecum*. Qualitative and Quantitative analysis of phytochemical constituents viz. Carbohydrate, Starch, Amino Acid, Protein, Lipid, Chlorophyll, Phenol and Alkaloid was performed by well-known test protocols available in the literature. Maximum amount of phytoconstituents was recorded in root part of plant. The results suggest that *Trigonella foenum-graecum* is a promising potential source of natural drug.

Keywords: *Trigonella foenum-graecum*, phytochemical constitution, Medicinal Herb, Natural drug.

INTRODUCTION

The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. Thus, the drug of plant origin served through the ages as mainstay in the treatment of human ailment and preservation of health. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs¹.

The most important of these chemically active (Bioactive) constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants.

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. 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)².

Legumes are rich in nutrients such as digestible protein with good array of amino acids and minerals. Leguminous seeds have been reported to be excellent sources of energy in animal and human

diets. This explains why considerable research has been directed to harnessing the potential of these seeds in animal or human diets³.

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family Leguminosae widely grown in Pakistan, India, Egypt, and Middle Eastern countries⁴. Fenugreek has been widely cultivated in Asia, Africa and Mediterranean countries for the edible and medicinal values of its seeds. In Chinese traditional medicine, the seeds of this plant have been prescribed as a tonic for stomach disorders, and the whole aerial part of the plant is used as a folk medicine for the treatment of renal diseases in the Northern-East region of China. Many phytochemical studies on constituents of the seeds have been reported⁵. The name fenugreek comes from foenum-graecum, meaning Greek hay, as the plant was traditionally used to scent inferior hay⁶.

The medicinal properties of some plants have been documented by some researchers. This study looks into the fundamental scientific bases for the use of medicinal plant such as *Trigonella graecum foenum* was investigated to better understand their properties and efficiency by determining the crude phytochemical constituents present in these plants. The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments. In the present study efforts was taken to assess the qualitative estimation of phytochemical compounds as well as quantitative analysis in *Trigonella foenum graecum*.

MATERIALS AND METHODS

Study Site

The study site is situated in northern Rajasthan which is under the influence of Great Thar desert. It is an agricultural field having a vast array of biodiversity. The experimental work was done in Department of Botany, Maharshi Dayanand College, Sriganganagar, Rajasthan.

Sample Collection

The sample was collected from the Areas of Maharaja Ganga Singh Stadium, Sriganganagar. The plant was transferred in department for further investigation and processing for the research. The taxonomical studies of weed plants were authenticated by Dr. Sudesh Dhingra, Professor of Botany.

Sample preparation

The plants were separated into leaf, stem, fruit and root. After this the plant parts (Leaf, Stem, Fruit & Root) were washed under running tap water and shade dried, after this the material were kept in hot air oven at 40-50°C. After that the dried plant materials were grinded into a fine powder with the help of a suitable grinder. Approximate half of the powdered sample was stored in air tight container for phytochemical estimation.

The plant extracts were prepared using the solvent ethanol. For the extraction of dried powdered sample parts (each 30g) weighed and put in the soxhlet thimble using Whatman filter paper No. 1 and 300 ml of ethanol (50%) in soxhlet flask. After that these sample were extracted at 50-60°C. After extraction the solvents were removed under pressure using rotary vacuum evaporator. All extracts were stored in air tight bottles in freeze at 4°C for evaluation the phytoconstituents.

QUALITATIVE ESTIMATION

Test for carbohydrates

To 2 ml of the plant extract, 1 ml of Molisch's reagent was added. A purple or reddish change in colour indicated the presence of carbohydrates⁷.

Test for Starch

Take 1g of dry powder in 50 ml of water boil for one minute and cool, thin and cloudily mucilage is produced, which gives thick and more transparent mucilage. To 10 ml of the mucilage add 0.05ml of 0.01 M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling⁸.

Ninhydrin Test

In the pH range of 4-8, all α - amino acids react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent to give a purple colored product (diketohydrin) termed Rhuemann's purple. All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The imino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively when subjected to the ninhydrin reaction⁹.

Biuret Test

Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent. In sample 5-6 drops of dilute Copper sulphate, 40% NaOH solution was added and observe the change⁹.

Test for Lipids

Sample with equal amount of water was added with Sudan Dye then colour change was observed¹⁰.

Test for alkaloids - Dragendroff's test (Harborne 1973)

To a few millilitre of the filtrate, 1 or 2 ml of Dragendroff's reagent was added. A prominent red or orange precipitate indicated a positive result for alkaloids¹¹.

QUANTITATIVE ESTIMATION

Carbohydrate Estimation

0.5g of sample (Stem, Leaf, Fruit and Peel) were homogenized with 10mL of 80% ethanol and centrifuged at 2000rpm for 20 minutes. 1mL of the supernatant was added with 5% phenol followed by the addition of 5mL H₂SO₄. Mixture was agitated and allowed to stand in water bath at 26-30°C for 20 minutes to develop color. The absorbance of the solution was read at 490nm wavelength using spectrophotometer (Systronics Model no. 2205). Standard solutions of dextrose were prepared at 0, 0.2, 0.4, 0.6, 0.8, 1mg/g respectively with the same treatment. Calibration curves of the absorbance values versus concentration of the standard were constructed and the value of carbohydrate in the sample was calculated¹².

Starch Estimation

0.5g of fresh plant tissue were homogenized with 10mL of 80% ethanol and centrifuged at 2000rpm for 20 minutes. After discarding the supernatant, Pellet was suspended in 5mL of distilled water by subsequently adding 6.5mL Perchloric acid (52%) to residue. The mixture was centrifuged for 20minutes at 2000rpm. Supernatant was decanted and collected by repeating this step thrice. Supernatant was transferred to 100mL volumetric flask and made up to 100mL mark with the distilled water. 1mL of this filtrate was analyzed followed by same procedure as that in carbohydrate estimation. Quantity of starch was calculated as glucose equivalent was used to convert the value of dextrose for starch estimation¹³.

Protein Estimation

0.5g of fresh weight of plant tissue was extract with 5mL of 5% TCA. The homogenized material was centrifuged at 2000rpm for 20 minutes. Pellet was dissolved in 10mL of 0.1N NaOH after discarding the supernatant. 0.1mL of this solution made up to 1mL by adding distilled water. By adding alkaline copper reagent residue was dissolved and allowed to stand for 10minutes followed by the addition of 0.5mL folin-ciocalteu reagent (50%). The optical density was measured at 750nm in spectrophotometer. The standard curve was prepared by using 0-1mg/ml solution of BSA in 0.1N NaOH and values of protein in the sample were calculated¹⁴.

Lipid Determination

1g of the fresh tissue was taken and homogenized with 10 mL CHCl₃ and methanol in 2:1 ratio. The crushed material was transferred to screw capped tubes and were kept overnight. The contents were filtered through sintered through glass funnel. Washing of mixture was done by CHCl₃ and methanol twice in times. Crude extract was added by one fifth of its volume of 1% NaCl to remove water soluble impurities. Now centrifugation step has taken at low speed, lower CHCl₃ layer containing lipids was then withdrawn by Pasteur pipette¹⁵.

Aliphatic Amino Acid Determination

0.5g of sample was crushed in 80% ethanol. This mixture was making up to 10mL and centrifuged at 2000rpm for 20minutes. 1mL of the supernatant is collected followed by the addition of 2mL ninhydrin. The tubes were kept at 100°C for 15minutes.

Tubes were cooled at room temperature and absorbance taken at 575nm wavelength⁹.

Phenol Determination

1 ml of sample (1 mg/ml) was mixed with 1 ml of folin-ciocalteu phenol reagent. After 5 min, 10 ml of a 7% Na₂CO₃ solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample¹⁶.

Determination of Alkaloids

5 g of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was

filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated¹⁷.

RESULTS & DISCUSSION

In *Trigonella foenum graecum* all selected phytoconstituents as mentioned in protocol are observed qualitatively. In Leaf Carbohydrate, Protein & Phenol are strongly present while Starch, Amino acid, Lipid & Alkaloid are moderately present. In Stem Carbohydrate & Protein is strongly observed but Starch, Amino Acid, Lipid, Phenol and Alkaloid are moderately observed. In roots Starch, Protein, Lipid, Phenol, Alkaloid are strongly present except Carbohydrate & Amino acid. In seed only, amino acid is moderately present, and others are strongly present (Table 1).

Table 1: Qualitative Phytoconstituents analysis in *Trigonella foenum graecum*

Phytoconstituents	Leaf	Stem	Root	Seed
Carbohydrate	++	++	+	++
Starch	+	+	++	++
Amino Acid	+	+	+	+
Protein	++	++	++	++
Lipid	+	+	++	++
Phenol	++	+	++	++
Alkaloid	+	+	++	++

++ = Strong presence + = Moderate presence - = Absence

Table 2: Quantitative Phytoconstituents analysis in *Trigonella foenum graecum* (in mg/g)

Phytoconstituents	Leaf	Stem	Root	Seed
Carbohydrate	0.310±0.13	0.280±0.16	0.390±0.42	0.240±0.14
Starch	0.208±0.27	0.243±0.22	0.285±0.33	0.324±0.16
Protein	0.112±0.54	0.140±0.34	0.164±0.68	0.136±0.23
Lipid	0.065±0.64	0.040±0.07	0.110±0.25	0.087±0.74
Chlorophyll a	0.870±0.15	0.412±0.57	-	-
Chlorophyll b	0.710±0.09	0.371±0.31	-	-
Total Chlorophyll	1.580±0.21	0.783±0.14	-	-
Phenol	0.122±0.32	0.154±0.18	0.289±0.29	0.182±0.07
Alkaloid	0.187±0.47	0.192±0.09	0.384±0.52	0.210±0.44

Results are shown in Mean±S.E.

In Primary Constituents Quantitative estimation Maximum Carbohydrate content was recorded in root part (0.390 mg/g) while minimum content of Carbohydrate was observed in Seed (0.240 mg/g). Starch was present maximum (0.324 mg/g) and minimum (0.208 mg/g) in Seed and Leaf respectively. Maximum Protein Content (0.164 mg/g) and Lipid Content (0.110 mg/g) was observed in Root. In leaf maximum chlorophyll (1.580 mg/g) was recorded.

In Secondary Metabolites Maximum and Minimum phenol content was recorded in Root (0.289 mg/g) and leaf (0.122 mg/g) respectively. Maximum alkaloid content was observed in root (0.384 mg/g) while minimum was observed in leaf (0.187 mg/g) part (Table 2).

CONCLUSION

The results revealed the presence of medicinally important constituents in plant studied. It is suggested from the present research work that further research work should be carried out to isolate, purify & characterize the active compounds responsible for activity of this plant.

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