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ORIGINAL ARTICLE

Assessment of Antioxidative Potential of seeds extract of *Trigonella foenum-graecum*

Himani Singh[1],Sonu Ambwani[1]*, Tanuj Kumar Ambwani[2], Sanjeev Agrawal[3]andAnil Kumar Gaur[1]

1. Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B.P.U.A.&T., Pantnagar, Uttarakhand, India
2. Department Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, G.B.P.U.A.&T., Pantnagar, Uttarakhand, India
3. Department of Biochemistry, College of Basic Sciences and Humanities, G.B.P.U.A.&T., Pantnagar, Uttarakhand, India

**sonuambwani@yahoo.co.in*

ABSTRACT

In Indian system of medicine Trigonella foenum-graecum is an important medicinal plant.Its leaves and seeds have been used in various ailments and as health tonic. To understand the mechanism of pharmacological actions, the qualitative phytochemical analysis and in vitro antioxidant activity of hydromethanolic seed extract of Trigonella foenum-graecum (TFE) was investigated through in vitro antioxidant assays, viz. total antioxidative capacity, reducing power capacity, hydrogen peroxide scavenging assay. Qualitative biochemical analyses showed presence of various phytochemicals in TFE. Various antioxidant activities were compared with suitable standard antioxidants such as ascorbic acid and quercetin. In every assay conducted the extract offered strong antioxidant activity in a concentration dependent manner. The hydromethanolic extract of fenugreek seeds showed significant total antioxidant capacity of 115.02mg ascorbic acid equivalents per g of extract. TFE exhibited potent H_2O_2 scavenging activity with IC_{50} values of 61.90µg/ml. The reducing power was increased in dose dependent manner from 0.022 at 10µg/ml TFE up to 0.305 at 200µg/ml TFE. The results clearly indicated that TFE has antioxidant potential and its therapeutic properties could be attributed to presence of various phytochemicals in the extract.

Keywords: *Trigonella foenum-gracecum*, antioxidant potential, TAC, H_2O_2 scavenging assay; Reducing Power; Phytochemicals

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INTRODUCTION

Trigonella foenum-gracecum is an annual herb that belongs to the Leguminaceae family. *Trigonella foenum-gracecum* (TFG) leaves are often used as vegetables owing to their high calcium, iron, β -carotene, and other phytochemical content, while the seeds are utilised as spice in culinary preparations due to their strong flavour. TFG is utilised as a dietary antioxidant supplement as well as to cure a variety of ailments due to its powerful therapeutic potential [1]. Over the last two decades, there has been a surge of interest in research into the prevention of uncontrolled oxidative processes that lead to a variety of diseases in humans, such as atherosclerosis, neurodegenerative diseases, carcinogenesis, chronic inflammatory diseases, ageing, radiation damage and a variety of other pathological disorders [2,3]. TFG has several medicinal properties, including anticancer, anthelmintic, antibacterial, anti-inflammatory, aphrodisiac, expectorant and wound healing [4]. Traditional herbal treatments are gaining popularity among professional healers as more people become aware of the dangers of modern pharmaceuticals [5]. TFG is one of the most well-known herbs among them, with a long history of usage as a spice as well as in Ayurvedic treatment. TFG seeds and leaves have a lower fat content. TFG also includes a high concentration of iron, making it effective in the treatment of anaemia. TFG's biological and pharmacological activities are primarily related to its chemical composition, which includes alkaloids

(gentianine, trigonelline, carpaine), saponins (diosgenin, fenugreekine), flavinoids, amino acids, insulin secretogogues (4-hydroxyisoleucine, arginine), mucilaginous fibres (galactomannan), coumarins, nicotinic acid, and other minerals and vitamins. Flavonoids exhibit a wide range of biological actions, including enzyme inhibition, protection against allergies, antibacterial, antifungal, antiviral, anti-malarial, antioxidant, anti-inflammatory, and anticarcinogenic characteristics. Flavonoids are benzo-gamma pyrone compounds with great pharmacological efficacy[6,7]. Due to the growing demand for plants as nutraceuticals and their ability in herbal medication formulations, it is critical to find medicinal plants with high antioxidant content. TFG is being promoted for its antioxidant effects, as a food additive, and as a therapeutic agent, needs to be scientific rationale of its efficacy in various *in vitro* and *in vivo* systems. The goal of this research was to look at fenugreek as a possible source of natural antioxidants. The present study was planned to explore the presence of phytoconstituents through biochemical analyses in the hydromethanolic seed extract of *Trigonella foenum-gracecum* (TFE). TFE was assessed by total antioxidative capacity, reducing power capacity and hydrogen peroxide scavenging assay.

MATERIAL AND METHODS

Sample (seeds) of *Trigonella foenum-graecum* was collected from Vegetable Research Center, G.B.P.U.A.&T, Pantnagar, India. The plant sample was collected, rinsed under running tap water, dried in shade, pulverised in a grinder, and kept in airtight container at 4^0C until further use. Biological Science Department G.B.P.U.A.&T, Pantnagar, India, validated the identification of *Trigonella foenum-graecum*. The voucher specimen was also submitted to Biological Science Department G.B.P.U.A.&T, Pantnagar, India.

Extraction procedure

The extraction technique described by [8]was used for the preparation of hydromethanolic *Trigonella-foenum graecum* seed extract (TFE). 100 g of this shade dried powder was mixed with 1000 ml of a solution of double distilled water and methanol (1:1). The solution was homogenised in an incubator cum shaker for 48 hours at 37°C. The mixture was filtered using muslin cloth; followed by Whatman filter paper No. 1. The extract was rotary evaporated at 45°C to remove excess solvent before being freeze dried. Finally, following lyophilization, the extract was weighed and kept at -20°C in a deep freezer until further usage. The percent yield was calculated by dividing quantity of plant extracts obtained by 100 gm of dry powder.

Phytochemical analysis

Qualitative phytochemical tests for the identification of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, steroids, tannins, terpenoids, saponins, reducing sugars, carbohydrate and proteins were carried out for TFE as per the methods described by [6,8-12].

Test for alkaloids

5ml of extract is mixed with 5ml of 2N HCl, heated, and filtered. A few drops of Mayer's reagent are added to this. The presence of alkaloids is indicated by a coloured precipitate.

Test for anthroquinones

5ml of extract was boiled with 10ml of sulphuric acid and filtered while still hot. 5ml of chloroform was added to the filtrate and agitated, then the chloroform layer was pipetted out to a separate test tube and 1ml of dilute ammonia was added. The presence of anthroquinones is shown by the colour shift.

Test for cardiac glycosides

Glacial acetic acid, a few drops of ferric chloride, and strong sulphuric acid were added to 1ml of extract. The presence of cardiac glycosides is indicated by the appearance of reddish brown at the intersection of two layers and green in the upper layer.

Test for flavonoids

To determine the presence of flavonoids, 2ml of extract is mixed with 2ml of 10% lead acetate. The presence of flavonoids is indicated by the yellowish green colour.

Test for phenols

2ml of ferric chloride solution is added to 2ml of plant extract to test for phenols. The presence of phenols is indicated by the presence of a blue green hue solution.

Test for steroids

2ml of acetic anhydride was added to 0.5 g of extract and 2 ml of Sulphuric acid was added via the test tube sides and the colour shift from violet to blue-green was noted.

Test for tannins

To 5ml of plant extract, add a few drops of 1% lead acetate. The presence of tannins is indicated by the presence of yellow precipitate.

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Test for terpinoids (Salkowski test)
To detect the presence of terpenoids, mix 2ml of extract in 2ml of chloroform and gently add strong sulphuric acid to produce a layer. The presence of terpenoids is indicated by the presence of a reddish brown colour.
Test for saponins
In a test tube, combine 5ml of extract with 10ml of distilled water and rapidly shake for 30 seconds. The presence of saponins is indicated by the formation of foam.
Reducing sugars
The extract was filtered after being agitated with distilled water. When the filterate is heated for a few minutes with Fehling's solutions A and B, an orange red precipitate indicates the presence of reducing sugars.
Carbohydrates
In a tiny test tube, 0.2ml of Molisch's Reagent was mixed with 1.0ml of extract. The tube was slanted after mixing. 0.5ml of strong sulphuric acid was gently poured down the edge of the test tube without stirring. Carbohydrates were detected as a red violet ring at the interface between the acid (bottom) and aqueous (upper) layers.
Proteins (Xenthoprotein test)
A few drops of nitric acid were carefully added to 1ml of extract by the sides of the test tube. The presence of protein in the sample was confirmed by the formation of yellow colour.
Antioxidant studies
Total Antioxidant Capacity estimation of the extract
Total antioxidant capacity of the extract was determined using the phospshomolybdenum technique [13] using ascorbic acid as standard and represented as mg ascorbic acid equivalent/g extract. For the calibration curve, ascorbic acid ranging from 2 to 20 g was employed. Plant extract reduced Molybdenum (VI) and form greenish phosphate Molybdenum (V) complex on reaction with freshly prepared phosphomolybdate reagent solution (0.6M sulphuric acid, 28mM tri sodium phosphate, 4 mM ammonium molybdate). The tubes were sealed, and the reaction mixture was incubated for 90 minutes in a boiling water bath at 95^0C. The samples were allowed to cool to room temperature and then absorption was measured in a UV- Visible spectrophotometer at 695 nm against a blank and results were recorded.
Reducing power estimation of the extract
Reducing power ability of the extracts was determined using a slightly modified method of the ferric reducing-antioxidant power assay [14]. The extract was incubated for 30 minutes at 50^0C with 2.5ml of phosphate buffer (0.1 mol/L, pH 6.6) and 2.5ml of 1 percent (w/v) potassium ferricyanide. At the end of the incubation time, 2.5ml of 10% (w/v) trichloroacetic acid (TCA) was added to the mixture to stop the reaction, and the mixture was centrifuged for 10 minutes at 3000g. The top layer (2.5 ml) was diluted with equal parts of water and agitated with 0.5ml of fresh 0.1% ferric chloride. A UV-Visible spectrophotometer was used to test absorbance at 700nm against a blank. The reference solution had been prepared.
Hydrogen peroxide scavenging (H_2O_2) activity
Quercetin was used as reference antioxidant to evaluate H_2O_2 radicals scavenging activity of extract [15]. Extract was mixed in 0.1 M phosphate buffer (pH 7.4) and then H_2O_2 (43 mM) solution prepared in same buffer was added. The concentrations of extract and reference chemical varied from 1 to 100g. After 10-minute incubation at room temperature, the absorbance was measured at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide using UV-Visible spectrophotometer. The percent H_2O_2 scavenging activity of the reference compound and extract was computed and the expressed in IC_{50} values.

RESULTS AND DISCUSSION
Antioxidants inhibit the generation of reactive oxygen species, either by inhibiting enzymes or by chelating trace elements. *Trigonella foenum-graecum* (TFE) hydromethanolic seed extract (TFE) was tested for photochemical analysis (alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, steroids, tannins, terpenoids, saponins, reducing sugars, carbohydrate and proteins) and *in vitro* antioxidant activity using three different methods, including total antioxidant capacity, reducing power, hydrogen peroxide scavenging activity.
A total of 14.89g of the hydromethanolic extract was prepared from 100 g seeds of *Trigonella foenum-graecum*with percent yield of 14.89% (Table 1). Preliminary qualitative phytochemical analysis of TFE revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, steroids, tannins, terpenoids, saponins, reducing sugars, carbohydrate and proteins. The results of phytochemical

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analysis are tabulated in Table 2. These secondary metabolites are reported to have many biological and therapeutic properties [16-19]. It was observed that anthraquinones and glycosides were absent in distilled water extraction and methanol extraction respectively [6]. Phytochemical screening of ethanolic seed extract of *Trigonella foenum-graecum* through maceration and Soxhlet method showed the presence of alkaloids, saponins, terpenoids, anthraquinone, glycosides, tannins, carbohydrate and phenol[19]. In contrast with the present findings [20]also revealed the absence of reducing sugars and flavanoids in ethanolic seed extract of *Trigonella foenum-graecum*.

A typical characteristic of total antioxidant capacity assay is that Mo (VI) is reduced to Mo (V) under acidic conditions forming Mo (V) complex which has dark bluish-green color. TFE has a total antioxidant capacity with value of 115.02 mg ascorbic acid equivalents g-1 extract(Table 3). It was revealed that fenugreek seeds have a total antioxidant capacity of 192 mg AAE/g extract [21]. The overall antioxidant activity of *Trigonella foenum-graecum* ethanolic seed extract was found to be 6g Tocopherol equivalent /mg [14]. Antioxidant properties in plants are linked to their phenolic and flavonoid content, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

The reducing power analysis is based on the concept that antioxidant molecules present in plant extract develop a colourful complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which absorbs the most at 700nm. Increasing absorbance was an indication of high reducing power, hence a greater absorbance value showed that the plant extracts had a higher reducing potential. Figure 1 shows the reducing action of TFE. The reducing power was an important measure of antioxidant activity. The reducing power of TFE was calculated and assessed in this work as a function of concentration. TFE was discovered to have a strong reducing capacity with value of 0.022 at 10µg/ml TFE up to 0.305 at 200µg/ml TFE. The extract's reducing power was lower than that of Ascorbic acid. The presence of different phytochemicals (most notably polyphenolic content) is responsible for the plant's antioxidative effects. The reducing power of *Trigonella foenum-graecum* extract rose as the amount of extract increased [14, 22]. The RPA values for Fenugreek seed aqueous ethanol extracts were much greater than those for methanol extracts [23]. Fenugreek contains volatile oil, phenolic acids, and flavonoids, making it a rich source of antioxidants [24].

Table 1: Percentage yield of the plant extract (TFE)

Plant name	Weight of dried plant material powder (g)	Weight of extract obtained (g)	Percent yield
Trigonella foenum-graecum (TFE)	100	14.89	14.89

Table 2: Results of phytochemical screening of TFE

S.No	Phytochemicals	TFE
1	Alkaloids	+
2	Anthraquinones	+
3	Cardiac glycosides	+
4	Flavonoids	+
5	Phenols	+
6	Steroids	+
7	Tannis	+
8	Terpenoid	+
9	Saponins	+
10	Reducing sugars	+
11	Carbohydrates	+
12	Proteins	+

Hydrogen peroxide acts as a weak oxidising agent, oxidising crucial thiol (-SH) groups and deactivating a few enzymes. Hydrogen peroxide may easily infiltrate biomembranes and combine with Fe(II) and perhaps Cu (II) ions to produce hydroxyl radicals once within the cell, which may explain many of its harmful effects. As a result, it is critical to regulate the amount of hydrogen peroxide that accumulates inside cells during stressful situations [25]. TAE inhibited H_2O_2 in a dose-dependent manner with IC_{50} value 61.90µg/ml [Figure 2]. The IC_{50} value of the *Trigonella foenum-graecum* leaves extract was found to be 17µg/ml [1].

The discrepancy in antioxidant properties may be attributed to the solvent solution used for the preparation of *Trigonella foenum-graecum* seeds extract.Because of the strong polarity of methanol,

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several phytochemicals that contribute to antioxidant activity, such as polyphenols and flavonoids, may absorb and extract effectively in methanol solvent systems compared to other solvents.Difference between the findings of the present study and the studies reported earlier may also be due to variation in environmental variables, such as temperature, location, climate, assay method and sampling time within the species.

Table 3: Total antioxidant capacity of TAE in terms of ascorbic acid equivalents. Each value represents mean ± SD (n=3)

Plant extract	Total Antioxidant Capacity (AAE mg/g of dry matter)			Mean Total Antioxidant Capacity (AAE mg/g of dry matter)
	I	II	III	
TFE	113.454	116.181	115.424	115.020 ± 1.40

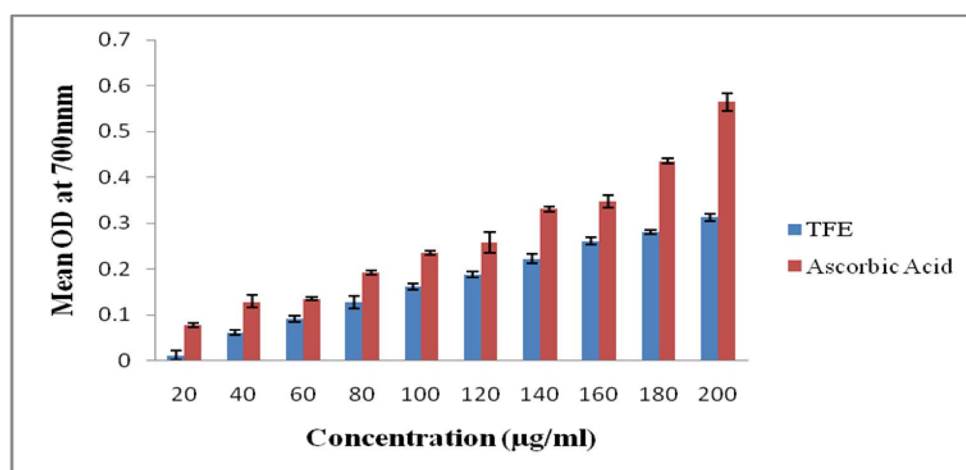

Fig 1: Reducing power for TFE and Ascorbic acid. Each value represents mean ± SD (n=3)

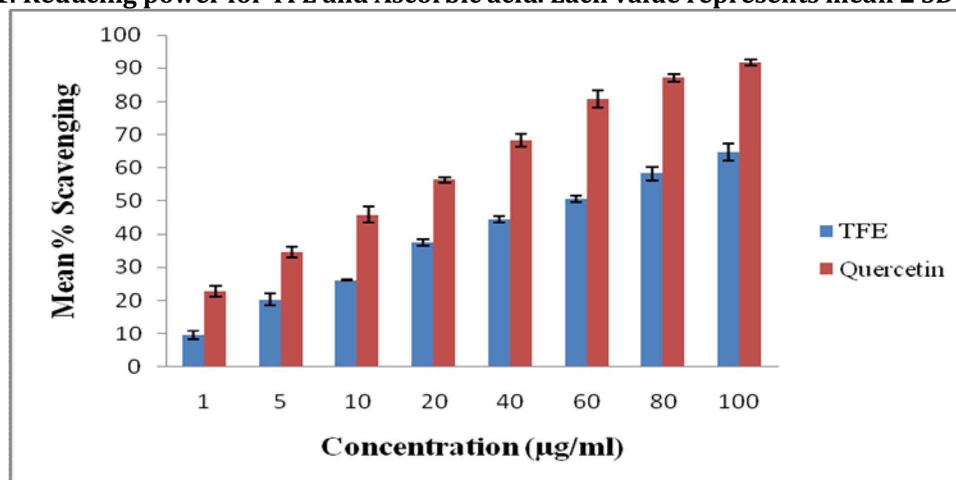

Fig 2: Hydrogen peroxide scavenging (H_2O_2) activity for TFE and Quercetin. Each value represents mean ± SD (n=3)

CONCLUSION

According to the present study, hydromethanolic extract of *Trigonella foenum-graecum* seeds showed presence of various phytoconstituents as well as significant antioxidant activity. The outcome of the study lays scientific foundation for the traditional usage of fenugreek for various medicinal benefits and as a source of natural antioxidants. Further validation of therapeutic potential of TFE should be conducted employing suitable assays.

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