The study of Antibacterial activity of fenugreek *(Trigonellafoenum-graecum)* Seeds extract.

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Abstract

It has been well known since ancient times that medicinal plants have antimicrobial activity because of the presence of substances, therefore they become important sources of drugs production. *Trigonellafoenum-graecum* is the scientific name of the plant and Fenugreek is the common name used in Iraq and local Arab people specilizied the seeds extract in many drugs to help

In this study on the seeds extract of Fenugreek, with used the determined by using agar diffusion method to mesurmant the antibacterial activity on bacterial isolates that isolate from different sources: *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeuroginosa, Escherichia coli, Proteus vulgaris ,Klebsiella pneumoniae.*

The aqueous extract of Fenugreek seeds with concentrations 1000, 500 and 250 mg/ml that inhibition growth of the bacteria *Streptococcus pyogenes*.

So the methanolic extract of fenugreek seeds with concentrations 1000, 500, 250 and 125 mg/ml that inhibition the growth of the bacteria *Staphylococcus aureus*.

So the aqueous extract for the fenugreek seeds was less active then methanolic extract against the growth of pathogenic bacteria.

From this study we support the use of fenugreek seeds as anatural extract was active against the pathogenic bacteria and may have arole as apharmaceutical and treatment from infectious diseases.

دراسة فعالية المضاد البكتيري لمستخلص بذور الحلبة *Trigonellafoenum-graecum*

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الخلاصة

عرفت النباتات الطبية ومنذ القدم بفعاليتها ضد الاحياء المجهرية الممرضة بسبب احتوائها على المركبات الفعالة مما جعلها مصدر مهم لانتاج العقار.

استخدم نبات الحلبة *Trigonellafoenum-graecum* في العراق والعديد من الدول العربية والعالمية. خصوصاً مستخلص البذور في العديد من المستحضرات الطبية والدوائية للمحافظة على صحة الانسان.

واظهرت نتائج هذه الدراسة التي اجريت على مستخلصات بذور الحلبة وباستخدام طريقة الانتشار في الحفر ضد الأنواع البكتيرية الممرضة والتي عُزلت من مصادر مختلفة:

Staphylococcus aurens, Streptococcus pyogenes, Pseudomonas aeuroginosa, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae.

ان المستخلص المائي لبذور الحلبة وبتراكيز 1000 و500 و250 ملغم/مل له فعالية مثبطة لنمو البكتريا المرضية *Streptococcus pyogenes*.

بينما اظهرت نتائج مستخلص الميثانول لبذور الحلبة وبتراكيز 1000 و500 و250 و125 ملغم/مل فعالية مثبطة لنمو البكتريا المرضية *Staphylococcus aureus*.

وقد تبين ان تأثير المستخلص الميثانولي لبذور نبات الحلبة اكثر تثبيطاً لنمو البكتريا المرضية من المستخلص المائي لبذور نبات الحلبة.

ومن هذه الدراسة وجد ان استخدام المستخلص الطبيعي لبذور الحلبة يكون فعال ضد البكتريا المرضية وبالتالي له مجال فعال في صناعة الأدوية ومعالجة الامراض المعدية.

Introduction

Intensive use of antibiotics is often followed by the development of resistant strains. Because this drug resistance, the search for new antibiotics continues unabated.

The interest in the study of medicinal plants as source of pharmacologically active compounds has increased worldwide (10).

Resistance in pathogens to the common therapeutic agents is increasing in recent years (5).

Antimicrobials of plant origin are efficient in the treatment of infectious diseases mitagation simultaneously many of the side effects that are often associated with synthetic antimicrobials (7).

(Fenugreek), ***Trigonellafoenum-graecum*** is an annual crop from the family leguminosae.

The seed of this plant grown in south Asia, has been known to have health potential with the ability to lower blood glucose and cholesterol levels, and hence in the prevention and treatment of diabetes and coronary heart diseases.

Fenugreek seeds are use as anti-diabetic activity and hypocholesterolaemic effects, so it has also to possess acurative gastric anti- ulcer action, anti- bacterial, anti-helminthic, anti-fertility effects and anti-nociceptive effects (14; 3).

The aim of this study is to evaluate the effect of methanolic and aqueous extracts of the seeds of fenugreek against various pathogenic bacteria growth in comparison with antibiotics (Streptomycin, Ciprofloxacin and Gentamycin)

Materials and methods

Fenugreek seeds:

The seeds brought from local herbal shops and dried in a oven for 24 hr at 40°C.

The dried seeds were grounded using home mixer and the course pieces of seeds were reground (9).

Preparation of extracts: the seeds were extracted by methanol or sterile distilled water (9).

A- methanolic extract:

Soaked twenty grams from fenugreek seed powder with 100 ml of 96% methanol for 20 min and blended in sterilized blender for 3 min extract was filtered by Whitman filter paper No. 4 and re filtered through micro filter 0.45 μm, then extract concentrated in a rotary evaporator at 50 °C and stored thereafter at 4 °C.

B- Aqueous extract:

Soaked twenty grams from fenugreek seed powder with 100 ml of sterilized distilled water for 20 min and blended in sterilized blender for 3 min, extract was filtered by Whitman filter paper No. 4 and re filtered through micro filter 0.45 μm, then extract concentrated in a rotary evaporator and stored thereafter at 4 °C (9).

Bacterial isolates:

We are collected 42 samples between February-march 2011 from Al-Yarmok hospital in Baghdad from different sources (wound infections, urine, pus, skin lesions) the swab samples were processed as soon as received by the Laboratory, and cultured in the suitable media (Blood agar and MacConkey agar), then detected by microbiological and biochemical test. (6; 8).

Preparation of concentrations:

The concentrations 1000, 500, 250 and 125 mg/ml of each of plant seeds methanolic extract was prepared in Dimethyl sulfoxide (DMSO) methanol (1:1 V/V) to achieve a decreasing range of extract concentrations from 1000 mg/ml to approximately 125 mg/ml and the plant seeds aqueous extract was prepared as the same as of methanolic extract concentrations except it dissolved in sterile distilled water (1:1 V/V).

Antibacterial activity:

The agar well diffusion method was used to determine antibacterial activity of extracts. (1; 8).

For bioassay a bacterial suspension in sterile normal saline was prepared (equally 0.5 McFarland).

The Muller Hinton agar used for this test six millimeter diameter wells were punched in to the agar using cork borer, and approximately 0.1 ml of each of the plant extract concentration mentioned above were administered to fullness in each well. Then the plates were incubated at 37 °C for 24hrs.

After incubation, bioactivity was determined by measuring the diameter of inhibition zones (DIZ) in mm. all samples were tested in duplicate. Controls containing sterile (DMSO) or sterile distilled water without plant extracts were also employed, as a negative control (10).

Antimicrobial susceptibility test:

All the bacterial isolates were cultured on Muller Hinton agar, the antibiotic discs:

Streptomycin 10 mcg/disc, ciprofloxacin 5 mcg/disc, Gentamicin 10 mcg/disc all form (Oxoid Company) were used as a positive control (10).

Results and discussion

Bacterial isolates:

We obtained 28 isolates as ***Staphylococcus aureus,***31 isolates as ***Pseudomonas aeuroginosa,*** 34 isolates as ***Escherichia coli***, 19 isolates as ***Proteus vulgaris***, 25 isolates as ***Klebsiella pneumoniae*** and 11 isolates as ***Streptococcus pyogenes*** .

Antibacterial activity:

The results showed that the methanolic extract of fenugreek seeds had the antibacterial activity. The different concentrations of methanolic extract of Fenugreek seeds (Table, 1) produced inhibition zone against bacterial isolates; ***Escherichia coli*** was sensitive to concentrations ranging from 1000-125 mg/ml. It's produced the largest inhibition zone, while ***Proteus vulgaris***, showed lower response 17, 16, 15 and 13 mm to various concentrations of methanolic seeds extract 1000, 500, 250 and 125 mg/ml ***Staphylococcus aureus*** was sensitive to methanolic fenugreek, seed extract to various concentrations 1000, 500, 250 and 125 mg/ml also ***Pseudomonas aeuroginos*** showed sensitive to methanolic fenugreek seed extract in various concentrations 1000, 500, 250 and 125 mg/ml ***Streptococcus pyogeenes*** also showed sensitive to concentrations of methanolic fenugreek seed extract 1000, 500, 250 and 125 mg/ml on the other hand ***Klebsiella pneumoniae*** was showed resistant to various concentrations of methanolic fenugreek seed extract 250 and 125 mg/ml with inhibition zone diameter 24 and 16 mm concentrations of methanolic, fenugreek seed extract 1000 and 500 mg/ml.

While other bacteria show response (Table, 2) against aqueous fenugreek seed extract ***Staphylococcus aureus*** produced inhibition zone against concentrations ranging 250 and 125 mg/ml ***Pseudomonas aeuroginosa*** and ***Escherichia coli*** are sensitive to aqueous fenugreek seed extract concentration 125 mg/ml ***Proteus vulgaris*** showed inhibition zone against the concentration 1000 mg/ml ***Streptococcus pyogenes*** was sensitive at concentrations 1000 and 250 mg/ml .

In (Table, 3) shows inhibition of antibiotics disc (Streptomycin, Ciproflaxacin and Gentamycin) against the same bacterial isolates, all them were sensitive to these antibiotics disc except ***Pseudomonas aeuroginosa*** was resist to streptomycin and ciprofloxacin, also ***Proteus vulgaris*** and ***Klebsiella pneumoniae*** were resist to streptomycin.

The solvents control, that were systematically run for all solvents did not exert any antibacterial activity. bacterial growth was observed. For the positive control while no growth was observed for the negative controls (12).

Fenugreek seeds are a rich source of the polysaccharide, galactomannan also a source of disogenin, yamogenin, gitogenin, tigogenin and neotigogens other bioactive constituents of Fenugreek include mucilage, volatile oils and alkaloids such as choline and trigonelline which could be of use as anatural antibacterial compounds (2).

Table (1): Antibacterial activity of methanolic extract of *Trigonellafoemum- graecum* seeds.

Bacterial isolates	Concentrations (mg/ml)	Inhibition zone diameter (mm)
Staphylococcus aureus	1000	24
	500	22
	250	20
	125	15
Pseudomonas aeuroginosa	1000	20
	500	18
	250	15
	125	13
Escherichia coli	1000	26
	500	19
	250	12
	125	11
Proteus vulgaris	1000	17
	500	16
	250	15
	125	13
Klebsiella pneumoniae	1000	24
	500	16
	250	-
	125	-
Streptococcus pyogenes	1000	17
	500	17
	250	16
	125	14

Table (2): Antibacterial activity of aqueous extract of *Trigonellafoemum- graecum* seeds.

Bacterial isolates	Concentrations (mg/ml)	Inhibition zone diameter (mm)
Staphylococcus aureus	1000	-
	500	-
	250	11
	125	9
Pseudomonas aeuroginosa	1000	–
	500	–
	250	–
	125	16
Escherichia coli	1000	–
	500	–
	250	–
	125	15
Proteus vulgaris	1000	12
	500	–
	250	–
	125	–
Klebsiella pneumoniae	1000	–
	500	–
	250	–
	125	–
Streptococcus pyogenes	1000	19
	500	–
	250	15
	125	–

Table (3): Antibacterial susceptibility test.

Bacterial isolates	Antibiotics discs mcg/disc	Inhibition zone diameter (mm)
Staphylococcus aureus	Streptomycin 10 mcg/disc	13
	Ciprofloxacin 5mcg/disc	27
	Gentamicin 10mcg/disc	16
Pseudomonas aeuroginosa	Streptomycin 10 mcg/disc	-
	Ciprofloxacin 5mcg/disc	-
	Gentamicin 10mcg/disc	8
Escherichia coli	Streptomycin 10 mcg/disc	18
	Ciprofloxacin 5mcg/disc	33
	Gentamicin 10mcg/disc	17
Proteus vulgaris	Streptomycin 10 mcg/disc	-
	Ciprofloxacin 5mcg/disc	15
	Gentamicin 10mcg/disc	10
Klebsiella pneumoniae	Streptomycin 10 mcg/disc	-
	Ciprofloxacin 5mcg/disc	25
	Gentamicin 10mcg/disc	14
Streptococcus pyogenes	Streptomycin 10 mcg/disc	10
	Ciprofloxacin 5mcg/disc	29
	Gentamicin 10mcg/disc	24

Most of researches were focused on the seeds of Fenugreek extracts (using different extract), for the paramagnetic studies, such as fluorescence analysis, extractive value loss by drying etc.

This comparative and multidisciplinary approach to the study of Fenugreek does help in understanding its identification, toxonomical, determination and medicinal importance in depth (4).

Extraction of secondary metabolities highly dependson using extractor techniques that depend on the chemical properties of these

compounds. Water- soluble compounds and proteins can be extracted in water or polar solvents where as water in soluble compounds can be extracted with organic solvents (16).

Results clearly indicate that further purification of this compounds can leads to isolation of potent antibacterial compound active against some pathogenic bacteria (15).

The microorganisms Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components this makes the cell wall impermeable to antimicrobial chemical substances.

Gram-positive bacterial on other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier, therefore the cell wall of Gram-negative organisms which are more complex than the Gram- positive ones act as a diffusion barrier and making them less susceptible to the antimicrobial agents than are Gram- positive. (11; 13).

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