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Trigonella emodi: A potential herb with antibacterial and antifungal activity

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ABSTRACT

The indiscriminate use of commercial antimicrobial drugs have led to the emergence of various multiple drug resistant (MDR) strains of pathogenic microbes, creating global health problems. Medicinal plants represent the vast untapped source for exploration of natural antimicrobial agents. The current study was performed with the aim to evaluate the antibacterial and antifungal potential of aqueous and methanolic extracts of *Trigonella emodi*, a traditionally used medicinal plant. Agar well diffusion method was used to perform antibacterial and antifungal assays. Bacterial strains employed for the study include *Staphylococcus aureus*; *Bacillus subtilis Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *escherichia coli* and the fungal strains employed include *Penicillium Chrysogenum*, *Aspergillus fumigatus*, *Candida albicans* and *Saccharomyces cerevisiae*. Methanolic extract was found to be more potent against the test pathogens. Among bacterial strains the most susceptible were *Bacillus subtilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and among fungal strains the most susceptible were *Candida albicans* and *Penicillium chrysogenum*. The highest antibacterial activity was shown by the methanolic extract against *Bacillus subtilis* (20mm) and the highest antifungal activity was exhibited by same extract against *Candida albicans* (24mm). The plant has promising potential to be used as a candidate for obtaining futuristic antimicrobial agents to combat bacterial and fungal infections.

Keywords: Antimicrobial resistance, Antibacterial and antifungal activity, phytochemicals.

I INTRODUCTION

The present healthcare system is badly hit by the growing menace of antimicrobial resistance. Many antibiotics are no longer effective against MDR microbes [1]. Common types of drug resistant bacteria include Methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), vancomycin resistant *Enteroococcus* (VRE) and multi-drug resistant *A baumannii* (MRAB). When antibiotics are used inappropriately, the target micro-organisms adapt and develop drug resistant genes, which pass on to their progeny through plasmid exchange, thereby leading to an elevated prevalence of multiple drug resistant infections [2]. The trend of obtaining

antibiotics from microorganisms has declined drastically over the last few decades. All the antibiotics that entered market during this period were the modifications of existing ones [3]. Moreover, the overuse of synthetic antibiotics is associated with serious side effects such as enterocolitis, hepatotoxicity and renal impairment. The current global programe may not be able to provide new effective antibiotics in next ten to twenty years [4]. Today, researchers all over the world are curiously searching for alternatives sources to develop future antimicrobials Medicinal plants with potential to combat microbial infections represent the best alternative [5,6]. Phytochemicals with antimicrobial properties are the emerging alternatives to conventional antibiotics [7,8]. The vast therapeutic potential of medicinal plants still remains undiscovered. Till now less than 1% of some 2.5 lakh higher plants have been screened for their phytochemistry or pharmacological effects [9]. The representatives of pharmaceutical industries are aware of huge therapeutic potential of medicinal plants and have started thorough screening for medicinal plants [10]. The current study was conducted with the objective to evaluate the antibacterial and antifungal potential of *Trigonella emodi*, an erect herb which belongs to the family

II MATERIALS AND METHODS

2.1. Collection and identification of plant material

The plant was collected from higher reaches of Kashmir Valley, India and identified in the Centre of Plant Taxonomy, Department of Botany, University of Kashmir, India. Specimen of the plant is retained in the KASH herbarium under a specific voucher specimen number 2055-KASH.

2.2. Preparation of extracts

Whole plant sample was allowed to shade dry at 30±2°C. The dried plant material was ground into coarse powder with the help of grinder and extracted using methanol and water as solvents, by using Soxhlet extractor (60-80°C). The extracts so obtained were concentrated with the help of rotary evaporator under reduced pressure and solid extract was stored in a refrigerator at 4°c.

2.3. Test micro-organisms

Six bacterial strains including two Gram positive bacteria namely *Staphylococcus aureus* (MTCC-2940), *Bacillus subtilis* (MTCC-441) *and four* Gram negative bacteria namely *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-139), *Escherichia coli* (MTCC-739), and *Pseudomonas aeruginosa* (MTCC-424) were employed for antibacterial assay. Four fungal strains, *Candida albicans* (MTCC-227), *Saccharomyces cerevisiae* (MTCC-170), *Aspergillus fumigatus* (MTCC-1811) and *Penicillium chrysogenum (*MTCC-947) were employed for antifungal assay. The Bacterial and fungal strains were obtained from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. Bacterial and fungal strains were maintained by

subculturing them on Mueller Hinton Agar (Himedia) and Sabouraud Dextrose Agar (Himedia) respectively after every fifteen days and then stored at 4ºC.

Gentamycin discs and Nystatin powder was obtained from EOS Laboratories, India and served as positive controls for antibacterial and antifungal assays respectively. 10%Dimethylsulfoxide (DMSO) was used as negative control.

2.4. Antibacterial Assay

Antibacterial assay was performed by Agar well diffusion method as described by Irshad et al [11] with some modifications. 100µl of standardized inoculum (0.5 Mc Farland) of each test bacterium was inoculated on molten Mueller Hinton Agar (Himedia), homogenised and then poured into sterile petri plates to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make uniform and equidistant wells into each petriplate. 100µl of each concentration (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) of plant extracts were loaded into different peripheral wells. Gentamycin (10µg/disc) disc was placed at the centre of each petriplate and served as positive control, while as 10%Dimethylsulfoxide served as negative control in a separate petri plate. The petri plates were then incubated at 37ºC for 18 to 24 hours in an incubator. The plates were then observed for the zones of inhibition. Antibacterial potential was evaluated by measuring the diameters of zones of inhibition in millimeters (mm) with the help of a standard measuring scale. The lowest concentration of the extract (between the range 10-100mg/ml) which does not permit the growth of test bacteria was considered as minimum inhibitory concentration (MIC).

2.5. Antifungal Assay

Antifungal assay was also performed by the method of agar well diffusion as described by Ahmad et al [12] with some modification 100µl of standardized inoculum (0.5 Mc Farland) of each test fungi were inoculated on sterile molten Sabouraud Dextrose Agar (Himedia), homogenised and poured into a sterile petri plate to yield a uniform depth of 4mm. The petriplates were allowed to solidify inside the laminar hood. Sterile cork borers of 5mm in diameter were used to make five wells at periphery and one well at centre of each petriplate. 100µl of each concentration (10mg/ml, 30mg/ml, 50mg/ml, 80mg/ml and 100mg/ml) of plant extract were loaded into five different peripheral wells. 100µl of Standard antibiotic Nystatin (0.5mg/ml) was loaded into the central well while as 10%Dimethylsulfoxide alone was used as negative control in a separate petri plate. The plates were then incubated at 32ºC for 24 to 36 hours. After incubation period, the plates were observed for the zones of inhibition. Antifungal potential was evaluated by measuring inhibition zone diameters in millimeters (mm) with the help of standard measuring scale. The lowest concentration of the extract (between the range 10-100mg/ml) which does not permit the growth of test fungi was considered as minimum inhibitory concentration (MIC).

2.6. Phytochemical Analysis

Preliminary phytochemical screening was done to detect the various types of phytochemicals present in the plant. Flavanoids were detected by lead acetate test while the rest of phytochemicals were detected by the methods described by Harborne [13].

2.6.1. Test for steroids

To 0.5 ml of solvent extract, 2ml of acetic acid was added and then 2ml of concentrated sulphuric acid (H_2SO_4) was added. Appearance of Blue or green colour was regarded as positive for the presence of steroidal compounds.

2.6.2. Test for tannins

To 5ml of solvent extract, two drops of 5% $Fecl_3$ were added. Formation of greenish precipitate confirmed the presence of tannins.

2.6.3. Test for terpenoids

To 5 ml of solvent extract, 2ml of chloroform was added and then 3ml of concentrated sulphuric acid (H_2SO_4) was added carefully. Appearance of reddish brown colouration at the interface confirmed the presence of terpenoids.

2.6.4. Test for flavonoids

To 2 ml of solvent extract, a few drops of lead acetate solution were added. Formation of yellow coloured precipitate confirmed the presence of flavonoids.

2.6.5. Test for alkaloids

To 2ml of solvent extract, a little amount of picric acid solution was added. Appearance of orange colour indicated the presence of alkaloids.

2.6.6. Test for saponins

About 1 ml of solvent extract was introduced into a tube containing 1ml of distilled water and the mixture was vigorously shaken for 2 to 3 minutes. Formation of froth confirmed the presence of saponins.

2.6.7. Test for anthraquinones

2ml of solvent extract was added to 10 ml of benzene, and then 0.5ml of ammonia solution was added. The mixture was shaken well. Appearance of violet colour in the layer phase confirmed the presence of anthraquinones.

2.6.8. Test for phenols

To 2 ml of solvent extract, 2ml of ferric chloride solution was added. Formation of deep bluish green solution confirmed the presence of phenols.

2.6.9. Test for cardiac glycosides

To 2ml of solvent extract, 2 ml of glacial acetic acid containing 1 drop of ferric chloride was added. Then 2ml of concentrated sulphuric acid (H_2SO_4) was added under layered. Formation of brown ring at the interface indicated the presence of cardiac glycoside.

2.6.10. Test for cardenolides

To 1 ml of solvent extract, 2ml of benzene was added. Formation of turbid brown colour confirmed the presence of cardenolides.

2.6.11. Test for phlobtannins

To 1 ml of solvent extract, few drops of 1% hydrochloric acid (HCl) were added and the mixture was boiled. Formation of red precipitate confirmed the presence of phlobtannins.

2.6.12. Test for volatile oils

To 2 ml of solvent extract, 0.1ml of sodium hydroxide and then small amount of diluted hydrochloric acid was added and shaken well. Formation of white precipitate indicated the presence of volatile oils.

III RESULTS

3.1. Phytochemical analysis

Phytochemical analysis revealed the presence of various secondary metabolites. Cardiac glycosides, flavonoids, phenols, steroids and tannins were detected in both the aqueous and methanolic extract. Saponins were detected only in aqueous extract while terpenoids were present only in methanolic extract. Alkaloids, anthraquinones, cardenolides, phlobtannins and volatile oils were found absent in both the extracts as shown in Table 1.

Table1: Preliminary phytochemical screening of aqueous and methanolic extracts

		Solvents	
S/N	Phytochemicals	Aqueous	Methanol
1	Alkaloids	-	-
2	Anthraquinones	-	-
3	Cardiac glycosides	+	+
4	Cardenolides	-	-
5	Flavonoids	+	+
6	Phenols	+	+
7	Phlobtannins	-	-
8	Saponins	+	-
9	Steroids	+	+
10	Tannins	+	+
11	Terpenoids	-	+
12	Volatile oils	-	-

NOTE: + (present), - (absent)

3.2. Antibacterial activity

As compared to aqueous extracts, methanolic extract showed maximum activity against all the tested bacterial strains with the zone of inhibition equal to 19mm against *Klebsiella pneumoniae* and pseudomonas aeruginosa, 15mm *Escherchia coli and Proteus vulgaris*, 17mm against *Staphylococcus aureus,* and 20mm against *Bacillus subtilis* at the concentration of 100mg/ml. The aqueous also showed considerable activity with the zones of inhibition equal to 14mm against *Klebsiella pneumoniae and Escherchia coli*, 12mm against *proteus vulgaris* and *Bacillus subtilis*, 16mm against *Staphylococcus aureus*, and 13mm against *Pseudomonas aeruginosa* at the concentration of 100mg/ml. 10%DMSO (negative control), showed no activity against any of the tested bacteriall strain. The results were compared to positive control (Gentamycin), which showed the zone of inhibition of 25 mm against *Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris* and *Pseudomonas aeruginosa,* 27mm against *Staphylococcus aureus* respectively and 20mm against *Escherichia coli* (Table 2, Figure 1).

Table 2: Zones of inhibition (in millimeter) of aqueous and methanolic extract of *Trigonella emodi* against bacterial strains

S/N	Bacterial strain	Solvent	Concentration of plant extract					Gentamycin (10µg/disc)
			10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml	
1	*Klebsiella pneumoniae*	Aqueous	-	11±0.27	12±0.13	13±0.31	14±0.02	25±0.32
		Methanol	11±0.25	12±0.26	14±0.35	17±0.38	19±0.23	
2	*Escherichia coli*	Aqueous	11±0.28	12±0.37	13±0.25	13±0.18	14±0.22	20±0.16
		Methanol	11±0.23	120.25	13±0.36	14±0.31	15±0.13	
3	*Proteus vulgaris*	Aqueous	-	9±0.11	10±0.26	10±0.23	12±0.34	25±0.32
		Methanol	-	10±0.28	12±0.27	13±0.21	15±0.13	
4	*Staphylococcus aureus*	Aqueous	-	12±0.13	13±0.22	15±0.24	16±0.78	27±1.29
		Methanol	10±0.30	13±0.29	14±0.34	15±0.28	17±0.04	
5	*Pseudomonas aeruginosa*	Aqueous	10±0.36	11±0.35	12±0.32	12±0.27	13±0.07	26±0.12
		Methanol	11±0.19	14±0.41	15±0.52	17±0.39	19±0.55	
6	*Bacillus subtilis*	Aqueous	-	10±0.16	11±0.36	11±0.31	12±0.34	25±0.32
		Methanol	12±0.37	14±0.30	16±0.19	18±0.20	20±0.98	

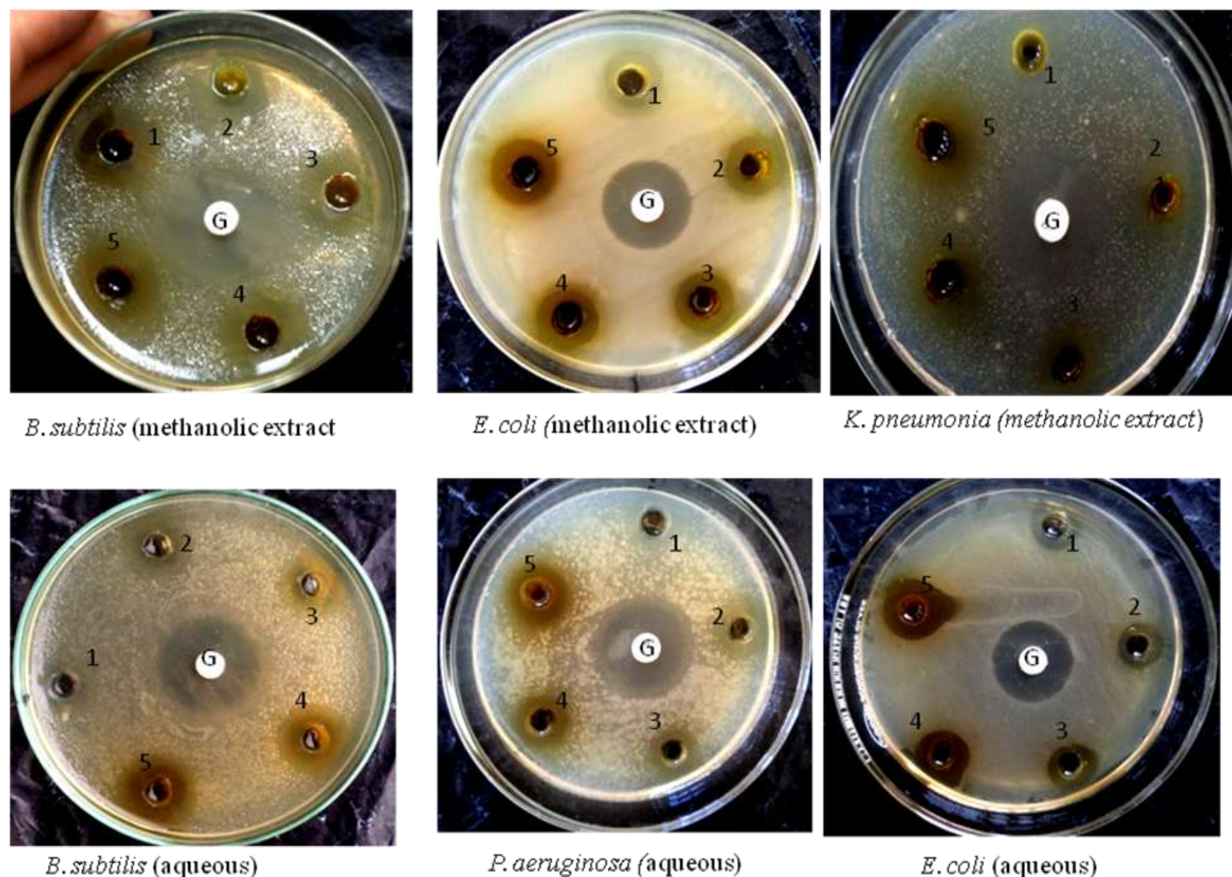

Fig. 1. Zones of inhibition of various methanolic and aqueous extracts of *Triginella emodi* against selected bacterial strains. 1, 2, 3, 4, 5 represent 10, 30, 50, 80 and 100 mg/ml concentrations of extract respectively. "G" represents Gentamycin (10µg/disc).

3.3. Antifungal activity

Methanolic extract showed the maximum antifungal activity against all the tested fungal strains with the zones of inhibition equal to 16mm against *Aspergillus fumigatus*, 24mm against *Candida albicans*, 18mm against *Penicillium chrysogenum*, and 19mm against *Saccharomyces cerevisiae* at the concentration of 100mg/ml. The aqueous extract showed the zone of 16mm against *candida albicans* and 15mm against *Aspergillus fumigatus, Penicillium chrysogenum and Saccharomyces cerevisiae, and* at the concentration of 100mg/ml. 10%DMSO (negative control), showed no activity against any of the tested fungal strain. The results were compared to positive control (Nystatin) which showed the zones of inhibition equal to 30mm against *Candida albicans* and *Saccharomyces cerevisiae,* 25mm against *Penicillium chrysogenum and* 27mm against *Aspergillus fumigates (*Table 3, Figure 2).

Table 3: Zones of inhibition (in millimeter) of aqueous and methanolic extract of *Trigonella emodis* against fungal Strains

S/N	Fungal strain	Solvent	Concentration of plant extract					Nystatin (0.5mg/ml)
			10mg/ml	30mg/ml	50mg/ml	80mg/ml	100mg/ml	
1	*Aspergillus fumigatus*	Aqueous	-	12±0.33	13±0.28	14±0.18	15±0.12	27±1.67
		Methanol	11±0.23	12±0.34	13±0.19	14±0.26	16±0.11	
2	*Candida albicans*	Aqueous	9±0.21	15±0.38	15±0.22	16±0.23	16±0.016	30±1.45
		Methanol	17±0.09	18±0.29	20±0.13	22±0.26	24±1.10	
3	*Penicillium chrysogenum*	Aqueous	-	11±0.16	12±0.14	13±0.32	15±0.12	25±1.09
		Methanol	12±0.28	13±0.10	15±0.39	16±0.29	18±0.98	
4	*Saccharomyces cerevisiae*	Aqueous	-	8±0.23	10±0.27	12±0.13	15±0.12	30±1.05
		Methanol	11±0.17	14±0.31	15±0.27	18±0.18	19±0.26	

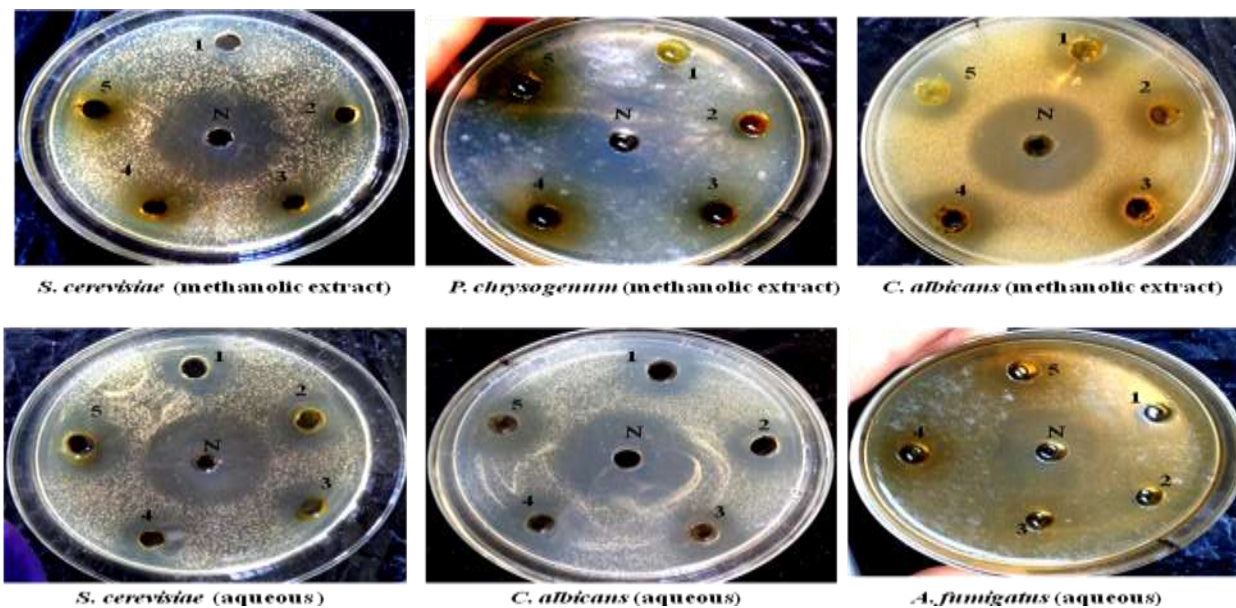

Fig. 2. Zones of inhibition of various methanolic and aqueous extracts of *Triginella emodi* against selected fungal strains. 1, 2, 3, 4, 5 represent 10, 30, 50, 80 and 100 mg/ml concentrations of extract respectively. "N" represents Nystatin (0.5mg/ml).

3.4. Minimum inhibitory concentration (MIC)

MIC of the aqueous extract was 30mg/ml against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus* *Bacillus subtilis*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Saccharomyces cerevissiae* but was undetected in case of *Pseudomonas aeruginosa*, *Escherichia.coli*, and *Candida albicans*. As far as methanolic extract is concerned, MIC was detected only in case of *Proteus vulgaris* (30mg/ml), but was undetected in rest test microbes as shown in Table 4.

Table 4: Minimum inhibitory concentration (MIC) of aqueous and methanolic extracts of *Trigonella emodi* against the tested bacterial and fungal strains.

S/N	Bacterial strain	Solvent	MIC (mg/ml)
1	*Klebsiella pneumoniae*	Aqueous	30
	(MTCC-139)	Methanol	ND
2	*Escherichia coli*	Aqueous	ND
	(MTCC-739)	Methanol	ND
3	*Proteus vulgaris*	Aqueous	30
	(MTCC-426)	Methanol	30
4	*Staphylococcus aureus*	Aqueous	30
	(MTCC-2490)	Methanol	ND
5	*Pseudomonas aeruginosa*	Aqueous	ND
	(MTCC-2940)	Methanol	ND
6	*Bacillus subtilis*	Aqueous	30
	(MTCC-441)	Methanol	ND
7	*Aspergillus fumigatus*	Aqueous	30
	(MTCC-1811)	Methanol	ND
8	*Candida albicans*	Aqueous	ND
	(MTCC-227)	Methanol	ND
9	*Penicillium chrysogenum*	Aqueous	30
	(MTCC-947)	Methanol	ND
10	*Saccharomyces cerevisiae*	Aqueous	30
	(MTCC-170)	Methanol	ND

ND= not detected within the observed range (10-100mg/ml)

IV DISCUSSION

Microbial infections have always been a serious threat to human health. The war between human beings and pathogenic microbes has taken a new turn due to the emergence of multiple resistant strains which are no longer affected by the old commercial antibiotics. Herbal medicine is the priceless gift of nature to man. In fact more than 80% of global population still depends on traditional herbal medicine for their healthcare needs [14]. Medicinal plants are rich in compounds with multiple therapeutic properties [15]. The present evaluated the antibacterial and antifungal potential of *Trigonella emodi.* Methanolic extract showed significant activity against the test microbial strains with prominent zones of inhibition against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans* and *Penicillium chrysogenum* in an almost dose dependent manner. Various studies done previously have also got maximum activity with methanolic extract [16-18]. The type of solvent used has an important role in determining the antimicrobial activity of an extract [19]. This could be due to the difference in the relative solubility of different phytochemicals in different solvents. The minimum inhibitory concentration of aqueous extract was found to be 30mg/ml against *Candida albicans* and most of the bacterial strains. The MIC of methanolic extract was detected only in case of *Proteus vulgaris* (i.e., 30mg/ml), which indicates the potency of the extract even at lower concentrations against most of the microbial strains. Analysis of Phytochemical composition of the plant shows the presence secondary metabolites such as of flavonoids, phenols, steroids, tannins, cardiac glycosides, saponins and terpenoids under study. Most of these active constituents have been reported for antimicrobial activity [20-21]. Therefore the plant may possess antimicrobial activity due to the presence of any of these bioactive constituents.

V CONCLUSION

This study reveals that *Trigonella emodi* contains some novel compounds which inhibited the growth of the test pathogens. The plant could prove an excellent candidate for obtaining future antibacterial or antifungal drugs. However, there is need of further study to isolate and characterize these novel compounds so as to understand their mechanism of action and effectiveness under in-vivo experimental conditions.

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