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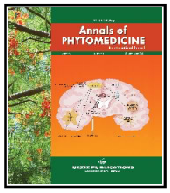

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Proximate composition, phytochemical analysis and antioxidant potency of *Trigonella foenum-graecum* L. seeds

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Abstract

The leguminous family of plants includes fenugreek (*Trigonella foenum-graecum* L.), one of the oldest and most promising therapeutic herbs. Fenugreek is a member of the Fabaceae family. The goal of the current study was to evaluate the proximate composition, mineral analysis, phytochemical analysis and antioxidant potency of fenugreek seeds belonging to variety Hisar Mukta (HM) 425. The moisture content (%), crude protein (%), crude fat (%), ash (%), crude fiber (%), total carbohydrates (%) and mineral content (ppm) in fenugreek seeds were evaluated. The acetone extract of seeds was examined for a number of phytochemicals, including total phenolics (1.35 mg GAE/g), total flavonoids (0.12 mg CE/g), total sugars (17.34 mg/g), non-reducing sugars (17.18 mg/g) and reducing sugars (0.16 mg/g). The antioxidant activity was evaluated using the DPPH free radical scavenging activity and the phosphomolybdenum assay. These findings support the notion that fenugreek seeds are an abundant source of phytochemicals and minerals with pharmacological and health benefits.

1. Introduction

The leguminous family of plants include fenugreek (*Trigonella foenum-graecum* L.), one of the ancient and most promising therapeutic herbs. Due to its culinary and medicinal applications as a herbal remedy, this plant has been used for over 2500 years. This plant's seeds have a long history of use in traditional medicine as an antidiabetic, antibacterial, anti-inflammation, anticancer and antioxidant agents (Brar *et al*., 2013; Aggarwal *et al*., 2022; Wani and Kumar, 2018; Goel *et al*., 2022). Moreover, fenugreek seeds have been shown to have potent free radical scavenging activities. Free radicals are generated naturally by the human body as a result of regular metabolism, other endogenous activities, or exposure to certain contaminants in the environment. The biological processes of the entire body depend on these radicals (Devi *et al*., 2020; Moond *et al*., 2023; Kumari *et al*., 2022). Yet, because of their high reactivity as oxidants and enzyme inhibitors, they cause the oxidation of macromolecules such as protein, lipids, DNA, and amino acids, which in turn causes cell damage and ultimately cell death. Free radicals and antioxidants must, therefore be in balance in order for the body to function physiologically and prevent oxidative stress. Oxidative stress, defined as an imbalance between oxidants and antioxidants, may be the cause of a wide range of human disorders, including cancer, inflammation, and diabetes (Yadav *et al*., 2011; Devi *et al*., 2023; Nehra *et al*., 2023). Dietary antioxidants are beneficial for defending the body and preventing chronic ailments, according to earlier studies.

The primary ingredients of fenugreek include carbohydrates, proteins, lipids, alkaloids, flavonoids, fiber, saponins as well as vitamins, amino acids, and minerals. Fenugreek contains large amounts of flavonoids like luteolin, apigenin, orientin, vitexin, quercetin, and isovitexin. These natural antioxidants promote immunological function, cellular health, and the slowing of effects of aging. The biggest factor of phenol and flavonoids' antioxidant action is their redox characteristics, which are essential in scavenging and neutralizing free radicals (Devi *et al*., 2023; Goel *et al*., 2022).

The present study's objectives were to determine the proximate composition, phytochemical constituents and evaluate the antioxidant capacity of dried *T. foenum-graecum* seeds acetone extract (Variety HM 425). To the best of our knowledge, no studies on *T. foenum-graecum* seeds from variety HM 425 have been reported. The seeds were found to be good source of total phenolics, total sugars, total flavonoids, non-reducing sugars and reducing sugars. Due to the presence of phenolics and flavonoids, seeds were found to have good antioxidant capacity using the DPPH free radical scavenging assay and phosphomolybdenum assay.

2. Materials and Methods

2.1 Collection of plant material

The *T. foenum-graecum* seeds belonging to variety Hisar Mukta (HM) 425 were obtained from Chaudhary Charan Singh Haryana Agricultural University's Vegetable Science Research Farm. The obtained seeds sample was verified by the Department of Botany and Plant Physiology at CCS HAU, Hisar, India, using a portal (Tropicos and IPNI). The Department of Genetics and Plant Breeding

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crops section of medicinal, aromatic and potential at CCS HAU Hisar assessed the validity of the voucher specimens using Voucher specimen number 20.

2.2 Proximate composition and mineral analysis

According to the Association of Official Analytical Chemists' (AOAC) recommended methods, the moisture, ash, crude fat, crude fibre, crude protein, and total carbohydrates were measured. Ash content and moisture content were estimated using the AOAC method (AOAC, 1995). To calculate crude fiber, the Maynard method was utilised (Maynard, 1970). The Micro-Kjeldahl technique was used to determine the nitrogen content (AOAC, 1990). The percentage of nitrogen content was multiplied by 6.25 to determine the crude protein content. The mineral content of Fenugreek seeds was analysed by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted acid digestion (Jackson, 1973; Ruig, 1986).

2.3 Preparation of Fenugreek seed acetone extract for phytochemical analysis and antioxidant activity

In a thimble of Whatman No. 1 filter paper, 10 g of powdered fenugreek seeds were ingested. A 500 ml round bottom flask and this thimble were both placed in a standard Borosil Soxhlet apparatus. 250 ml of acetone was added. As a result, acetone was used as a solvent to percolate the powdered seed sample. All of the following were calculated: total phenolic content, total sugars, total flavonoid content, reducing sugars, non-reducing sugars, DPPH free radical scavenging activity and total antioxidant capacity.

2.4 Quantitative analysis of phytochemicals

2.4.1 Total phenolic content

Total phenolic content of an acetone seed extract was determined using the Folin-Ciocalteu method, with gallic acid serving as the standard (Singleton and Rossi, 1965).1 ml of extract was added to 2 ml of Na_2CO_3 (20% w/v) and 1 ml of Folin-Ciocalteu reagent. The volume was then increased to 10 ml using distilled water. This mixture was centrifuged for 10 min at 6000 rpm after standing for 8 min. A UV-Vis double beam spectrophotometer (Model UV 1900 Shimadzu) was used to measure the absorbance of the supernatant solution at 730 nm. Similar steps were taken to prepare the blank, but as a substitute of the extract, the blank was prepared by the proper solvent.

2.4.2 Total flavonoids

Catechin served as the standard in an aluminium chloride colorimetric assay used to determine the total flavonoids in an acetone seed extract (Marinova *et al*., 2005). After 5 min, 1 ml of extract was thoroughly mixed with 4 ml of distilled water, 0.3 ml of 5% $NaNO_2$ solution, and 0.3 ml of 10% $AlCl_3$ solution. Immediately, distilled water was used to increase the volume to 10 ml after the addition of 2 ml of 1 M NaOH. A UV-Vis double beam spectrophotometer was used to measure the solution's absorbance at 510 nm. Similar steps were taken to prepare the blank, but as an alternative of the extract, the blank was prepared using the proper solvent.

2.4.3 Total sugars

The Dubois method was used to calculate the total sugars in an acetone seed extract, with D-glucose serving as the reference (Dubois *et al*., 1956). A phenol solution of 2.0 ml was added to 1 ml of extract. The reaction mixture was then immediately given 5.0 ml of concentrated H_2SO_4, and the solution was allowed to cool for 30 minu. A UV-Vis double beam spectrophotometer was used to calculate the reaction mixture's absorbance at 490 nm. Similar steps were taken to prepare the blank, but instead of the extract, the blank was prepared using the proper solvent.

2.4.4 Reducing sugars

Reducing Sugars of an acetone seed extract was determined using Method of Nelson as modified by Somogyi with D-glucose as standard (Nelson, 1944; Somogyi 1952). To 1 ml of seed extract, 1 ml of alkaline copper reagent was added. The mixture was thoroughly combined, wrapped in aluminium foil, and heated in a hot water bath for 20 to 25 min. Allow it to cool to room temperature after that. Arsenomolybdate reagent (1 ml) was added, and the solution was then diluted with distilled water to a final volume of 10 ml. A UV-Vis double beam spectrophotometer was used to gauge the reaction mixture's absorption at 520 nm. Similar steps were taken to prepare the blank, but instead of the extract, the blank was prepared using the proper solvent.

2.4.5 Non-reducing sugars

The difference in concentration between total sugars and reducing sugars was used to calculate the non-reducing sugars.

2.5 Evaluation of antioxidant activity

2.5.1 DPPH free radical scavenging activity

The antioxidant activity of acetone seed extract was assessed using the DPPH free radical scavenging assay (Hatano *et al*., 1988). In a typical experiment, 2 ml of DPPH solution at various concentrations were mixed with 1 ml of seed extract (100-1100 g/ml). After incubating the reaction mixture in the dark for 30 min, the absorbance at 517 nm was measured with a UV-Visible spectrophotometer. As a reference, ascorbic acid (10-110 µg/ml) was used as a standard and measured in a consistent manner.

The percentage of scavenging activity was calculated using the formula below:

$$\%\ \text{DPPH free radical scavanging activity} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

where As is the absorbance of sample and Ac is the absorbance of control

2.5.2 Phosphomolybdneum assay

With Ascorbic acid as the reference, the phosphomolybdenum method was used to assess the total antioxidant capacity (Prieto *et al*., 1999). Then 0.3 ml of extract was mixed with 3 ml of phosphomolybdenum reagent. The solution was capped and incubated at 95°C for 90 min. A UV-Visible spectrophotometer was used to measure the absorbance at 695 nm. The preparation of the blank was similar, except it contained the applicable solvent in place of the extract.

3. Results

3.1 Proximate composition and mineral analysis

Proximate composition and mineral content in Fenugreek seeds were reported in Table 1. In proximate composition, seeds of Fenugreek had moisture content (9.07%), ash content (3.55%), crude fat

(7.77%), crude fiber (9.25%), crude protein (25.57%) and total carbohydrates (44.79%). In mineral analysis, seeds of Fenugreek consist of iron (28.77 ppm), copper (1.05 ppm), zinc (68.65 ppm) and manganese (1.93 ppm).

Table 1: Proximate composition and mineral content in Fenugreek seeds

Proximate composition (%)	Moisture content	9.07
	Ash content	3.55
	Crude fat	7.77
	Crude fiber	9.25
	Crude protein	25.57
	Total carbohydrates	44.79
Mineral content (ppm)	Iron (Fe)	28.77
	Copper (Cu)	1.05
	Zinc (Zn)	68.65
	Manganese (Mn)	1.93

3.2 Phytochemical analysis

Table 2 summarises quantitative analysis of various phytochemicals, including total phenolic content, total flavonoids, total sugars, non-reducing sugars and reducing sugars of acetone extract of Fenugreek seeds.

Table 2: Phytochemicals in acetone extract of the Fenugreek seeds

Phytochemicals	Concentration
Total phenolic content	1.35 mg GAE /g
Total flavonoids	0.12 mg CE/g
Total sugars	17.34 mg/g
Reducing sugars	0.16 mg/g
Non-reducing sugars	17.18 mg/g

3.2.1 Total phenolic content

Total phenolic content of seed extract was calculated using a standard curve that included gallic acid as the standard (Figure 1). There was 1.35 mg GAE/g of total phenols in the acetone extract of the seeds.

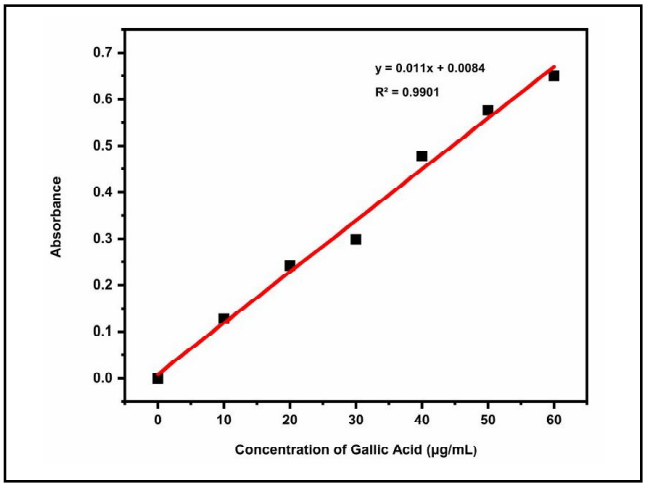

Figure 1: Total phenolic content standard curve using gallic acid as a standard.

3.2.2 Total flavonoids

Total flavonoids of seed extract were calculated using a standard curve with catechin as standard (Figure 2).The amount of flavonoids in the acetone extract of the seeds was 0.12 mg CE/g.

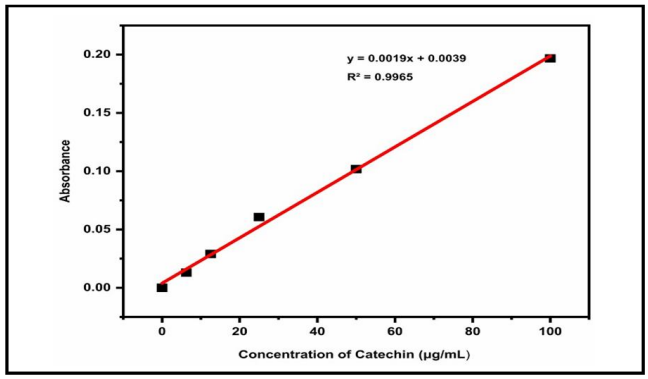

Figure 2: Total flavonoids standard curve with catechin as the standard.

3.2.3 Total sugars

Total sugars of seed extract was calculated using a standard curve with D-glucose as the standard (Figure 3).17.34 mg/g of total sugars were discovered in the acetone extract of the seeds.

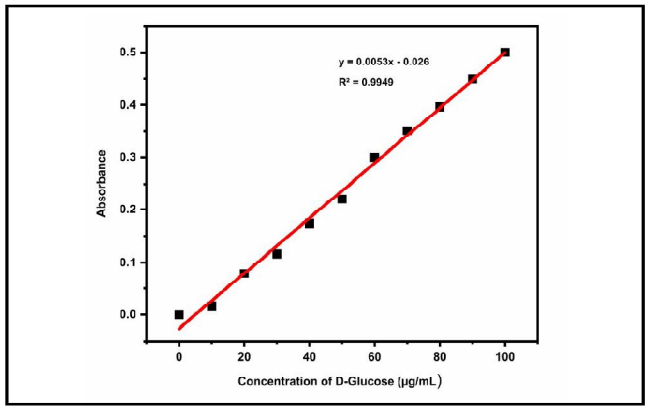

Figure 3: Standard curve of total sugars using D-glucose as standard.

3.2.4 Reducing sugars

Reducing sugars of seed extract was calculated using a standard curve with D-glucose as the standard (Figure 4). Reducing sugars in acetone extract of seeds were found to be 0.16 mg/g.

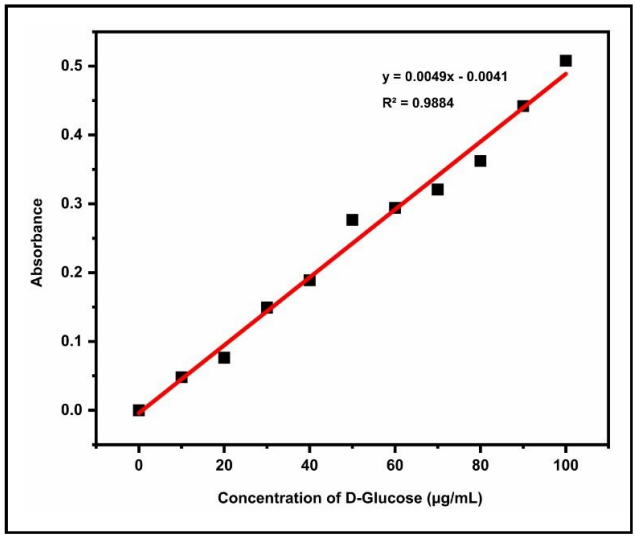

Figure 4: Standard curve of reducing sugars using standard as D-glucose.

3.2.5 Non-reducing sugars

The non-reducing sugars in the acetone extract of the fenugreek seeds were calculated using the difference between the concentration of total sugars and that of reducing sugars. There were 17.18 mg/g of non-reducing sugars in the acetone extract of the seeds.

3.3 Antioxidant activity

3.3.1 DPPH free radical scavenging activity

Ascorbic acid's ability to scavenge DPPH free radicals was 90.51% at a concentration of 110 µg/ml, 86.12, 71.14, 63.52, 45.26, and 27.84% at concentrations of 90, 70, 50, 30, and 10 µg/ml, respectively. Acetone seed extract had the highest DPPH free radical scavenging activity at 1100 µg/ml, which was followed by concentrations of 54.18, 47.69, 31.81, 19.11, and 4.53%, respectively (Table 2). With an IC_{50} of 41.32 µg/ml compared to 825.00 µg/ml for acetone seed extract, ascorbic acid demonstrated greater antioxidant efficacy. The quadratic regression equation for the IC_{50} (g/ml) value of the DPPH free radical scavenging activity is shown in Figure 5.

Table 3: DPPH free radical scavenging activity of acetone extract of Fenugreek seeds

Concentration of acetone seed extract (µg/ml)	% DPPH free radical scavenging activity
100	4.53
300	19.11
500	31.81
700	47.69
900	54.18
1100	65.43

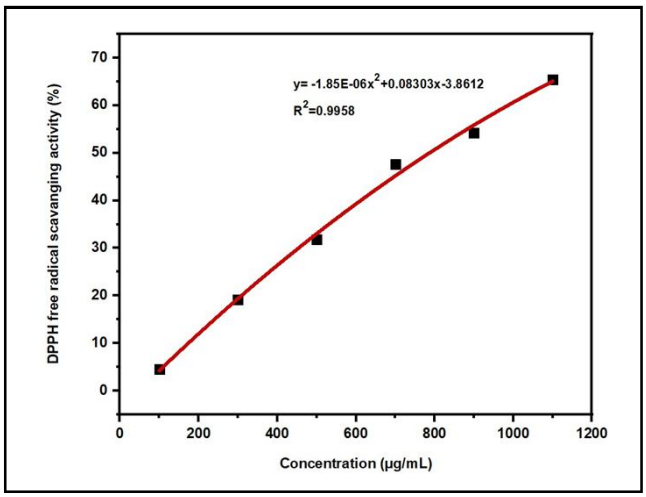

Figure 5: Equation of quadratic regression for IC_{50} (µg/ml) of the DPPH free radical scavenging activity.

3.3.2 Phosphomolybdneum assay

A standard curve for ascorbic acid was used to evaluate the total antioxidant strength of seed extract (Figure 6). It was discovered that the acetone extract of seeds contained 21.14 mg AAE/g of total antioxidant activity.

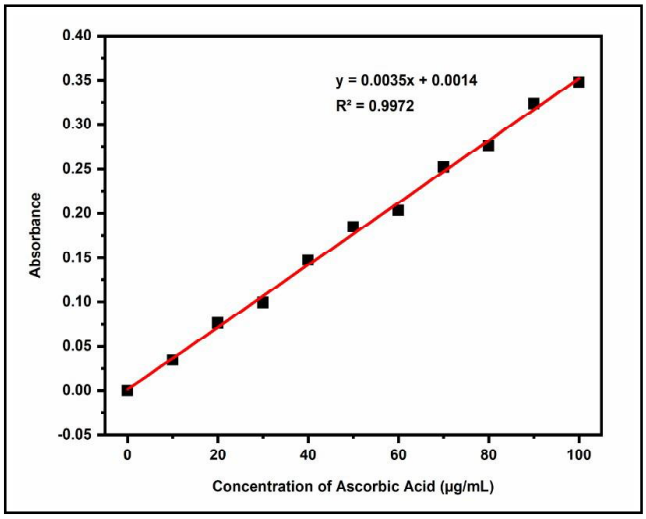

Figure 6: The ascorbic acid standard curve for total antioxidant capacity.

4. Discussion

All over the globe, medicinal plants are recognised as a significant source of organic antioxidants. Because of the phytochemicals in plants, which have distinct physiological effects on people, medicines can be made from them.

The moisture content (9.07 %), ash content (3.55%), crude fat (7.77%), crude fiber (9.25%), crude protein content (25.57%) and total carbohydrates (44.79%) in seed powder of Fenugreek were measured in current study. Mahmood and Yahaya (2017) reported that Fenugreek seeds had 6.833 ± 0.531 (%) moisture content, 3.566 ± 0.478 (%) ash content, and 28.45 ± 0.15 (%) crude protein which is in close agreement with present findings. Waniand Kumar (2016) reported 6-7 % crude fat in Fenugreek seeds. Results of the present

investigation were in close agreement with the estimation of Saini *et al.* (2016) who reported 9.26% of crude fiber content in Fenugreek seed powder.

The evaluation of phenolic compounds is based on oxidation of Folin-Ciocalteu reagent. After oxidising phenols, phosphotungstic acid and phosphomolybdic acid are combined to create this reagent, which is then reduced to a mixture of blue tungsten and molybdenum oxides (Moond *et al.*, 2023). The amount of phenolic content in the extract directly correlates with the intensity of the blue colour that is produced, which has a maximum absorption around 730 nm.

The evaluation of total flavonoids is based on an acid-stable complex formed by the C-3, C-5, and C-4 keto groups of flavones and flavanols with $AlCl_3$. Furthermore, orthodihydroxyl groups on the A or B ring in flavonoids can react with $AlCl_3$ to form acid labile complexes.

The evaluation of total sugars is based on formation of hydroxymethyl furfural, which was created by the dehydration of D-glucose in an acidic medium, combined with phenol to form a yellow-brown solution with a maximum absorbance (max) at 490 nm.

During evaluation of reducing sugars, cupric ions are converted to cuprous ions when reducing sugars are heated in the presence of alkaline copper tartrate, which results in the formation of cuprous oxide. When cuprous oxide is combined with arsenomolybdic acid, it is reduced to molybdenum blue which is measured at 520 nm using a UV-Vis spectrophotometer.

In DPPH free radical scavanging assay, when seed extract was added, the DPPH solution's purple hue turned yellow. This happened as a result of the DPPH molecule being scavenged after the hydrogen atom was given in order to stabilise it. The concentration of antioxidants has an impact on how much purple colour fades.

In total antioxidant capacity evaluation, molybdenum (VI) can be converted by the antioxidants in the extract to the green phosphomolybdate (V) complex (Moond *et al.*, 2023).The antioxidant potential of Fenugreek seeds is due to the presence of flavonoids and phenolics.

5. Conclusion

In the current study, it is found that Fenugreek seeds from variety HM 425 contain phytochemicals that may be essential in scavenging species that cause oxidative stress. In order to comprehend the pharmacological effects of Fenugreek seeds, a quantitative analysis of phytochemicals and antioxidant capacity would be useful. The pharmaceutical, medicine, and dietary supplement industries stand to benefit greatly from the current findings. To develop applications for the food and pharmaceutical industries, more research must be done to identify the precise substances that make up the antioxidant system.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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