WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 10, 1474-1484. Research Article ISSN 2277–7105

INVESTIGATION OF ANTIBACTERIAL ACTIVITY IN METHANOL AND AQUEOUS SOLVENT OF *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK) SEED

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Article Received on
10 April 2025,

Revised on 01 May 2025,
Accepted on 21 May 2025

DOI: 10.20959/wjpr202510-36978

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ABSTRACT

The herb fenugreek (*Trigonella foenum-graecum*) is used extensively in agriculture, culinary, and traditional medicine. It has antibacterial, anti-inflammatory, and antioxidant qualities due to the presence of bioactive substances like as alkaloids, flavonoids, and saponins. As a spice, food additive, and nutritional supplement, fenugreek seeds and leaves help with lactation, blood sugar regulation, and digestion. It is used to treat respiratory, skin, and diabetic conditions in Ayurvedic and Unani therapy. It is also utilized in animal feed and improves soil fertility. This study reveals the antibacterial activity of *Trigonella foenum-graecum* seeds against different varieties of gram-positive and gram-negative bacteria. The antibacterial activity of methanol and aqueous extracts were determined by using agar well and disc diffusion methods. The results showed that aqueous and methanolic extracts have strong antibacterial action against pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilus* and *Staphylococcus aureus*. The study also reveals the efficacy of boiling water extracts which included active antimicrobial components as opposed to cold water extract. The result shows the importance of fenugreek seed for it's a potential natural antibacterial agent activity.

KEYWORDS: Antibacterial, Antimicrobial, Fenugreek seeds, Methanolic extract and Aqueous extract.

INTRODUCTION

Fenugreek seeds is an imperative medicinal herb belong to the Leguminosae family.[1] Seeds and Plant derived products are used in food as a flavouring agent and as a source of medicine from ancient time.[2] Their seeds contain strong aromatic chemicals that provide food colour, taste, and aroma to food.[3] The seeds are used as spices all throughout the world, while the leaves are used as green leafy vegetables in the diet.[4] *Trigonella foenum graceum* is grown in Mediterranean nations, Egypt, Turkey, China, India, and Pakistan. Fenugreek has been mentioned as having use in the pharmaceutical, nutraceutical, and medical domains.[5] Nowadays India currently produces between 45,000 and 55,000 tonnes of fenugreek annually, making it the world's largest producer.[6] Seeds of *Trigonella foenum graceum* have medicinal properties such as antibacterial, antioxidant, antidiabetic, anticancer, hypocholesterolemic, lactation aid, gastric stimulant, for anorexia, galactagogue (milk producing agent) and hepatoprotective effect.[7] Fenugreek seeds are used as a dietary supplement and very useful in the management of diabetes atherosclerosis, liver protection, cardioprotect-ive and renal production.[8] Fenugreek seeds are rich in proteins that contain lysine and L-tryptophan, along with mucilaginous fiber and a variety of unique chemical compounds, including saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, and trigonelline.[9] Steroidal saponin–diosgenin, which is found in fenugreek seeds, is used to make progesterone and other medications. The alkaloid trigonelline present in fenugreek seed is converted into niacin when the seed is roasted. Fenugreek seeds contain compounds that stimulate the pancreas to release digestive enzymes that aid in digestion, according to research findings. Because of its calming properties, the seeds can be used to treat gastritis and stomach ulcers.[10]

Table 1: Botanical Classification of *Trigonella foenum graecum*.[11]

Kingdom	Plantae
Order	Fables (Leguminales)
Family	Fabaceae (Leguminosae)
Subfamily	Faboideae (Papilionaceae)
Tribe	Trifolieae
Subtribe	Trigonellinae
Genus	*Trigonella*
Species	*Trigonella foenum-graecum Linn*.

Application of fenugreek

In addition to its medicinal properties, fenugreek is also recognized for its culinary value. The plant is widely used as a spice that not only improves the taste of food, but also contributes to

metabolic functions and overall health. Food supplements containing fenugreek have hypoglycemic properties and are recommended for diabetic patients.[12], fenugreek extracts exhibit antimicrobial activity against numerous bacteria. Fenugreek roots, seeds and shoots have antifungal properties. Fenugreek seed are used the treatment of gastrointestinal disorders. Aqueous solutions and macerated Fenugreek oils exert protective effects on the mucosa in ulcer disease and prevent colon cancer. Fenugreek leaves are used in the treatment of eye diseases and gynaecological disorders. Fenugreek seeds contain the neuroprotective alkaloid trigonelline which can be effectively used in the prevention and treatment of neurodegenerative disease. Fenugreek seeds also have anti-inflammatory, antipyretic and analgesic properties. Fenugreek extracts effectively prevent and inhibit the progression breast cancer. Flavonoids could also significantly contribute to fenugreek's anticarcinogenic properties. It can also be used in the production of oral hormones and steroids. Fenugreek seed extracts lower blood glucose level. Water and alcohol extracts, tinctures, meads, tonics with antidepressant and psychotonic properties, and muscle growth supplements. Fenugreek is used in the treatment of seborrhea, acne and dermatitis. The plant is widely used in cosmetology.[13]

MATERIALS AND METHODS

Collection: Fresh fenugreek seeds were collected from Grocery shop of Main Road, Ranchi, Jharkhand, India. The plant is Authenticated from Botanical Department at YBN University, Ranchi, Jharkhand. the Ref No. of certificate (Ref. No. YBN/1/UNIV/BOT/12Q25/2025-25). The fresh seeds were washed under running tap water in order to remove soil and dirt. Seeds were rewashed with distilled water and subsequently dried at room temperature for 3 days. The dried seeds were ground well with mortar pestle. The sample powder was stored in air tight container at room temperature ready to be used for further experiment.[14]

Preparation of extraction: The extracts from fenugreek seed were prepared by maceration method by utilizing 1 solvent methanol. 10 grams of dried powder of fenugreek seeds was dissolved in 100 ml of methanol for 72 h at room temperature. Then mixture solution is filtered with Whatman no.1 filter paper. Filtrate allowed to evaporated at room temperature. Dried extract was stored for further use in experiment after dilution as per requirements.[15]

Preparation of sample from extracts: To prepare the sample for the evaluation of antibacterial activity, in this experiment we used two fraction of sample one is methanolic sample and one other is aqueous sample. 538 mg of extract dissolved in 1000 µl of methanol.

470mg of extract dissolved in 900µl of distilled water. Mixed the extract from solvent by using Vortex mixer and microcentrifuge are used to separate or remove the debris from sample. After separation, well samples are ready to use in experiment.

Test microorganisms used in the study: Microorganisms were procured from the NCMR, NCCS Pune, India. Out of four bacterial strain used in the study, two Gram-negative bacteria were *Escherichia coli* (ACC-3099) and *Pseudomonas aeruginosa* (ACC-3973) and two Gram-positive bacteria were *Bacillus subtilus* (ACC-2511) and *Staphylococcus aureus* (ACC-2408). All the bacterial strains used in the investigation were maintained at 4°C on nutrient agar medium.

Procedure: Taken 1.3gm nutrient broth powder and 1.5gm agar-agar powder by using a digital balance. Added the weighed powder into a 250ml conical flask containing 100ml distilled water. Stirred the mixture using a glass rod. Covered the flask with cotton plug and aluminium foil. Sterilization was done by autoclave at 121^0C for 15 min at 15 psi.

Figure No. 1: Culture Media.

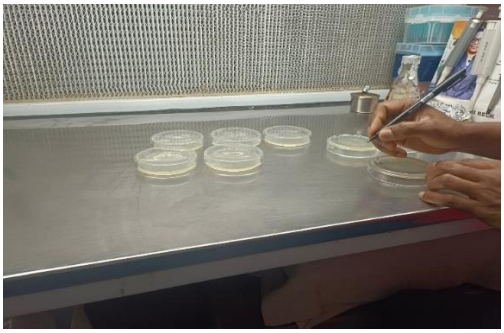


Figure No. 2: Pouring of Culture Medium And Labelling

Pouring plates: Allowed the sterilized culture media to cool 45-50^0C and poured 15-20ml of culture medium into sterile petri dishes under aseptic condition (laminar air flow chamber) and labelled concentration of sample.

Plating Procedure: For each bacterial isolate, 0.1 mL of the desired dilution was poured into a separate sterile Petri dish and spreaded bacteria in petri plates.

Disc Preparation: Discs are mainly made from whatmaan filter paper of diameter 6mm. using the micropipette, dispance a accurate volume (05µl, 10µl, 15µl and 20µl) sample solution onto each disc and ensured the solution is to be absorbed fully and evenly. Allowed the loaded disc to air dry in a sterile enviroment to remove solvent and enure stability.

Antimicrobial assay: The disc-diffusion and well difusion method was followed to test the antimicrobial potential of *Trigonella foenum graecum* seeds. Four different microorganisms were used to test the antimicrobial capacity of this plant seeds extract, which include both Gram-positive; bacterial culture collection *Bacillus subtilus* (ACC-2511) and *Staphylococcus aureus* (ACC-2408) and Gram-negative bacteria; *Escherichia coli* (ACC-3099) and *Pseudomonas aeruginosa* (ACC-3973). Bacteria were inoculated in nutrient agar media. 100 µl of microorganism inoculum was spread (glass rod) uniformly into sterilized petri plates (121°C, 20lbs pressure for 20 min in autoclave). After spreading the inoculum, 6 mm discs were placed into the media with the help of sterilized forceps. Methanol and water excerpts were loaded in different petri plates using a micropipette. Three different volumes of 538mg/1000 µL (5 µL, 10 µL, 15 µL and 20uL) were used to test the potential of excerpts as an antimicrobial against selected microorganisms and placed inside the incubator for 18-24 hrs to promote the maximum growth of microorganisms. Tetra-cycline (30mcg) was used as the positive control. Methanol, and Aqeous were used as the negative control.[15, 16]

Calculations: For standard calculation, each experiment was conducted in triplicate. Mean (mm), standard deviation, and standard error (±) are calculated. The area of the inhibited zone was calculated in mm2 . The P value was calculated. All the calculations were done in MS Excel. Zone of inhibition (mm2) = Area of inhibited zone ($\pi r_1 2$)-Area of disc ($\pi r_2 2$). The percentage of inhibition of leaves excerpts of this plant is calculated by using the formula.

RESULT AND DISCUSSION

Antibacterial activity (Well diffusion and Disc diffusion methods)

Now a day bacteria develop resistance is the major problem to antibacterials, and synthetic antibacterial have many side effects; in such conditions, the development of new antibacterial is essential, which are herbal and give maximum benefits without any side effects. The present investigation focuses on the antibacterial potential of seed extract of *Trigonella*

foenum-graecum. In this experiment we used two solvent methanolic solvent and aqueous solvent in which all the two extracts show response against selected microorganism the result.

Two different solvent extracts (Aqueous and Methanol,) were used against a series of bacterial strains including *Escherichia coli* (ACC-3099) and *Pseudomonas aeruginosa* (ACC-3973) *Bacillus subtilus* (ACC-2511) and *Staphylococcus aureus* (ACC-2408). The methanolic and aqueous extract of fenugreek seed had been evaluated for anti-bacterial activity by using the disc diffusion and well diffusion method.[15,16]

In well diffusion method, the methanolic seed extract showed moderate anti-bacterial activity with the highest zone of inhibition (16 mm) against *E. coli* (ACC-3099) at 100µl. Minimal activity was observed against *P. aeruginosa* (ACC-3973) (table 2 and graph 1). In well diffusion method, the Aqueous seed extract showed stronger activity, particularly against *Staphylococcus aureus* (ACC-2408), with a 33 mm inhibition zone at 100 µl. Minimal activity was observed against *P. aeruginosa* (ACC-3973), with 12 mm inhibition (table 3 and graph 2).

In disc diffusion method, the methanolic seed extract showed moderate anti-bacterial activity with the highest zone of inhibition (17 mm) against *Bacillus subtilus* (ACC-2511) at 20µl. Minimal activity was observed against *Escherichia coli* (ACC-3099) (table 4 and graph 3). In disc diffusion method, the aqueous extract is failed to show any inhibition in this method, likely due to poor diffusion of active compounds in the agar matrix (table 5 and graph 4).

Table No. 2: Inhibitory effect by *Trigonella foenum graecum* seed extract (methanolic).

	Zone of inhibition in mm					
Bacterial Strains	**25µl**	**50 µl**	**75 µl**	**100 µl**	**Positive control**	**Negative control**
ACC-2511	11	11	10	10	35	0
ACC-3973	0	0	0	0	30	0
ACC-2408	8	10	10	12	48	0
ACC-3099	12	12	12	16	33	0

Table No. 3: Inhibitory effect by *Trigonella foenum graecum* seed extract (aqueous).

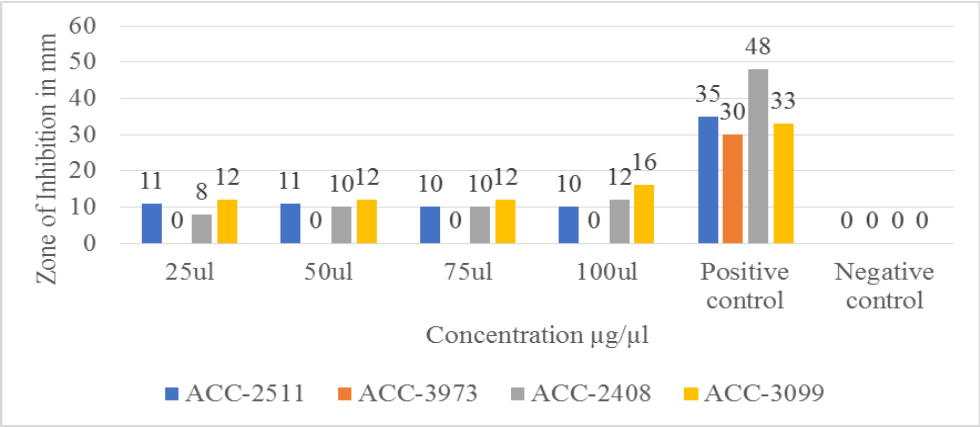
	Zone of inhibition in mm					
Bacterial Strains	**25 µl**	**50 µl**	**75 µl**	**100 µl**	**Positive Control**	**Negative Control**
ACC-2511	11	12	13	15	43	0
ACC-3973	10	11	12	11	35	0
ACC-2408	25	28	30	33	30	0
ACC-3099	10	11	13	15	30	0

Table No. 4: Inhibitory effect by *Trigonella foenum graecum* seed extract (methanolic).

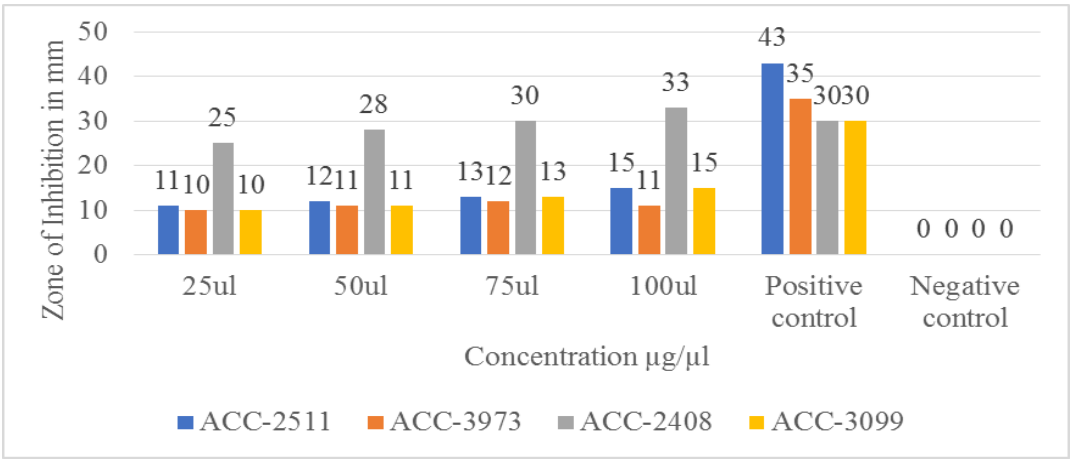
	Zone of inhibition in mm					
Bacterial Strains	**5µl**	**10µl**	**15µl**	**20µl**	**Positive control**	**Negative control**
ACC-2511	8	10	15	17	55	0
ACC-3973	7	10	12	16	45	0
ACC-2408	0	0	0	8	43	0
ACC-3099	0	0	6	7	28	0

Table No 5: Inhibitory effect by *Trigonella foenum graecum* seed extract (aqueous)

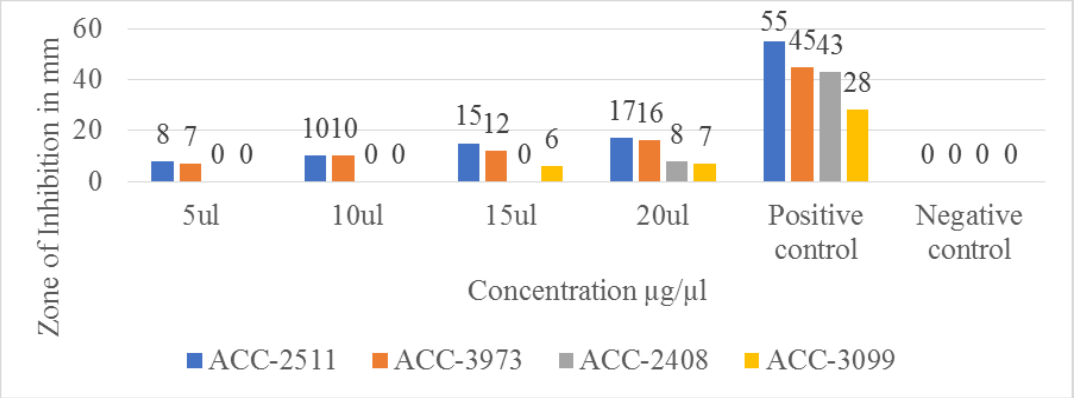
	Zone of inhibition in mm					
Bacterial Strains	**5µl**	**10µl**	**15µl**	**20µl**	**Positive Control**	**Negative Control**
ACC-2511	0	0	0	0	47	0
ACC-3973	0	0	0	0	43	0
ACC-2408	0	0	0	0	42	0
ACC-3099	0	0	0	0	36	0



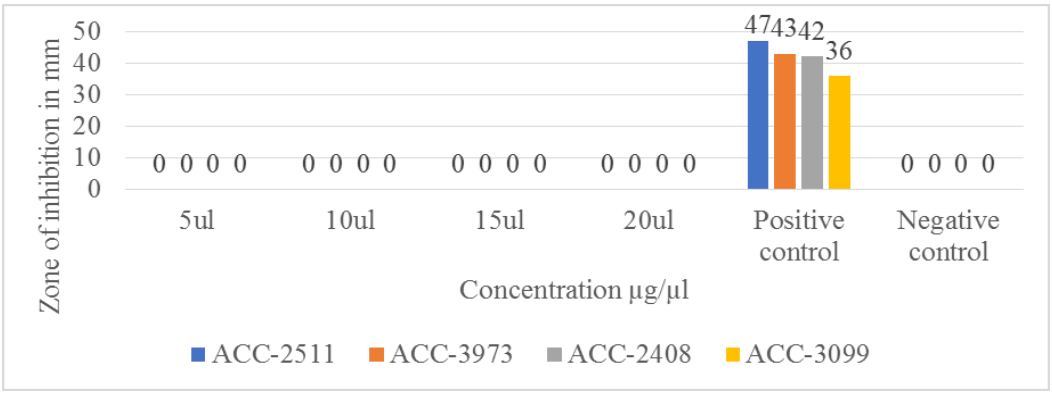
Graph 1: Showed anti-microbial activity (Zone of inhibitions) by well diffusion method (Methanolic).



Graph 2: Shows anti-microbial activity (Zone of inhibitions) by well diffusion method (aqueous).



Graph 3: Shows anti-microbial activity (Zone of inhibitions) by disc diffusion method (Methanolic).



Graph 4: Shows anti-microbial activity (Zone of inhibitions) by disc diffusion method (aqueous).

CONCLUSIONS

The present study aimed to evaluate the antimicrobial and antioxidant potential of fenugreek (*Trigonella foenum-graecum*) seeds, exploring their applications in health and food preservation. The findings demonstrate significant bioactive properties of fenugreek seed extracts, supporting their traditional use in medicine and potential for modern therapeutic and preservative applications.

The methanolic and aqueous extracts of fenugreek seeds exhibited notable antimicrobial effects against both Gram-positive and Gram-negative bacteria. In the well diffusion method, the aqueous extract showed stronger activity, particularly against *Staphylococcus aureus* (33 mm inhibition zone at 100 µl), while the methanolic extract was more effective against *Escherichia coli* (16 mm inhibition zone at 100 µl). The disc diffusion method revealed moderate activity for the methanolic extract against *Bacillus subtilis* (17 mm inhibition zone at 20 µl), but the aqueous extract failed to show inhibition, likely due to poor

diffusion of active compounds in the agar matrix. These results align with previous studies highlighting fenugreek's antimicrobial properties, attributed to bioactive compounds like saponins, flavonoids, and alkaloids, which disrupt microbial cell membranes and interfere with enzyme activity. The variability in efficacy between solvents underscores the importance of extraction methods in optimizing bioactive compound recovery.

ACKNOWLEDGEMENTS

The authors would like to thanks the Eminence Biopath, Research and Training Centre Laboratory, Ranchi, Jharkhand for providing research opportunity and for the use of laboratory facilities to carry out this study. I thank to Mrs. Nilam Kumari Beck (Director, Eminence Biopath) and Assistant Professor Dr. Vinay Oraon (R. U.) for frequent helpful discussions during this work.

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