

INDIAN MEDICINAL PLANT *TRIGONELLA FOENUM-GRÆCUM*: PHYTOCOMPOUNDS  
DETECTION AND PHARMACOLOGICAL STUDIESAFAQUE AHMAD<sup>1</sup>, HIRDAY N VERMA<sup>1</sup>, KAPIL DEV<sup>2\*</sup><sup>1</sup>School of Life Sciences, Jaipur National University, Jaipur - 302 017, Rajasthan, India. <sup>2</sup>Department of Biotechnology, Jamia Millia Islamia, New Delhi - 110 025, Delhi, India. Email: kdev@jmi.ac.in

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## ABSTRACT

**Objective:** Fenugreek (*Trigonella foenum-graecum*) is a medicinal plant of culinary origin commonly used as spice and vegetable. The present study was focused to identify the immunomodulatory applications of *T. foenum-graecum*.

**Methods:** The presence of phytochemicals and many biological activities such as antioxidant, antibacterial, and antifungal and anti-inflammatory activities were identified by various standard protocol respectively.

**Results:** The phytochemical analysis was flavonoids, alkaloids, phenols, etc., whereas no traces of carboxylic acid were found. Results showed that *T. foenum-graecum* aqueous and methanolic extract (TfgAE and TfgME) highly inhibited *Escherichia coli* (percentage mean growth inhibition [% MGI] of 77.012±1.732% and 78.428±1.448%) and least effective against *Staphylococcus aureus* (percentage MGI of 46.661±0.707% and 78.428±0.0076%), respectively. TfgAE, TfgME, and ascorbic acid demonstrated highest percentage 2,2-diphenyl-1-picrylhydrazyl radical scavenging of 94.19316±0.0047%, 94.7942±0.0050%, and 99.67775±0.0053% and lowest of 17.18339±0.0059%, 24.47114±0.0052%, and 61.81149±0.0046%, respectively. Ferric reducing antioxidant power assay of TfgAE and TfgME showed maximum of 1.0527±0.065 mM and 1.0067±0.041 mM and minimum value of 0.3027±0.052 mM and 0.275±0.057 mM, respectively, as compared to the ascorbic acid (1.1847±0.049 mM and minimal at 0.373±0.032 mM). TfgAE and TfgME showed anti-inflammatory activity with the highest inhibition of albumin denaturation of 44.93771±0.0071% and 39.14707±0.0068% and the lowest of 21.32969±0.0075% and 15.53905±0.0065%, respectively. The other assay revealed that TfgAE and TfgME demonstrated the highest proteinase inhibition of 29.94818±0.0071% and 74.337±0.0073% and the lowest of 14.47679±0.0082% and 25.327±0.0079%, respectively, at extract volume or amount of 1000 µl and 100 µl.

**Conclusion:** Results showed *T. foenum-graecum* might act as strong natural antibiotic agent and antioxidant agent and can be used against inflammatory diseases.

**Keywords:** Food chemistry, Phytochemicals, Antibacterial, Antioxidant, Anti-inflammatory, Fenugreek, *Trigonella foenum-graecum*.

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## INTRODUCTION

Food is just a major concern of human health that controls the metabolism and functioning of the human body. The food ingredients, namely proteins, fat, carbohydrates, vitamins, antioxidants, fibers, and minerals prevent the various chronic diseases and metabolic disorders [1]. Plant-based natural antioxidants are drawing attention of the researchers, industrialist, and users toward them because they cure the body of various diseases such as cancer, atherosclerotic heart disorders, and other epidemics [2]. The secondary metabolites found in the herbal plants possess various biofunctional compounds which could be used as drugs, food additives, colorants, flavorants, and as pesticide agents [3].

Fenugreek (*Trigonella foenum-graecum* L.) being the ancient medicinal herbs that belong to the family Fabaceae possesses the highest medicinally important bioactive compounds. There is an abundant dietary fiber in fenugreek seeds which help in the downregulation of blood sugar levels in diabetic patients. Fenugreek (*T. foenum-graecum*) plant as a whole or its plant parts are used to prepare the powdered form and that can be used for solvent extraction and can prepare various types of extracts that possess bioactive compounds of medicinal use. There are high toxic oils, and other bioactive ingredients in the fenugreek seed include volatile oils and alkaloids show toxic effects on bacteria, parasites, and fungi [4]. In modern Egypt, it may be used as wheat and maize flour to prepare bread [5]. Fenugreek might be used as lactation stimulant and flavorant seeds in India [6]. It might be exploited

in labor pain and delivery in the old Rome while they might be used as a tonic for the treatment of edema and legs weakness in the China [7]. Other uses of fenugreek seeds include anti-diabetic property [8,9].

Fenugreek plant is a reservoir of several ethnomedicinal properties that include antimicrobial, laxative, uterine tonic, expectoral, restorative, galactagogue, anti-carcinogenic, anti-inflammatory, anticholesterolemic, antiviral, and antioxidant [39,10]. This plant also possesses several other potentials to fight against fever, body pain and fat, swelling, and might induce lactation and sex hormones. Fenugreek compounds are biofunctional in nature that could act as a safeguard against various diseases such as malaria, cancer, bacterial, and viral diseases [11, 3]. Fenugreek possesses abundant of polyphenolics compounds that are known to prevent peroxidation and hence decreases the oxidative hemolysis of erythrocytes [12,13]. Moreover, their normal consumption may reduce triglycerides and cholesterol concentrations in the blood [14], prevent cancer [15], and regulate diabetes mellitus [16].

This culinary herb is being used in pharmacology and is used for the treatment of diseases. It may also be used as a dietary antioxidant because it might reduce the hydrogen peroxide-induced peroxidation in the mitochondria of liver and provide protection to the cellular organelles from oxidative stress [17]. These days, fenugreek is used in pharmacology as a medicinal plant and might provide treatments for the fatal diseases. There were some reports available (to the best of my knowledge) on the seeds of fenugreek which were tested against various human pathogenic

bacterial strains. In the recent study, we have evaluated the antimicrobial activity of fenugreek against pathogenic bacteria. This study revealed that methanolic seed extracts are having strong antibacterial activities against some bacterial pathogens. The present study is however intended toward the identification of the phytochemical ingredients found in the extracts followed by the comparative appraisal of *in vitro* antibacterial, antioxidant, and anti-inflammatory activities of the aqueous and methanolic extract of *T. foenum-graecum*.

## METHODS

### Sample collection

The seed samples of fenugreek (*T. foenum-graecum*) were obtained from the local ayurvedic clinic.

### Chemicals and reagents

Various chemicals used for antioxidant assays 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) and trypsin were purchased from Sigma-Aldrich, USA. Other chemicals (analytical grade) which were used for antibacterial, antifungal, and anti-inflammatory assay were obtained from SRL, Himedia, and Merck.

### Test organisms

The tested bacterial strains were obtained from NCCS, Pune, India. The bacterial cultures were then maintained at 4°C on slants of nutrient agar.

### Preparations of aqueous and methanolic extract of *T. foenum-graecum* seeds

The extracts from *T. foenum-graecum* were prepared by dissolving powdered *Trigonella* in distilled water (DW) and methanol to obtain aqueous and methanolic extracts, respectively. The plant sample was soaked for 48–72 h in the solvents for the extraction of almost all valuable phytochemicals. Further, the samples were centrifuged at 6000 rpm for 15 min for the settling down of debris at the bottom in the form of a pellet, and the supernatants were collected. The supernatant was named as *T. foenum-graecum* aqueous extract (TfgAE) and *T. foenum-graecum* methanolic extract (TfgME) and again filtered through a membrane filter (0.25 µm) and used for the further bioactivities.

### Identification of phytochemicals from TfgAE and TfgME

The extracts TfgAE and TfgME were screened qualitatively to determine the presence of phytochemicals including amino acids, flavonoids, tannins, quinones, alkaloids, phenols, carbohydrates, glycosides, steroids, carboxylic acids, resins, proteins, saponins, and terpenoids according to the method described by Harborne with some minor modifications [18].

### Evaluation of antimicrobial activity

#### Determination of antibacterial activity by broth microdilution assay

Antibacterial activity of the TfgAE and TfgME was evaluated against various Gram-positive and Gram-negative strains (*Bacillus subtilis* [MTCC 736], *Pseudomonas aeruginosa* [MTCC 2453], *Staphylococcus aureus* [MTCC 902], and *Escherichia coli* [MTCC 443]) as described by Barbade and Datar with some modifications [19]. The bacterial primary cultures were prepared by inoculating bacterial colony from agar plate into the Luria broth (LB) media followed by incubation at 37°C for 12 h. The fresh LB media were used to dilute the overnight cultures, and then TfgAE and TfgME extracts were added and incubated at 37°C for 12–14 hrs. Results were compared to the positive control culture (LB media and bacterial inoculums) and negative control (media+bacteria+ampicillin). The experiment was repeated thrice for the confirmation. The absorbance was recorded at 600 nm. Using the given formula, the percentage inhibition was calculated:

$$\text{Percentage mean growth inhibition (\% MGI)} = [(Ac-At)/Ac] \times 100,$$

Ac and At here represent the absorbance of control and test sample, respectively.

### Evaluation of antioxidant potential of TfgAE and TfgME

#### DPPH assay

TfgAE, TfgME, and the ascorbic acid were checked for their antioxidant potential due to the basic effect of free radical scavenging of the stable DPPH as per the method of Goveas and Abraham with minor modifications [20]. TfgAE and TfgME extracts were serially diluted in DW and methanol, respectively. DPPH (0.1 mM) was prepared in 80% methanol and mixed with the serially diluted sample and standard ascorbic acid solutions separately. The reaction mixtures were placed in the dark for about 5–10 min and the absorbance had been recorded at 517 nm spectrophotometrically. DPPH solution was used as a control. The sample was taken as their respective blank. Using the formula given below the DPPH scavenging was calculated as:

$$\text{DPPH scavenging Activity (\%)} = [(Ac-At)/Ac] \times 100,$$

Here Ac and At represent the absorbance of control and test sample, respectively.

#### Ferric reducing antioxidant power (FRAP) assay

This FRAP assay to determine the antioxidant potential of TfgAE and TfgME was assessed using the method of Sudha *et al.* with few modifications [21]. Serially diluted TfgAE and TfgME were added to the freshly prepared FRAP reagent and the incubation of reaction mixture for 4–10 min in the dark. The formation of colored complex, i.e., ferrous tripyridyltriazine complex increased the absorbance which was recorded at 593 nm. The FRAP reagent was used as blank in the cuvette. The absorbance of each diluted extract and standard was expressed in mM of FRAP value.

#### Superoxide dismutase (SOD) assay

SOD assay was performed using the protocol as described by Kakkar *et al.* with few modifications [22]. The extracts, i.e., TfgAE and TfgME were mixed with Phenazine methosulfate (PMS), sodium pyrophosphate buffer, and nitro blue tetrazolium chloride (NBT). Further, the addition of nicotinamide adenine dinucleotide (NADH) to the above mixture, the whole reaction was initiated and then incubated at 30°C for 10–15 min. The glacial acetic acid was used to terminate the reaction by adding it to the assay mixture. n-butanol was also added to the assay mixture, and the intensity at 560 nm of the chromogen developed in a layer of butanol was recorded.

#### Reduced glutathione (GSH) assay

Reduced GSH was determined according to the method as described by Moron *et al.* with some modification [23]. TfgAE and TfgME of different concentration were mixed with 0.2 M sodium phosphate buffer (pH 8.0). Standard GSH was also prepared with concentrations of 2–10 nm range. Further, 5,5'-Dithiobis 2-nitrobenzoic acid (DTNB) freshly prepared solution was mixed to the reaction mixture, and then the solution was incubated for 10 min. The intensity of the yellow color developed was recorded at 412 nm. The results were expressed in µM/min/µl of both the extracts.

#### Catalase assay

Catalase activity was assayed as described by Jambunathan *et al.* with certain modifications [24]. H<sub>2</sub>O<sub>2</sub> and phosphate buffer were taken in a cuvette, and the few amount of TfgAE and TfgME extracts was added continuously and then mixed thoroughly. The H<sub>2</sub>O<sub>2</sub> degradation by the extracts was recorded as a drop in the absorbance by 0.05 units and noted to be at 240 nm. The H<sub>2</sub>O<sub>2</sub>- Phosphate buffer was used as control in this assay. Results were expressed by the amount of enzyme present in the extracts which decreased the absorbance at 240 nm by 0.05–0.06 units and termed as one enzyme unit. The ultraviolet (UV) absorption of H<sub>2</sub>O<sub>2</sub> recorded at 240 nm was due the degradation by the enzyme catalase.

## Assessment of anti-inflammatory activity

### Albumin denaturation inhibition

The anti-inflammatory assay was performed by the method of Mizushima *et al.* with some changes [25]. The TfgAE and TfgME were serially diluted and then mixed with 1% aqueous solution of bovine serum albumin (BSA) fraction. The assay mixtures were incubated at 37°C for 30 min and then heat was given at 60°C for 15 min. BSA was used as control and water was taken as blank. Aspirin (100 µg/ml) was used as standard anti-inflammatory drug. When the samples were cooled down, the turbidity was recorded at 660 nm. Using the below formula percentage inhibition of albumin denaturation was calculated as:

$$\text{Percentage of inhibition} = (\text{Abs}_c - \text{Abs}_t) \times 100 / \text{Abs}_c$$

Abs<sub>c</sub> and Abs<sub>t</sub> were the absorbance of control and test sample, respectively.

### Proteinase inhibitory action

The bioactivity was done by Oyedepo and Femurewa with some minor modifications [26]. The reaction mixture contained 20 mM Tris-HCl buffer (pH 7.4) and serially diluted extracts, i.e., TfgAE and TfgME. Further, approximately 0.05 mg trypsin was added to it. At 37°C, the sample was incubated for 15–25 min, and then some amount of 1% casein was added followed by the additional incubation for 25 min. Perchloric acid was added to the sample mixture to arrest the reaction. The absorbance of the supernatant was read at 210 nm. The percentage of proteinase inhibitory activity was calculated by the given formula.

$$\% \text{ proteinase inhibition} = (A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}}$$

Where A<sub>control</sub> and A<sub>sample</sub> are the absorbance at 210 nm for control and sample respectively.

### Determination of antifungal activity

TfgAE and TfgME extracts were tested for their antifungal activity against the *Candida* cells (ATCC 10261 and ATCC 90028) by assessing the minimum inhibitory concentration using the broth microdilution method [27]. Using the extracts or without any extract in the media, the fungal cultures were grown. First, the 2-fold dilutions of the extracts were carried out. Then, by the disc diffusion method, the antifungal activity of TfgAE and TfgME was determined in the solid YEPD agar plate. The fungal cells, i.e., *Candida* strains (10<sup>5</sup> cells/ml) were inoculated in the YEPD agar (molten ~ 40°C) and then poured into a Petri plates. Further, the discs were kept on the solid agar plates and different concentrations of TfgAE and TfgME extracts were poured on the disc in 10 µl volume. The plates were then incubated in an incubator for 36–73 h. The zone of inhibition was recorded with its average diameter in mm.

### Statistical analysis

The experiments were done in triplicates. Results were expressed as graphs representing mean ± standard error of mean using the software GraphPad Prism 5.

## RESULTS

### Identification of phytochemicals from TfgAE and TfgME

Qualitative phytochemical analysis of aqueous and methanolic extracts of *T. foenum-graecum* plant revealed the presence of phenolics and non-phenolics phytochemicals such as total phenols, tannins, and flavonoids, alkaloids, sterol, resins, terpenoids, xanthoproteins, quinines, glycosides, and saponins while the steroids, tannins, and carboxylic acids were absent in both types of extracts. Other compounds were found in fairly detectable quantity (Table 1).

### Assessment of antimicrobial activity

#### Antibacterial activity

Antibacterial potential of the TfgAE and TfgME extracts was determined against various bacterial strains by evaluating the percentage MGI in the

presence of these extracts. Results were compared to the control sample where only LB media and bacterial inoculum were added. The results obtained suggested that TfgAE and TfgME possessed the bactericidal property and showed inhibition of growth of microorganisms in their presence. It was found that TfgAE was most effective against *E. coli* (ATCC 25922) with percentage MGI of 77.012±1.732% and was least effective against *S. aureus* with percentage MGI of 46.661±0.707%. TfgME showed maximum percentage MGI of 78.428±1.448% against *E. coli* whereas least against *S. aureus*, i.e. percentage MGI of 78.428±0.0076%. The standard antibiotic ampicillin showed almost complete inhibition against all the bacterial strains at the final concentration of 0.5 mg/ml (Fig. 1).

### Evaluation of Antioxidant Potential of TfgAE and TfgME

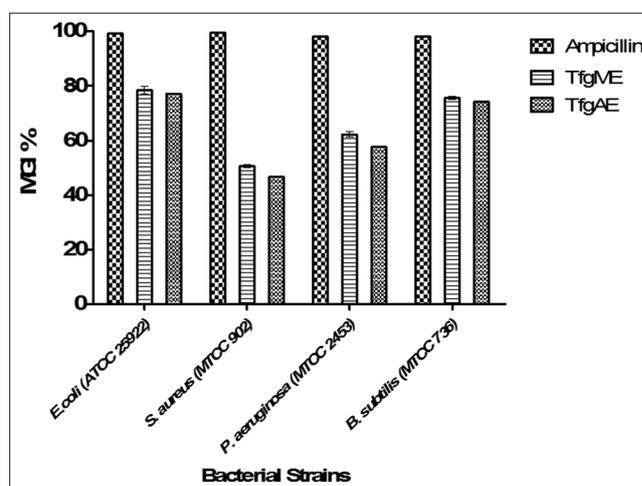
#### DPPH Assay

*T. foenum-graecum* showed scavenging of DPPH free radical in a dose-dependent manner which was shown in Fig. 2. The DPPH radical scavenging activity by TfgAE and TfgME was shown with the highest and lowest scavenging at amount 500 µl and 50 µl of the extracts. TfgAE demonstrated the highest percentage DPPH radical scavenging of 94.19316±0.0047% and the lowest of 17.18339±0.0059% while the TfgME showed the highest % DPPH radical scavenging of 94.7942±0.0050% and the lowest of 24.47114±0.0052%. The results

**Table 1: Tabular representation of the presence of phytochemicals extracted from the *T. foenum-graecum* methanolic and aqueous extract**

Phytochemical constituents	TfgAE	TfgME
Alkaloids	+	++
Flavonoids	+	++
Steroids	+	++
Saponins	+	++
Phenols	+	++
Resins	+	++
Tannins	++	+
Terpenoids	+	++
Xanthoproteins	++	+
Quinones	+	++
Glycosides	+	++
Carboxylic acid	-	-

*T. foenum-graecum*: *Trigonella foenum-graecum*, TfgAE: *Trigonella foenum-graecum* aqueous extract, TfgME: *Trigonella foenum-graecum* methanolic extract



**Fig. 1: Antibacterial potential of a methanolic and aqueous extract of *Trigonella foenum-graecum* was calculated, and the results were expressed in percentage mean growth inhibition. Experiment was performed in triplicates. Results were expressed in the form of means ± standard error of mean**

were quite comparable to that of standard, i.e., ascorbic acid with maximum percentage DPPH scavenging of  $99.67775 \pm 0.0053\%$  and minimum of  $61.81149 \pm 0.0046\%$ .

#### FRAP assay

The results showed that FRAP value of *T. foenum-graecum* increases in according to the concentration-dependent manner. The FRAP assay showed the highest absorbance at 500  $\mu\text{l}$  for both TfgAE and TfgME extracts, and the lowest was recorded at 50  $\mu\text{l}$  with a maximum FRAP value  $1.0527 \pm 0.065$  mM and  $1.0067 \pm 0.041$  mM and a minimum value of  $0.3027 \pm 0.052$  mM and  $0.275 \pm 0.057$  mM, respectively, as compared to standard maxima at  $1.1847 \pm 0.049$  mM and minimal at  $0.373 \pm 0.032$  mM, respectively. These extracts reacted with ferric tripyridyltriazine ( $\text{Fe}^{\text{III}}$ -TPTZ) complex which produced a blue colored ferrous tripyridyltriazine ( $\text{Fe}^{\text{II}}$ -TPTZ) complex. It had been cleared that *T. foenum-graecum* showed fair antioxidant activity which was comparable to ascorbic acid, a standard antioxidant (Fig. 3) from the given observations.

#### SOD assay

SOD assay is dependent on inhibition of formation of NADH-PMS-nitroblue tetrazolium formazon. The conversion of superoxide free radicals to hydrogen peroxide and molecular oxygen is due to SOD assay. It is a major protection for aerobic cells in resisting the toxic effects of superoxide radicals. They represent the main enzymatic source of peroxides and belong to a large family of isoenzymes that relate cellular response to the oxidative stress. The amount of extract which may inhibit NBT reduction up to 50% in 1 min termed as one unit of enzyme activity. The degradation of superoxide radicals per minute was evaluated, and this radical was degraded on an average unit of 0.006 by TfgAE and 0.023 units by TfgME (Fig. 4).

#### Reduced GSH assay

GSH peroxidase catalyzes the reduction of hydrogen peroxides and functions to protect the cell from oxidative damage. GSH is the necessary part of non-enzymatic antioxidants, and it can reduce peroxytrite with the formation of oxidized glutathione, which results in the formation of GSH by NADPH-dependent GSH reductase. DTNB was used as a control, and the DTNB with different concentration of TfgAE and TfgME was used as a test sample. The concentration of GSH was expressed in  $\mu\text{M}/\text{min}/\mu\text{l}$  extract and estimated up to 200  $\mu\text{l}$  of both extracts. GSH was found in low concentration at 50  $\mu\text{l}$  and in high concentration at 200  $\mu\text{l}$  volume of both TfgAE and TfgME (Fig. 5).

#### Catalase assay

The results revealed that the absorbance of UV light by hydrogen peroxide decreases when scavenged or deteriorated by the enzyme catalase found in the extracts which can be measured at 240 nm. When the absorbance reduced, the enzyme activity can though be portrayed as the concentration of hydrogen peroxide is decreasing significantly in a dose-dependent manner. The 100% hydrogen peroxide was degraded to  $75.16246 \pm 0.0063\%$  and  $71.58474 \pm 0.0067\%$  when the initially small amount of TfgAE and TfgME was added to the cuvette, respectively. Degradation per minute observed up to 10 min, and finally, there was approximately  $7.889728 \pm 0.0068\%$  and  $2.229903 \pm 0.0059\%$  radical was remained (Fig. 6).

#### Evaluation of anti-inflammatory activity

##### Albumin denaturation inhibition

Infection or damage in the body tissue shows response against this infection through inflammation. Protein denaturation is said to be one of the major causes of inflammation. Due to this, we have tried to find out the ability of *T. foenum-graecum* to inhibit protein denaturation. The results showed that TfgAE and TfgME were responsible for inhibiting thermally induced albumin denaturation at different concentrations. The TfgAE showed the highest percentage inhibition of albumin denaturation of  $44.93771 \pm 0.0071\%$  and the lowest of  $21.32969 \pm 0.0075\%$  while TfgME resulted in maximum percentage

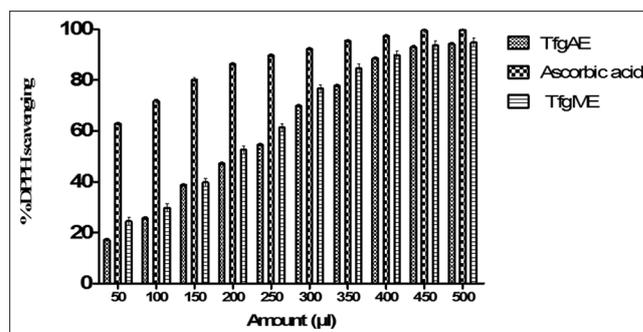


Fig. 2: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity of methanolic and aqueous extract were calculated and compared to ascorbic acid, i.e., standard. The activity increased in time- and concentration-dependent manner. Experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.

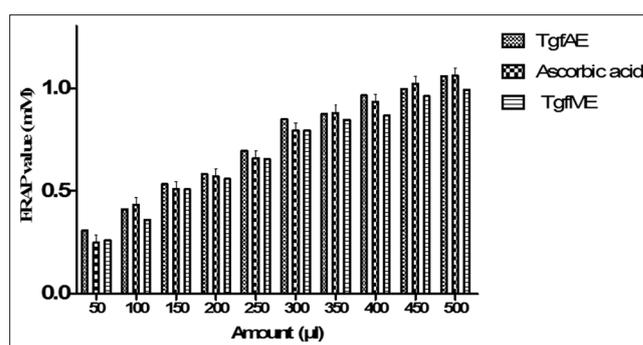


Fig. 3: Ferric reducing the antioxidant power of methanolic and aqueous extract of *Trigonella foenum-graecum* was estimated and compared to ascorbic acid as standard. The results were expressed in mM. Experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.

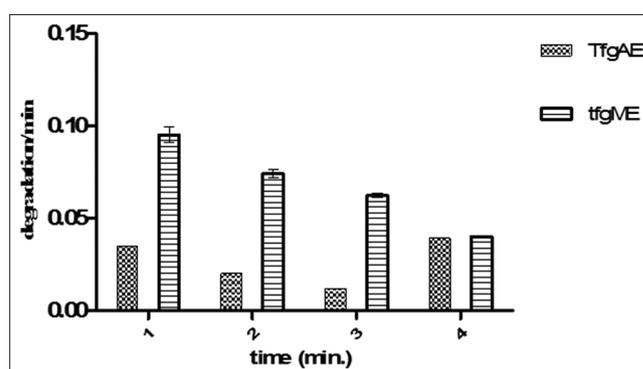


Fig. 4: Superoxide radical level significantly decreased with increase in the concentration of methanolic and aqueous extract. The results were expressed in degradation/min. Experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.

inhibition of  $39.14707 \pm 0.0068\%$  and minimum percentage inhibition of  $15.53905 \pm 0.0065\%$  at extract volume or amount of 1000  $\mu\text{l}$  and 100  $\mu\text{l}$ , respectively (Fig. 7).

#### Proteinase inhibitory activity

Results exhibited significant anti-proteinase activity at different concentrations of TfgAE and TfgME. The TfgAE demonstrated the

highest percentage proteinase inhibition of  $29.94818 \pm 0.0071\%$  and the lowest of  $14.47679 \pm 0.0082\%$  while TfgME resulted in maximum proteinase inhibition of  $74.337 \pm 0.0073\%$  and minimum inhibition of  $25.327 \pm 0.0079\%$  at extract volume or amount of  $1000 \mu\text{l}$  and  $100 \mu\text{l}$ , respectively (Fig. 8).

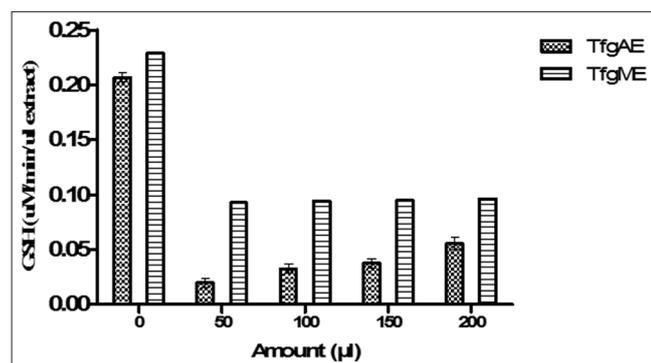


Fig. 5: Reduced glutathione (GSH) concentration in  $\mu\text{M}$  was estimated in the presence of methanolic and aqueous extract. The amount of reduced GSH increased with increase in the amount of extract. The experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.

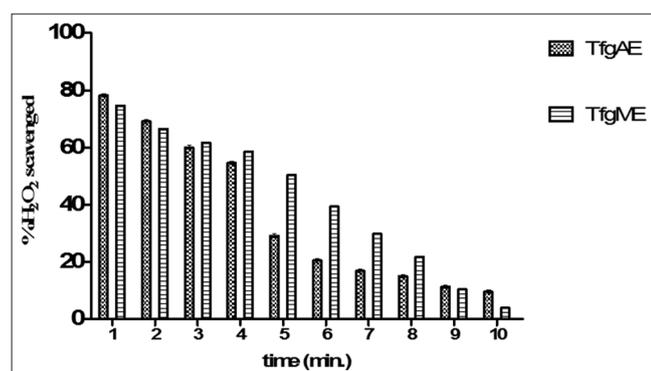


Fig. 6: Hydrogen peroxide scavenging (%) was assessed with increasing amount of methanolic and aqueous extract. The hydrogen peroxide degradation increased in concentration-dependent manner. The experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.

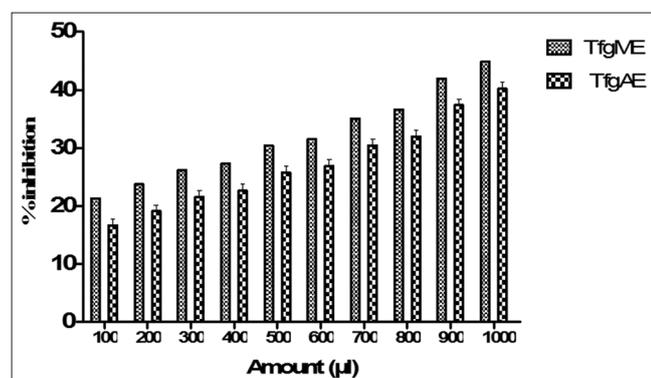


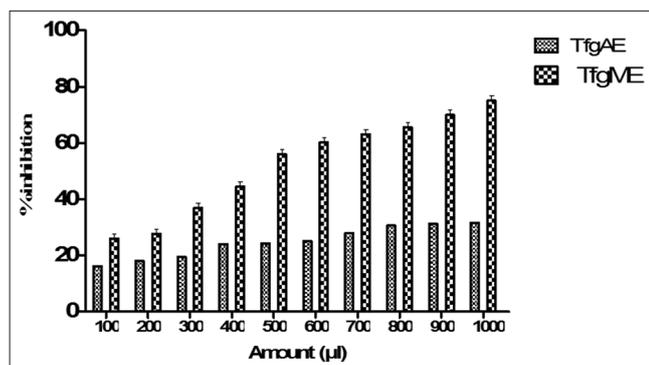
Fig. 7: Inhibition of albumin denaturation activity of a methanolic and aqueous extract of *Trigonella foenum-graecum* was checked at various concentrations taking aspirin ( $100 \mu\text{g/ml}$ ) as standard. The experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean

## DISCUSSION

*T. foenum-graecum* has been mostly used in ayurvedic medicines, and its seeds possess various kinds of bio-functional properties such as antimicrobial, anticholesterolemic, anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, hypotensive, laxative, uterine tonic, restorative, and galactagogue [10,39]. In addition, it provides several enzymatic properties, mitigates fever, reduces body pain and fat, augments appetite, alleviates swelling and induces lactation, and sex hormones. In the present study, the phytochemical analysis of extracts of powdered *T. foenum-graecum* seeds showed the presence of phenolic (tannins, total flavonoids, and phenols), and non-phenolics (alkaloids, sterol, resins, terpenoids, glycosides, saponins xanthoproteins, and quinines) phytochemicals while only carboxylic acid was not found. The medicinal importance of *T. foenum-graecum* plant was revealed by the presence of these secondary metabolites or bioactive compounds in the extracts. Furthermore, the methanolic extract gave a firm and excessive presence of bioactive compounds as compared to the aqueous extract. *T. foenum-graecum* plant depicts the therapeutic role in many diseases and does have various pharmacological properties that may be due to the presence of these bioactive compounds. Furthermore, the phytoconstituents which we have observed and reported here during the research work might be used for medicinal purpose. The presence of phytochemicals such as total phenols, flavonoids, and tannins may be responsible for nutraceutical and pharmacological activities such as anti-inflammatory, anti-oxidant antineoplastic, and antimicrobial [28-33].

Mostly plants possess potent antioxidant activity. Tannins and flavonoids are capable of scavenging free radical species and hence act as a classic antioxidant group in plants [34]. Free radicals are known to cause the aging of skin due to the failure action mechanism of natural antioxidants. Various *in vivo* and *in vitro* studies proved that the main cause of aging is the loss in the activity of enzymatic and non-enzymatic agents which enhance the level of reactive oxygen species (ROS) inside the cellular system. Ascorbic acid is one of the most important natural antioxidants as it oxidizes ascorbate to dehydroascorbate and therefore stamps out ROS. It may also be used to perform other several important physiological functions such as it might act as the cofactor for prolyl hydroxylase activity, which helps in the hydroxylation of prolyl and leads to the formation of procollagen and elastin [35,40]. It is common assumption that the cellular aging process can be prevented by plants' phenolic substances, which has galvanized the investigation of *T. foenum-graecum* metabolites and its possible, feasible action in the prevention and reduction of cellular aging. The result depicted the similar pattern of antioxidant activity by TfgAE, TfgME, and ascorbic acid (TfgME being higher than TfgAE) suggesting *T. foenum-graecum* as a potent antioxidant source.

Inflammation is just a common phenomenon after the reaction of living tissues toward injury. Some inflammatory vasodilator such as histamine, bradykinin, and prostaglandin- $E_2$  acts as an effective vasodilator which elicits the blood flow and redness in the region of acute inflammation [36]. Tannins are not only meant for their antiseptic property but also are important for their astringent action. This astringent property provides them the therapeutic value in arresting hemorrhage by constricting blood vessels and in protecting wounds, inflammation, and ulcer from external irritation by the participating surface protein which form an impervious protective coating on them. Thus, it is certain that the constituents are enough to cure the infection and tannins are also responsible to cure inflammatory diseases. The results obtained provide support for the use of *T. foenum-graecum* extract locally against inflammation, ROS related diseases, fever, ulcer, infection, etc. Alkaloids being bitter substance provide eminent antimicrobial action. Hence, it is immediate justifiable alkaloid-containing plants that show effectiveness against the microbial diseases. The results show that TfgAE and TfgME both are having antibacterial activity in concentration-dependent manner which was sufficiently comparable to that of the standard drug.



**Fig. 8: Percentage inhibition of trypsin activity in the presence of a methanolic and aqueous extract of *Trigonella foenum-graecum* was evaluated. Experiment was performed in triplicates. Results were expressed in the form of means  $\pm$  standard error of mean.**

The present work depicts homology with the previous study which validated that the constituents obtained from the seeds of *T. foenum-graecum* exhibited the potent antimicrobial activity. This may be due to the presence of competent bioactive compounds in the seed extracts of *T. foenum-graecum* which may be responsible for the destruction of the pathogenic microbes [37,38]. Finally, it can be concluded that these bioactive compounds can help in the treatment of bacterial infections. Results depicted from this study encouraging that this herb should be extensively studied and explored its utility in the prevention of several oxidative stressed, inflammatory diseases, etc.

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#### CONFLICTS OF INTEREST

All the authors have no conflicts of interest. The manuscript has neither published nor simultaneously submitted for publication elsewhere. All the authors agree to the submission to the journal.

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