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## ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF *TRIGONELLA FOENUMGRAECUM* SEED EXTRACT USING DISK DIFFUSION METHOD

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### ABSTRACT

The pharmaceutical industry has produced a large number of new antibiotics over the last three decades, but resistance to these antibiotics by microorganisms has increased. The WHO estimates that medicinal plants are the primary medicine for 2/3 of the world's population. Today the recent studies focus on plant research all over the world to extract appropriate and efficient antimicrobial drugs. Fenugreek (*Trigonella foenumgraecum* Linn.) is an annual herb which has widely been consumed throughout the world as a food, a food additive and in the traditional remedies science civilizations. This study was aimed preliminary to investigate in vitro antimicrobial activity of fenugreek seeds against Gram-negative and Gram-positive bacteria such as Gram positive bacteria; *Bacillus subtilis*, *Bacillus cereus*, *Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli*, *Entrobacter*, *Klebsiella*, *Escherichia coli*. Using hydro methanol extraction. The antimicrobial activities were evaluated using agar disc diffusion method. The results indicated that the antimicrobial of *Trigonella foenumgraecum* Linn inhibit the growth of microorganism's as dose dependently manner. The appear results confirm that the antibacterial activity of *Trigonella foenumgraecum* seed extract in present test system.

### KEYWORDS

Fenugreek, Seeds, Antibiotics, Solvent extraction and Antimicrobial.

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### INTRODUCTON

Plants have formed the basis for traditional medicine systems in most societies and have been used for thousands of years in countries like China and India. Plant-based systems still play a vital role in health care, and WHO has estimated about 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care. The various medicinal and aromatic plants (whole or some parts) are known to be useful use for human benefit and welfare. In context of medicinal plants these

benefits, is the capacity of some plant seeds, fruits, leaves and other parts exerting pharmacological activities such as, analgesics, diuretics, antispasmodics, antimicrobial activity etc. *Trigonella foenumgraecum* is an annual herb which has widely been consumed throughout the world as a food, a food additive and in the traditional remedies science civilizations. Fenugreek, being rich in photochemicals, has traditionally been used as a food, forage and medicinal plant (Raghuram *et al*, 1994)<sup>1</sup>.

Active components of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components (Lai and Roy, 2004)<sup>2</sup>. Many contain of plants included lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, saponins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects (Michael *et al*, 2003)<sup>3</sup>. The active components as steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis. Locally, fenugreek seeds have traditionally and commonly been used to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis, to ease labor pains and menstruation pain, and as an appetite stimulant (Alkofahi *et al*, 1996)<sup>4</sup>. This plant has also been used as an external poultice to control inflammation and dandruff. Modern medicine is beginning to provide confirmation of many of the traditional medicinal applications of fenugreek seeds (Puri, 1998<sup>5</sup>, Billaud, 2001)<sup>6</sup>. To the best of our knowledge, no previous studies have been reported for antimicrobial activity of fenugreek using these bacterias. The present study was aimed to evaluate the antimicrobial activity of fenugreek seeds in order to be used in some infectious diseases.

## MATERIAL AND METHODS

### Plant Materials

The *Trigonella foenumgraecum* seed were collected from local market of Raipur, Chhattisgarh and dried

for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

### Extract Preparation

*Trigonella foenumgraecum* seed (20g) was extracted in 50% of methanol and Millipore water solvent the supernatant was collected and concentrated in water bath at 40-50 C .The dried powder was kept in air tied box.

### Microorganisms

The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis*, *Bacillus cereus*, *Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli*, *Entrobacter*, *Klebsiella*, *Escherichia coli*. These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

### Antibacterial Assay

Antibacterial activity of *Trigonella foenumgraecum* seed extract was determined by agar disk diffusion method (Nair *et al*, 2005)<sup>7</sup> at four concentrations i.e., 100, 75, 50 and 25mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus*, *Acetobacter*, *Citrobacter* and *Escherichia coli*). Discs of 6mm diameter were prepared from Whatmann filter paper No.24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX – Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX–Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime). Bacteria were used as standard. The plates were incubate at 37°C for 24hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

## RESULTS AND DISCUSSION

The microbial growth was considered as zero hour and further accordingly reading were taken our present day show the antibacterial activity of extract of *Trigonella foenumgraecum* against *E.coli* is best in 75% concentration after 12hrs of 12.2mm zone of inhibition although 100% concentration is having

13.7mm. In *Entrobacter* is best in 50% concentration of extract is 11.2 and in 100% concentration is 13.8mm in *Klebsiella* 25% concentration is having 11.2mm. and 75% concentration is having 11.08mm zone of inhibition. In *Bacillus subtilis* 75% and 50% concentration show good activity of zone of inhibition 12.2mm and 11.4mm. *Bacillus cereus* 100% concentration is 13.96mm and in 50% concentration is 12.2mm. In *Bacillus frimicutes* 50% concentration is having 12.6mm zone of inhibition and 25% concentration is having 12.2mm respectively.

The above observation suggest that different concentration (25%, 50%, 75%, 100%) were having good antibacterial activity against *E.coli*, *Bacillus subtilis*, *Entrobacter*, *Klebsiella*, *Bacillus aureus*, *Bacillus frimicutes* the development of antibiotic resistance. Thus the extract is showed in Table 5 varying activity against all organisms. On comparing the zone of inhibition of extract to that of standard antibiotics extract showed better than Ciprofloxan (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions.

Being annul herb plant it can be grown in any part of the word as it required moderate day length for its cultivation. It can be therapeutic drug for curing many step of disease as well as its extracted compounds can be used individually in drug designing and discovery. The consumption of fenugreek has provide safe and secure for human and may be simply implemented for health benefit as a dietary component, through its rich full fiber packaged and other bioactive component. We observed the number of antibacterial compounds present in extracts by bioautography and the correlation between cytotoxicity and potency against Gram-negative and Gram-positive bacteria. The result is also showed whether extracts with high activity against Gram-negative bacteria would have higher activity against other Gram-negative bacteria than against Gram-positive bacteria.

Our result for antibacterial activity of plant *Trigonella foenumgraecum* shows that antibacterial activity of methanolic extract of *Trigonella foenumgracum* against *E.coli* is best in 75% concentration after 12hrs of 12.2mm zone of inhibition .Although 100% concentration is having 13.7mm. In *Entrobacter* is best in 50% concentration

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The use of medicinal plants as antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. Medicinal plants have a long history of usage with low side effects (Karimi, Moradi, 2013)<sup>8</sup>. It can be reported that *Trigonella foeanumgraecum* could be a potential source of antibacterial agents so further studies should be conducted to identify the active constituents responsible for this activity.

## SUMMARY

Herbal medicines are the natural plants and their parts which are being used as medicinal purpose. This is one of the oldest types of medicine in human history (Mushiwokufa, 2016<sup>9</sup>, Leung 2016)<sup>10</sup>. The seeds of *Trigonella foenumgraecum* have been used as an orally as insulin substitute for reduction

in blood glucose, and the extracts from seed have been reported to lower blood glucose levels (Madar and Stark, 2002)<sup>11</sup>.

Antibacterial are used to treat bacterial infections. The drug toxicity to humans and other animals from antibacterial is generally considered low. (Depends) Prolonged use of certain antibacterial can decrease the number of gut flora, which may have a negative impact on health. Consumption of probiotics and reasonable eating can help to replace destroyed gut flora. Stool transplants may be considered for patients who are having difficulty recovering from prolonged antibiotic treatment, as for recurrent *Clostridium difficile* infections. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Shaik D 1994)<sup>12</sup>.

The above observation suggest that different concentration (25%, 50%, 75%, 100%) were having good antibacterial activity against *E.coli*, *Bacillus subtilis*, *Entrobacter*, *Klebsiella*, *Bacillus aureus*, *Bacillus frimicutes*. the development of antibiotic resistance. Thus the extract is showed varying activity against all organisms. The development of antibiotic resistance has become a global public health challenge which is causing in effectiveness of antibacterial agents leading to increase in diseases and death rate. Therefore this study intends to investigate the antibacterial action of *Trigonella foeanumgraecum* (fenugreek) extract against multidrug pathogens.

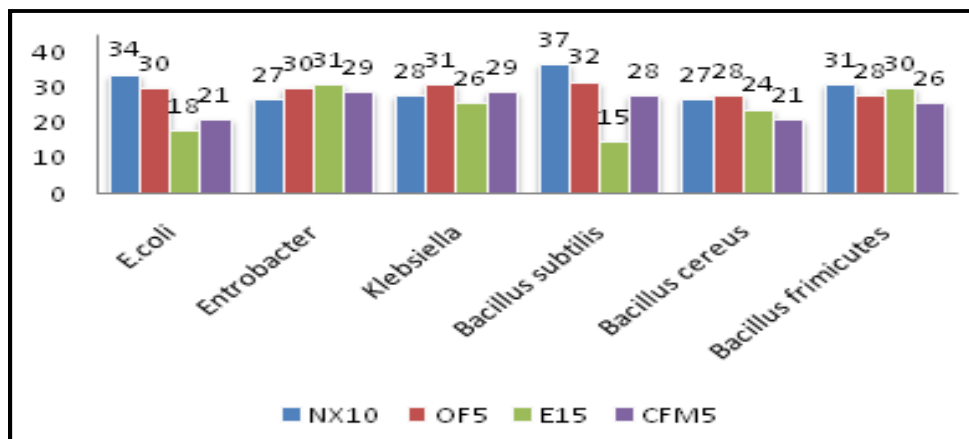
*Trigonella foenumgraecum* is a rich source of vitamin B1, iron, silicon, sodium, saponins, dietary fibers, protein, amino acids and fatty acid contents (Sharma, 1990)<sup>13</sup>. *Trigonella foenumgraecum* contains phenolic and flavonoids compounds which help to enhance its antioxidants capacity. (Dixit P, Ghaskadbi S, 2005)<sup>14</sup>. The hepatic lipids in body because of its potential to modify the activities of several enzymes such as enzymes related to glucose and lipid metabolism (Madar Z, Shomer IJ 1990)<sup>15</sup>. Scientists have reported several medicinal uses of *Trigonella foenumgraecum* seeds such as remedies for diabetes and hypercholesterolemia, hepatoprotective protection against free radicals, and protection against breast and colon cancer (M. M. Al-Oqail 2013)<sup>16</sup>. These protective roles are possible due to the nonnutritive secondary metabolites also known as phytochemicals. The major constituents that are present in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fibers, saponins, steroidal saponins, vitamins, and minerals, nitrogen compounds which can be categorized under nonvolatile and volatile constituents. So, in future it can be used as an alternate to synthetic antibiotics. Much effort has needed to increase plant extract as a dietary supplement in food to resist the human pathogenic bacterial disease. This work has revealed further potentials of this plant in the area antimicrobial agent. As a result of the high antimicrobial activity, the extract of *Trigonella foeanumgraecum* would be considered a safe antimicrobial agent.

**Table No.1: The study of antibacterial activities of *Trigonella foenumgraecum* seed extract using Disk Diffusion method (Mean ± SE)**

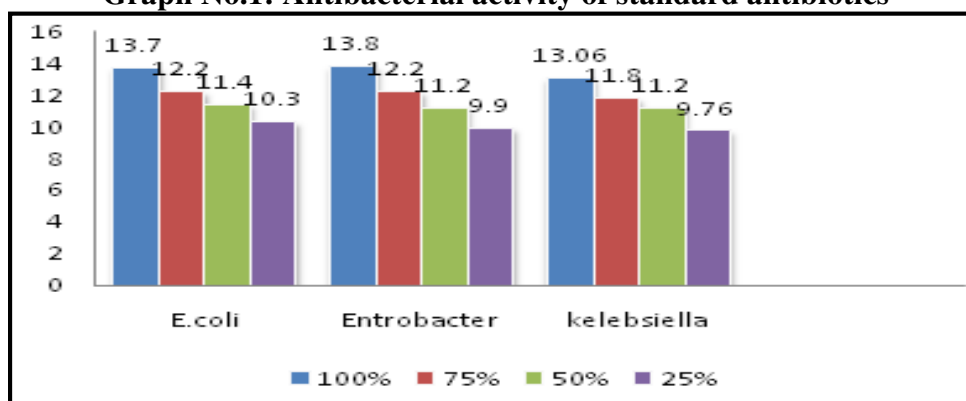
S.No	Bacterial Stain	Bacteria Use	Zone of Inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram Negative (-)	<i>E. coli</i>	13.7±0.20	12.2±0.24	11.4±0.069	10.3±0.40
		<i>Entrobacter</i>	13.8 ± 0.31	12.2 ± 0.40	11.2±0.23	9.96±0.341
		<i>Klebsiella</i>	13.06±0.651	11.8±0.138	11.2±0.346	9.76±0.603
2.	Gram Positive (+)	<i>Bacillus subtilis</i>	13.4 ± 0.705	12.2 ± 0.245	11.4 ± 0.173	10.4±0.705
		<i>Bacillus cereus</i>	13.96 ± 0.23	12.3 ± 0.473	12.2 ± 0.578	10.4 ± 0.346
		<i>Bacillus frimicutes</i>	13.3 ± 4.815	12.6 ± 0.23	12.2 ± 0.670	9.9 ± 0.346

**Table No.2: The study of antibacterial activities of standard antibiotics using Disk Diffusion method**

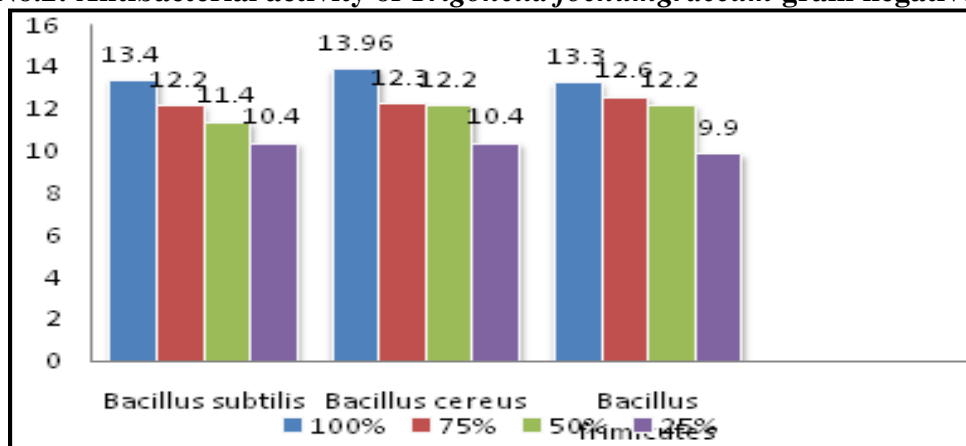
S.No	Bacterial Stain	Bacteria Use	Zone of Inhibition (In MM)			
			NX10	OF5	E15	CFM5
1	Gram Negative (-)	E. coli	34.00	30.00	18.00	21.00
		Entrobacter	27.00	30.00	31.00	29.00
		Klebsiella	28.00	31.00	26.00	29.00
2	Gram Positive (+)	Bacillus subtilis	37.00	32.00	15.00	28.00
		Bacillus cereus	27.00	28.00	24.00	21.00
		Bacillus frimicutes	31.00	28.00	30.00	26.00



**Graph No.1: Antibacterial activity of standard antibiotics**



**Graph No.2: Antibacterial activity of *Trigonella foenumgraecum* gram negative bacteria**



**Graph No.3: Antibacterial activity of *Trigonella foenumgraecum* gram positive bacteria**

## CONCLUSION

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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