Study of Antibacterial Activities of Seeds Extract of Fenugreek (Trigonella foenum-graecum)

Alhan M Alwan (MSc)[1], **Izdehar M Jassim** (PhD)[2] **and Ghssan M Jasim** (MSc)[3]

Abstract

Background: The antibacterial activity of seeds methanol extract and hot aqueous extract of fenugreek (Trigonella foenum-graecum) showed the presence of activity against four species of bacteria and the methanol extract have higher activity than other extract.

Objective: To examine the antimicrobial activities of the crude methanolic extract and hot aqueous extract of seeds of *Trigonella foenum-graecum.*

Patients and Methods: We prepared three different concentrations for all extracts (75% ,50%, 25%, v/v). The study detected antibacterial activity by using well diffusion in agar technique .The study applied on the Four species of bacteria includes :*Staphylococcus aureus , Bacillus subtilus, Pseudomonas aeruginosa* and *Escherichia coli.* The study continued from September 2016 to January 2017 in the Laboratory of Biotechnology in College of Science - University of Diyala.

Results: The study showed highest antibacterial activity of methanolic extract of seeds of *Trigonella foenum –graecum* that attained highest zone of inhibition (30 mm) against P. aeruginosa when using (75%) concentration,While attained lowest zone of inhibition (10 mm) against E.coli when using (25%) concentration. Whereas the hot aqueous extract of seeds of *Trigonella foenum –graecum* that attained highest zone of inhibition (24 mm) against *P. aeruginosa* when using (75%) concentration, while don't give any effect against bacterial spp. when using (25%)concentration.

Conclusion: Antibacterial activity of methanol extract of seeds of *Trigonella foenum-graecum* was higher than the hot aqueous extract also higher than commercial antibiotic (Amikacin).

Key words: Trigonella Foenum –Graecum, Antibacterial Activity, Antibiotics.

Corresponding Author: Tulibaflower@yahoo.com.

Received: 16th April 2017

Accepted: 4th June 2017

[1,2] Biotechnology Department - College of Science - University of Diyala - Diyala-Iraq.
[3] Biology Department - College of Education for Pure Science -University of Diyala- Diyala-Iraq.

Introduction

Plant extracts are identified for their have several pharmaceutical and nutraceutical chattels. *Trigonella feonum-graecum* commonly known as Fenugreek in England, in Japan koroha, in India Methi and in China Ku. Tou, Fenugreek goes to the family of Fabaceae [1] . Fenugreek is one such plant that possess phytoconstituent such as flavonoids, alkaloids steroids, terpenoids, steroids, saponins, anthocyanin and tannins [2].

Its seeds and green leaves are used in food as well as in medicinal application it offers natural food fiber and other nutrient required in human body [3]. This also assistances in nitrogen obsession and soil improvement

[4]. The seeds of the Fenugreek possess toxic oils ,volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi [5]. Also the seeds of fenugreek used as cancer therapy in china medicine. The seeds also contains some active sex substances like trimethyl amine [6].

Fenugreek seeds have hypoglyaemic and hypocholesterolemic outcome it advances marginal glucose consumption, contributing to enhancement in glucose acceptance and applies hypoglycemic influence by substitute at the insulin receptor level as well as at the gastrointestinal level [7] . The seeds also use in treatment of stomach ulcer, enteritis , Urinary tract infection [6]. Fenugreek seeds and sprouts was found to be operative against a change of gram negative such as E.coli and gram positive such as *Staphylococcus aureus* [8].

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 moreover contains fiber and high quantity of calcium, zinc and iron. Seeds of fenugreek have maple flavor and bitter taste but by the process of roasting, their bitterness can be condensed and flavor can be improved. Seeds contain volatile oil and fixed oil in small amounts [9]. These are rich source of soluble nutritional fibers contain [10]. 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 [11] .

Fenugreek has phenolic and flavonoids composites which assistance to rise its antioxidants ability [12]. It likewise have an capability to lower the hepatic lipids in body because of its latent to alter the actions of several enzymes such as enzymes associated to glucose and lipid metabolism [13].

The diversity of compounds produced in plants can show different bioactivity potential. Hence the current training was done to investigate the antibacterial action of methanolic and hot aqueous extracts of *Trigonella foenum-graecum* seeds were measured and compared with sensitivity of bacterial species against antibiotics.

Materials and Methods

Source of Fenugreek seeds: Fenugreek's seeds *(Trigonella foenum -graecum*) were purchased from the indigenous markets of Diyala city ,Iraq.

Bacterial isolates:Four species of bacteria were used in present study, two isolates are gram positive *(Staphylococcus aureus and Bacillus subtilus)* and others two are gram negative *(Pseudomonas aeruginosa and Escherchia coli)* were obtained from Laboratory of Biotechnology in College of Science - University of Diyala.

Sensitivity to antibiotics:The susceptibility of four isolates were used in this training were confirmed by the standard disk diffusion technique on Mueller Hinton(MH) agar plates and using the breakpoints defined by the Clinical and Laboratory Standards Institute (2012)[14]. Nine sensitivity disks of antibiotics from (Himedia) were used including: Ampicillin - sulbactam (20 µg/ml), Ciprofloxacin (5 µg/ ml), Cefotaxime (10µg /ml), Augmentin (10µg /ml), Nitrofourantoin (15µg/ ml), Ceftazidime (15µg/ml), Gentamicin (10µg/ml), Piperacillin (5µg /ml) and Amikacin (30µg/ml).

Preparation of hot aqueous extract of seed of fenugreek: The aqueous extraction are applied of fenugreek seeds for which, evaluated 15 gm of seeds powdered, add 250 ml of sterile distilled water to it, kept the mixture for 7 days and filtered it with muslin textile and again filtered with filter paper (Whatman No.1), filtrate was permissible for hot extraction process on water bath at 40°C.

Preparation of methnol extracts of seed of fenugreek: In prepared the methanol extract ,weighed 10 gram from dry seeds powdered and put it in flask ,add 100 ml of 95% methanol put it in rotatory shaker for 24hours, filtrate filtered it with muslin cloth and again with Filter paper (Whatman No.1), Make centrifugation 5000 rpm for 15 minutes ,collect the supernatant and allowed for evaporate the solvent by using rotary evaporator at 40 °C, kept it yet to use at 4 °C.

Preparation of Minimum inhibitory concentration (MIC): The minimum inhibitory concentration was prepared to noticed anti bacterial activities of both extracts of seeds of fenugreek (hot aqueous extract and methanol extract).We prepare three different concentration of all them (25% ,50% ,75% v/v).

Antimicrobial activity of seeds of fenugreek: The antimicrobial activity of fenugreek seeds was determined against four species of bacteria comprises (*Staphylococcus aureus, Bacillus subtilus, Pseudomonas aeruginosa and Escherchia coli)* by the well diffusion method. Well diffusion method was measured the inhibition zone to know the antibacterial activity of seeds of fenugreek.

Statistical analysis

Data were analyzed by Statistical package software -SPSS [15].The results are presented as mean ± standard error of three replicates, The data were considered significant when P-value was > 0.05.

Result

Through the study of antibacterial action of different antibiotics on four bacterial species under test the results revealed that Staphylococcus aureus out of 9 antibiotics cefotaxime ,amikacin and piperacillin give maximum zone of inhibition whereas B.subtilis was found to be more sensitive to Ciprofloxacin and Ceftazidime . And in the instance of E. coli and P. aeruginosae amikacin give maximum zone of inhibition in both bacterial species as shown in table (1).

Table (1): The suscepitibility of bacterial isolates to antibiotics.

Antibiotics	Code	Conc.µg/ disk	Susceptibility of isolates (zone of inhibition(mm) S/R			
			S.aureus	***B.subtilis***	***P.aeruginosae***	***E.coli***
Ampicillin–sulbactam	Amps	20	(12mm)S	(11mm)S	(13mm)S	(16mm) S
Piperacillin	Pip	5	(14mm) S	(7mm)R	(6mm)R	(8mm) R
Cefotaxime	CTX	10	(16mm) S	(14mm)S	(7mm)R	(10mm) S
Ciprofloxacin	CIP	5	(6mm) R	(17mm)S	(11mm)S	(21mm)S
Augmentin	AG	10	(12mm)S	(14mm)S	(12mm)S	(17mm) S
Nitrofourantoin	N	15	(0) R	(14mm)S	(6mm) R	(14mm) S
Ceftazidime	CAZ	15	(9mm) R	(17mm)S	(10mm) R	(14mm)S
Gentamicin	CN	10	(14mm)S	(9mm)R	(0) R	(15mm) S
Amikacin	AK	30	(16mm)S	-	(17mm) S	(22mm) S

S=sensitive, R =resistance

The result of antibacterial action of methanol and hot aqueous extracts of fenugreek seeds via agar well diffusion technique. Determined inhibition was detected in methanol extract of seeds shown in table 2 and figure 1.

Table (2): Antibacterial action of Methanol extract of seeds of fenugreek in different concentrations at 100 µl.

Bacterial isolates	Zone inhibition mm		
	25%	50%	75%
Staphylococcus aureus	13	18	20
Bacillus subtilus	14	20	25
Pseudomonas aeruginosa	18	22	30
Escherichia coli	10	19	20

*

GRACE:GlobalRegistryofAcuteCoronaryEvents

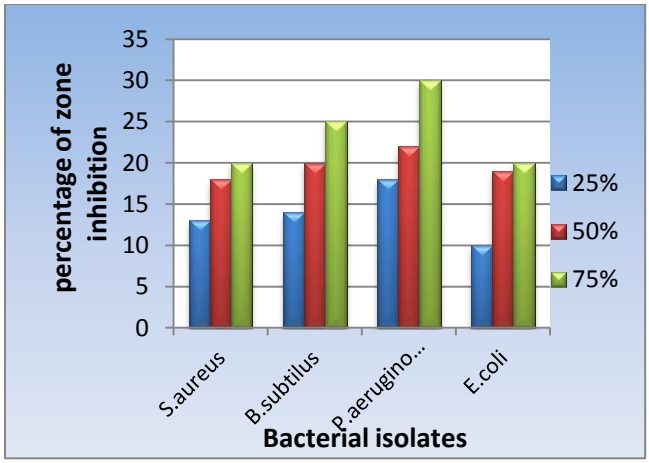

Figure (1): The percentage of zone inhibition of methanol extract of seeds of fenugreek to four bacterial spp. in different concentrations.

Whereas the hot aqueous extract give low activity against bacterial isolates comparison with methanol extract the activity increase with increase the concentration, the concentration 25% Donot appear any activity against bacteria. While the highest activity was at 75% conc. against *P. aeruginosa* give highest inhibition zone 24 mm. The results was shown in table 3 and figure 2.

Table (3): Antibacterial action of aqueous extract of seeds of fenugreek in different concentrations at 100 µl.

Bacterial isolates	Zone inhibition mm		
	25%	50%	75%
Staphylococcus aureus	-	13	17
Bacillus subtilus	-	17	20
Pseudomonas aeruginosa	-	14	24
Escherichia coli	-	15	22

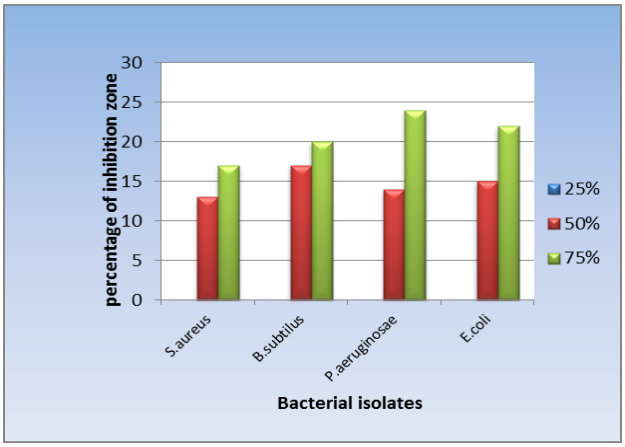

Figure (2): The percentage of zone inhibition of hot aqueous extract of seeds of fenugreek to four bacterial spp. in different concentrations.

Discussion

The antibacterial action of methanol and hot aqueous extracts of fenugreek seeds via agar well diffusion technique, Determined inhibition was detected in methanol extract of seeds ,The resultswhich is in agreement with Sharma et al., 2017 [16], but the results was more than the result experimental by Premananth et al. 2011 [17]. In another study by Mawahib et al., 2015 ,observed that methanol extract of seeds of fenugreek give highest inhibition zone when applied on some bacteial species and fungi [18]. Another study by Salah et al., 2010 were observed both extraction of aqeuous and methanol donot appear any effect on bacterial spp. Like *E. coli, S.aureus, P.aeruginosa* and *Klebsilla spp.*[19].

In conclusion, methanol extract exhibit the highest zone of inhibition as compared to the aqueous extract. In comparison of two extracts, the methanol extracts was found to be maximum as compare to the aqeuous extract of seeds of fenugreek . 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 two solvent extracts (Methanol and aqueous) the antibacterial activities of methanol extract of Fenugreek seeds is higher than hot aqueous extract.

References

[1] Coot N. In :Fenugreek- Trigonella foenum- graecum. (2004).Townssend letter.PP.88.

[2] Sumayya AR, Sivagami S, Nabeelah A. Screening and biochemical quantification of phytochemicals in fenugreek (Trigonella foenum-graecum).RJPBCS. 2012; 3(1):165-169 .

[3] Thomas JE, Bandara M, Lee EL, Driedge D, Acharya S. Biochemical monitoring in fenugreek to develop functional food and medicinal plants variants. N Biotechnol. 2011; 28: 110-117.

[4] Montgomery J. The potential of fenugreek (Trigonellafoenum- graecum) as a forage for dairy herbs in central Alberta.University of Alberta. 2009: 4-15.

[5] Fraenkel D, Gottfried S. The raison of secondery plant substances. Science .2007;129(3361):1466-1470.

[6] Barners D, Anderson LA, Phillipson JD. Herbal Medcines: Aguid for health care professionals, 2nd ed., pharmaceutical press. London.2002.

[7] Singh V, Garg AN. Availability of essential trace elements in Indian cereals,

vegetables and spices using INAA and the contribution of spices to daily dietary intake. Food Chem. 2006; 94: 81-89.

[8] Thomas JE, Basu SK, Achraya SN. Identification of Trigonella accessions which lack antimicrobial activity and are suitable for forage development. Can J Plant Sci. 2006; 86:727-732.

[9] Sowmya P, Rajyalakshmi P . Hypocholesterolemic effect of germinated fenugreek seeds in human subjects.Plants foods Hum Nutr. 1999;53: 359-365.

[10] Sharma RD, Raghuram TC. Hypoglycaemic effect of fenugreek seeds in non-insulin dependent diabetic subjects. Nutri Res. 1990; 10: 731-739.

[11] Mathur P, Choudhary M. Consumption pattern of fenugreek seeds in Rajasthani families. J Hum Ecol. 2009; 25: 9-12.

[12] Dixit P, Ghaskadbi S, Mohan H, Devasagayam TP. Antioxidants properties of germinated fenugreek seeds. Phyto ther Res. 2005; 19: 977- 983.

[13] Madar Z, Shomer IJ. Polysaccharide composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats. J Agric Food Chem. 1990; 38: 1435- 1539.

[14]Clinical Laboratory Standards Institute(C.L.S.I). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow aerobically ;Approved standard ninth edition M07-A9. Clinical and Laboratory Standards Institute ,Wayne, PAUSA. 2012;32(2):1-88.

[15] Sabine, L. And Brian ,S.E.Ahand book of statistical analyses using spss.Boca Raton London Newyork Washington,D.C.2004.

[16] Sharma V, Singh P, Rani A. Antimicrobial Activity of Trigonella foenum –graecum L.(fenugreek). Euro J. Experim Bio. 2017;7(1):l 5-9.

[17] Premanath R, Sudisha J, Lakshmi Devi N, Aradhya SM . Antibacterial and antioxidant activity of Fenugreek (*Trigonella foenum-graecum* L.) leaves. Res J Med Plants.2011; 5: 695-705.

[18] Mawahib EME, Ammar MAA, Badr EAS. Antimicrobial activities and phytochemical screening of callus and seeds extracts of fenugreek (*Trigonella foenum-graecum*). Int J Curr Microbiol App Sci. 2015; 4(2):147-157.

[19] Salah SZ, Bestoon MF, Osman JN. Antibacterial effect of fenugreek (*Trigonella foenum –gracum*).Bas J Vet Res. 2010;10(2):134-138.