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Phytochemical and Antibacterial studies of Fenugreek *Trigonella foenum-graecum* L.-A Multipurpose Medicinal Plant.

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family Fabaceae and it is small annual herb. The Fenugreek seed contains a number of medicinally important compounds such as volatile oils, alkaloids, flavonoids, saponins, fatty acids and rich source of polysaccharide galactomannan. In the present study, we evaluated the phytochemical analysis for the presence of various secondary metabolites and antibacterial activity of the seed extracts of *Trigonella foenum-graecum* L. against pathogenic bacteria like gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia*) bacteria by *in vitro* agar well diffusion method. The Ethanol seed extracts of fenugreek showed pronounced inhibition than Chloroform, Water, benzene and Acetone extracts. Seed extracts showed more inhibitory action on *Klebsiella pneumonia* and *Pseudomonas aeruginosa* than *Escherichia coli*, *Staphylococcus aureus* and *salmonella typhii*.

Key words: *Trigonella foenum-graecum* L., antibacterial activity, seed extracts, Zone of inhibition.

INTRODUCTION

Higher and aromatic plants have been used traditionally in folk medicines as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts[1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases[2]. Fenugreek (*Trigonella foenum-graecum* L.) is one of the world's oldest medicinal herbs belongs to the family Fabaceae. The fenugreek seeds are rich in dietary fiber, that it can lower blood sugar levels in diabetes. Fenugreek seed is widely used as a galactagogue that is often used to increase milk supply in lactating women and cure breast cancer[3]. Fenugreek seed is useful for tuberculosis, diabetes, atherosclerosis, constipation, highcholesterol, hypertriglyceridemia and externally it is used as a poultice for abscesses, boils, carbuncles, etc[4]. Insulin is used to replace fat and reduce the calories of food. It is suitable for consumption by diabetes[5]. The seeds of the fenugreek herb possess toxic oils, and other bioactive constituents of the fenugreek seed include volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi[6]. Recent pharmocological investigation of the seed extract of this plant revealed anticancer properties[7, 8]. The antifungal activity of fenugreek was also reported[9]. Based on the studies carried out in fenugreek, world wide report shows that the seeds of this plant possess strong antibacterial activity[10]. However, to the best of our knowledge, very few reports are available on antibacterial properties of fenugreek seed against the important human pathogenic bacteria so far. In the present study we established antimicrobial activity of fenugreek against pathogenic bacteria. The study confirms that organic solvent seed extracts possess strong antibacterial properties against various pathogens Viz., *Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, E.coli,* and *Staphylococcus aureus.*

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MATERIALS AND METHODS

I. Preparation of seed extracts:

Apparently healthy plant seeds were collected, washed thoroughly in tap water and dried in room temperature for 20 days**.** The dried 25 g seed was powdered and soaked separately in 100 ml Chloroform, Acetone, Ethanol, Benzene, and Water by keeping it in a shaker for 3 days. The extracts were filtered into a petri plate using Whatman filter paper. It is set for evaporation. The dried sample is evaluated for its antimicrobial activity.

II. Phytochemical screening of Fenugreek seed extracts

The phytochemical components of the fenugreek seed extracts were screened by using the methods of Brindha et al.,[11] and Harbone [12]. The components analysed were alkaloids, volatile oils, fatty acids, emodins, flavonoids, triterpenoids, antharacene glycosides, tannins, phenolics and sponins.

III. Seperation of the compounds

The compounds present in the fenugreek seed extracts were qualitatively analysed by using thin layer chromatography, for which commercially available sheets were used, TLC aluminium sheets with silica gel $60F_{354}$ were used. The isolation and seperation of monoterpenes and sesquiterpenes was done by using the procedure of Brindha et al.,[11] and Janusz Malarz[13].

IV. Inoculums

The test microorganisms gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aerogenosa*, *Klebsiella pneumonia*) bacteria were obtained from culture repository of best biotech culture collection, Bangalore, India. The organisms were inoculated onto NB (Nutrient Broth), (0.5% Peptone, 0.5% Sodium chloride, 0.15% Yeast extract; pH 7.4) and incubated at 37° C for overnight. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed

was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10^5 CFUml^{-1} before use[14].

V. Determination of antibacterial activity:

The antibacterial activity of the seed extracts was determined using agar well diffusion method following published procedure with slight modification[15]. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8 mm diameter) were punched in the agar and filled seed extracts. Control wells containing solvents (negative control) in the plate[16]. The plates were incubated at 37°C for 18h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of standard drug chloromphenicol.

VI. Statistical analysis

The resultant clear zones around the discs were measured in mm. The antibacterial activity of seed extracts were indicated by clear zones of growth inhibition. Data of three independent experiments represented by five replicates from each experiment were subjected to statistical analysis (Mean±SE), according to New Ducan's Multiple Range Test[17].

RESULTS AND DISCUSSION

Phytochemical screening of fenugreek seed extracts:

The preliminary phytochemical screening of the fenugreek seed extracts using different solvents was reported (Table 1). All the four organic solvents such as ethanol, chloroform, acetone and benzene showed positive result for the presence of volatile oils and fatty acids which were absent in the water extract. In the water extract, flavonoids and tannins were present which were absent in the organic solvent extracts.

Table 1: Preliminary phytochemical analysis of fenugreek seed extracted with different solvents.

Sl. No	Name of the compound	B	C	A	E	W
1	Alkaloids	-	-	-	+	+
2	Volatile oils	+	+	+	+	-
3	Fattyacids	+	+	+	+	-
4	Emodins	-	-	-	-	-
5	Flavonoids	-	-	-	-	+
6	Triterpenoids	-	-	+	-	-
7	Antharacene glycosides	-	-	-	-	-
8	Tannins	-	-	-	-	+
9	Phenolics	-	-	-	-	-
10	Saponins	+	+	-	+	+

+ Present, - Absent, A-Acetone, B-Benzene, C-Chloroform, E-Ethanol, W-Water.

Antibacterial activity of different solvent extracts:

The antibacterial activity of the fenugreek seeds was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated (Table 2 & 3). The results showed that the organic solvent extracts possessed strong antibacterial activity. In Ethanol and Acetone the highest antibacterial activity was retained in 50µl and 100µl concentration of seed extracts. We found that ethanol and acetone extracts of seeds were successful in killing the bacteria in a dose dependent manner. At 50µl concentration, the ethanol extract showed pronounced inhibition against all the tested organisms, the maximum inhibition was observed against *Pseudomonas aerogenosa* (9.9±0.23 mm), *Klebsiella pneumonia* (9.4±0.23 mm) *and Staphylococcus aureus* (7.3±0.30 mm) and moderate inhibition was observed against *E.coli* (6.1±0.14 mm) and *Salmonella typhi* (7.2±0.29 mm). The other solvent extracts did not actively inhibit the growth of the bacteria at 50 µl concentration except acetone against *Pseudomonas aerogenosa* (8.4±0.28 mm) (Table 2). The growth of *Pseudomonas aerogenosa* was inhibited by all the seed extracts at 100 µl concentration and maximum inhibition was observed with ethanol extract as (16.3±0.35 mm) zone of inhibition, which was higher than the zone of inhibition caused by the standard drug chloromphenicol (14.0±0.37 mm). Similarly ethanol seed extract produced maximum inhibition (15.1±0.16 mm) to the growth of *Klebsiella pneumonia* than chloromphenicol (13.5±0.18 mm) where as other extracts showed less inhibitory activity than chloromphenicol. *Pseudomonas aerogenosa* was inhibited by all the seed extracts and the maximum inhibition was observed with ethanol, acetone and chloroform where as benzene and water extract showed least inhibition at 100µl concentration (Table 3). We found that organic extracts of the seeds were successfully in inhibiting the bacteria in a dose dependent manner. Besides the 50µl concentration of seed extracts, the 100µl concentration of seed extracts was found to possess maximum inhibition (Table 2 & 3).

Table 2: Antimicrobial activity of seed extract of fenugreek (*Trigonella foenum-graecum* L.) 50µl concentration against various microorganisms.

Extract	Zone of inhibition in (mm)(Mean ± SE)				
	P.aerogenosa	*K.pneumonia*	*E.coli*	*S.typhi*	*S.aureus*
Water	6.3±0.17	5.2±0.21	3.3±0.13	4.0±0.41	3.2±0.31
Chloroform	6.2±0.25	3.4±0.23	2.4±0.28	1.6±0.28	4.2±0.32
Acetone	8.4±0.28	2.3±0.27	6.4±0.30	4.3±0.41	3.2±0.25
Benzene	4.2±0.25	3.4±0.23	4.2±0.23	2.3±0.35	3.0±0.33
Ethanol	9.9±0.23	9.4±0.23	6.1±0.14	7.2±0.29	7.3±0.30
Chloromphenicol	8.3±0.23	8.1±0.23	6.0±0.14	7.1±0.29	7.0±0.30

The negative control control wells were exposed with the neat solvent and the positive control was chloromphenicol (50 µg ml^{-1}). Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments.

Table 3: Antimicrobial activity of seed extract of fenugreek (*Trigonella foenum-graecum* L.) 100µl concentration against various microorganisms

Extract	Zone of inhibition in (mm) (Mean ± SD)				
	P.aerogenosa	*K.pneumonia*	*E.coli*	*S.typhi*	*S.aureus*
Water	9.2±0.16	13.2±0.39	5.3±0.33	7.3±0.33	6.45±0.32
Chloroform	11.4±0.24	10.2±0.21	4.5±0.32	3.4±0.30	7.1±0.31
Acetone	14.0±0.20	9.2±0.17	5.4±0.29	6.2±0.26	7.4±0.30
Benzene	9.4±0.28	7.3±0.27	6.1±0.56	5.08±0.23	5.26±0.28
Ethanol	16.3±0.35	15.1±0.16	11.2±0.30	13.2±0.33	13.9±0.25
Chloromphenicol	14.8±0.37	13.5±0.18	10.1±0.33	12.2±0.37	13.0±0.23

The negative control wells were exposed with the neat solvent and the positive control was chloromphenicol (50 µg ml^{-1}). Each value represents the mean±standard error (SE) of five replicates per treatment in three repeated experiments

We used the organic solvents for the extraction from seeds of Fenugreek plant. Fenugreek seed is reported to have anti-microbial activity. The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. The seed extracts of fenugreek showed significant activity against all the bacteria tested. Similar to our result, the biological activity of *Mentha piperita* against the pathogenic bacteria were reported[18]. Based on that we used five different solvent extracts of seeds of fenugreek showed activity against all bacteria at all dosages. The seed extracts of fenugreek exhibited antibacterial activity only in water, chloroform, acetone, benzene and ethanol against the bacteria tested in agar well diffusion method at 50µl and 100µl concentration by the following method of[16]. We observed maximum activity at 100µl concentration against *Pseudomonas aerogenosa* than *Staphylococcus aureus, Klebsiella pneumonia, Salmonella*

typhi and E.coli. The present work was similar to[18] a study which showed that the compounds from fenugreek possess potent antimicrobial activity and suggesting that the fenugreek seed extracts contains the effective active constituents responsible for eliminating the bacterial pathogens. Finally it can be concluded that the active chemical compounds present in fenugreek should certainly find place in treatment of various bacterial infections. The results from the present study are very encouraging and indicate this herb should be studied more extensively to explore its potential in the treatment of many infectious diseases.

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