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Antimicrobial Activity of *Trigonella foenum-graecum* L. (Fenugreek)

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Abstract

Traditional uses of plants have led to investigating their bioactive compounds which had resulted in the detection of a significant number of therapeutic properties. *Trigonella foenum-graecum* L. is believed to be native to the Mediterranean region or Asia; it is one of the most ancient medicinal herbs. Fenugreek contains different alkaloids, flavonoids and saponins. In present studies the antimicrobial activity of Fenugreek leaves, seeds and stem extract (Methanol, Acetone and aqueous extract) against *E. coli* and *Staphylococcus* were determined by the well diffusion method. The maximum zone of inhibition was given by methanol i.e. 20 mm and 19 mm against *E. coli* and *Staphylococcus* respectively, followed by Acetone extract which give the equal zone of inhibition for both organism i.e. 16 mm while the aqueous extract shows nil zone of inhibition. Thus from bacteriological point of view Fenugreek leaves and stem appears to play a great role in clinical as well as antibacterial agents.

Keywords: Fenugreek; Antibacterial; *E. coli*; *Staphylococcus*; Extract

Introduction

The nature had been a source of medicinal plants for thousands of years since the beginning of man, over the past 20 years; interest in medical plants has grown enormously from the use of herbal products as natural cosmetics and self-medication by the general public for their biological effects. According to the WHO more than 80% of the world's population relies on plant based herbal medicines for their primary health care needs [1].

Presently there has been an amplified interest worldwide to identify antioxidants compounds which are pharmacologically effective or have low or no side effects for use in preventive medicines and food industries [2]. Herbs and spices been extensively used as food additives for natural antioxidants. *Trigonella foenum-graecum* L. commonly known as Fenugreek, belongs to the family of Fabaceae. It is the most promising medicinal herb known from ancient time.

Its seeds and green leaves are used in food as well as in medicinal application it provides natural food fiber and other nutrient required in human body [3]. This also helps in nitrogen fixation and soil enrichment [4].

Fenugreek leaves has been found to have ascorbic acid of 220.97 mg/100 g leaves and β - carotene of 19 mg/100 ml of leaves. It also contains fiber and high amount of calcium, zinc and iron. Seeds of fenugreek have maple flavor and bitter taste but by the process of roasting, their bitterness can be reduced and flavor can be enhanced. Seeds contain volatile oil and fixed oil in small quantities [5]. These are rich source of soluble dietary fibers contain [6]. Fenugreek contains saponins, hemicelluloses mucilage, tannins and pectin and these compounds help to decrease the level of low density lipoprotein cholesterol (LDL) in blood by inhibiting bile salts re- absorption in the colon. Fenugreek seeds are rich in proteins such as globulin, histidine, albumin and lecithin [7].

Fenugreek is a promising protective medicinal herb in cancer patients under chemotherapeutic intervention because fenugreek extract shows a protective effect by modifying the cyclophosphamide induced apoptosis and free radical mediated lipid peroxidation in the urinary bladder of mice [8].

Diosgenin (C₂₇ H₄₂ O₄₃) is a crystalline steroid saponins found in fenugreek and used as a starting material for the synthesis of steroid hormones such as cartison and progesterone, it has been found to be potentially in treatment of cancer [9]. Fenugreek seeds have hypoglycaemic and hypocholesterolemic effect it improves peripheral glucose utilization, contributing to improvement in glucose tolerance and exerts hypoglycemic effect by acting at the insulin receptor level as well as at the gastrointestinal level [10].

Fenugreek contains phenolic and flavonoids compounds which help to enhance its antioxidants capacity [11]. It also have an ability to lower 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 [12]. Based on several medicinal past reported scientific findings Fenugreek can be various health benefits can be drawn from this natural herb but in some extreme cases like patients suffering from chronic asthma, etc., it should avoided or its consumption should be minimized.

Materials and Methods

Collection of sample

For the isolation of bacteria spoiled cabbage and fresh plants and for antimicrobial activity seeds of *Trigonella foenum-graecum* were collected from the local market of Haridwar, India.

Isolation and identification of bacteria

Desired bacteria were isolated from spoiled cabbage by serial dilution method. Selective media such as Mac Conkey, EMB and Mannitol salt agar media were used for the isolation of *E. coli* and *Staphylococcus*. Identification was carried out on the bases of morphological and biochemical characteristic through gram staining, catalase test, indole production test, methyl red test, voges- proskauer test, starch hydrolysis, sugar fermentation.

Determination of bacterial growth

The bacterial were inoculated in the broth and incubated in the shaking incubator at 120 rpm and then determine the optical density of nutrient broth at 640 nm after every 2 hrs [13].

Preparation of extract of fenugreek plant material

The aqueous and methanol/acetone extraction are prepared of fenugreek plant material for which, weighed 15 gm of leaves, seeds and stems powdered, add 250 ml of sterile distilled water to it, kept the mixture for 7 days and filtered it with muslin cloth, filtrate was allowed for hot extraction process on water bath at 40°C.

Antimicrobial activity of fenugreek plants

The antimicrobial activity of fenugreek plants was determined against the *E. coli* and *Staphylococcus* by the well diffusion method. Well diffusion method was measured the inhibition zone to know the antimicrobial activity of fenugreek.

Results and Discussion

Enumeration of microorganism

Total bacterial colonies were enumerated from the vegetable sample was calculated as CFU on Mac Conkey as well as MSA.

Table 1 CFU/ml (on Mac conkey).

S.no	Dilution	CFU ± SE
1.	10 ⁻³	15.67 ± 0.33
2.	10 ⁻⁴	9.67 ± 0.33
3.	10 ⁻⁵	5 ± 0.58
4.	10 ⁻⁶	0.67 ± 0.66

Maximum number of bacteria found in 10⁻³ on Mac Conkey agar and MSA that is 15.67 ± 0.33 and 17 ± 0.58 respectively

(Tables 1 and 2). These isolates was identified on the bases of Organisms are characterized on morphological and biochemical characterization (Table 3).

Table 2 CFU/ml on MSA plates.

S.no	Dilution	CFU ± SE
1.	10 ⁻³	17 ± 0.58
2.	10 ⁻⁴	13 ± 1.16
3.	10 ⁻⁵	8 ± 0.58
4.	10 ⁻⁶	5 ± 0.58

Table 3 Morphological characterization and biochemical characterization.

S no.	Characteristics	<i>E. coli</i>	<i>Staphylococcus</i>
1	Colour	Pinkish, metallic sheen colony	Yellow halo zone around colonies
2	Cell shape	bacilli	Cocci
3	Cell arrangement	single	Irregular cluster
4	Gram's reaction	-ve	+ve
5	Catalase test	+ve	+ve
6	Fermentation	Acid+gas	Acid
7	Starch hydrolysis	-ve	-ve
8	Indole	+ve	-ve
9	Citrate utilization	-ve	-ve
10	MR	+ve	+ve
11	VP	-ve	+ve

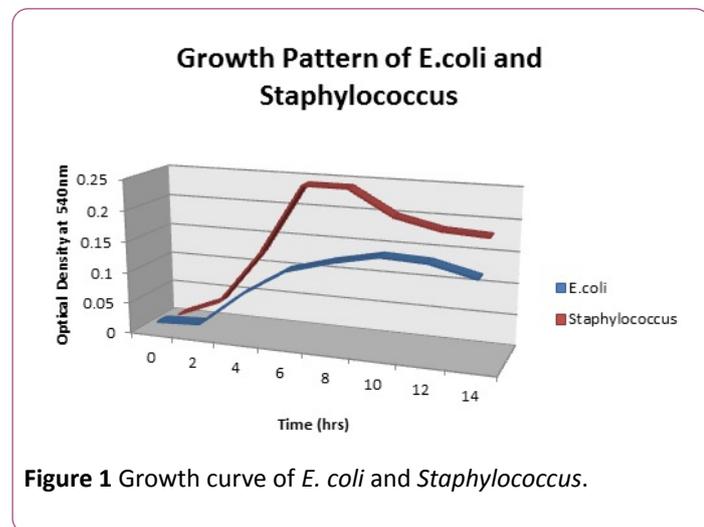
Table 4 Showing OD of bacterial isolate at 540 nm.

S no.	Time (hrs)	Isolates	
		<i>E. coli</i>	<i>Staphylococcus</i>
1	0	0.016	0.007
2	2	0.020	0.036
3	4	0.076	0.124
4	6	0.120	0.240
5	8	0.140	0.240
6	10	0.155	0.200
7	12	0.152	0.184
8	14	0.135	0.180

After Gram staining and biochemical configuration test the bacteria as Gram negative bacilli (*E. coli*) and Gram positive cocci in bunches (*Staphylococcus*) which was isolated from spoiled cabbage.

Growth curve

The growth pattern of the isolates was observed by using nutrient broth after every 2 hrs of interval, the following growth pattern was found (Table 4 and Figure 1).



Antimicrobial activity of multidrug

During the study of antibacterial activity of different antibiotics on *Staphylococcus* out of 12 antibiotics ciprofloxacin and Linezolid give maximum zone of inhibition and in the case of *E. coli*, Amikacin give maximum zone of inhibition (Tables 5 and 6).

Table 5 Antibiotics sensitivity against *Staphylococcus* spp.

S.no	Antibiotics	code	zone of inhibition (mm) R/S
1.	Ciprofloxacin	CP	21S
2.	Cephalexin	PR	16S
3.	Linezolid	LZ	21S
4.	Levofloxacin	QB	18S
5.	Roxythromycin	AT	16S
6.	Lincomycin	LM	15S
7.	Tetracycline	TE	15S
8.	Co Trimaxazole	BA	14S
9.	Ampicillin	AS	10R
10.	Cloxacillin	CX	12S
11.	Gentamicin	GM	16S
12.	Cefotaxime	CF	9S

Antibacterial activity of Trigonella extract

The result of antibacterial activity of Acetone, Methanol and Aqueous extract of *Trigonella*'s leaves, stem and seeds by agar well diffusion method. Maximum inhibition was observed in

methanol extract of leaves shown in Table 7 which is more than the result observed by Premananth et al. in 2011 [14].

Table 6 Antibiotics sensitivity against *E. coli*.

S.no	Antibiotics	Code	Zone of inhibition (mm) R/S
1.	Amikacin	AK	15S
2.	Cefitaxime	CF	14S
3.	Gatifloxacin	GF	14S
4.	Gentamicin	GM	13S
5.	Levofloxacin	QB	13S
6.	Sparfloxacin	SF	13S
7.	Ceftriaxone	CF	12S
8.	Tobramycin	TB	12S
9.	Amphicillin	AS	11R
10.	Cefadroxil	CD	8R
11.	Olfloxacin	OF	7R
12.	Ciprofloxacin	RC	5R
13.	Cefixime	CX	0R
14.	Cefazolin	CZ	0R
15.	Norfloxaicn	NF	0R

Table 7 Antibacterial activity of aqueous extract of fenugreek at 100 µl.

S.no	Plant's Part	Zone of inhibition (mm)	
Aqueous extract		<i>E. coli</i>	<i>Staphylococcus</i>
1	Leaves	4	2
2	Seeds	5	5
3	Stem	2	-
Methanol extract		<i>E. coli</i>	<i>Staphylococcus</i>
1	Leaves	20	19
2	Seeds	-	-
3	Stem	13	11
Acetone extract		<i>E. coli</i>	<i>Staphylococcus</i>
1	Leaves	16	16
2	Seeds	-	-
3	Stem	17	17

Conclusion

The present study carried out to determine the antibacterial activity of Fenugreek's leaves, seeds and stem in aqueous, methanol and acetone extract against *E. coli* and *Staphylococcus* isolated from to be spoiled cabbage which is mostly available at very low cost and widely used by the local street food corners. In further study Methanol extract exhibit the highest zone of

inhibition as compared to the aqueous extract. In comparison of all the extracts the leaves extracts was found to be maximum as compare to the extracts of seeds and stems. The results were obtained during this experiment were highly effective and comparable with the commercial antibiotic at 100 µl concentration of Fenugreek extract. We conclude that out of three solvent extract (Methanol, Acetone and aqueous) the order of antibacterial activities of solvent extract of Fenugreek is Methanol>Acetone>Aqueous.

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