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EVALUATION OF PHYTOCHEMICAL AND NUTRITIONAL COMPOSITION OF FENUGREEK (TRIGONELLA FOENUM-GRAECUM L.)

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ABSTRACT

India is a major producer of fenugreek and also a major consumer of it for its culinary uses and medicinal application. For medicinal and cosmetic purpose, its leaf and seed are used to prepare extract and powder. With this background, the current study aimed for evaluation of phytochemical and nutritional composition of Fenugreek leaves. The Fenugreek leaves were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. Results revealed that the major phytochemicals found in aq. Fenugreek leaf extract were phenolic compounds, flavonoids, glycosides, alkaloids, and tannins. Quantitative estimation of phytochemicals in aq. Fenugreek leaf extract estimated highest quantities of total sugars i.e., 28.64 mg/g followed by non-reducing sugars (27.97 mg/g), total phenolics (2.96 mgGAE/g), total flavonoids (1.19 mgQE/g), and reducing sugars (0.67 mg/g). Furthermore, nutrient composition evaluation revealed that aq. Fenugreek leaf extract composed of moisture (85.69%), Fiber (Soluble: 0.61%; Insoluble: 3.89%), Fat (0.98%), and protein (3.86%), and Ash (9.54%). In conclusion, aq. Fenugreek leaf extract composed of considerable quantities of phytochemicals such as total sugars, reducing sugars, non-reducing sugars, phenolic compounds, and flavonoids. Hence, aqueous Fenugreek leaf extract may be explored in development of natural pharmaceutical drug agents.

Keywords: Trigonella foenum-graceum, Fenugreek, Leaf, Phytochemicals, Nutrients, Sugars

INTRODUCTION

India is a major producer of fenugreek and also a major consumer of it for its culinary uses and medicinal application. Majority of the Indian population belongs to vegetarian class. In such a situation, fenugreek is of utmost importance due to its high nutritive value, medicinal importance and industrial uses. It is used in functional food, traditional food, nutraceuticals as well as in physiological utilization such as antibacterial, anticancer, antiulcer, antioxidant and antidiabetic agent. It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, Middle East and Argentina.[1,2]

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In India its cultivation is concentrated mainly in Rajasthan, which has share of 83% of the total fenugreek production in the country.[3]

Trigonella foenum-graecum (fenugreek) belongs to the family Fabaceae is an annual plant with leaves consisting of three small obovate to oblong leaflets (Figure 1). The species name "foenum-graecum" means "Greek-hay" indicated its use as a forage crop in the past.[4] It is a medicinal herb used to treat various diseases such as diabetes, inflammation, cancer, hypercholesteremia, reproductive dysfunction, and neurodegenerative disorders. For centuries, fenugreek seeds have been used as carminative, demulcent, expectorant, laxative, and stomachic agents. Numerous clinical and pre-clinical studies have revealed its anti-diabetic, anti-sterility, and anti-fertility effects. Moreover, it regulates the production of enzymes that control blood sugar levels and help in reducing cholesterol.[5]

Figure 1. Showing leaves of Fenugreek (Trigonella foenum-graecum)

In India and other countries of the Mediterranean region, it is primarily used as a spice crop to the flavour and nutritive value of food.[6] Its fresh tender leaves and stems are consumed as curried vegetable. Commercially, it has high economic value as food, fodder and medicine. For medicinal and cosmetic purpose, its leaf and seed are used to prepare extract and powder. The leaves both fresh and dried are used in meat curries and several vegetable dishes. Hence, in the current study we aimed for evaluation of phytochemical and nutritional composition of Fenugreek leaves.

MATERIALS AND METHODS

Collection Fenugreek Leaves

The Fenugreek leaves were procured from local vegetable market, and thoroughly washed with running tap water to remove the dirt particles and wiped off, and then shade dried. The dried Fenugreek leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered Fenugreek leaves was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of double distilled water. The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Phytochemical screening was carried out on the aqueous (aq.) Fenugreek leaf extract by using standard procedures to detect phytoconstituents as described by Sofora,[7] Trease and Evans[8] and Herborne.[9]

Test for alkaloids

Approximately 0.2g of aq. Fenugreek leaf extract was warmed with 2% H_2SO_4 (2.0mL) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for tannins and phenolic compounds

The aq. Fenugreek leaf extract in small quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins and phenolic compounds.

Test for glycosides

About 0.6g of aq. Fenugreek leaf extract was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for saponins

About 0.2g of aq. Fenugreek leaf extract was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for flavonoids

0.2g of aq. Fenugreek leaf extract was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for steroids

2 mL of acetic anhydride was added to 0.5g of aq. Fenugreek leaf extract and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for terpenoids

0.3g of aq. Fenugreek leaf extract was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Formation of reddish-brown coloration at the interface indicates positive results for the presence of terpenoids.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the aq. Fenugreek leaf extract was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[10] The phenolic content of the aq. Fenugreek leaf extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mgGAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in aq. Fenugreek leaf extract.[11] The flavonoid content was determined from extrapolation of

calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mgQE/g).

Total sugars

The total sugar content of aq. Fenugreek leaf extract was determined using method as described Dubois et al. Briefly, 1 mL of aq. Fenugreek leaf extract was taken in test tube and added 2.0 mL of phenol solution to it. Then 5.0 mL of conc. H_2SO_4 was poured directly in the reaction mixture followed by cooling of solution for 30 minutes and then UV-Vis double beam Spectrophotometer was used to measure absorbance of the reaction mixture at 490 nm against a blank prepared in a similar way but having acetone in place of extract. The total sugars concentration in extract was calculated from the regression equation obtained from the standard curve of D-glucose and results obtained was expressed as mg/g extract powder.[12]

Reducing sugars

The reducing sugar content of aq. Fenugreek leaf extract was determined using modified method of Nelson. Briefly, 1 mL aq. Fenugreek leaf extract was taken in test tube and 1.0 mL of distilled water was added to it. Thereafter, 1.0 mL of alkaline copper reagent was added, properly mixed and covered with aluminium foil and heated in hot water bath for 20-25 minutes. After the boiling, tubes had cooled to room temperature, 1.0 mL of the arsenomolybdate reagent was added and then properly mixed and the reaction mixture was diluted with distilled water up to 10.0 mL volume. As a result, the reaction mixture's absorbance was measured at 520 nm using a UV-Vis double beam spectrophotometer against a blank prepared in a similar manner but having 1.0 mL distilled water in place of extract. The reducing sugars concentration present in the acetone extract was calculated from the standard curve of D-glucose and the result was expressed as mg/g extract powder.[13,14]

Non-reducing sugars

The non-reducing sugar content of aq. Fenugreek leaf extract was determined as the difference between the concentration of total sugars and that of reducing sugars as follows;

Non-reducing sugars = Total sugars - Reducing sugars

Nutrient Composition

Moisture

The moisture content of the Fenugreek leaves was determined as per the method described in Association of Official Analytical Chemists (AOAC, 2000).[15]

$$\text{Moisture content (\%)} = \frac{(w_2 - w_1) - (w_2 - w_3) \times 100}{(w_2 - w_1)}$$

Where,

W1: Initial weight of the empty petridish (g)

W2: Weight of the petridish + Fenugreek leaf sample before drying (g)

W3: Weight of the petridish + Fenugreek leaf sample after drying (g)

Crude fat content determination

The crude fat content of the Fenugreek leaves was determined as per the method described in Association of Official Analytical Chemists (AOAC, 2000).[15] Soxhlet apparatus was used to determine crude fat content of the Fenugreek leaf samples. The crude fat (%) was calculated using the following formula:

Crude fat (%) = Weight of ether extract (g)/Weight of sample (g) X 100

Protein

The protein content of the Fenugreek leaf sample was determined as per the method described in Association of Official Analytical Chemists (AOAC, 2000).[15]

- Place the samples of Fenugreek leaves (0.5-1.0 g) in digestion flask.
- Add 5 g Kjedahl catalyst and 200 ml of conc. H_2SO_4.
- Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently until frothing ceases. Boil briskly until solution clears.
- Cool and add 60 ml of distilled water cautiously.
- Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.
- Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Percentage of nitrogen and protein was calculated by the following equations:

Nitrogen (%) = T_S-T_B x Normality of acid x 0.014)/ Weight of sample (g) X 100

Where,

T_S - Titre volume of the sample (ml)

T_B - Titre volume of Blank (ml),

0.014-M eq. of N

Protein (%) = Nitrogen x 6.25

Where,

6.25-The protein-nitrogen conversation factor

Crude fibre

The crude fibre content of the Fenugreek leaf sample was determined as per the method described in Association of Official Analytical Chemists (AOAC, 2000).[15] The crude fibre (g) was calculated using the following formula:

Crude fibre (%) = (100-(moisture + fat)) x A x 100/W1

Where;

W1 = Weight of the Fenugreek leaf samples

W2 = Weight of the crucible + Fenugreek leaf samples before heating at 600°C

W3 = Weight of the crucible + Fenugreek leaf sample after heating at 600°C

W2–W3 = A = Weight of crude fibre

Ash

The ash content of Fenugreek leaves was determined as per the method described in Association of Official Analytical Chemists (AOAC, 2000).[15] Drying the Fenugreek leaf samples (5g) at 100°C and churned over an electric heater. It was then ashes in muffle furnace at 550°C for 5 hrs. Ash content was calculated using the following formula:

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Ash content (%) = Weight of ash (g)/Weight of sample (g) X 100

RESULTS AND DISCUSSION

Majority of the Indian population belongs to vegetarian class. In such a situation, a leafy vegetable such as fenugreek is of utmost importance due to its high nutritive value, medicinal importance, and industrial uses. Recent researchers have identified fenugreek as a valuable medicinal plant with a potential for multipurpose uses and also as a source for preparing raw materials of pharmaceutical industry.[16] Therefore, the current study was conducted with the main purpose of assessment of phytochemical and nutritional composition of aq. Fenugreek leaf extract.

The major phytochemicals found in aq. Fenugreek leaf extract were phenolic compounds, flavonoids, glycosides, alkaloids, and tannins. Whereas, phytochemicals such as saponins, steroids, and terpenoids were found to be absent in aq. Fenugreek leaf extract (Table 1).

Table 1: Photochemical screening of aq. Fenugreek leaf extract

Phytochemical Components	Aq. Fenugreek Leaf Extract
Phenolic compounds	+
Flavonoids	+
Glycosides	+
Saponins	-
Steroids	-
Alkaloids	+
Tannins	+
Terpenoids	-

+: Present; -: Absent

The results of quantitative estimation of phytochemicals in aq. Fenugreek leaf extract was represented in Table 2. Results revealed that aq. Fenugreek leaf extract contains highest quantities of total sugars i.e., 28.64 mg/g followed by non-reducing sugars (27.97 mg/g), total phenolics (2.96 mgGAE/g), total flavonoids (1.19 mgQE/g), and reducing sugars (0.67 mg/g).

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Table 2: Quantitative estimation of phytochemicals in aq. Fenugreek leaf extract

Phytochemicals	Aq. Fenugreek Leaf Extract
Total Phenolics (mgGAE/g extract)	2.96
Total flavonoids (mgQE/g extract)	1.19
Total sugars (mg/g extract)	28.64
Reducing sugars (mg/g extract)	0.67
Non-reducing sugars (mg/g extract)	27.97

Values are expressed mean; n=3

Literature reports evidenced that Fenugreek leaves are quite rich in protein, iron, calcium, carotene and ascorbic acid as well as minerals and vitamins.[17,18] In concurrence with literature reports in our study aq. Fenugreek leaf extract composed of moisture (85.69%), Fiber (Soluble: 0.61%; Insoluble: 3.89%), Fat (0.98%), and protein (3.86%), and Ash (9.54%).

Table 3: Nutrient composition of aq. Fenugreek leaf extract

Nutrient Composition	Aq. Fenugreek Leaf Extract
Fiber (%)	
Soluble	0.61
Insoluble	3.89
Fat (%)	0.98
Protein (%)	3.86
Moisture (%)	85.69
Ash (%)	9.54

Values are expressed mean; n=3

The term phytochemical is usually used to those chemicals that may have biological significance but are not established as important nutrients. But in narrower sense the term phytochemical describes the number of secondary metabolic compounds found in plants. The scientists estimate that approximately 10,000 different phytochemicals having the potential therapeutic effects on various ailments.[19]

CONCLUSION

This preliminary pilot study delineated that aqueous Fenugreek leaf extract composed of considerable quantities of phytochemicals such as total sugars, reducing sugars, non-reducing sugars, phenolic compounds, and flavonoids. Hence, aqueous Fenugreek leaf extract may be explored in development of natural pharmaceutical drug agents.

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