

Evaluating the Antibacterial Activity of Fenugreek (*Trigonella foenum-graecum*) Seed Extract against A Selection of Different Pathogenic Bacteria

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In the present study, Fenugreek plants obtained from two different cultivars, one from Saudi Arabia and another from Yemen, were screened for phytochemical active constituents and investigated for antimicrobial properties against a selection of gram-positive and gram-negative pathogenic bacteria. Five different solvent seed extracts from each cultivar were tested. The results of the study demonstrated that the chloroform and methanolic extracts possessed significant antibacterial activity against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* ATCC14027 and *Klebsiella pneumonia* ATCC700603. The antimicrobial activity of the extracts was investigated using the agar well diffusion method. The results of the antimicrobial analysis identified *Shigella sonnei* as the most sensitive pathogen to the crude extracts of Fenugreek seeds obtained from Saudi Arabia and with the largest zones of inhibition.

Key words : *Trigonella foenum-graecum*, Seed Extract, Antibacterial Activity.

Due to their broad chemical diversity, medicinal plants are regarded as the basic building blocks for a significant number of highly effective pharmaceutical drugs, and are continuously considered the primary source for the discovery of new molecular components. In plants, these molecular compounds are usually in the form of secondary metabolites that have the ability of establishing a definite physiological action of the body, such as alkaloids, steroids, tannins and phenol compounds, flavonoids, resins, and fatty acids (Erdogrul, O. 2002). Today, traditional medicines are used for primary health care for nearly 80% of the world's population.

Fenugreek (*Trigonella foenum-graecum*, Family: Leguminosae) is an annual herbaceous

plant, 30 to 60 cm in height. The plant is native to North Africa, as well as the countries bordering the eastern Mediterranean, and is widely cultivated in India. Fenugreek is one of the oldest widespread natural medicinal plants used in the treatment of a variety of ailments. The leaves and seeds of Fenugreek plants possess blood sugar level reducing properties (Raghuram *et al.*, 1994), as well as anthelmintic, antibacterial (Bhatti *et al.*, 1996), anti-inflammatory, antipyretic (Ahmadianiet *al.*, 2001), and antimicrobial (Alkofahiet *al.*, 1996) properties. Fenugreek also contains lecithin and choline, which have been observed to help in dissolving cholesterol and fatty substances, minerals, B. Complex, iron, Phosphates, PABA (Para-Amino Benzoic Acid), and vitamins A and D. The plant can be used as an appetite stimulant due to containing neurin, biotin and trimethylamine (Michael and Kumawat, 2003). In recent years, the indiscriminate use antimicrobial agents in the treatment of infections as led to the development

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of multiple antibiotic resistance. This, in turn, has led researchers to investigate plants as a source of new antimicrobial agents (Patrick, G. 2005; Shito, M. 2001). The World Health Organization (WHO) indicated that medicinal plant research should include an identification of the studied plant's chemical constituents, as well as a determination of the plant's biological activities (WHO, 1997). In the present study, Fenugreek plants obtained from two different regions in the Middle East, namely Saudi Arabia and Yemen, were screened for phytochemical active constituents and investigated for antimicrobial properties against a selection of gram-positive and gram-negative pathogenic bacteria.

MATERIALS AND METHODS

Collection and Storage of Fenugreek Seeds Samples

Fresh Fenugreek seeds from two cultivars, one from Saudi Arabia (Al-Qassim) and another from Yemen, were obtained. The samples were labelled and stored at 4°C in polythene bags, whilst awaiting processing. The fresh seeds were individually washed under running tap-water in order to remove soil and dirt particles. Samples were then rewashed with distilled water and subsequently, dried at 40°C for 2 days in an oven (Memmert, Germany). The dried seeds were ground well into a fine powder with a mixer grinder (Philips, Brazil). The powder was stored in an airtight sealed plastic container at room temperature ready to be used for further experimentation.

Extraction

The method described in Alade and Irobi (1993) was used for the preparation of plant extracts with some modifications. Twenty grams of powdered plant material was soaked separately in 100ml distilled water, acetone, chloroform, ethanol and methanol for 48 hours. At intervals of 24 hours, each mixture was stirred using a sterile glass rod. At the end of extraction process, each extract was passed through Whatman No.1 filter paper (Whatman, England). The resulting filtrate was reduced to dryness by removing the solvent in an air-dried oven at 40°C. Each dried crude extract was dissolved in 2ml distilled water and stored in Eppendorf tubes at -18°C.

Antibacterial Bioassay

The crude extracts were screened against a number of selected pathogenic bacteria by agar well diffusion (Petropoulos, G. A. (2002); Raghvendra, D. *et al* (2010)). In this method, 10ml aliquots of nutrient broth (Sigma-Aldrich, Germany) was inoculated with the test organism, and incubated at 37°C for 24 hours. Sterile cotton swabs were dipped in the bacterial suspension and evenly streaked over the entire surface of the agar plate to obtain uniform inoculums. Six wells per plate were made with the reverse side of a sterilised micropipette. 50µl of crude extract were then poured into the respective wells using a micropipette. Distilled water was used as negative control. Respective solvents were used as the positive control. Each extract was analyzed in triplicate. All plates were incubated for 24 hours at 37°C. The antibacterial activity was determined by measuring the diameter of the zone of inhibition to the nearest (mm) as observed from the clear zone surrounding the well.

Minimum Inhibitory Concentration (MIC)

The MIC was determined using the streak method (Hancock, 1997). Extracts were dissolved in distilled water and serially diluted in Eppendorf tubes in a laminar flow cabinet. The same volume of an actively growing culture of the tested pathogen was added. The tubes were cultured overnight in an incubator at 37°C. The following morning, streaking was performed on all samples in nutrient agar plates. MIC was found by determining the lowest concentration of the test solution that inhibited growth.

Determination of Fenugreek Antimicrobial Activity on Test Organisms

The following bacterial strains were obtained from the Department of Microbiology, King Saud University, Riyadh, Saudi Arabia: *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Shigella sonnei* ATCC 25931 (clinical isolate), *Staphylococcus aureus* ATCC25923, *Salmonella typhi* ATCC14027 and *Klebsiella pneumoniae* ATCC700603 (clinical isolate). All bacterial strains were maintained on nutrient agar plates (Merk, Germany).

Phytochemical Analysis

The methanolic extracts of the Fenugreek seeds extracted previously were screened for phytochemicals.

Phytochemical Analysis Tests Performed

The identification of alkaloids was carried out using the Mayer's test. A portion of the plant extract was mixed with 5ml of sulphuric acid in 50% ethanol. 1ml of Mayer's reagent was added drop by drop. The formation of a greenish coloured or cream precipitate indicated the presence of alkaloids.

The identification of flavonoids was carried out using the Sodium Hydroxide test. 5ml of plant extract was mixed with few magnesium chips and 2 drops of concentrated hydrochloric acid were added and warmed. The presence of a pink/red colour indicated the presence of flavonoids.

Tannins were identified using the Bromine Water test. 5ml of plant extract was extracted with 20ml of 50% alcohol and then filtered. A few drops of bromine water was added to the resulting filtrate. The formation of a buff/white precipitate indicated the presence of tannins.

Saponins were identified via the Frothing test. 3ml of the plant extract was added to 10ml distilled water and shaken vigorously for 30 seconds. Froth formation indicates the presence of saponins.

The identification of terpenoids was performed using Noller's test. The test plant extract was warmed along with a tin piece and 3 drops of thionyl chloride. Terpenoids are present if the solution turned a purplish colour.

Finally the presence of steroids was detected using the Libermann- Burchard test. 2ml of the test plant extract were mixed with 2 drops of chloroform and 2ml of acetic anhydride, along with 1ml of concentrated sulphuric acid added down

the side of the tube. The formation of a reddish ring at the contact zone of the two liquids and a greenish colour in the separation layer indicates the presence of steroids.

RESULTS AND DISCUSSION

Medicinal plants are being investigated extensively for their use as building blocks of many pharmaceutical drugs. Fenugreek seed (*Trigonella foenum-graecum*) is easily available in most of the agricultural and non-agricultural fields and the usage of this extract for medicinal purpose was reported by several researchers. This study reported the extract's effectiveness against several gram positive and gram negative bacterial isolates including *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Salmonella typhi* ATCC14027 and *Klebsiella pneumonia* ATCC700603. The *Trigonella foenum-graecum* extract investigated were obtained from Fenugreek seeds from two cultivars, one from Saudi Arabia, and the other from Yemen.

The aqueous extract of Fenugreek seed from both cultivars exhibited antibacterial activity against six clinical isolates of bacteria (Table 1 and 2). Both cultivars showed maximum antibacterial activity against *Pseudomonas aeruginosa* and lowest activity against both *Klebsiella pneumonia* and *Shigella sonnei*.

The cultivar from Saudi Arabia demonstrated greater activity against the bacterial isolates when dissolved in either doxycycline or chloroform solvent. For the Yemenis cultivar, doxycycline, chloroform as well as methanolic

Table 1. Antibacterial activity of Fenugreek seed extract (Al-Qassim)

Bacteria	Inhibition zone(mm)						
	F. seed(Al-Qassim) Extract						
	DC	W	A	Ch	M	E	DW
<i>Pseudomonas aeruginosa</i>	34.5	6.8	22.3	35.6	29.7	28.4	0
<i>Escherichia coli</i>	30.6	5.2	18.2	28.4	25.2	22.6	0
<i>Salmonella typhi</i>	37.5	-	22.4	34.6	28.8	-	0
<i>Staph aureus</i>	28.6	3.6	18.7	27.4	30.3	16.2	0
<i>Klebsiella pneumonia</i>	26.8	9.2	25.7	36.3	25.5	11.1	0
<i>Shigella sonnei</i>	27.5	9.6	-	38.4	27.3	19.1	0

W,A,Ch,M and E : SOLVENTS Standard DC: (Doxycycline):-: Not active against tested microorganisms

solvents exhibited greater activity compared to acetone, water and ethanolic extracts.

An earlier study on the antibacterial activity of fenugreek seed extracts revealed its antibacterial potential against *E. coli*, *B. cereus*, *L. acidophilus* and *Pneumococcus*, with the choleform extract demonstrating high antibacterial activity (Upadhyay, R. K. et al. 2008). Khanra et al. (2010) have also demonstrated that Fenugreek seed extracts exhibited antimicrobial activity against *E. coli*, *S. typhi*, *V. cholerae*, *S. sonnei*, *S. aureus*, *M. hitea*, *B. subtilis*, and *L. bacillus*. These are in

agreement with the present study.

Phytochemical analysis (Table 3) and previous studies (Khursheed, R. et al. 2012) have revealed and supported the presence of alkaloids, flavonoids, tannins, saponins, terpenoids and steroids, which may individually or collectively, attribute to the antibacterial properties of Fenugreek seed extracts. Khursheed et al. have found that Fenugreek seeds also contained proteins, carbohydrates and triterpens.

It can be concluded that fenugreek seed extracts exhibits antibacterial activity against many

Table 2. Antibacterial Activity of Fenugreek seed extract (Yemen)

Bacteria	Inhibition zone(mm) F. seed (Yemen) Extract						
	DC	W	A	Ch	M	E	DW
<i>Pseudomonas aeruginosa</i>	38.3	9.6	34.5	33.4	37.3	19.1	0
<i>Escherichia coli</i>	34.5	6.8	22.3	35.6	29.7	28.4	0
<i>Salmonella typhi</i>	30.6	-	18.2	28.4	36.2	22.6	0
<i>Staph aureus</i>	37.5	7.4	22.4	36.7	28.8	-	0
<i>Klebsiella pneumonia</i>	28.6	3.6	20.4	29.4	30.3	16.2	0
<i>Shigella sonnei</i>	26.8	9.2	25.7	39.8	25.5	17.8	0

W,A,Ch,M and E : SOLVENTS Standard DC: (Doxycycline)-: Not active against tested microorganisms

Table 3. Phytochemical screening of methanolic extracts of Fenugreek *Trigonella foenum-graecum* obtained from Saudi Arabia and Yemen

Phytochemical	Methanolic Plant Extract	
	Fenugreek (Saudi Arabia)	Fenugreek (Yemen)
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Steroids	+	+

+ = Positive, - = Negative

gram positive and gram negative bacterial isolates. The cultivar from Yemen, however, demonstrated greater antibacterial activity, in general, against all six bacterial isolates compared to the cultivar from Saudi Arabia.

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