JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov. 2013. Vol. **7**(Spl. Edn.), p. 417-422

Phytochemical Screening and Antimicrobial Activity of EthOH/Water *Trigonella foenum-graecum* (Fenugreek) Extracts

Sherif H. Abd-Alrahman[1]* and Monir M. Salem-Bekhit[2]

[1]Biochemistry Department, College of Science, King Saud University, PO Box, 2455, Riyadh, 11451, Saudi Arabia.
[2]Department of Pharmaceutics, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh, Saudi Arabia.

(Received: 13 August 2013; accepted: 24 October 2013)

EthOH/Water extract of fenugreek ground seeds was assayed to determine their antimicrobial activity against six bacterial strains *Staphylococcus aureus, Bacillus cereus* as Gram positive bacterium. *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia and Salmonella typhi* as Gram negative bacterium. Fenugreek extract showed antifungal potential and the magnitude of their inhibitory effects was species dependent. The results of phytochemical screening of fenugreek seeds extract revealed the presence of various chemical compounds such as alkaloids, saponins, flavonoids, phenols, glycosides, Anthocyanin, betacyanin, and steroids. Results showed that the growth of *S. aureus* and *S. typhi* was inhibited at a MIC value of 2mg/ml followed by *E. coli, P. vulgaris* and *K. pneumonia*, while *Ps. aeruginosa* showed highest MIC value of 2mg/ml. The study findings provide supportive evidence for the use of *Trigonella foenum* in traditional medicines and it could be an important source of biologically active compounds useful for developing better new antibacterial drugs.

Key words: Phytochemical Screening, Antibacterial activity, *Trigonella foenum*, Fenugreek, Medicinal plants.

Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections[1-3]. WHO recognized that medicinal plants played an important role in the health care of about 80% of the world population in developing countries and depend largely on traditional medicine[4,5]. It is estimated that about 75% of the 120 biologically active plant derived compounds, presently in the use worldwide, have been derived through follow up researches to verify the authenticity of the data from folk and ethnomedicinal uses. So, there is a great scope for new drug discoveries based on traditional plant uses[6,7].

Trigonella foenum-graecum, known as fenugreek, is an annual plant in the family of Fabaceae, has long been used as a spice and an herbal remedy across the Middle East. People harvest and roast dried seeds of the plant for food flavoring and medicinal purposes. Various components of fenugreek are responsible for its beneficial effects, including blood sugar regulation and cholesterol reduction[8]. The seeds of fenugreek are used by people in Asia, Africa and Mediterranean countries as one of the ingredients in daily diet (Basch, Ulbricht, Kuo, Szapary, & Smith, 2003). The fenugreek seeds contain polyphenolic compounds, which have been

* To whom all correspondence should be addressed.
Tel: +966 561776615;
E-mail: drsherif_hussein@yahoo.com

correlated to the beneficial health effects of fenugreek (Saleh, Torgils, &Øyvind, 2010). Fenugreek seeds also have a long history of medicinal uses in Ayurvedic and Chinese traditional medicines for the amelioration of hypocholesterolemic effects (Sharma, 1986), diabetes (Raju, Gupta, Rao, Yadava, & Baquer, 2001), lack of appetite (Petit *et al*., 1993), fever (Agababyan, Gevorgyan, Tumadzhyan, Akopyan, &Aristakesyan, 2009), microbial infection (Randhir, Lin, & Shetty, 2004), oxidative effects (Dixit, Ghaskadbi, Moha, &Devasagayam, 2005), and cancer (Shabbee *et al*., 2009). Today, it is considered as one of the leading functional foods in the market with anecdotal traits ranging from ameliorating diseases to improving health. Further investigations concerning the protective effect of Trigonella foenum graecum L. on the histological structures and function of liver, kidney and pancreas of induced diabetes were studied[9-12].

The aim of this study was to investigate the phytochemical potential and antimicrobial activities of *Trigonella foenum-graecum*. Various extracts such as methanol, methanol/water and aqueous extracts of seeds of *I. Trigonella foenum-graecum* (fenugreek) were tested against different microbial species.

MATERIALS AND METHODS

Materials

Fresh Fenugreek (*Trigonella foenum-graecum*) seeds were obtained from the local market, Riyadh, Saudi Arabia. The seeds were cleaned, then ground to a fine powder using an electric grinder to pass a 0.4 mm screen. All chemical reagents used in this study were of analytical grade. Deionized water was produced with a Milli-Q system from Millipore (Saint-Quentin-en-Yvelines, France). All solutions were stored at 4°C in the dark.

Methods

Preparation of extracts

About 10 g of fenugreek seeds powder were soaked in 100 ml solvent with agitation at 40°C overnight, as previously reported (Mazza *et al*., 2002). The extract was filtered and dried over anhydrous sodium sulfate and finally evaporated under steam of nitrogen using sample concentrator model Techne DB.3 (Techne, UK). Several solvents (Ethanoland Ethanol /water (1:1)) were tested. Ethanol was tested in our study as it is less toxic than methanol, and so it could be an interesting solvent for the extraction of polar volatile compounds from fenugreek seeds. The yield of the aqueous-ethanol extract was 1.8 g, the extract was stored at -20°C until further use**.** Aliquot of the extract was resolved in Dimethyl sulfoxide (DMSO) to a final concentration of 1.0 mg/mL.

Test Microorganisms

Six bacterial strains used in this study, including *Staphylococcus aureus, Bacillus cereus* as Gram positive bacterium. *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Salmonella typhi* ATCC 0650 as Gram negative bacterium. These organisms were obtained from ATCC (American Type and Collection Center). The bacteria rejuvenated in Mueller-Hinton broth MHB (Difco, USA) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar MHA (Sigma, USA).

Phytochemical Profiling

The phytochemical screening of the sample was carried out as described by[14, 15]. The samples were screened for the following components

Test for Carbohydrates Molisch's Test

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for Tannins

Ferric Chloride Test

To 1ml of plant extract, 2ml of 5% greenish black indicates the presence of tannins.

Test for Saponins

Foam Test

To 1ml of plant extract, 5-10ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

Sulphuric Acid Test

A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange color.

Test for Alkaloids

Mayer's Test

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then

few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Anthocyanin and Betacyanin

Sodium Hydroxide Test

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added and heated for 5minutes at 100°C. Formation of bluish green color indicates the indicates the presence of betacyanin.

Test for Glycosides

Sulphuric Acid Test

To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated sulphuric acid were added. Presence of greenish blue color indicates the presence of glycosides. Ferric chloride was added. Formation of dark blue or presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Test for Phenols

Ferric Chloride Test

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Antibacterial assay

The method reported by Baqir *et al*. 1985[13] has been adopted. The tests were run in triplicate. Petri plates (23x23 mm) were prepared with Trypticase soy agar and an adequate amount of inoculum was flooded onto each plate, excess inoculum was removed and the plates were dried for 30 min at 37°C. Holes (6 mm diameter) were made in the inoculated agar and filled with samples of plant extracts, plates were incubated for 24 hrs at 37°C. Inhibition zones when present were measured in millimeter (Table 2).

Testing for Antimicrobial Activity

Antibacterial activity of the extracts were determined using a modified Kirby-Bauer[14] disc diffusion method. All the bacterial test strains maintained on NA were freshly subcultured for 24-48hrs at 37 °C. Saline suspension of each test strain was prepared and turbidity matched to 0.5 McFarland standard to yield a bacterial suspension of 1.5×10cfu/ml. freshly prepared Mueller Hinton Agar 8MHA plates were seeded with the test inoculums to obtain a lawn culture. Sterile Whatman No. 1 filter paper discs (5mm diameter) impregnated with different concentrations of plant extracts (50 and 100 µg/disc) were placed on the inoculated MHA plates. 5% DMSO served as negative control, Norfloxacin (10µg) and Tetracycline (30µg) were used as standard antibiotics. Post incubation at 37°C for 24-48h, plates were read for zone of inhibition around the disc. Antifungal susceptibility testing was carried out as described for antibacterial testing with SDA as the assay medium and Amphotericin B (100U/D) as standard antifungal agent.

Determination of Minimum Inhibitory Concentrations (MIC)

The antimicrobial activity of the *Trigonella foenum* extract, that shows antimicrobial activity, were determined using micro dilution broth method as described by Brantner and Grein, 1994[15]. Different antibiotics [Ampicillin, amikacin, gentamicin, kanamycin, and tetracycline (10–32 µg/ml)] were used as reference standards (CLSI, 2011). The *Trigonella foenum* extract solution was prepared to obtain final concentrations of 0.25-2.0 mg/ ml for antibacterial testing. One microliter of an overnight culture of each bacterial strain, containing approximately 10^4 CFU, was applied onto a 96-well plate in the presence of MHB. The microtiter plates were incubated at 35°C for 18 h. Observations were performed at least in replicate and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth, MIC.

RESULTS AND DISCUSSION

The chemical composition of Fenugreek seeds is given in (Table 1). These seeds are a rich source of fiber and protein. The fiber may be further classed as gum (gel fiber) and neutral detergent fiber. The protein fraction contains the amino acid

Table 1. Proximate Composition (%) of Fenugreek Seeds

Component	Whole Seeds
Moisture	9.0
Ash	3.0
Lipids	8.0
Protein	26.0
Starch	6.0
Total Fiber	48.0
Gum	20.0
Neutral Detergent Fiber	28.0

4-hydroxyisoleucine, which has been proven to stimulate insulin production.

The preliminary phytochemical screening of seeds extracts of *Trigonella foenum-graecum* (Table 2) revealed the presence of various chemical compounds such as alkaloids, saponins, flavonoids, phenols, glycosides, Anthocyanin, betacyanin, and steroids, some of which have been previously associated with antibacterial activity[16]. Since there are no reports available exclusively on *Trigonella foenum-graecum*, the phytochemical content of *Trigonella foenum-graecum* in the present study was comparable with the available literature on phytochemical content of related species[17-19] respectively.

Table 2. Phytochemical analysis of Fenugreek (*Trigonella foenum-graecum*) seeds extract

Phytochemical	EthOH/Water extract
Alkaloid	+
Tannins	-
Flavonoids	+
Saponins	+
Triterpenes	+
Glycosides	-
Anthocyanin	Betacyanin
cholesterol	-

potential of the compounds of Fenugreek. Hence Fenugreek may yield potential molecules in the treatment of infections caused by pathogenic bacteria which have developed resistance against the known antibiotics, Singleton, 1999[20].

In comparison the aqueous extract showed less pronounced antimicrobial activity

Results showed that the growth of *S. aureus* and *S. typhi* was inhibited at a MIC value of 2mg/ml followed by *E. coli*, *P. vulgaris* and *K. pneumonia*, while *Ps. aeruginosa* showed highest MIC value of 2mg/ml. The poor activity of the 50 % ethanol/water extract against most bacterial strains investigated in this study is in agreement with previous reports[21,22,23]. This could be due to the insolubility of the active compounds in water or the hot water could have caused denaturation of the active compounds. It is also observed from

Fenugreek 50% aqueous-ethanol were screened for their antimicrobial activity at two different concentration (50 µl and 100 µl) against *S. aureus, B. cereus, E. coli, P. aeruginosa,K. pneumonia, and S. typhi* (Table 3). The results were given in table 2. Aside from concerns with food quality degradation, these microorganisms may be causal agents of intestinal infections in humans. The extract inhibited all the tested bacteria, suggesting a broad antimicrobial activity of fenugreek extract in a concentration-dependent manner.

The MIC as low as µg mL^{-1} of a semi-purified fraction against gram negative and positive bacteria is suggestive of good antibacterial

Table 3. Antimicrobial activity of different 50% aqueous-ethanol of Trigonella foenum graecum (50 and 100 µl concentration) against various microorganisms

Microorganism	Activity	
	50 µl	100 µl
S. aureus	5 ± 0.4	8 ± 0.4
B. cereus	5 ± 0.0	7 ± 0.0
E. coli	4 ± 0.8	5 ± 0.8
Ps. Aeruginosa	4 ± 0.8	5 ± 0.8
K. pneumonia	3 ± 0.7	7 ± 0.7
S. typhi	5 ± 0.2	9 ± 0.2

Table 4. Minimal inhibitory concentration (MIC) of solvent extracts of *Trigonella foenum-graecum* seeds by broth microdilution method against different strains. (µg/ml^{-1})

Bacteria	0.25	0.5	1	2	Control
S. aureus	0.22	0.17	0.04	0.08	0.22
E. coli	0.25	0.27	0.20	0.25	0.30
Ps. aeruginosa	0.25	0.28	0.20	0.22	0.14
K. pneumonia	0.22	0.20	0.19	0.23	0.25
P. vulgaris	0.30	0.23	0.27	0.20	0.25
S. typhi	0.22	0.17	0.05	0.07	0.20

the results that the ethanol/water extract had wide antibacterial activity (Table 2) against both gram positive and gram negative bacteria *S. aureus* and *S. typhi*, respectively, (Table 4). The activity of the extracts against the Gram negative bacteria is noteworthy as these bacteria are known to exhibit high degree of resistance to conventional antibiotics[24]. The few variations in results between the disc diffusion and MIC results can be due to the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of seeds extract diffusion[25,26].

To the best of our knowledge this isone of the studies evaluated the phytochemical and antimicrobial effects of the 50 % ethanol/water extracts of fenugreek seeds against various pathogens are reported. Finding of this present study constitute supportive evidence to validate folkloric use of this plant as a remedy for various infections. Further investigations are required to isolate the active constituents responsible for the observed antimicrobial activity.

CONCLUSION

It may be concluded from this study that *Trigonella foenum-graecum* (fenugreek) extract has antimicrobial activity against various microorganisms. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

ACKNOWLEDGMENTS

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

REFERENCES

1. Caceres AL, Lopez BR, Giron MA, Logemann H., Plants used in Guatemala for the treatment of dermatophytic infection in Screening for antimycotic activity of 44 plant extracts. *Journal of Ethnopharmacology* 1991; **31**:263-276.
2. Nweze EL, Okafor JI, Njoku O., Antimicrobial activities of methanolic extracts of Trema guinensis (Schumm and Thorn) and Morinda Lucida Benth used in Nigeria. *Biological Research* 2004; **2**:39-46
3. Vineela CH, Elizabeth KM., Antimicrobial activity of marine algae of Visakhapatna city, Andhra Pradesh. *Asian Journal of Microbiology, Biotechnology & Environmental Science* 2005; **7**:209-2012.
4. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z., Medicinal plants in therapy. *Bull World Health Organ* 1985; **63**(6):965-981
5. NRC NRC., Neem: A tree for solving global problems. National Academy Press, . Washington, DC, 1992.
6. Govindarajan R, Vijayakumar M, Pushpangadan P., Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. *J Ethnopharmacol* 2005; **99**(2):165-178. doi:S0378-8741(05)00228-X [pii] 10.1016/j.jep.2005.02.035
7. Patwardhan B, Warude D, Pushpangadan P, Bhatt N., Ayurveda and traditional Chinese medicine: a comparative overview. *Evid Based Complement Alternat Med* 2005; **2**(4):465-473. doi:10.1093/ecam/neh140
8. http://www.livestrong.com/article/206838-what-is-the-composition-of-fenugreek/.
9. Hamden K, Jaouadi B, Carreau S, Aouidet A, El-Fazaa S, Gharbi N, Elfeki A., Potential protective effect on key steroidogenesis and metabolic enzymes and sperm abnormalities by fenugreek steroids in testis and epididymis of surviving diabetic rats. *Arch Physiol Biochem* 2010; **116**(3):146-155. doi:10.3109/13813455.2010.486405
10. Hamden K, Masmoudi H, Carreau S, Elfeki A ., Immunomodulatory, beta-cell, and neuroprotective actions of fenugreek oil from alloxan-induced diabetes. *Immunopharmacol Immunotoxicol* 2010; **32**(3):437-445. doi:10.3109/08923970903490486
11. Jelodar GA, Maleki M, Motadayen MH, Sirus S., Effect of fenugreek, onion and garlic on blood

glucose and histopathology of pancreas of alloxan-induced diabetic rats. *Indian J Med Sci* 2005; **59**(2):64-69

12. Thakran S, Siddiqui MR, Baquer NZ., Trigonella foenum graecum seed powder protects against histopathological abnormalities in tissues of diabetic rats. *Mol Cell Biochem* 2004; **266**(1-2):151-159
13. Baqir SNS, S. Dilnawaz and S., Screening of Pakistani plants for antibacterial activity. *Pak J Sci Ind Res* 1985; **28**(4):269-275.
14. Bauer AW, Kirby WM, Sherris JC, Turck M., Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; **45**(4):493-496
15. Brantner A, Grein E., Antibacterial activity of plant extracts used externally in traditional medicine. *J Ethnopharmacol* 1994; **44**(1):35-40. doi:0378-8741(94)90096-5 [pii]
16. Nweze ET, Okafor JI, Njoku O., AntimicrobialActivities of Methanolic extract of Trumeguineesis (Schumm and Thorn) and Morinda lucinda Benth used in Nigerian Herb. Medicinal Practice., *J Bio Res Biotechnol* 2004; **2**(1): 34-46
17. Ikewuchi CC, Ikewuchi CJ, Ayalogu OE., Proximate and Phytochemical Profile of Sansevierialiberica Gérôme and Labroy. *Journal of Applied Sciences and Environmental Management* 2010; **14**(2):103-106
18. Ogukwe CE, Oguzie EE, Unaegbu C., PhytochemicalScreening on the leaves of Sansevieria trifasciata. *J Chem Soc Nigeria* 2004; **29**: 8-9
19. Sunilson J, Jayaraj P, Varatharajan., Analgesic and Antipyretic Effects of Sansevieria Trifasciata Leaves African *Journal of Traditional Complementary and Alternative medicines* 2009; **6**(4):529-533
20. Singleton P., Bacteria in Biology. Biotechnology and Medicine in Africa. John Wiley and Sons, Chichester, 1999.
21. Igbinosa OO, Igbinosa EO, Aiyegoro OA., Antimicrobial activity and phytochemical screening of stem barkextracts from Jatropha curcas (Linn). *African Journal of Pharmacy and Pharmacology* 2009; **3**(2):058-062
22. Koduru S, Grierson DS, Afolayan AJ., Antimicrobial activity of Solanum aculeastrum (Solanaceae). *Pharamacol Biology* 2006; **44**: 284-286
23. Jameel A. Khader, Farman Ullah, Muhammad Ullah, Riazullah, Naser M. AbdEIslam. Phytochemical and Antimicrobial Activity of *Sonchus eruca*. *J Pure Appl Microbiol*, 2013; **7**(1); 249-252.
24. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ., Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. *BMC complementary and alternative medicine* 2008; **8**: 53. doi:10.1186/1472-6882-8-53
25. Ntombeziningi SM., Antimicrobial activity testingof traditionally used plants for treating woundsand sores at Ongoye area KwaZulu-Natal, SouthAfrica. University of Zululand, 2009.
26. Sanjay Kumar, Atul Kumar Singh, Santosh Kumar Verma, Richa Misra and Chandrabhan Seniya. Antibacterial and Phytochemical Analysis of Some Medicinal Plants and their Efficacy on Multidrug Resistant Bacteria. *J Pure Appl Microbiol*, 2013; **7**(3): 2191-2204.