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IN-VITRO EVALUATION OF ANTIOXIDANT AND ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT OF *TRIGONELLA FOENUM-GRAECUM*

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ABSTRACT

Trigonella foenum-graecum (Fenugreek) is a medicinal plant traditionally known for its various health-promoting properties, yet its antioxidant and antiulcer activities require further evaluation. The present study aimed to investigate the phytochemical profile, in vitro antioxidant potential, and antiulcer activity of the ethanolic extract of *Trigonella foenum-graecum* leaves. Fresh leaves were collected, shade-dried, powdered, and extracted with ethanol using the maceration technique. Qualitative phytochemical screening revealed the presence of carbohydrates, proteins and amino acids, flavonoids, phenolic compounds, and saponins. Antioxidant activity was evaluated using DPPH radical scavenging, ferric reducing power, and phosphomolybdenum assays, along with determination of total phenolic content. The extract demonstrated strong concentration-dependent antioxidant activity, with significant free radical scavenging, reducing, and electron-donating capacities. The presence of phenolic compounds further supported its antioxidant potential. In addition, antiulcer activity was evaluated through in vitro pepsin enzyme inhibition, protein denaturation inhibition, and acid neutralization assays. The extract exhibited significant inhibitory effects on pepsin activity and protein denaturation, indicating potential mucosal protective and antiulcer properties, while comparatively weak activity was observed in the acid neutralization assay. These findings suggest that the ethanolic extract of *Trigonella foenum-graecum* leaves possesses notable antioxidant and antiulcer activities, which may be attributed to its phenolic compounds, flavonoids, saponins, and other phytoconstituents, highlighting its potential as a natural therapeutic agent for managing oxidative stress and ulcer-related disorders.

INTRODUCTION:

Trigonella foenum-graecum (Fenugreek) is a nutrient-dense cruciferous vegetable belonging to the family Brassicaceae. Its primary center of origin is believed to be Northern Europe. In India, it is commonly cultivated in regions such as Jammu and Kashmir and West Bengal[1]. Similar to other cruciferous vegetables, Fenugreek is rich in vitamins, minerals, dietary fiber, and bioactive phytochemicals, including glucosinolates, flavonoids, and phenolic acids, which contribute to its antioxidant and disease-preventive properties [2]. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the body's

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antioxidant defense capacity. This imbalance can cause damage to vital biomolecules such as DNA, proteins, and lipids, thereby contributing to aging and the development of various diseases, including cancer, diabetes, and cardiovascular disorders. Therefore, maintaining a balance between ROS production and antioxidant defense is essential for maintaining overall health. Antioxidants are compounds that protect the body by preventing or minimizing ROS-induced damage to lipids, proteins, carbohydrates, and DNA [3].

An ulcer is a break or lesion in the gastrointestinal mucosal lining that develops when aggressive factors such as gastric acid, pepsin, and *Helicobacter pylori* overpower natural protective mechanisms, including mucus, bicarbonate, prostaglandins, and mucosal blood flow. Peptic ulcers are among the most common gastrointestinal ulcers and may occur in the stomach as gastric ulcers or in the duodenum as duodenal ulcers. Antiulcer agents act through various mechanisms, including reducing gastric acid secretion, inhibiting pepsin activity, neutralizing gastric acid, or enhancing the protective mechanisms of the gastric mucosa. These agents include proton pump inhibitors, histamine H_2-receptor antagonists, antacids, mucosal protective agents, prostaglandin analogues, and herbal preparations with gastroprotective activity [4].

Although cruciferous vegetables are well recognized for their antioxidant properties, scientific evidence regarding the antiulcer potential of Fenugreek remains limited. Considering its rich phytochemical composition, Fenugreek may possess combined antioxidant and antiulcer properties.

Therefore, the present study was designed to evaluate the *in vitro* antioxidant and antiulcer activities of Fenugreek extracts. The antioxidant potential was investigated using DPPH radical scavenging, ferric reducing power, phosphomolybdenum, and total phenolic content assays, while antiulcer activity was evaluated using pepsin enzyme inhibition, protein denaturation inhibition, and acid neutralization assays. The study aims to provide preliminary scientific evidence supporting the potential therapeutic role of Fenugreek in managing oxidative stress and ulcer-related disorders.

MATERIALS & METHODOLOGY:

Collection and Authentication of Fenugreek:

Fresh leaves of *Trigonella foenum-graecum* (Fenugreek) were procured from K. M. Doddi Market, Mandya District, Karnataka, India. The collected plant material was identified and authenticated by Dr. Thejesh Kumar M. P., HOD and Assistant Professor, Department of Botany, Bharathi College, Bharathinagara, Maddur Taluk, Mandya District, Karnataka, and was used for further experimental studies.

2.2 Preparation of Extract[22]:

The collected fresh leaves of *Trigonella foenum-graecum* were thoroughly washed with tap water followed by rinsing with distilled water. The leaves were shade-dried at room temperature and coarsely powdered using a mixer grinder. About 65 g of coarse leaf powder was macerated with 70% ethanol in a 1:10 ratio (w/v), using 650 ml of solvent, for 48 hours with intermittent shaking and magnetic stirring for 30 minutes. The mixture was filtered, and the filtrate was dried at room temperature to obtain a semi-solid extract, which was stored in an airtight container under refrigeration until further use.

Solubility Analysis:

The solubility of the *Trigonella foenum-graecum* leaf extract was evaluated in different solvents, including water, DMSO, chloroform, ethanol, methanol, ethyl acetate, and acetone, to determine the most suitable solvent or vehicle for conducting *in vitro* experiments.

Phytochemical Screening[23,24]:

Preliminary phytochemical screening of the extract was carried out using standard qualitative tests to identify the presence of primary and secondary metabolites, including carbohydrates, proteins and amino acids, alkaloids, glycosides, flavonoids, phenolic compounds, and saponins.

Antioxidant Activity:

DPPH Free Radical Scavenging Assay:

The antioxidant activity of the *Trigonella foenum-graecum* leaf extract was evaluated

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using the DPPH radical scavenging assay. The method is based on the reduction of the stable DPPH free radical by hydrogen-donating anti- oxidants, resulting in a decrease in absorbance.

Different concentrations of the extract (20–100 µg/ml) were prepared, and the volume was made up to 3 ml with 0.1 mM DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 20 minutes, and the absorbance was measured at 517 nm using a UV–visible spectrophotometer. The percentage radical scavenging activity was calculated, and the IC_{50} value was determined.

Ferric Reducing Power Assay:[26]

The ferric reducing power assay was performed to evaluate the electron-donating and reducing ability of the extract. The assay is based on the reduction of Fe^{3+}/ferricyanide to Fe^{2+}, followed by formation of a Prussian blue complex with ferric chloride, which is measured at 700 nm.

Different concentrations of the extract and standard (20–100 µg/ml) were mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 minutes and cooled, followed by addition of 2.5 ml of 10% trichloroacetic acid. The mixtures were centrifuged at 3000 rpm for 10 minutes. Subsequently, 5 ml of the supernatant was mixed with 5 ml of distilled water and 1 ml of freshly prepared 0.1% ferric chloride. Absorbance was measured at 700 nm using a UV–visible spectrophotometer, and the reducing power was expressed as ascorbic acid equivalents (AAE).

Phosphomolybdenum Assay: [27]

The total antioxidant capacity of the extract was determined using the phosphomolybdenum assay, which is based on the reduction of Mo(VI) to Mo(V) by antioxidants, resulting in the formation of a green-colored phosphomolybdenum complex with maximum absorbance at 695 nm.

Different concentrations of the extract and standard (6–30 µg/ml) were mixed with 3 ml of phosphomolybdenum reagent and incubated in a water bath at 95 °C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against a reagent blank using a UV–visible spectrophotometer. The antioxidant activity was calculated using a standard calibration curve of ascorbic acid and expressed as ascorbic acid equivalent (AAE).

Antiulcer Activity:

Pepsin Enzyme Inhibition Assay: [29,30]

The pepsin enzyme inhibition assay was performed to evaluate the ability of the extract to inhibit the proteolytic activity of pepsin. The assay is based on the inhibition of pepsin-mediated hydrolysis of casein under acidic conditions.

Different concentrations of the extract (5–25 µg/ml) were incubated with 1 ml of 0.5% casein solution and 1 ml of pepsin at 37 °C for 20 minutes. The reaction was terminated by adding 2 ml of 10% trichloroacetic acid, followed by filtration to remove precipitated proteins. The absorbance of the clear supernatant was measured at 280 nm against an appropriate blank. The percentage inhibition of pepsin activity was calculated by comparing the absorbance of the test sample with the control.

Protein Denaturation Inhibition Assay: [31]

The protein denaturation inhibition assay was used to evaluate the protective effect of the extract against heat-induced protein denaturation. Egg albumin was used as the protein source, and the inhibition of denaturation was determined by measuring the reduction in turbidity at 660 nm.

Different concentrations of the extract were mixed with 1 ml of 1% egg albumin, and the pH was adjusted to 6.4. The mixtures were incubated at room temperature for 20 minutes and subsequently heated at 55 °C for 30 minutes in a water bath. After cooling, the absorbance was measured at 660 nm using a spectrophotometer. Egg albumin without extract served as the control, and the percentage inhibition of protein denaturation was calculated relative to the control.

2.6.3 Acid Neutralization Assay: [32]

The acid neutralization assay was performed to evaluate the ability of the extract to neutralize gastric acid. The assay is based on the neutralization of hydrogen ions by substances possessing antiulcer potential. The test extract was mixed with 10 ml of 0.1

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N hydrochloric acid and incubated at room temperature for a fixed period. After incubation, the mixture was titrated against 0.1 N NaOH using phenolphthalein as an indicator until a persistent light pink color appeared. The volume of sodium hydroxide required for neutralization was recorded, and the percentage of acid neutralization was calculated relative to the control containing acid without extract.

RESULTS

Solubility analysis:

The extract exhibited good solubility in water, butanol, acetic acid and DMSO. This evaluation facilitated the identification of appropriate solvents for *in vitro* studies. Among these, water was selected as the vehicle for conducting the assays.

Phytochemical Investigation:

Qualitative phytochemical screening of the ethanolic extract of *Trigonella foenum-graecum* was carried out using standard methods. The results are presented table 1:

Antioxidant activity:

DPPH Radical Scavenging Assay

The Fenugreek extract exhibited a concentration-dependent increase in radical scavenging activity. The IC_{50} value was determined to be 57.2 µg/mL.The DPPH radical scavenging activity of extract is presented in Table 2 and Figure 1.

Ferricyanide Reducing Power Assay (FRAP)

The extract exhibited reducing power in a concentration-dependent manner. The reducing activity of the extract is compared to the standard ascorbic acid.The 100µg extract showed activity equivalent to 74.4µg of ascorbic acid. The ferric reducing activity of the extract is shown in Table 3 and Fig 2.

Phosphomolybdenum Assay

The phosphomolybdate ion reducing activity of both the extract and the standard (ascorbic acid) increased proportionally with concentration. At 30 µg, the extract demonstrated antioxidant activity equivalent to 23.37 µg of ascorbic acid. The results are presented in Table 4 and Figure 3.

Antiulcer Activity

Pepsin enzyme Inhibition Assay

The extract exhibited dose-dependent inhibition of pepsin activity. The IC_{50} value of the extract was determined to be 10.33 µg. The pepsin inhibition potential of the Fenugreek extract is presented in Table 5 and Figure 4.

Protein Denaturation Assay

The extract exhibited concentration-dependent inhibition of protein denaturation. The IC_{50} value of the extract was 96.12 µg. The protein denaturation inhibitory activity of the extract is presented in Table 6 and Figure 5.

Acid neutralisation assay:

The pH of the Fenugreek extract was initially measured using pH paper and found to be in the range of 6–7, indicating a slightly acidic nature. To further confirm this, an acid neutralisation assay was performed. In the standard titration, 30 ml of 0.1 N HCl required 24.6 ml of 0.1 N NaOH for neutralisation. When 100 mg of the extract was dissolved in 30 ml of 0.1 N HCl, it consumed 28.4 ml of NaOH for neutralisation. These results confirm that the Fenugreek extract is weakly acidic in nature. Since it does not possess significant gastric acid neutralisation capacity, it is unlikely to act through an antacid mechanism.

Table 1: Phytochemical screening of Fenugreek extract

SL NO	Phytochemical compounds	Ethanolic extract of Fenugreek
1.	Alkaloids	-
2.	Glycosides	-
3.	Flavonoids	+
4.	Phenolic compounds	+
5.	Saponins	+

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6.	Carbohydrates	+
7.	Proteins and amino acids	+

Note:(+) indicates presence of phytochemicals , (-) indicates absence of phytochemicals

Table 2: Inhibitory effect of Fenugreek extract on DPPH radical scavenging activity

S.NO	Concentration(µg)	% inhibition	IC50 value (µg)
1.	50	6	412
2.	100	14	
3.	150	20	
4.	200	23.68	
5.	250	29.55	

Table 3: Concentration and absorbance values of ascorbic acid and Fenugreek extract

Sample	Concentration(µg)	Absorbance
Standard (Ascorbic acid)	20	0.340
	40	0.447
	60	0.542
	80	0.657
	100	0.667
Extract	20	0.281
	40	0.354
	60	0.429
	80	0.515
	100	0.609

Table 4: Concentration and absorbance values of ascorbic acid and Fenugreek extract

Sample	Concentration(µg)	Absorbance
Standard (Ascorbic acid)	6	0.348
	12	0.451
	18	0.562
	24	0.635
	30	0.734
Extract	30	0.619

Table 5: Inhibitory effect of Fenugreek extract on Pepsin enzyme activity..

S. NO	Concentration (µg)	% inhibition	IC50 value (µg)
1.	5	38.3	

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2.	10	49.9	10.33
3.	15	51.4	
4.	20	54.1	
5.	25	54.9	

Table 6: Inhibitory effect of Fenugreek extract on Protein denaturation.

S. NO	Concentration (µg)	% inhibition	IC50 value (µg)
1	20	15.0	96.12
2	40	29.6	
3	60	37.3	
4	80	45.1	
5	100	51.2	

Fig 1: Scatter graph of DPPH radical scavenging activity of Fenugreek extract

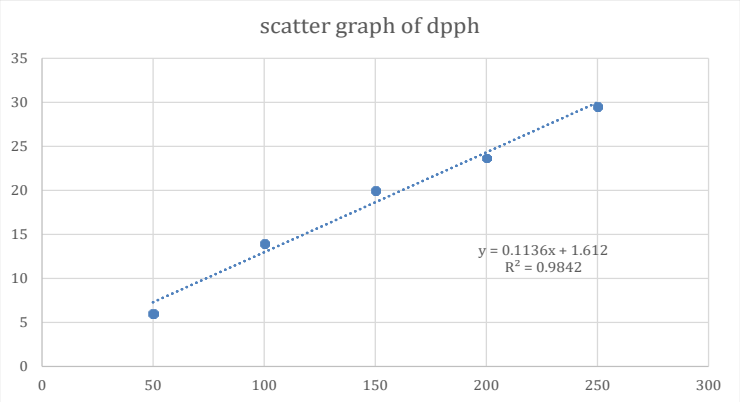


Fig 2: Scatter graph of Ferric Reducing Power Assay of ascorbic acid and Fenugreek extract

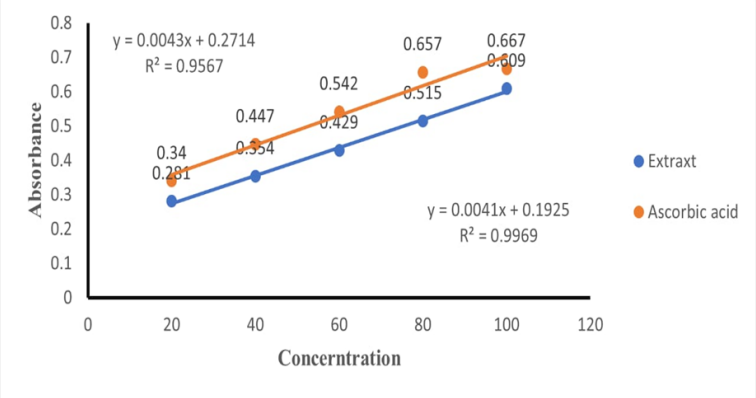


Fig 3: Scatter graph of phosphomolybdenum assay of ascorbic acid.

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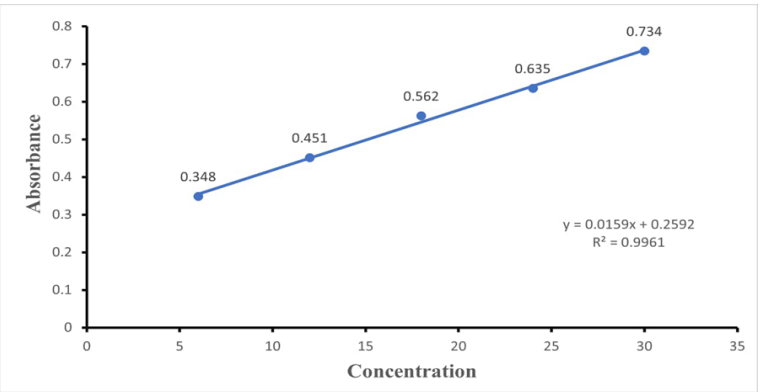


Fig 4: Scatter graph of Pepsin inhibition assay of Fenugreek extract.

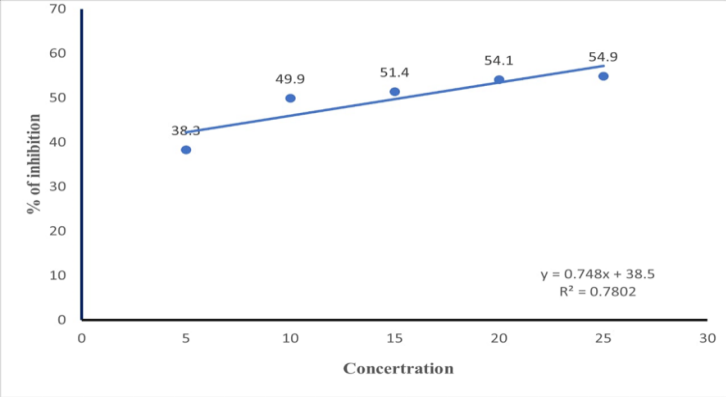
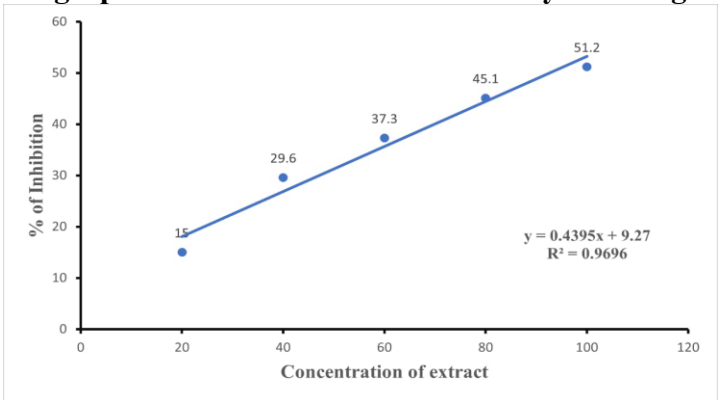


Fig 5: Scatter graph of Protein Denaturation Assay of Fenugreek extract.



DISCUSSION :

The etiology of peptic ulcer is largely unknown, but it is attributed to an imbalance between mucosal defence and aggressive factors. Excessive gastric acid secretion, denaturation of proteins in the gastric mucosa, and increased proteolytic activity of pepsin can contribute to ulcer formation. Compounds that counteract these effects are considered potential antiulcer agents.[16]

Qualitative phytochemical screening of the ethanolic extract of Fenugreek revealed the presence of carbohydrates, proteins and amino acids, flavonoids, phenolic compounds, and saponins. These phytoconstituents are known to play important roles in biological activities, particularly antioxidant and antiulcer effects.

The antioxidant activity of the extract was confirmed by multiple *in vitro* assays. The DPPH radical scavenging assay demonstrated a concentration-dependent activity with an IC_{50} of 412 µg/mL, indicating significant radical-quenching potential. This effect is likely attributed to the phenolic and flavonoid components, which can donate hydrogen atoms to stabilize free radicals. The ferric reducing power assay further substantiated the antioxidant nature of the extract, showing a strong electron-donating ability. At a concentration of 100 µg, the extract exhibited activity equivalent to 74.4 µg of ascorbic acid. Likewise, the phosphomolybdate assay confirmed its total antioxidant capacity, where 30 µg of extract showed activity

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equivalent to 23.37 µg of ascorbic acid. The high total phenolic content, expressed as 79.2 µg gallic acid equivalents per 100 µg of extract, emphasizes the central role of polyphenols in mediating these effects.

Beyond antioxidant activity, the extract also exhibited antiulcer potential. The pepsin inhibition assay showed a strong inhibitory effect with a low IC_{50} value of 10.33 µg, suggesting that the extract can modulate proteolytic activity in the gastric environment. In addition, inhibition of protein denaturation with an IC_{50} of 96.12 µg supports its ability to protect proteins from structural damage under stress conditions, a mechanism often implicated in ulcer formation. These findings are consistent with reports on other cruciferous vegetables, where secondary metabolites such as flavonoids and phenolics are known to exert gastroprotective effects through antioxidant and enzyme-modulating mechanisms.

CONCLUSION:

The present study demonstrated that *Trigonella foenum-graecum* extracts exhibit significant *in vitro* antioxidant and antiulcer activities. Antioxidant effects, confirmed by DPPH radical scavenging, ferric reducing power, and phosphomolybdenum assays, estimation of total phenolic content. The antiulcer potential, evidenced by pepsin enzyme inhibition assay and protein denaturation inhibition assays, suggests a protective effect against gastric ulcer. These findings highlight Fenugreek as a promising source of natural therapeutic agents. However, further *in vivo* studies and clinical evaluations are required to validate its efficacy and support its potential therapeutic applications.

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Conflict of Interest: The authors declare no conflict of interest.

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