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Chemical Composition of *Trigonella foenum-graecum* Seeds and Inhibitory Activity of Their Seeds Oil Against Some Microbes

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Abstract: The study aimed to know the chemical composition of fenugreek seeds and test the inhibitory effectiveness of the extracts of the seed oil on the types of bacteria and yeast. The results of the chemical analysis of the circuit showed that the moisture content of 4.90%, fiber 6.55%, ash 3.35%, protein 27.50%, fat 4.50%, and carbohydrates 42.26%. The physiochemical properties of fenugreek seed oil were similar to edible oil. Plant extracts were obtained by extracting oil with hexane at concentrations 40, 80 and 100%. The inhibitory activity was tested against the experimental cells that included the Gram negative: *Escherichia coli* O157: H7s, *Samonella. typhimurium* ,. Gram positive: *Staphylococcus aureus Aspergillus niger*. Antibody activity was highly effective against living organisms, specially against *E.Coli* at which the aortic diameter reached the highest concentration (100%) 21 mm.

Keywords: Inhibitory activity, fenugreek seeds, fenugreek seed oil, bacteria, fungus.

Introduction

Trigonella foenum-graecum seeds belong to the *Papilionaca*e family of the Leguminosae Fabaceae family, a herbaceous plant widely cultivated in the Mediterranean, Asia and Egypt (1,2,3). It is used in Arabic, Chinese and Indian as a folk medicini and it has a nutritional benefit used in the treatment of diabetes and high blood cholesterol. The seeds contain 5-7% of oil, which is composed of linoleic acid, linolenic acid and oleic. It also has an anti-cancer and diuretic roles (4). It is useful for patients suffering from tuberculosis and constipation (5,6). It is used to produce breast milk and to treat breast cancer (7,8). It also have

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antibacterial (9), and fungal activity (10). The seeds of the fenugreek being used as active compounds in biology research work to heel some infectious diseases (11). They are also being used as food supplements that have the potential to be anti-oxidant (12). They have a role in the digestion process if they are useful for the digestive system and ulcers. It is also used as a seasoning in cooking in small quantities and is recognized and safe by the US Food and Drug Administration (13, 14). The aim of this study was to evaluate the antimicrobial activity of *Trigonella foenum-graecum* plant used in Iraq and traditional medicinal system for antimicrobial activity.

Materials and methods

The seeds of the *Trigonella foenum-graecum* were obtained from the Iraqi market. The samples were sampled from the university garden and placed in plastic cans at 4 ^{o}C until they were used. The samples were analyzed for moisture, ash, protein, fat and fiber. The carbohydrates was calculated by subtracting the previous components from 100 different sample (16). After that, the oil was extracted by n-hexan using the method (17). The experiment included the measurement of acid value, solubility, esters, free fatty acid, Oleic acid and refractive index. The samples were then prepared to test antiboitic effect of fenugreek seed oil, samples were prepared in three different concentrations of oil 40, 80, 100%. Three bacterial species and one type of fungus were selected to detect the inhibitory activity of plant extracts. The pathogenic microorganisms isolated from garden of University of Baghdad for research proposal (Table 1)

Table 1. Shows the isolates and sources of pathogenic microorganisms

Microscopic object	**Source**
Escherichia coli	Faculty of Agriculture - University of Baghdad

Staphylococcus aureus	Faculty of Agriculture - University of Baghdad
Salmonella typhimrum	Faculty of Agriculture - University of Baghdad
Aspergillus niger	Faculty of Agriculture - University of Baghdad

in vitro fertilization of experimental microorganisms: The method described as a standart was followed by a test tube containing 5 cm^3 concentration of Moeller-Hinton broth (Sigma, USA) by a loopfull vector of bacteria growing on the concentration of the nutritious nut (Himedia, India). As for the yeast, a test tube containing the concentration of the (Oxoidoid, America), was tested with a number of cells that had already grown in the same medium after the addition of the macrophage. The tube was well incubated at 37 °C for three hours. 30 °C for 24 hours. The growth curve was measured using the Macfarland Solution by reading the optical density using the optical spectrometer and the 525nm wavelength for the test bacteria. The active isolates were selected with the McFarland solution. As for the fungus samples were prepared by taking 1 cm^3 and diluting it after which the density of the implanted plant was read to obtain the number of cells 4×10^8 (19). The effect of both fry seed extracts on the bacteria and fungi under study, as follows: To fertilize the nutritious surface of the bacteria with each experimental bacterial cell, While in the center of the saberoid agar by sterile steriliswab bacteria from the pods were pitted with bacteria and fungus. A 6 mm diameter was drilled on the surface of the medium implanted by the cork hole. Concentrates of graduated oil were collected (40, 80, 100%). Seed extracts were extracted individually and 0.1 cm 3 in each hole with one hole containing distilled distilled water (Control) for comparison. The dishes were left in the refrigerator at 4 °C for half an hour and then incubate the dishes at 37 °C for 18-20 hours. The diameter of the Dimeter Inhibition zone is estimated around each hole. The procedure was done three times for each treatment and repeated the

experiment two times. The dishes containing bacterial implants were prepared and amputations only on the same grounds mentioned above for the purpose of comparison (20).

Statistical analysis: Areas of inhibition (mm) were measured the activity of the seed oil extract was clear against the microorganisms subjected to three replicates of each experiment according to (21).

Results and Discussion

Extraction of plant of antibactarial activity have been widely investigated in many studies (33). Three different types of experimental bacteria were used in this study, five of which were negative for the *Escherichia coli, Samonell,. Typhimurium*. One of them is positive for the chroma dye: *Staphylococcus aureu, Aspergillus niger*. These microorganisms have been chosen as common causes of some human and animal diseases and are contaminants that cause damage to certain foods and their resistance to antibiotics (22). The results in Table 2 show the chemical content of the seeds of the fenugreek if the moisture content is 4.90 % Was less than the result obtained by (23) 9.3% and that (24) received a result of 3.4%, which is less than our results. The raw fiber content of the fenugreek seeds is 6.55 % if the results are agreed with (25) if it is 6.70%. Ash content 3.35 % The result was agreed with (26) if it was 3.15 % and differed with (27) if it was 7.6 % more than it was due to the diversity of the fenugreek and soil characteristics (28). The protein content was 27.50%. If it was more than the value obtained (26) if it was 24.70 %, the fat content was 4.50 %, which is less than 27 % if it was 7%, the carbohydrate content was 42.26 % (29) if it is 40.60 %.

Table 2. Chemical composition of the seeds *Trigonella foenum-graecum*

Chemical composition of the seeds of the fenugreek	**%**
Protein	27.50
Fats	4.50
Humidity	4.90
Carbohydrate	42.26
Fiber	6.55
Ash	3.35

The results showed that the seeds contain a high percentage of protein and carbohydrates and low fiber, moisture and ash .The physical properties of fenugreek seed oil as shown in Table 3, if the value of the volume of amalgam of potassium hydroxide / g for oil was 5.00 and the number of sapon 200 and the value of Ester 192.50, and the content of free fatty acids estimated as alolic acid 2.50 and refractive index 1.4700 These results showed that the use of fenugreek seed oil, Oil is suitable for human consumption and for eating, but we must get rid of the sharp smell in it.

Table 3. Chemical properties of *Trigonella foenum-graecum* seed oil

Acid value mgKOH/g of	5.00
Saponification value mgKOH/g of oil	200
Ester value	192.50
Free fatty acid value (Oleic acid) /100g	2.50
Refractive index (at 37 ^{0}C)	1.4700

Anti-microbial properties results shown in Table 4 show that the seeds of the oil of the fenugreek have an antimicrobial effect they are all sensitive to oil. The highest activity against *E.coli* is shown if the inhibition zone reached 21 mm when the concentration was 100 % (30) if it had a 23 mm inhibitory area and *Staphylococcus* had a ND, ND, 17 mm

concentration at 40, 80 and 100% respectively. *Staphylococcus aureus* caused food poisoning in milk and cooked meat (30). *Salmonella* At 100% concentration, 16 mm was approaching what he found (31).

Table 4. Anti - oil properties against microorganisms

Oil concentration	**Inhibition zone Diameter (mm)**		
	Escherichia Coli	*Staphylococcus aureua*	*Salomonella Typhimurim*
40%	ND	ND	ND
80%	ND	ND	ND
100%	21	17	16

ND = Not detected

The results of the statistical analysis of fenugreek seed oil showed that activity against *E.Coli* and against *Aspergillus niger* as shown in Table 5 shows that concentrated oil improves the shelf life of food.

Table 5. Anti-oil properties of Aspergillus niger

Oil concentration	**Inhibition zone Diameter (mm)**
40%(w/v)	ND
80%(w/v)	ND
100%(w/v)	23

ND = Not detected

Comparison of the seed oil of the fenugreek 100 % with antibiotics: The results showed in Table 6 that the test microbes used in the study had a clear resistance to most of the antibiotics used despite the high concentration used Table 6 shows superiority in the inhibitory effectiveness of 100 % seed oil against the test isolates used. Consistent with what I

mentioned (32) Plant extracts outweigh antibiotics in inhibiting pathogenic bacteria if used correctly.

Table 6: The inhibitory effect of the alcoholic extract compared to some antibiotics

Isolates	**VN**	**T**	**GN**	**AX**	**Abstract**
	Inhibition zone Diameter (mm)				
Escherichia coli	**-**	**17**	**18**	**-**	**21**
Staphylococcus aureus	**14**	**-**	**15**	**-**	**17**
Salmonella typhimiurum	**-**	**14**	**11**	**14**	**16**
Aspergillus niger	**-**	**-**	**-**	**-**	**23**

The rates represent replicates, - there is no inhibition
Ax: Amoxicillin, GN: Gentamycin, T: Tetracycline, VN: Vancomycin

Aksoy et al. (33) described an some phonelic compounds from local tomato plant which was bacteriostatic for *Clavibacter michiganensis* subsp. Michiganensis. Also Aksoy et al (34) showed that some of bacteria can be biological agent for insects. The screenings of these the plants showed that some of the screened plants are potential source of antibacterial agents. This in vitro study corroborated the antimicrobial activity of the plant in Iraq. These results are promising and may contribute to the future development of natural bio pesticides for the control of *Trigonella foenum-graecum* for the microorganisms.

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