

EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*) EXTRACTS

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ABSTRACT

Objective: The present investigation was performed to evaluate the antimicrobial potential of *Trigonella foenum-graecum* leaves extracts and to elucidate the presence of phytochemicals responsible for its biological activity.

Methods: The plant extracts were prepared by sequential cold maceration method by using four solvents viz., hexane, ethyl acetate, methanol and distilled water. Antimicrobial activity of extracts was carried out by agar well diffusion method against four bacteria and five fungi. Minimum inhibitory concentration (MIC) of different extracts was determined using the broth dilution method. Thin layer chromatography (TLC), TLC bioautography and phytochemicals analysis were also performed.

Results: The antibacterial activity of *T. foenum-graecum* leaves extracts was found maximum on *Serratia marcescens* with a zone of inhibition (ZOI) of 12.33±0.57 mm by aqueous extract followed by inhibition of *Bacillus cereus* (ZOI = 11.50±0.50 mm) by the methanol extract. The range of MIC of different extracts recorded was 6.25 to 25 mg/ml. Results of the antifungal activity showed that methanol extract showed a maximum zone of inhibition against *Trichoderma viridae* (ZOI = 14.5±0.5 mm) followed by ethyl acetate extract (ZOI = 12.0±1.0 mm). The MIC value for methanol extract against *T. viridae* was 6.25 mg/ml. The result of TLC bioautography revealed that compounds eluted at R_f 0.58 and R_f 0.67 exhibited strong while compounds eluted at R_f 0.31 and R_f 0.37 showed moderate antibacterial activity against *S. marcescens*. Phytochemicals analysis indicated that methanol and aqueous extracts contain more phytochemicals as compared to hexane and ethyl acetate extracts.

Conclusion: The results obtained in this study clearly indicate that *T. foenum-graecum* leaves extracts to have a significant potential to be used as an antimicrobial agent due to the presence of different phytochemicals.

Keywords: Antimicrobial activity, Phytochemicals analysis, TLC bioautography, Minimum Inhibitory Concentration (MIC), *Trigonella foenum-graecum*

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INTRODUCTION

The problem of drug resistance in microbes is increasing day by day [1, 2]. Continuous and overuse of synthetic antibiotics often produce tolerance in human and create resistance in microbes for a particular antibiotic. Being synthetic antibiotics have a number of side effects over agents of natural origin. So, researchers strive to find out a drug that could be therapeutically efficacious towards a wide range of microorganisms with minimal or no side-effects [3]. The alarming increase in the rate of infectious diseases with antibiotic resistant microorganisms and side-effects of some synthetic antibiotics has led to an increasing interest towards medicinal plants as a natural alternative to synthetic drugs [4, 5].

In recent years, there has been a gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries in utilization to develop antimicrobial drugs, because plant-derived drugs have been reported to be safe and without side-effects [6]. Traditionally the people of India have a long-standing practice of using a wide variety of herbal products in the treatment of diseases or as preservatives in foods. Spices are indispensable components of Indian cuisines since ancient times. Spices are considered as a rich source of bioactive antimicrobial compounds [7].

Trigonella foenum-graecum commonly known as fenugreek is an annual herb indigenous to the countries touching on the eastern shores of the Mediterranean and widely cultivated in India, Egypt, and Morocco [8]. The plant parts like leaves and seeds are widely consumed in Indo-Pak subcontinent as well as in other oriental

countries as a spice in food preparations and as an ingredient in traditional medicines [9]. A wide range of uses was found for fenugreek in ancient times. Fenugreek being rich in antioxidants and phytochemicals has been traditionally used as food, forage and medicinal plant [10, 11]. Medicinally it was used in the treatment of wounds, abscesses, arthritis, bronchitis, ulcer and digestive problems. Thus, fenugreek is food and a spice commonly eaten in many parts of the world for many years. Fenugreek is also used traditionally as a demulcent, laxative, lactation stimulant and exhibits hypocholesterolemic, hypolipidemic and hypoglycemic activity in healthy and diabetic animals and humans [9]. The pharmacological uses of different plant parts of fenugreek have been reported by different researchers. The seeds of fenugreek have been reported to have anti-diabetic [12, 13], anti-cancerous [14], anti-inflammatory [15] and antioxidant activity [16]. Its leaves have been reported to possess potential antibacterial activity [7, 17-20], antifungal activity [21], anti-diabetic [22] and antioxidant property [18, 23, 24]. Fenugreek is an ancient medicinal plant as the plant contains active constituents such as alkaloids, flavonoids, steroids, saponins, etc. [8].

The potential for developing antibacterial into medicine appears rewarding from both the perspective of drug development and the perspective of phytomedicine [25]. Most of the work regarding the antimicrobial activity of *T. foenum-graecum* was carried out using seeds as a plant material, but the use of leaves as a plant material was very limited. It is the first report for the evaluation of antimicrobial activity of *T. foenum-graecum* leaves against some microorganisms including two bacteria viz., *Serratia marcescens* and *Bacillus cereus* as well as all the fungal strains used in the present

study. TLC bioautography of aqueous extract of *T. foenum-graecum* leaves against *S. marcescens* was also evaluated for the first time. Thus, to fulfil the aim of the present investigation, we evaluated the antimicrobial potential of *Trigonella foenum-graecum* leaves extracts and to elucidate the possible class of main components responsible for its biological activity.

MATERIALS AND METHODS

Plant material used in the study

Fenugreek (*Trigonella foenum-graecum*) plants were collected from local market of Anand, Gujarat, India. The taxonomic identities of these plants were confirmed by the taxonomist. Fresh plant materials (leaves) were washed with tap water followed by air drying and then homogenized to a fine powder. The powder was stored in airtight bottles at 4 °C.

Preparation of extracts

The extracts from plant leaves were prepared by sequential cold maceration method by utilizing four solvents viz., hexane, ethyl acetate, methanol and distilled water [26]. Fifty grams of dried powder of plant material was soaked in 250 ml hexane for 24 h at room temperature under shaking condition at 120 rpm. The mixture was filtered with whatman no. 1 filter paper. The filtrate was collected in Petri dishes and allowed the solvent to be evaporated at 37 °C. The dried extract was stored and further used for antimicrobial assay after dilution as per requirement. The filter cake was dried at room temperature and stored separately. The dried powder of filter cake was sequentially re-suspended in 250 ml ethyl acetate, methanol and distilled water to prepare dried extracts of plant material in each solvent. After extraction in each solvent, remaining filter cake was dried and further used with next solvent for extraction. All the extracts were concentrated to dryness and kept at 4 °C.

Preparation of sample from extract

To prepare the samples for the evaluation of antimicrobial activity, 100 mg of each extract were dissolved in 1 ml of dimethyl sulphoxide (DMSO).

Test microorganisms used in the study

Microorganisms were procured from the Department of Microbiology, ARIBAS, New V. V. Nagar, India. Out of four bacteria used in the study, three Gram-negative bacteria were *Escherichia coli* (MTCC No. 448), *Pseudomonas aeruginosa* (MTCC No. 424) and *Serratia marcescens* (local isolate) and a Gram-positive bacterium was *Bacillus cereus* (MTCC No. 135). All the bacterial strains used in the investigation were maintained at 4 °C on nutrient agar medium. All the test fungal strains, including *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium chrysogenum* and *Trichoderma viridae* were maintained at 4 °C on potato dextrose agar (PDA) slants.

Preparation of bacterial suspension

Colonies of different strains of bacteria (*E. coli*, *S. marcescens*, *P. aeruginosa* and *B. cereus*) were transferred to the different fresh nutrient broth in sterile conditions and were incubated at 37 °C for 24 h. These suspensions were preserved in 250 ml sterile flasks for further use.

Antibacterial activity

The antimicrobial activity of the test extracts was carried out by agar well diffusion method [26, 27]. For the antibacterial assay, initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 °C for 18 h. The plates of the Muller Hilton's agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the bacterial suspension (10^5 - 10^6 colony forming unit "CFU"/ml) which was spread evenly on the surface of the medium of the plate. After 15 min, wells with 6 mm diameter were made with the help of a sterile cork borer in the solid medium and filled with the test samples of 100 mg/ml concentration. The positive and negative control wells were filled with Gentamicin (standard drug of 10 µg/ml concentration) and DMSO, respectively. All the plates were

incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (ZOI) in mm. Triplicates were carried out for each extract against each of the test organism.

Antifungal activity

The antifungal activity the plant extracts was performed by agar well diffusion method [28]. For assessment of the antifungal activity of the extracts, the stock cultures of fungi were revived by inoculating in broth media and grown at 27 °C for 72 h. The plates of the potato dextrose agar (PDA) media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the fungal suspension (10^3 spores/ml) which was spread evenly on the solid media. After 15 min, the wells with 6 mm diameter were made by using sterile cork borer and filled with test samples of 100 mg/ml concentration. The positive and negative control plates were also prepared with Fluconazole (standard drug of 10 mcg/disc concentration) and DMSO, respectively. All the plates were incubated at 27 °C for 5-7 d and then the diameter of the zone of inhibition was recorded. Triplicates were carried out for each extract against each of the test organism.

Determination of Activity Index (AI)

Activity index of all the extracts was calculated using following formula [26].

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

Determination of relative percentage inhibition (RPI)

The relative percentage inhibition of all the test extracts with respect to the positive control was calculated by using the following formula [26].

$$\text{RPI} = \frac{100 (X-Y)}{(Z-Y)}$$

Where X= Total area of inhibition of the test extract; Y= Total area of inhibition of the solvent and Z= Total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using the area = πr^2 ; where r = radius of the zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of different extracts with respect to different bacteria and fungi was determined using broth dilution method [26]. Mueller-Hinton broth (HiMedia, Mumbai, India) was used only for the antibacterial study and potato dextrose broth (HiMedia, Mumbai, India) for the antifungal study. All the extracts dissolved in DMSO were first diluted to the highest concentration (100 mg/ml) to be tested and then two-fold serial dilution was made in the concentration range from 1.56 to 100 mg/ml. For broth dilution, 0.1 ml of standardized suspension of a strain (10^6 CFU/ml) separately was added to each tube containing various extracts at concentrations of 0 (control), 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml in broth medium. The tubes were incubated at 37 °C for 24 h for bacterial strains and 27 °C for 48 h for fungal strains and observed for visible growth after vortexing the tubes gently. The lowest concentration of extract in a tube that failed to show any visible growth was considered as the MIC. A tube containing nutrient broth and inoculum without extract was taken as a control. Bacterial and fungal suspensions were used as negative control while broth containing standard drugs was used as positive control.

Analytical thin layer chromatography (TLC)

Analytical TLC was performed to find out a suitable solvent system for the development of chromatogram. Different solvent systems were tried on pre-coated TLC plates (Merck, silica gel 60 F254 plate, 0.25 mm) to develop chromatogram. Among all, toluene: acetone: the glacial acetic acid (45:45:10) solvent system was found best and used for subsequent bioautography analysis. The components were visualized under visible light, and R_f values were recorded.

TLC Bioautography

The aqueous extract of *T. foenum-graecum* leaves was used for TLC bioautography against *S. marcescens*. Plates were developed with

toluene: acetone: glacial acetic acid (45:45:10), which separated components into a wide range of R_f values. The components were visualized under visible light. Developed TLC plates were carefully dried for complete removal of the solvents and overlaid by nutrient agar seeded with an overnight culture of *S. marcescens*. The plate was incubated for 24 h at 37 °C and then sprayed with an aqueous solution of 5 mg/ml of methyl thiazol tetrazolium (Sigma). Inhibition zones were observed as clear spots against a purple background, and their R_f values were compared with the reference plate [29, 30].

Preliminary phytochemicals analysis

The extracts were tested for the presence of alkaloids, tannins, saponins, cardiac glycosides, steroids, phenols and flavonoids according to standard protocols for detecting the presence of different phytochemicals in the plant extracts [31].

RESULTS

Antibacterial activity

Antibacterial activity (in terms of the zone of inhibition, activity index and relative percentage inhibition) of the plant extracts was assessed against some selected pathogenic bacteria by agar well diffusion method. In the present study, total four extracts of *T. foenum-graecum* leaves having a concentration of 100 mg/ml were tested.

All the extracts showed antibacterial potential against test different bacteria. The inhibitory effect of *T. foenum-graecum* leaves extracts was found maximum on *S. marcescens* (ZOI = 12.33±0.57 mm and AI = 0.648) by aqueous extract followed by inhibition of *Bacillus cereus* (ZOI = 11.50±0.50 mm and AI = 0.761) by the methanol extract.

Hexane extract could only inhibit the growth of *S. marcescens*. While ethyl acetate extract showed a zone of inhibition against *B. cereus*. Methanol and aqueous extracts showed significant antimicrobial activity against all the test bacteria used in the study. *P. aeruginosa* was found most resistant bacteria which were slightly inhibited by methanol and aqueous extracts. The detailed result of the zone of inhibition for the antibacterial assay is represented in table 1 and results of the activity index along with the relative percentage inhibition are given in table 2.

MIC values of different extracts with respect to different bacteria were determined using a broth dilution method (table 3). Extracts showing antibacterial activity in agar well diffusion assay were further evaluated for determination of MIC values. The range of MIC of different extracts recorded was 6.25 to 25 mg/ml. In the present study, 6.25 mg/ml was recorded as the MIC for aqueous extract against *S. marcescens*. MIC for hexane and ethyl acetate was 25 mg/ml while MIC for methanol extract was 12.5 mg/ml against the most sensitive bacteria.

Table 1: Antibacterial activity (ZOI) of *T. foenum-graecum* leaves extracts

Extract	Conc. (mg/ml)	Bacterial strains and zone of inhibition (mm)			
		<i>S. marcescens</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
Hexane	100	9.66±0.57	-	-	-
Ethyl Acetate	100	-	10.33±0.57	-	-
Methanol	100	10.00±1.00	11.50±0.50	9.50±0.50	9.33±0.57
Aqueous	100	12.33±0.57	11.33±1.15	9.33±0.57	10.50±0.50
Gentamicin	10 µg/ml	19.00±1.00	15.10±0.76	15.00±1.00	18.10±1.04
DMSO	-	-	-	-	-

(-): No zone of inhibition, DMSO: Dimethyl sulphoxide, ZOI: Zone of inhibition (including 6 mm well diameter), The test was done in triplicate, Diameter of the zone of inhibitions is given here as mean±standard deviation

Table 2: Antibacterial activity (AI and RPI) of *T. foenum-graecum* leaves extracts

Extract	Conc. (mg/ml)	<i>S. marcescens</i>		<i>B. cereus</i>		<i>P. aeruginosa</i>		<i>E. coli</i>	
		AI	RPI	AI	RPI	AI	RPI	AI	RPI
Hexane	100	0.508	25.84	-	-	-	-	-	-
Ethyl Acetate	100	-	-	0.684	46.40	-	-	-	-
Methanol	100	0.526	27.70	0.761	58.00	0.633	40.11	0.515	26.57
Aqueous	100	0.648	42.11	0.750	56.30	0.622	38.68	0.580	33.65

AI: Activity Index, RPI: Relative Percentage Inhibition, (-): No inhibitory activity

Table 3: Minimum inhibitory concentration (MIC) of *T. foenum-graecum* leaves extracts against bacteria

Test organism	MIC in mg/ml			
	Hexane	Ethyl Acetate	Methanol	Aqueous
<i>Serratia marcescens</i>	25.0	NA	25.0	6.25
<i>Bacillus cereus</i>	NA	25.0	12.5	25.0
<i>Pseudomonas aeruginosa</i>	NA	NA	25.0	25.0
<i>Escherichia coli</i>	NA	NA	25.0	12.5

MIC: minimum inhibitory concentration (mg/ml), NA: not assessed

Antifungal activity

Antifungal activity (in terms of zone of inhibition, activity index and relative percentage inhibition) of different extracts was evaluated against some fungi by agar well diffusion method. Results of the investigation showed that methanol extract of *T. foenum-graecum* leaves was able to give a maximum zone of inhibition against *T. viridae* (ZOI = 14.5±0.5 mm and AI = 0.649) followed by ethyl acetate extract (ZOI = 12.0±1.0 mm and AI = 0.537). Hexane and aqueous extracts slightly inhibited *T. viridae*. Growth of *A. niger* (ZOI =

10.33±0.57 mm and AI = 0.639) and *A. flavus* (ZOI = 9.16±0.28 mm and AI = 0.429) were inhibited by only hexane extract of *T. foenum-graecum* leaves whereas other extracts failed to give a zone of inhibition. *A. oryzae* was sensitive to only ethyl acetate extract while it was resistant to other three extracts. All the extracts of *T. foenum-graecum* leaves used in the study were failed to give any inhibitory effect against *P. chrysogenum*. The detailed result of the zone of inhibition for antibacterial assay is represented in table 4 and results of the activity index along with the relative percentage inhibition are given in table 5.

Table 4: Antifungal activity (ZOI) of *T. foenum-graecum* leaves extracts

Extract	Conc. (mg/ml)	Fungal strains and zone of inhibition (mm)				
		<i>A. niger</i>	<i>A. flavus</i>	<i>A. oryzae</i>	<i>P. chrysogenum</i>	<i>T. viridae</i>
Hexane	100	10.33±0.57	9.16±0.28	-	-	9.33±0.57
Ethyl Acetate	100	-	-	8.33±0.57	-	12.0±1.00
Methanol	100	-	-	-	-	14.5±0.50
Aqueous	100	-	-	-	-	9.66±0.57
Fluconazole	10 mcg/disc	16.16±1.04	21.33±1.15	16.00±1.00	18.66±1.52	22.33±0.57
DMSO	-	-	-	-	-	-

(-): No zone of inhibition, DMSO: Dimethyl sulphoxide, Zone of inhibition including 6 mm well diameter, The test was done in triplicate, Diameter of the zone of inhibitions is given here as mean±standard deviation.

Table 5: Antifungal activity (AI and RPI) of *T. foenum-graecum* leaves extracts

Extract	Conc. (mg/ml)	<i>A. niger</i>		<i>A. flavus</i>		<i>A. oryzae</i>		<i>P. chrysogenum</i>		<i>T. viridae</i>	
		AI	RPI	AI	RPI	AI	RPI	AI	RPI	AI	RPI
Hexane	100	0.639	40.86	0.429	18.44	-	-	-	-	0.417	17.45
Ethyl Acetate	100	-	-	-	-	0.520	27.10	-	-	0.537	28.87
Methanol	100	-	-	-	-	-	-	-	-	0.649	42.16
Aqueous	100	-	-	-	-	-	-	-	-	0.432	18.71

AI: Activity Index, RPI: Relative Percentage Inhibition, (-): No inhibitory activity

MIC values of different extracts with respect to different fungi were determined using broth dilution method. Extracts showing antifungal activity in agar well diffusion assay were further evaluated for MIC values. The MIC value for methanol extract against *T. viridae* was 6.25 mg/ml followed ethyl acetate extract

having 12.5 mg/ml and against the same fungus. Hexane and aqueous extracts have MIC value of 50 mg/ml against *T. viridae*. MIC of hexane extract was 50 mg/ml against *A. niger* and 100 mg/ml against *A. flavus*. The results of MIC values determination are shown in table 6.

Table 6: MIC of *T. foenum-graecum* leaves extracts against fungi

Test organism	MIC in mg/ml			
	Hexane	Ethyl acetate	Methanol	Aqueous
<i>A. niger</i>	50.0	NA	NA	NA
<i>A. flavus</i>	100	NA	NA	NA
<i>A. oryzae</i>	NA	100	NA	NA
<i>P. chrysogenum</i>	NA	NA	NA	NA
<i>T. viridae</i>	50.0	12.5	6.25	50.0

MIC: minimum inhibitory concentration (mg/ml), NA: not assessed

Analytical TLC and TLC bioautography

The R_f values and colour of bands of the aqueous extract of *T. foenum-graecum* leaves separated by TLC have been summarized in table 7. TLC bioautography assay allowed outlining the chemical profile of the aqueous extract thus detecting the active substances having antimicrobial activity. The result of this assay revealed that compounds eluted at R_f 0.58 and R_f 0.67 exhibited strong while compounds eluted at R_f 0.31 and R_f 0.37 showed moderate antibacterial activity against *S. marcescens*, suggesting thereby the presence of antimicrobial active substances (fig. 1).

Phytochemical analysis

The qualitative phytochemicals analysis is useful in determining the presence of significant chemical constituents of the extracts. The results of preliminary analysis of phytochemicals are given in table 8. Results indicated that methanol and aqueous extracts contain more phytochemicals as compared to hexane and ethyl acetate extracts.



Fig. 1: TLC bioautography of aqueous extract of *T. foenum-graecum* leaves against *S. marcescens*

Table 7: R_f value and colour of band of the aqueous extract of *T. foenum-graecum* leaves separated by Thin Layer Chromatography (TLC)

R_f value	Band colour in visible light
0.31	Greenish yellow
0.37	Yellow
0.58	Light green
0.67	Light green
0.86	Green
0.93	Dark green

Table 8: Qualitative analysis for phytochemicals present in various extracts of *T. foenum-graecum* leaves

Name of phytochemical	Extracts			
	Hexane	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	-	-	+	-
Sterols	-	+	+	+
Cardiac glycoside	+	+	+	+
Flavanoids	-	-	-	+
Phenol	+	-	+	+

(+): Present, (-): Absence

DISCUSSION

Infectious diseases are the main cause of mortality worldwide. The number of multidrug-resistant microbial strains and strains with reduced susceptibility to antibiotics are continuously increasing due to indiscriminate use of antibiotics and other synthetic antibacterial agents in treatment [31, 32].

Plants have been used for medicinal purposes and are the primary source of phytochemicals present in conventional medicaments since the existence of human being. The relationships between cultures and the traditional use of plants have been described by various Ethnobotanical studies. These studies are of the utmost importance and provide essential information that allows the development of scientific research more oriented to explore and prove the therapeutic potential of plants [33]. Plants used in traditional medicine contain a vast array of substances that can be utilized to treat chronic and even infectious diseases. More than 80% of world's populations depend on traditional medicines derived from plants for their primary health care needs [43]. The plant-based traditional medicines were proven highly effective for their utilization as a source of antimicrobial compounds [32]. Plants are rich in a wide variety of secondary metabolites such as terpenoid, tannins, alkaloids, and flavonoids, which have been found to have medicinal properties [20].

Agar well diffusion techniques have been widely utilized to assay the antimicrobial activity of plant extracts [7, 26, 31, 33]. In the present study, we found that aqueous and methanol extracts of *T. foenum-graecum* leaves possess higher antibacterial activity. One of the very few previous studies on the antimicrobial activity of *T. foenum-graecum* also showed that methanol extract of fenugreek has antibacterial activity against *Pseudomonas* sp., *E. coli*, *Shigella dysenteries* and *Salmonella typhi* [7]. In another study, high antimicrobial activity against peptic ulcer-linked *Helicobacter pylori* was found in the fenugreek sprout extract [18]. It was reported that the inhibitory effects of *T. foenum-graecum* on *Escherichia coli* and *Klebsiella pneumonia* were only with the aqueous fraction. Whereas, crude extract, petroleum ether, and dichloromethane fractions did not show any inhibitory effect [19]. *T. foenum-graecum* also displayed antibacterial activity against *Bacillus subtilis*, *Sarcina lutea*, *Xanthomonas campestris*, *Proteus vulgaris* and *Pseudomonas denitrificans* [20]. Thus, present and previous studies lead us to conclude that methanol and aqueous extracts have maximum inhibitory action on different bacterial strains. According to a previous report, the MIC values of acetone extract were found to be 64 µg/ml against *Shigella dysenteriae* and *Pseudomonas* sp. The MIC value was found 16 µg/ml against *E. coli* [7]. The range of MIC was found to be between 1.25 and 80 µg/µl. Most of the test microorganisms were inhibited by 80 and 40 µg/µl of the crude extracts of *T. foenum-graecum*. [19]. Hence, *T. foenum-graecum* showed a great potential of antibacterial activity against gram positive and gram negative bacteria.

Results of the present investigation showed that all the extracts of *T. foenum-graecum* leaves inhibit the growth of *T. viridae* and failed to prevent the growth of *P. chrysogenum*. The growth of *A. niger*, *A. oryzae* and *A. flavus* was inhibited by an only single extract of *T. foenum-graecum* leaves. Methanol extract possessed the most effective antifungal activity in the present study. Results of a previous study suggested that the constituents of *T. foenum-graecum*

have potential against harmful pathogenic fungi viz., *Fusarium graminearum*, *Alternaria* sp. and *Rhizoctonia solani* [21]. The same study indicated that the antifungal activity of *T. foenum-graecum* resided mainly in the methanol fraction. The minimum inhibitory concentration (MIC) of methanol fraction which caused total inhibition of *Rhizoctonia solani* and *Alternaria* sp. was found to be 60 µg/ml [21]. Therefore, *T. foenum-graecum* could be an important source of biologically active compounds useful for developing better new antifungal drugs.

Based on the results of the antibacterial assay and TLC bioautography, it is concluded that *T. foenum-graecum* have a stronger antibacterial activity against bacteria tested in the present study. Further work can be done to isolate and identify antimicrobial substances present in the extracts to prepare highly competent antimicrobial drugs.

Results of the present study suggested that methanol and aqueous extracts contain more phytochemicals as compared to hexane and ethyl acetate extracts. Almost similar results were found in past work for the qualitative analysis of fenugreek seeds for the presence of phytochemicals in different extracts [35]. The knowledge of the extent and mode of action for antifungal activity of specific compounds, present in the plant extracts, may lead to the successful utilization of such natural compounds for the treatment of infections caused by pathogenic microorganisms. Further identification and purification of active chemical constituents from the crude plant extracts will be helpful to develop drugs against pathogenic microorganisms.

CONCLUSION

From the present study, it is concluded that a potent antimicrobial activity against various pathogenic microorganisms by *T. foenum-graecum* leaves extracts especially methanol and aqueous extracts. Natural substances which are part of daily diet nutritional supplement with antimicrobial property constitute a new source of herbal drugs. The antimicrobial potential of this plant is a remarkable relevance as *T. foenum-graecum* leaves are edible and generally consumed and no extra processing is needed for its administration. Further isolation and exploration on the isolated chemical constituents on antimicrobial activity may lead to chemical entities for clinical application.

CONFLICT OF INTERESTS

Declare none

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