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Antibacterial Activity of *Trigonella Foenum-Graecum* Leaf Extracts on Clinical Isolates

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Abstract: *Trigonella foenum-graecum*, commonly called as Fenugreek is an aromatic herb belonging to the family Fabaceae. The leaves and seeds of the plant are used as common ingredients in most of the Indian foods for their flavour and medicinal properties. The leaves are known to have applications in different disease conditions like diabetes, digestive and renal disorders, tuberculosis, skin infections etc. and the seeds are a rich source of lipids, mucilage and proteins. In the present work, the aqueous and hexane extracts of the leaves of *Trigonella foenum-graecum* are analysed for their activity on ten clinical isolates viz. Methicillin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter divergens*, *Shigella flexneri*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Both the extracts have shown antibacterial activity against all isolates at various concentrations tested by agar well diffusion method, using Streptomycin as standard. The inhibition zone diameters were found to be in the range of 5.0 mm for MRSA to 21.67 mm for *Salmonella paratyphi A* with aqueous extract and 5.0 for *Shigella flexneri* to 20.67 mm for *Salmonella paratyphi B* with hexane extract. The standard error was found to be on the order of 0.0 to 1.53 and 0.0 to 2.0 as a function of increasing concentration of aqueous extract and hexane extract respectively. *Shigella flexneri* and *Citrobacter divergens* were found to be resistant to aqueous extract at all concentrations tested. The Activity index and Relative percentage inhibition of the extracts were also calculated. These findings indicate that both the extracts can be used for treatment of infections caused by the above clinical isolates.

Keywords: *Trigonella foenum-graecum*, clinical isolates, antibacterial activity, aqueous extract, hexane extract

I. INTRODUCTION

Plant extracts have been used since ancient times as medicines for treatment of many ailments. Now-a-days nosocomial infections have risen due to development of drug-resistance in bacterial pathogens. To overcome this problem, many medicinal plants and their components are used as natural drugs. Among the various phytochemicals that are in use, fenugreek is the one. Fenugreek or *Trigonella foenum-graecum* is an annual herb indigenous to the countries touching on the Eastern shores of Mediterranean and widely cultivated in India, Egypt and Morocco [1]. The leaves and fried and ground seeds of this plant (Telugu-menthi) are used as spices in the preparation of various foods which are tasty and healthy. Phytochemicals are natural and non-nutritive bioactive compounds produced by plants, which act as protective agents against external stress and pathogenic attack and could be used as single therapeutic agent or as combined formulation in drug development [2]. Fenugreek is rich in antioxidants and phytochemicals and is used traditionally as a food, forage and as a medicinal plant [3] [4]. It possesses the phyto constituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin and tannins [5]. Fresh fenugreek leaves contain ascorbic acid (220.97 mg/100 g) and β -carotene (19 mg/100 g) [6] and are a rich source of calcium, iron and zinc [7]. The leaves of this plant have antibacterial [8] [9], antifungal [10], anti-diabetic [11] [12], anti-helminthic [13] and antioxidant property [14] [15]. In the present study, ten bacterial isolates viz. the Gram positive Methicillin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and the Gram negative *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter divergens*, *Shigella flexneri*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Proteus mirabilis* obtained from various clinical samples were tested for their sensitivity to aqueous and hexane extracts of *Trigonella foenum-graecum* leaves and the results are furnished in this paper.

II. MATERIALS AND METHODS

A. Sample collection

The seeds of *Trigonella foenum-graecum* are collected and cultivated in a field by using natural fertilizers like cow dung, neem oil etc. After 20-30 days leaves are collected from the plants. The leaves are shade dried and powdered. The powder was stored at 4⁰ C for further use.

B. Preparation of aqueous extract of *Trigonella foenum-graecum* leaves

15 g of shade dried Fenugreek leaf powder was soaked in 150 ml of sterile distilled water and loaded on an orbital shaker at 120 rpm for 24 hours. The mixture was filtered by using Whatmann No. 1 filter paper and the filtrate was concentrated through Rotary evaporator. The left over liquid was dried on water bath [16] and the extract was used for the preparation of different concentrations.

C. Preparation of Hexane extract of *Trigonella foenum-graecum* leaves

15 grams of shade-dried leaves of Fenugreek powder was soaked in 75 ml of hexane and loaded on an orbital shaker at a speed of 120 rpm for 24 hrs at room temperature. The mixture was filtered through Whatmann No. 1 filter paper. The filtrate was collected into a China dish and the solvent was evaporated at room temperature. The process was repeated with the left over filter cake for three times [17]. The dried extract was collected and different concentrations of the extract was prepared in hexane.

D. Preparation of the bacterial culture:

The bacterial cultures Gram positive Methicillin Resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and the Gram negative *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter divergens*, *Shigella flexneri*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Proteus mirabilis* isolated from clinical samples were obtained from SVS Medical College, Mahabubnagar, Telangana State, India. Conventional bacteriological methods such as colony morphology, gram staining and biochemical tests were used for identification of isolates [18]. The test organisms were inoculated in Mueller Hinton broth (pH 7.4.) for 8 hours. The concentration of the suspensions was adjusted to 0.5 Mc Farland standard [19] to reach an optical density of 0.08 – 0.10 at 625 nm by adding sterile distilled water. This gives a bacterial suspension containing 1.5×10^8 CFU/ml [20]. Isolates were seeded on Mueller Hinton agar plates by using sterilized cotton swabs.

E. Antibacterial Sensitivity Testing:

Antimicrobial sensitivity testing was done by Agar well diffusion method. Mueller Hinton agar plates were prepared and wells of diameter 2 mm were cut. The bacterial culture was spread with a sterilized cotton swab and 50 µl of various concentrations of the compound viz. 50 to 300 mg/ml. Triplicates were maintained for all the tests.

F. Determination of Activity Index (AI):

Activity index of all the extracts was calculated using following formula [21]

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

G. Determination of relative percentage inhibition (RPI):

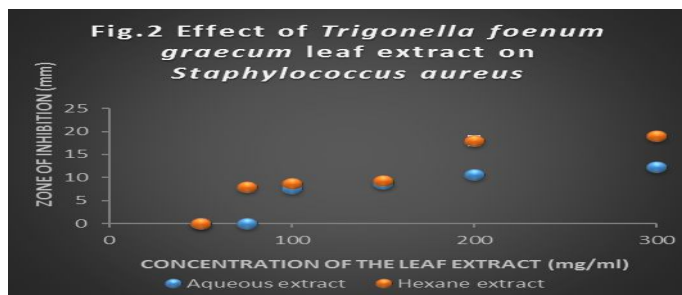
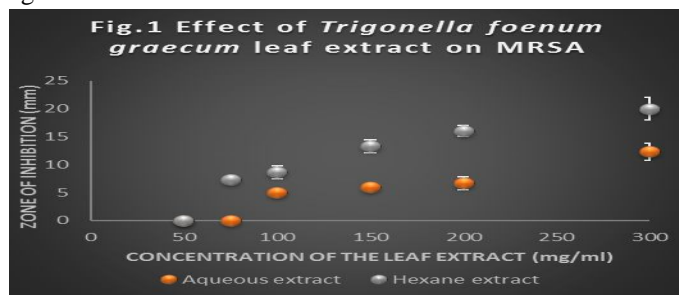
The relative percentage inhibition of all the test extracts with respect to the positive control was calculated by using the following formula [22]

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

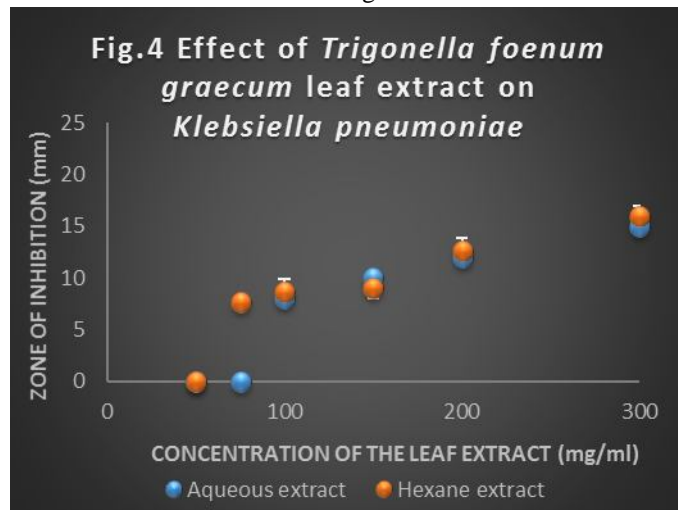
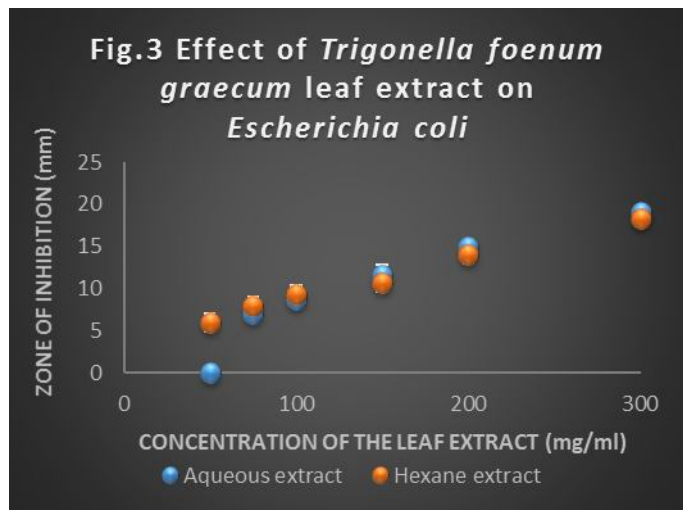
Where X= Total area of inhibition of the test extract; Y= Total area of inhibition of the solvent and Z= Total area of inhibition of the standard drug. The total area of the inhibition was calculated by using the area = πr^2 ; where r = radius of the zone of inhibition.

III. RESULTS AND DISCUSSION

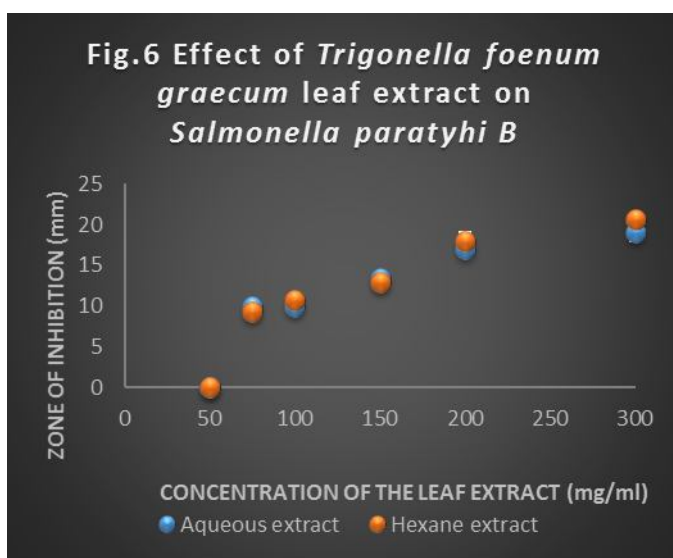
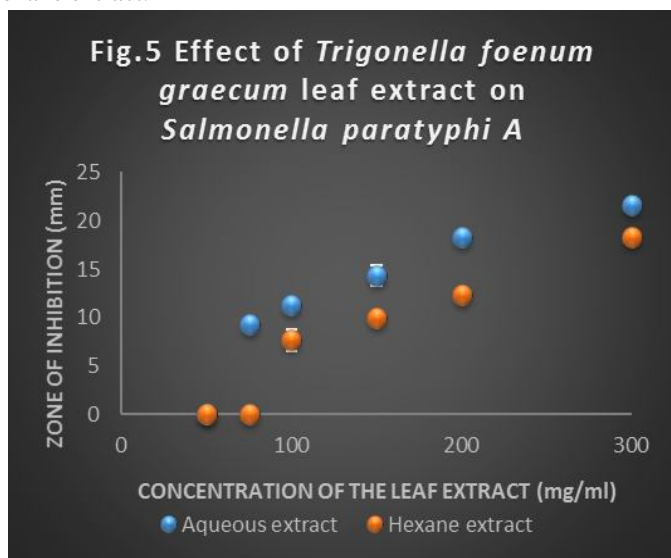
The aqueous and hexane extracts of *Trigonella foenum graecum* were found to be active against both Gram positive and Gram negative bacteria at different concentrations and the results are depicted below.



The aqueous extract of Fenugreek is active against Methicillin Resistant *Staphylococcus aureus* starting from the concentration of 100 mg/ml whereas the hexane extract has shown the inhibitory activity from the concentration of 75 mg/ml (Fig. 1). A minimum inhibitory zone of 5 mm with aqueous extract at a concentration of 100 mg/ml and a maximum of 20 mm inhibition was observed with hexane extract at 300 mg/ml with this bacterium. *Staphylococcus aureus* is resistant to lower concentrations of both the extracts but sensitive to increasing concentrations of the extracts, starting from 100 mg/ml with aqueous and 75 mg/ml with hexane extracts (Fig 2). The minimum inhibitory zone of 7.67 ± 0.58 mm with aqueous extract at a concentration of 100 mg/ml and maximum zone of inhibition was found to be 19 mm with hexane extract at a concentration of 300 mg/ml.



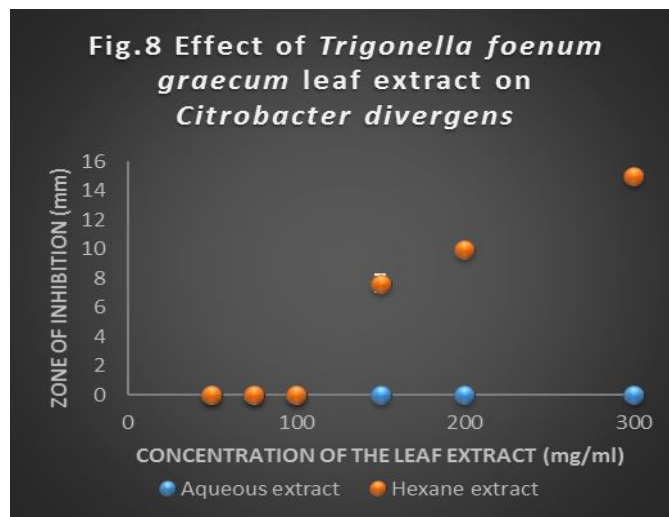
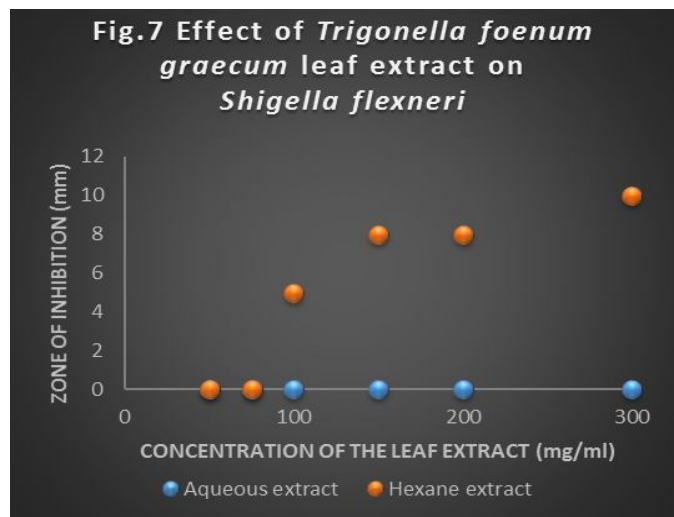
The aqueous extract of Fenugreek is active against *Escherichia coli* from the concentration 75 mg/ml whereas, the hexane extract is active at all concentrations tested (Fig. 3). The minimum zone of inhibition shown against this bacterium is 6 mm with hexane extract at 50 mg/ml and a maximum of 19 mm with aqueous extract at a concentration of 300 mg/ml. *Klebsiella pneumoniae* is resistant to aqueous extract up to 75 mg/ml and at 50 mg/ml with hexane extract but sensitive to increasing concentrations of the extracts (Fig. 4) with a minimum inhibitory zone of 7.67 ± 0.58 mm at 75 mg/ml and a maximum of 16 ± 1 mm at 300 mg/ml with hexane extract.



A. *Salmonella paratyphi*

A is resistant to aqueous extract at 50 mg/ml and up to 75 mg/ml with hexane extract (Fig. 5). Increasing concentrations of both the extracts have shown increasing zones of inhibition with a minimum of 7.67 ± 1.16 mm with hexane extract at 100 mg/ml and a maximum of 21.67 ± 0.58 mm with aqueous extract of the plant at a concentration of 300 mg/ml. The aqueous and hexane extracts

of the plant inhibited *Salmonella paratyphi B* from concentrations 75 mg/ml (Fig. 6) with a minimum zone of inhibition of 9.33 ± 0.58 mm at a concentration of 75 mg/ml and a maximum of 20.67 ± 0.58 mm at a concentration of 300 mg/ml with hexane extract.



B. *Shigella flexneri*

Is completely resistant to all the concentrations of aqueous extract tested and up to 75 mg/ml with hexane extract (Fig. 7). A minimum inhibitory zone of 5 mm at a concentration of 100 mg/ml and a maximum of 10 mm at a concentration of 300 mg/ml was observed with hexane extract of the plant. Similarly, *Citrobacter divergens* was also resistant to all concentrations of aqueous extract tested and up to 100 mg/ml of hexane extract (Fig. 8). A minimum zone of inhibition of 7.67 ± 0.58 mm at a concentration of 150 mg/ml and a maximum of 15 mm at a concentration of 300 mg/ml was observed with hexane extract.

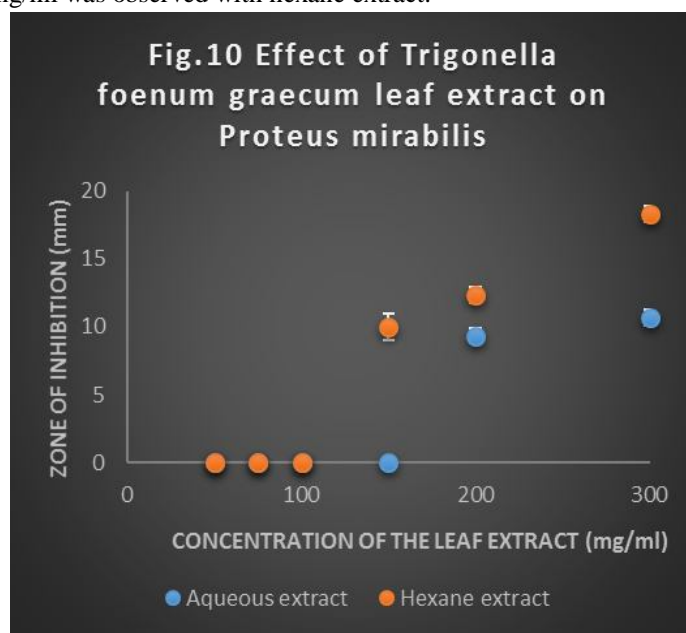
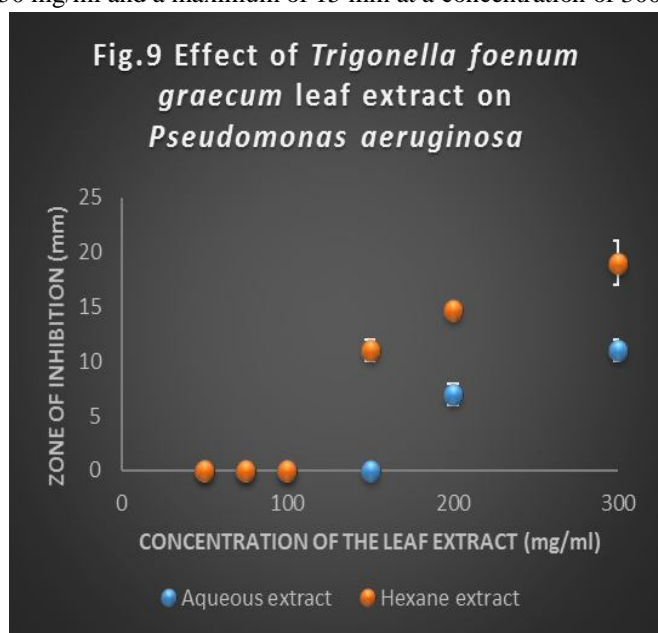


Fig. 9 indicates that *Pseudomonas aeruginosa* was resistant to concentration up to 150 mg/ml of aqueous extract and 100 mg/ml of hexane extract of *Trigonella foenum graecum* leaves. A minimum In hibitory zone of 7.0 ± 1.0 mm with aqueous extract at a concentration of 200 mg/ml and a maximum of 19.0 ± 2.0 mm with hexane extract at a concentration of 300 mg/ml was observed. *Proteus mirabilis* was also found to be resistant to aqueous extract up to a concentration of 150 mg/ml and up to 100 mg/ml with hexane extract (Fig.10). A minimum zone of inhibition was found to be 9.33 ± 0.56 mm with aqueous extract at a concentration of 200 mg/ml and a maximum of 18.33 ± 0.58 mm with hexane extract at a concentration of 300 mg/ml.

The Activity indices of the extracts were calculated and have shown a minimum of 0.19 for MRSA at a concentration of 100 mg/ml and a maximum of 0.59 for Staphylococcus aureus at a concentration of 300 mg/ml against Gram positive bacteria with aqueous extract (Table 1). For Gram negative bacteria, minimum AI was found to be 0.23 and maximum of 0.63 for Escherichia coli.

Table 1. Activity Index of Aqueous Extract

Concentration	50 mg/ml	75 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	Streptomycin (Control) Inhibitory zone (mm)
Bacterium							
Gram Positive Bacteria							
MRSA	-	-	0.19	0.23	0.26	0.47	26.0 ± 00
Staphylococcus aureus	-	-	0.37	0.41	0.51	0.59	21.0 ± 0.0
Gram Negative Bacteria							
Escherichia coli	-	0.23	0.29	0.40	0.50	0.63	30.00 ± 0.0
Klebsiella pneumoniae	-	-	0.27	0.33	0.40	0.50	30.00 ± 0.0
Salmonella paratyphi A	-	0.29	0.35	0.44	0.57	0.67	32.33 ± 0.58
Salmonella paratyphi B	-	0.31	0.31	0.41	0.52	0.58	32.67 ± 2.30
Shigella flexneri	-	-	-	-	-	-	19.00 ± 0.0
Citrobacter divergens	-	-	-	-	-	-	35.00 ± 0.0
Pseudomonas aeruginosa	-	-	-	-	0.30	0.48	23.00 ± 0.00
Proteus mirabilis	-	-	-	-	0.37	0.43	25.00 ± 0.00

The hexane extract has shown a minimum AI of 0.28 for MRSA and a maximum of 0.90 for Staphylococcus aureus with Gram positive bacteria, whereas, for Gram negative bacteria, a minimum of 0.20 for Escherichia coli and a maximum of 0.83 for Pseudomonas aeruginosa was observed (Table 2).

Table 2. Activity Index of Hexane Extract

Concentration	50 mg/ml	75 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	Streptomycin (Control) Inhibitory zone (mm)
Bacterium							
Gram Positive Bacteria							
MRSA	-	0.28	0.33	0.51	0.62	0.77	26.0 ± 00
Staphylococcus aureus	-	0.38	0.41	0.44	0.86	0.90	21.0 ± 0.0
Gram Negative Bacteria							
Escherichia coli	0.20	0.27	0.31	0.36	0.47	0.61	30.00 ± 0.0
Klebsiella pneumoniae	-	0.26	0.29	0.30	0.42	0.53	30.00 ± 0.0
Salmonella paratyphi A	-	-	0.23	0.31	0.38	0.57	32.33 ± 0.58
Salmonella paratyphi B	-	0.29	0.33	0.40	0.55	0.63	32.67 ± 2.30
Shigella flexneri	-	-	0.26	0.42	0.42	0.53	19.00 ± 0.0
Citrobacter divergens	-	-	-	0.22	0.29	0.43	35.00 ± 0.0
Pseudomonas aeruginosa	-	-	-	0.48	0.64	0.83	23.00 ± 0.00
Proteus mirabilis	-	-	-	0.40	0.49	0.80	25.00 ± 0.00

The Relative percentage inhibition of both the extracts were calculated which showed the maximum activity of 34.53 for Staphylococcus aureus and a minimum of 3.70 for MRSA with aqueous extract on Gram positive bacteria (Table 3). On the other hand, on Gram negative bacteria, RPI of this extract was found to be in the range of 5.44 for Escherichia coli at a concentration 75 mg/ml to 44.94 for Salmonella paratyphi A at a concentration of 300 mg/ml. The hexane extract has shown a minimum RPI of 4.44 for MRSA at a concentration of 75 mg/ml and a maximum of 80.25 for staphylococcus aureus at a concentration of 300 mg/ml with Gram positive bacteria (Table 4). For Gram negative bacteria, a minimum RPI was found to be 2.66 for Klebsiella pneumoniae at a concentration of 75 mg/ml and a maximum of 65.92 for Pseudomonas aeruginosa at a concentration of 300 mg/ml.

Table 3. Relative Percentage Inhibition of Aqueous Extract

Concentration	50 mg/ml	75 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	Inhibitory zone diameter (mm)	
							Streptomycin (Control)	Water (Solvent)
Bacterium								
Gram Positive Bacteria								
MRSA	-	-	3.70	5.33	6.60	22.53	26.0 ± 0.0	0.0 ± 0.0
Staphylococcus aureus	-	-	13.38	17.09	25.87	34.53	21.0 ± 00	0.0 ± 0.0
Gram Negative Bacteria								
Escherichia coli	-	5.44	8.37	15.16	25.0	40.11	30.00 ± 0.0	0.0 ± 0.0
Klebsiella pneumoniae	-	-	7.11	11.11	16.0	25.0	30.00 ± 0.0	0.0 ± 0.0
Salmonella paratyphi A	-	8.34	12.30	19.66	32.16	44.94	32.33 ± 0.58	0.0 ± 0.0
Salmonella paratyphi B	-	9.36	9.36	16.66	27.06	33.80	32.67 ± 2.30	0.0 ± 0.0
Shigella flexneri	-	-	-	-	-	-	19.00 ± 0.0	0.0 ± 0.0
Citrobacter divergens	-	-	-	-	-	-	35.00 ± 0.0	0.0 ± 0.0
Pseudomonas aeruginosa	-	-	-	-	9.26	22.87	23.00 ± 0.00	0.0 ± 0.0
Proteus mirabilis	-	-	-	-	13.97	18.25	25.00 ± 0.00	0.0 ± 0.0

Table 4. Relative Percentage Inhibition of Hexane Extract

Concentration	50 mg/ml	75 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	300 mg/ml	Inhibitory zone diameter (mm)	
							Streptomycin (Control)	Hexane (Solvent)
Gram Positive Bacteria								
MRSA	-	4.44	7.73	23.5	35.48	57.61	26.0 ± 0.0	5.0 ± 0.0
Staphylococcus aureus	-	6.91	9.71	12.65	71.11	80.25	21.0 ± 0.0	6.0 ± 0.0
Gram Negative Bacteria								
Escherichia coli	-3.35	0	2.78	5.99	15.79	32.54	30.00 ± 0.0	8.0 ± 0.0
Klebsiella pneumoniae	-	2.66	4.55	5.22	14.39	25.46	30.00 ± 0.0	6.0 ± 0.0
Salmonella paratyphi A	-	-	-0.51	3.67	8.99	27.74	32.33 ± 0.58	8.0 ± 0.0
Salmonella paratyphi B	-	4.96	7.56	12.89	27.91	37.95	32.67 ± 2.30	6.0 ± 0.0
Shigella flexneri	-	-	-7.69	4.81	4.81	16.35	19.00 ± 0.0	7.0 ± 0.0
Citrobacter divergens	-	-	-	-1.92	1.66	12.59	35.00 ± 0.0	9.0 ± 0.0
Pseudomonas aeruginosa	-	-	-	17.24	36.41	65.92	23.00 ± 0.00	6.0 ± 0.0
Proteus mirabilis	-	-	-	12.50	21.21	51.89	25.00 ± 0.00	5.0 ± 0.0

The results indicate that both extracts are exhibiting inhibitory effect on the clinical isolates at higher concentrations. Similar effect has been shown by the leaf extracts of *Trigonella foenum-graecum* on *Serratia marcescens* and *Bacillus cereus* [23]. The hexane extract has shown to be more potent compared to the aqueous extract which is in accordance with the previous studies where the hexane extract demonstrated good in vitro antimicrobial profile especially against MRSA, *S. faecalis*, *S. pyogenes*, *B. cereus* and *P. aeruginosa* which are known to cause infections that are extremely difficult to treat due to multiple drugs resistance [17].

IV. CONCLUSIONS

Trigonella foenum graecum was found to be a broad spectrum antibacterial agent which acts on both Gram positive and Gram negative bacteria. The hexane extract has shown to be more potent compared to the aqueous extract. The extracts are shown to be more promising for the treatment of infections caused by *Staphylococcus aureus* and *Salmonella paratyphi A*. The Gram negative bacterium *Citrobacter divergens* was found to be completely resistant to the aqueous extract and lower concentration of hexane extract, but sensitive to higher concentrations of the hexane extract. Similarly, *Pseudomonas aeruginosa* and *Proteus mirabilis* were also resistant to lower concentrations of both the extracts but became sensitive with increasing concentrations of the extracts. This indicates that the plant can be exploited for the treatment of various intestinal and hospital acquired infections.

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