

Evaluation of Antibacterial and Antifungal Activity of *Trigonella Foenum Graecum* Seeds and leaves Against Some of Clinical Bacteria and Fungi

Zeena G. Faisal

Al-Iraqia university / college of Education - department of biolog

Abstract:

The present study investigates the inhibitory effect of *Trigonella foenum-graecum* leaves and seeds against three pathogenic bacteria (*Escherichia coli*, *Serratia marcescens* and *Bacillus cereus*), and three clinical fungal species (*Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viride*) collected from laboratories of College of Science /Baghdad university, by well diffusion method. The results showed that methanol and aqueous extracts of fenugreek seeds and leaves have inhibitory activity against subjected bacterial isolates and fungal species under study. Bacterial pathogens are more susceptible to methanol and aqueous extracts of fenugreek seeds and leaves than fungal species as well as methanol and aqueous extracts of fenugreek leaves are more effective than seeds extracts. In conclusion, present study showed that fenugreek seeds and leaves methanol and aqueous extracts have antimicrobial activity against various pathogenic bacteria and fungi, so it is used as a medicinal herb.

Key words: *Trigonella foenum-graecum*, antibacterial and antifungal activity.

تقييم الفعالية المضادة للبكتيريا والفطريات لبذور *Trigonella Foenum Graecum*

وأوراقها ضد بعض البكتيريا والفطريات السريرية

زينة غازي فيصل

الجامعة العراقية / كلية التربية - قسم علوم الحياة

الخلاصة:

تبحث الدراسة الحالية في التأثير التثبيطي لأوراق وبذور *Trigonella foenum-graecum* ضد ثلاثة أنواع من البكتيريا المسببة للأمراض (*Escherichia coli* و *Serratia marcescens* و *Bacillus cereus*) وثلاثة أنواع من الفطريات السريرية (*Aspergillus flavus* و *Aspergillus niger* و *Trichoderma viride*) تم جمعها من مختبرات كلية العلوم / جامعة بغداد، باستخدام طريقة الانتشار بالحفر. أظهرت النتائج أن مستخلصات الميثانول والمستخلصات المائية لبذور الحلبة وأوراقها لها تأثير مثبط ضد العزلات البكتيرية والفطريات المستخدمة في الدراسة. تعتبر البكتيرية المرضية أكثر تأثراً بالمستخلص الميثانولي والمستخلصات المائية لبذور الحلبة وأوراقها من الأنواع الفطرية المستخدمة، إلى جانب أن مستخلص الميثانول والمستخلص المائي لأوراق الحلبة أكثر فعالية من مستخلصات البذور. في الختام، أظهرت الدراسة الحالية أن مستخلصات الميثانول والمستخلصات المائية لبذور الحلبة وأوراقها لها نشاط مضاد للميكروبات ضد البكتيريا والفطريات المرضية المختلفة، لذلك فهي تستخدم كعشبة طبية.

الكلمات المفتاحية: *Trigonella foenum-graecum*. الفعالية الحيوية ضد البكتيريا والفطريات المرضية.

Introduction

The use of plants for medicinal purposes has existed for thousands of years, and the practice goes back to the beginning of man. Interest in medicinal plants has increased over the past few years due to their wide application as herbal products for self-medication, natural cosmetics and their general biological effect[1]. Research on the use of medicinal plants should continue especially because of the alarming increase in the incidence of new infectious diseases and its reemergence. In addition, scientists are witnessing the issue of antibiotic resistance from microbes in current clinical use due to the misuse and overuse of synthetic antibiotics[2]. Beyond the medicinal or pharmaceutical approach to plants, herbal products are increasingly being used to supplement people's diet to improve the quality of life[3].

The *Trigonella foenum graecum* which is popularly referred to as fenugreek belongs to the Leguminosae family which is an annual, herbaceous, and aromatic plant[4]. The leaves of the plant are edible and are a common vegetable in world while the seeds of the fenugreek are used as a spice in many parts of the world[5]. It is claimed that the fenugreek seed has an anti-diabetic, anticancer, anti-fertility, anti-parasitic, anti-microbial hypocholesterolemic and lactation stimulant effects. Both the fenugreek

seeds and leaves are used in Ayurveda to make extracts or powder for medicinal use[1].

Previous research has shown the antioxidant and antibacterial activities of fenugreek leaves and seeds[3]. However little comparison has been done on the antifungal and antibacterial activity in fenugreek seeds and leaves extracts with the antibacterial and antifungal activity from prescription antibiotics[6]. This study aims to investigate the quantity and quality of antifungal and antibacterial components in aqueous and methanol extracts of fenugreek seeds and leaves extracts.

Material and Methods

Sample Collection and Preparation: Fresh fenugreek (*Trigonella Foenum Graecum*) seeds and leaves were collected from local markets. The fresh materials of seeds and leaves were washed with tap water and dried in room temperature for 25 days.. The leaves and seeds were then separately homogenized into a fine powder and then stored in airtight tubes at 4 °C.

Fifty grams of seeds and 50g of leaves powder are then soaked separately in 100ml of methanol and water. The solvents were kept in closed flasks for three days. The extracts were then filtered using Whatman filter paper into sterile Petri plates. It was set for evaporation and samples were dried so that they could be assessed for antimicrobial activity[7].

Microbial Strains: Three different isolates of clinical bacteria (*Escherichia coli*, *Serratia marcescens* and *Bacillus cereus*), and three clinical fungal species (*Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viride*). These strains were collected from laboratories of College of Science / Baghdad university. The identity of isolates was confirmed in the central health laboratories in Baghdad.

Determination of Antibacterial and Antifungal Activity: The antibacterial and antifungal activity the leaves and seeds extracts was determined using the agar well diffusion method[8]. Mueller-Hinton agar (MH) was used. Microbial cultures that freshly grown at 37°C for bacteria and 30°C for fungi , were appropriately diluted in dimethyl sulphoxide to obtain cell suspension at (1.5×10^8 cfu/ml). 100µl of suspensions were spread on(MH), allow the inoculums to dry. Five wells of 5 mm diameter were made into the agar medium. Four wells were filled with 100 µl of different aqueous and methanol extracts of fenugreek seed and leaves. The fifth well was filled with 100 µl of sterile distilled water. The inculam was allowed to diffuse into the medium for 1 h at room temperature. Plates of test organisms were incubated for 24 h at 37°C for bacteria and 30°C for fungi. Antimicrobial activity was estimated by measuring the inhibition zone(ZOI) diameter against the test organisms.

Results and Discussion

The antibacterial activity from both methanol and aqueous extracts of fenugreek leaves and seeds were described in table-1. The results revealed that methanol extract of fenugreek leaves by well method showed inhibitory activity against *S. marcescens*, *B.cereus* and *E.coli*. It observed the highest inhibition in *S. marcescens* (ZOI 10.0 ± 1.0 mm), while the lowest inhibition in *E.coli* (ZOI 9.33 ± 0.57 mm). The aqueous extract of fenugreek leaves was observed to have a maximum inhibition zone within *S. marcescens* (ZOI 12.33 ± 0.57 mm), while the aqueous and methanol extracts of fenugreek leaves have approximately the same inhibition zone within *B.cereus* (ZOI 11.50 ± 0.50 mm for methanol extract and 11.33 ± 0.15 for aqueous extract). *E.coli* has less susceptibility pattern for both methanol and aqueous extracts of fenugreek leaves, as compared with *S. marcescens* and *B.cereus*.

The methanol extract of seeds have a greater zone of inhibition compared to the aqueous extract when similar amounts were used for the test, as described in table-1. The largest inhibition zone of seeds methanol extract was observed within *B.cereus* (ZOI 17.50 ± 0.50). Aqueous extract of seeds was observed to have inhibitory effect against *S. marcescens* (ZOI of 6.3 ± 0.24), and there is no effect against *B.cereus* and *E.coli*.

Table -1: The susceptibility pattern of aqueous and methanol extracts for fenugreek leaves and seeds against bacteria under study

Bacterial strains	Leaves extract (100 mg /ml)		seeds extract (100 mg /ml)	
	Methanol	Aqueous	Methanol	Aqueous
<i>S. marcescens</i>	10.00±1.00	12.33±0.57	14.00±0.24	6.3±0.24
<i>B. cereus</i>	11.50±0.50	11.33±0.15	17.50±0.50	-
<i>E.coli</i>	9.33±0.57	10.50±0.50	13.55±0.57	-

On the other hand, The antifungal activity for the leaves and seeds extracts was measured by analyzing the zone of inhibition through the agar well diffusion method. Aqueous and methanol extracts gave different results during the measurement of antifungal activity against fungal species under study, as described in table-2. For the leaves extracts, the methanol sample had a maximum inhibition zone against *T. Viride* (ZOI 14.5±0.5mm) as compared to aque-

ous extract (ZOI 9.66±0.57mm).

There is no effect of both aqueous and methanol extracts against *A. Flavus* and *A. Niger*. Methanol extracts of seeds had a higher antifungal activity on *T. Viridae* and *A. Flavus* (ZOI 18.1±0.65 and 10.3±0.24 respectively), compared to methanol extract of the leaves. There was no antifungal activity when aqueous extracts for fenugreek seeds were immersed in *T. Viridae* and other fungal strains.

Table -2: The susceptibility pattern of aqueous and methanol extracts for fenugreek leaves and seeds against fungi under study

fungal strains	Leaves extract (100 mg /ml)		seeds extract (100 mg /ml)	
	Methanol	Aqueous	Methanol	Aqueous
<i>A. Flavus</i>	-	-	10.3±0.24	-
<i>A. Niger</i>	-	-	-	-
<i>T. Viride</i>	14.5±0.50	9.66±0.57	18.1±0.65	-

The current study proven that alcohol and aqueous extracts of seeds and leaves have a high antibacterial and antifungal activity due to presence of high percentage of metabolites like, alkaloids, flavonoids, tannins, saponins, terpenoid and steroids,

which may individually or collectively, attribute to the antimicrobial properties of fenugreek seeds and leaves extracts [9]. It was observed that bacterial pathogens are more susceptible to methanol and aqueous extracts of fenugreek seeds and

leaves than fungal species. These results are in agreement with studies who evaluated the antimicrobial activity of fenugreek seeds and leaves extracts against different types of the microorganisms [3,5]. This can be attributed to the ability of secondary metabolites that extracted by methanol or water were able to break down the peptide bonds of prokaryotic proteins [6]. The knowledge of the extent and mode of action for antimicrobial activity of specific compounds, present in the plant extracts, may lead to the successful utilization of such natural compounds for the treatment of infections caused by pathogenic microorganisms. Further identification and purification of active chemical constituents from the crude plant extracts will be helpful to develop drugs against pathogenic microorganisms.

As a conclusion, *Trigonella foenum-graecum* leaves and seeds extracts have clear antimicrobial activity against pathogenic bacteria and fungi. So, it can be used in the production of therapeutic agents, thus reducing the incidence of resistance among pathogens that appear as a result of the misuse and overuse of antibiotics. Methanol and aqueous extracts of fenugreek leaves are more effective than seeds extracts, this means that fenugreek leaves have more metabolites than seeds. Further isolation and exploration of the isolated chemical constituents and studying their antimicrobial activity may lead to ex-

plorate chemical entities for clinical application.

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