

ANTIBACTERIAL EFFECTS OF FENUGREEK (*Trigonella foenum-graecum*)

*Salah Salman Zaen Al-abdeen, **Bestoon Mohammad Faraj,

***Osman Jamal Nasrulla

*Department of Biology, College of Science, University of Kerkuk, Kerkuk, Iraq

**Department of Conservative, College of Dentistry, University of Sulaimany, Sulaimany, Iraq.

***Department of Medicine, College of Veterinary Medicine, University of Sulaimany, Sulaimany, Iraq.

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ABSTRACT

Fenugreek has a long history of medical uses in Chinese medicine and Kurdish area. The antibacterial activity of aqueous and some organic compounds extracts of stems, leaves, seeds and roots of fenugreek were tested against three Gram-negative and one Gram-positive bacteria by the well diffusion and colony account methods. The microorganisms used were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp. All extracts of the plant did not exhibit any inhibitory activity against any of the microorganisms tested by each well diffusion and colony account technique.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L. *Leguminosae*) is one of the oldest medicinal plants, originating in India and Northern Africa, and is grown native in Kurdistan (Iraq). The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use, having properties of reducing blood sugar level. Leaves, bark, flowers and fruits of plants derived phytoalexin(1) isothiocyanates(2) allicins, anthocyanins (3) and essential oils (4)tannins and polyphenols and terpenoids (5,6,7) have demonstrated antibacterial and/or antifungal activities. These compounds are bactericidal and/or bacteriostatic influencing lag time, growth rate and maximum growth of microorganisms (8), anthelmintic, antibacterial (9) anti-inflammatory, antipyretic (10) and antimicrobial (11).

In Kurdistan region, fenugreek is still used as a supplement in wheat and maize flour for bread-making.

New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms. Interest in plant-derived drugs has been increasing, mainly due to the current widespread belief that "green medicine" is safer and more dependable than

costly synthetic drugs, many of which have adverse side effects(12).Antimicrobial compounds of plant origin may occur in stems, roots,

The general belief that the advent of antibiotics will bring end to the occurrence of infectious diseases was cut short with the occurrence of resistance to antimicrobial drug. The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase.

A significant opportunity exists to identify new, natural plant derived antimicrobial agents for treatment of diseases or as food or cosmetic preservatives. Our objective was to evaluate the antibacterial activity of aerial parts of native Fenugreek plant cultivated in Sulaimany city against strains of bacteria isolated from sputum samples of hospitalized patients.

MATERIALS AND METHODS

2.1. Samples

1. The aerial parts (leaves, stems , seeds and an occasional root of fenugreek plants used were taken from the College of Agricultural- University of Sulaimany in 2009.

2. The microorganisms tested in this study were isolated from 22 sputum samples from hospitalized patients in the Shores Hospital of Sulaimany city- Iraq. The samples were collected and transported to the laboratory for analysis using prescribed procedures (13)

2.2. Antibiotic sensitivity test:

Five antibiotics used to determinate the resistance of the bacterial isolates erythromycine, doxycycline, neomycin, ciprofloxacin and streptomycin.

2.3. Preparation of the Fenugreek extracts:

Fenugreek infusion was prepared according to the method described by (14). Plant parts were dried for 24 hours at 37⁰ C in oven. Exposure to sunlight was avoided to prevent the loss of active components. Dry seed were then ground in a grinding machine; 60gm of dried ground seed were taken in a non-metallic jar and one liter of hot boiled distilled water were poured on it and was kept at room temperature for 5-8 hours to prepare an infusion.The same method using socsolate and organic solvent ethanol, benzene, chloroform, hexan and petroleum ether were performed on seeds only.

All the extracts were filtered through sterile gauze and the pH were neutralized. Finally the extracts were sterilized by autoclaving. All extracts were stored in the dark until use.

2.4. Antibacterial activity

Antibacterial assay was done by agar well diffusion method and colony counting methods

2.5. Agar well diffusion technique and counting /reading of colonies:

The antimicrobial activities of the water extracts of the Fenugreek were evaluated by means of agar-well diffusion assay (15, 16, and 17) with some modifications. Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø 90 mm).50 µl from each selected bacterial isolates of the swabs (Cell suspensions containing 10⁸ CFU/ml cells) according to (McFarland standard 0.5)(18) , were taken separately and evenly spread

onto the surface of the agar plates of Mueller- Hinton agar (Oxoid, UK), using a micropipette. Once the plates had been aseptically dried, 6 mm wells were bored using a sterile cork borer. Extracts (75 µl) were placed into the wells and the plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameters of the polymicrobial growth inhibition zones.

Counting was done by examined the plates directly after removing the plates from the incubator. Count enumerated using automated colony counters, by the aid of lenc and low power binocular microscope (19)

RESULTS

An *in vitro* agar well diffusion assay was performed to test the susceptibility of 22 clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Kliebsilla*.to Fenugreek water extract. The results depicted in Table 1 show that 100% of the strains were not inhibited by test solution of aquatic extraction and organic compounds extractions that showed in table (1) and figures 1,2and 3

The sensitivity test of all bacterial type show inhibitions zone by all type of antibiotics as shown in table (2)

DISCUSSIONS

Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. The use of higher plants and preparations made from them to treat infections is a longstanding practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of ailments (20). All *in vitro* experimental methods have advantages and disadvantages. In the agar diffusion test, the size of the microbial inhibition zone depends upon the solubility and diffusibility of the test substance and, therefore, may not express its full effective potential.

Our results are in agreement with (21), who refers that seed extraction not has any antibacterial effect. Hot water extraction of fenugreek don't has any effect on *Helicobacter pylori* (22)

In contrast to our findings numerous authors have reported various degrees of bacteriostatic activity of Fenugreek water extract against gram positive and gram negative bacteria (9). The cause of deference in antibacterial influence due to variation in plant components that's gets from deferent area (23)

This variation may be due to the aspects in this study, the method of extraction of medicinal plants, the method of antibacterial study, culture medium, pH, temperature, incubation period, and the genetic variation of plant, age of the plant or the environmental factors that make the comparison of published data challenging (24).

In the other hand the uses of fenugreek in most lines used in food products (25).

In conclusion and from the bacteriological point of view, fenugreek doesn't appear to play any role in clinical medicine, dentistry and veterinary medicine as an antibacterial agent.

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Table 1: Antibacterial effect of extracts

Extract	<i>E.coli</i>	<i>Klebsiella</i>	<i>pseudomonas</i>	<i>St. aureus</i>
Seed water	-	-	-	-
Seed ethanol	-	-	-	-
Seed benzene	-	-	-	-
Seed petroluim eather	-	-	-	-
Seed hexan	-	-	-	-
Stem ethanol	-	-	-	-
Stem water	-	-	-	-
Stem benzene	-	-	-	-
Stem petroluim ether	-	-	-	-
Stem hexan	-	-	-	-
Root water	-	-	-	-
Root ethanol	-	-	-	-
Root benzene	-	-	-	-
Root hexan	-	-	-	-
Root petroluim ether	-	-	-	-
Leave ethanol	-	-	-	-
Leave benzene	-	-	-	-
Leave petroleum ether	-	-	-	-
Leave hexan	-	-	-	-
Leave water	-	-	-	-

Table 2: Antibiotic sensitivity test.

Antibiotics Bacteria	Ciprofloxacin	Doxycycline	Erythromycin	Neomycin	Streptomycin
<i>E. coli</i>	+++++	+++++	+++	++	+++
<i>Klebsiella</i>	+++++	+++	+++	++	+
<i>Pseudomonas</i>	++++	++	+++	++	+
<i>St.aureus</i>	++++	+++	++++	+++	++

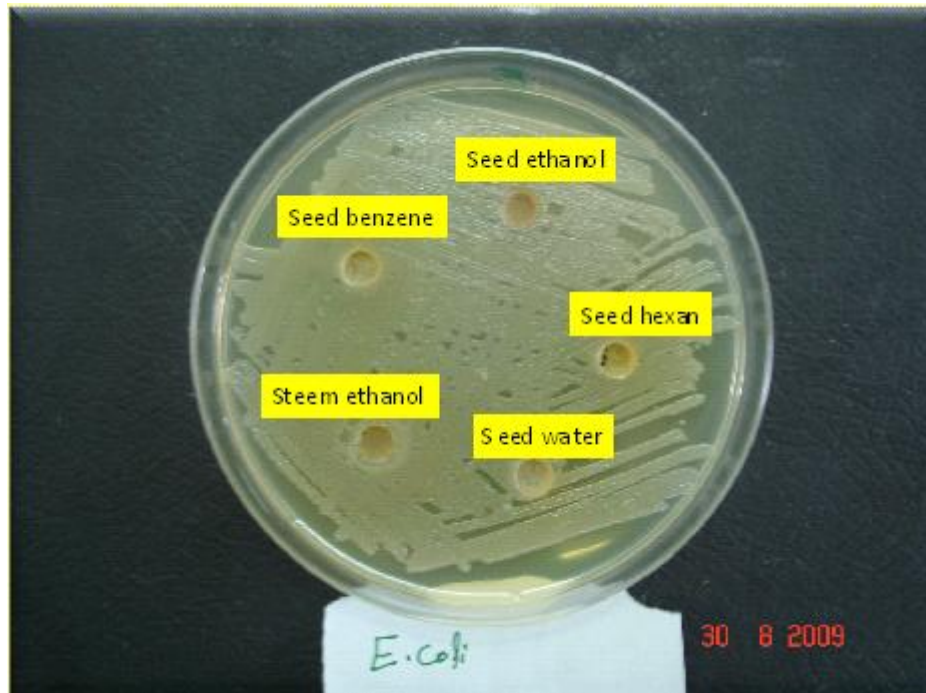


Figure 2: Negative response of extracts to *E. coli*

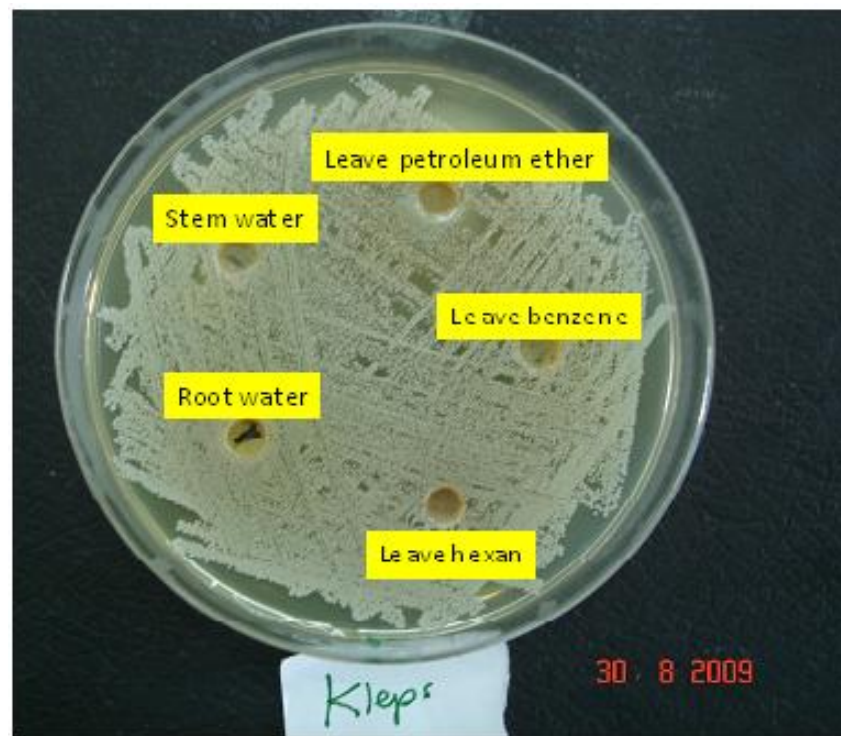


Figure 2; Negative response of extracts to *Klebsiella* spp.

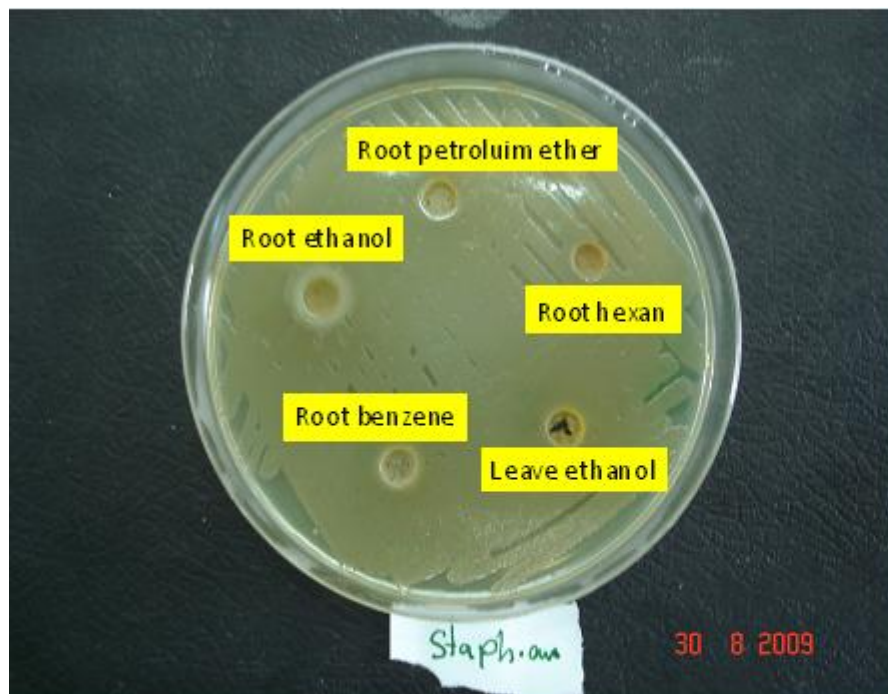


Figure 3: Negative response of extracts to *St. aureus*

التأثير المضاد للبكتريا لنبات الحلبة (*Trigonella foenum-graecum*)

صلاح سالم زين العابدين* ، بستون محمد فرج** ، عثمان جمال نصر الله***

* قسم علوم الحياة ، كلية العلوم ، جامعة كركوك ، كركوك ، العراق.

** كلية طب الاسنان ، جامعة السليمانية ، السليمانية ، العراق

*** كلية الطب البيطري ، جامعة السليمانية ، السليمانية ، العراق

الخلاصة

تعتبر الحلبة (Fenugreek) ذات تاريخ طويل في الاستعمالات الطبية في عدة دول مثل الطب الصيني والعراق والمناطق الكردية. جربت الفعالية المضادة للبكتريا للمستخلصات المائية ومستخلصات بعض المركبات العضوية للسيقان والاوراق والبذور والجنور لنبات الحلبة ضد ثلاثة انواع من البكتريا السالبة لصبغة كرام وواحدة موجبة لصبغة الكرام بطريقة الانتشار في الحفر وعد المستعمرات. انواع البكتريا المستعملة كانت المكورات العنقودية الذهبية والاي كولاى وزوائف الايروجنوزا والكليسيلا . جميع المستخلصات لم تظهر اية فعالية تثبيطية لاي نوع من انواع البكتريا ومن كلا الطريقتين الانتشار في الحفر وعد المستعمرات .

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