THE ANTIMICROBIAL ACTIVITY OF THE FENUGREEK SEEDS AND CORN SILK EXTRACTS

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ABSTRACT

Fenugreek seeds extract included separation of trigonelline alkaloid, crude saponin, oil, cold and hot alcohol extract. Also corn silk extracts included separation of crude saponin, cold and hot alcohol extracts as well as cold and hot water extract. All these extracts were tested for their antibacterial effects. The oil and trigonelline of fenugreek seeds inhibited the growth of both *Proteus mirabilis* and *Klebsiella aerogenes* at concentration of 80 and 8mg/ml, respectively. Crude saponin showed more potent inhibitory effect against the growth of *Escherichia coli* and *Klebsiella aerogenes* at concentrations of 50 and 100mg/ml, respectively. Crude alkaloids did not show any activity against uropathogenic bacteria that were tested. Hot alcohol extract of corn silk showed more potent effect than cold alcohol extract against growth of *Proteus mirabilis, Klebsiella aerogenes and E. coli* at a concentrations of 50 and 100mg/ml. Hot and cold water extracts of corn silk showed a slight inhibitory effect against *Proteus mirabilis* and *Klebsiella aerogenes* and a mild inhibitory effect against *E. coli* at a concentration of 25mg/ml for hot water extract and 50mg/ml and 100mg/ml for cold water extract.

Key words: Fenugreek seeds, Corn silk, Antimicrobial activity.

الفعالية المضادة للبكتريا لمستخلصات بذور الحلبة وشعر الذرة

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الخلاصة

تضمنت مستخلصات بذور الحلبة: قلويد الترايكوناللين، الصابونين الخام، الزيت، مستخلص كحولي حار وبارد. أما مستخلصات شعر الذرة فتضمنت الصابونين الخام المعزول، مستخلص كحولي حار وبارد فضلاً عن مستخلص مائي حار و بارد. تم اختبارجميع تلك المستخلصات كمضادات للبكتريا. ثبط الزيت والترايكونيللين لبذور الحلبة نمو كلا من *K. aerogenes* و *P. mirabilis* عند التركيزين 80 و 8 ملغم/مليلتر على التوالي. أظهر الصابونين الخام تأثيراً عالياً ضد نمو *K. aerogenes* و *E. coli* عند التراكيز 50 و 100 ملغم/مليلتر على التوالي. لم تظهر القلويدات الخام أي فعالية ضد الممرضات البكتيرية التي تم اختبارها. أظهر المستخلص الكحولي لشعر الذرة تأثيراً عالياً ضد نمو كلاً من *E. coli* و*K. aerogenes* و*P. mirabilis* عند التركيزين 50 و 100 ملغمِ/مليلتر بينما أظهر المستخلص الكحولي البارد تأثير قليل ضد تلك البكتريا. ولم يظهر المستخلص المائي الحار والبارد أي تأثير ضد بكتريا *P. mirabilis* و *K. aerogenes* وتأثير قليل ضد بكتريا *E. coli* عند التركيز 25 ملغم/مليلتر للمستخلص المائي الحار وعند 50 و 100 ملغم/مليلتر للمستخلص المائي البارد.

INTRODUCTION

The human body carries a wide range of bacteria but few of these are able to cause infection in immune competent host. Indeed, the majority are considered commensal or normal flora and can play an important role in host defense. On other hand, a commensally in the wrong place can be just as harmful as a true pathogen, for example, if *E. coli* or *Proteus mirabilis* an essential component of the intestinal flora enters the bladder, urinary tract infection can ensue (1).

Bacterial pathogens have developed many strategies for survival in higher organisms which, during their evolution, have formed very sophisticated defense mechanisms. Common bacterial properties involved in the infection process include adhesion to epithelial surface, invasion (penetration) of host cell, intracellular multiplication of pathogen, colonization in the cell tissue or transmission to a new susceptible host, production of enzymes, which damage the host defense system, and synthesis of toxins (2).

E. coli is the most common cause of acute, uncomplicated urinary tract infection. *Klebsiella aerogenes* are fairly common cause of urinary tract infection and occasionally give rise to cause of severe bronchopneumonia (3).

Fenugreek is an annual herb belongs to the family Leg. (leguminosea), it is widely grown in Mediterranean countries and Asia (4,1). The seeds and leaves of this plant have along history in traditional medicine (5). Its properties are found in the ripe dry seeds. Fenugreek exhibits pharmacological properties such as anti tumor, antiviral, antimicrobial, anti-inflammatory, hypertensive antioxidant activity, lactation stimulant, and treat weakness (6,4). Fenugreek is used as seeds, powdered seeds or packed in capsule because seeds of fenugreek are somewhat bitter; fenugreek preparation is best taken in a capsule form (7).

Corn silk is an outer thread- like part of corn. It is the fresh styles and stigmas of *Zea mays* L. It has been used to treat pathological swelling and asthma; also, it is used as diuretic and demulcent to the lining of bladder and ureters in oriental medicine (7,9).
This study was performed to test the inhibitory effect of fenugreek seeds and corn silk extracts against uropathogenic bacteria.

MATERIALS AND METHODS

Plant material

1- Fenugreek seeds: were purchased from the local market and identified by a botanist Prof. Dr. Ali-Al-Musawi in the College of Science, Baghdad University. These seeds belong to *Trigonella foenum graecum* L. The seeds were cleaned, dried and finally powdered.

Corn silk of *Zea mays* was collected from the farm during March 2005.

Bacterial isolates

Proteus mirabilis, Klebsiela aerogenes and *E. coli* were obtained from Dr. Sareaa, Biology Department, College of Science, Baghdad University. These bacteria were isolated from urinary tract infection (UTI) patients.

Preparation of extracts (Extraction of alkaloid)

Trigonelline and other alkaloids were extracted from fenugreek seeds. A sample of 250g of defatted seeds was refluxed with 600ml of 96% ethanol for 4hrs. After cooling and filtration, the filtrate was evaporated and concentrated to a small volume by using rotary evaporator then reflexed with 5% HCl for 2hrs, then cooled and partitioned with chloroform three times(10). Two layers were formed, chloroform layer contain (primary, secondary and tertiary) alkaloids and the aqueous acidic layer contains quaternary alkaloids like trigonelline and choline. This layer was evaporated under vacuum to a small volume then tested by dragendorff's spray reagent. Trigonelline was isolated from all extracts by preparative thin layer chromatography (PTLC), two mobile phases were used, methanol: water: HCl (50:50:2) and n-butanol-acetic acid-water (100:20:50). The silica gel zone containing trigonelline and choline were scrapped out, collected in a beaker, eluted with several portion of ethanol with gentle heating, and filtered(11). The filtrate was evaporated to dryness to give a yellow precipitate.

Saponins extraction

A volume of 100ml of (5% HCl in 70% ethanol) was added to 10g of both fenugreek seeds and corn silk and refluxed for 4hrs. The filtrate was extracted with ethyl acetate. Two layers were formed; ethyl acetate layer was concentrated to dryness using rotary evaporator. The dried residues were weighed and dissolved in chloroform. The crude saponins were detected by using TLC and benzene-acetone (2:1) as mobile phase(12).

Cold alcohol extracts

A sample of 10g of both fenugreek seeds and corn silk was macerated in 100ml of 75% ethanol, shaken in a shaker incubator for 24 hour at room temperature, and then the extract was filtered using Whatman No.1 filter paper and evaporated to dryness using a rotary evaporator. The weight of crude alcohol extract was estimated and stored in a deep freezer(13).

Hot alcohol extracts

A sample of 10g of both fenugreek seeds and corn silk was weighed and refluxed with 75% ethanol using soxhlet apparatus for 8 hours, then the extract was evaporated to dryness using a rotary evaporator and the weight of the crude alcohol extract was determined and stored in a deep freezer(14).

Hot water extracts

A sample of 10g of corn silk was refluxed with 100ml of distilled water for 60 minutes, the extract was filtered using Whatman No.1 filter paper and evaporated to dryness using a rotary evaporator then the weight of crude extract was determined and stored in a deep freezer(15).

Cold water extracts

Aliquot of 100ml of distilled water was added to 10g of corn silk in conical flask, shake for one day using a shaker incubator(16). The extract was filtered using Whatman No.1 filter paper and evaporated to dryness using rotary evaporator; then the weight of crude extract was determined and stored in a deep freezer.

The antimicrobial activity

Agar diffusion procedure (wells method)

Four isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a wire loop and transferred to a

universal bottle containing 5-10ml of sterilized normal saline (0.85%).The suspension was shaken and the turbidity of suspension compared with McFarland standard tube 0.5(17). This suspension should not be allowed to standing for more than 15-20 minutes to avoid separation of any layer before inoculation of the plates. Muller-Hinton agar was dispensed into plastic Petri plates on a level of horizontal surface so as to give a uniform depth of approximately 4mm, this requires approximately 25ml in 100mm plates. The wells were made on the agar (3-4 wells in each plate). Sterilized cotton swab was dipped into standardized suspension and the excess of inoculum removed from the swab by rotating it several times with a firm pressure on the inside wall of the test tube above the fluid level. The dried surface of Muller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This streaking procedure was repeated and the plate approximately rotated 60 degrees each time so as to ensure an even distribution of inoculum. The plant extracts were applied in wells in different concentrations and left at room temperature for less 2-4hrs in order to allow the substance to diffuse in the agar. The plates were incubated at 37˚C for 24hrs. After incubation, the plates were examined.

Broth dilution techniques

The suspension for each *P. mirabilis*, *K. aerogenes* and *E. coli* bacteria was prepared by transferring single colonies at the same morphological type which were cultured on plate nutrient agar into universal tubes containing normal saline solutions and standardized with McFarland tube 0.5.Nutrient broth media were prepared for the dilution of the plant extracts. Stock solution of each plant extract was prepared and diluted with nutrient broth to yield different concentration of extract. Control solution was without extract containing distilled water. A volume of 1ml of bacterial suspension was added, the total volume was 2ml. The universal tubes of culture were incubated at 37˚C for 24hrs. After incubation, the cultures of bacteria were re-cultured on agar plate to observe the amount of growth and inhibition(18).

RESULTS AND DISCUSSION

Extracts showed no any antibacterial activity against the tested bacteria when using wells method. The size of the zone may be affected by the density or viscosity of the culture medium, the rate of diffusion of the extract, the concentration of the extract on the well, the sensitivity of the microorganism to the extracts, and the interaction between the extract and the medium. The rate of compounds diffusion depends on the molecular weight of the substance (19).

Antibacterial activity of fenugreek seed and corn silk extracts were tested against *P. mirabilis*, *K. aerogenes* and *E. coli* (Table 1) using a broth dilution technique.

Fenugreek seeds oil (80mg/ml) was effective against *P. mirabilis*, and *K. aerogenes* whereas 100mg/ml of crude saponin was more effective against *E. coli*. Trigonelline and choline were effective against *P. mirabilis* at 8mg/ml concentration but it was unable to inhibit other bacteria used in this study.

Hot alcohol extract of fenugreek showed some inhibitory activity against *P. mirabilis* but showed no activity against the other tested bacteria at concentration of 100mg/ml, also crude alkaloid was tested against *P. mirabilis* and showed no activity.

Table(1): Effect of fenugreek extracts on bacteria using broth dilution technique

Extract	Concentration mg/ml	*P. mirabilis*	*K. aerogenes*	*E. coli*
Oil	25	———	+	+
	50	+ +	+ +	+ +
	80	+ + +	+ + +	+
Cold alcohol	25	——	——	——
Hot alcohol	50	——	——	+
	100	+ +	——	——
Cold methanolic alcohol	25	+	+	+
Crude saponins	50	——	+ +	+ + +
	100	+	+ + +	+ + +
Crude alkaloid	25	——	-------	-------
	50	——	-------	-------
	100	——	-------	-------
Trigonelline and Choline	0.5	——	-------	-------
	1	+	-------	-------
	2	+	------	-------
	4	+	-------	-------
	8	+ + +	-------	-------

Hot alcohol extract of corn silk showed more potent inhibitory activity than cold alcohol extract when tested against *P. mirabilis*, *K. aerogenes*, and *E. coli* at concentration 100mg/ml and a slight activity at 50mg/ml whereas other extracts of corn silk were less active against the tested bacteria (Table 2).

It appears generally that the test microorganisms were not sensitive to hot water and cold water extracts of corn silk compared with the other extracts as determined by broth dilution. The reasons for this, may be due to the identified components, from plants are active against microorganisms. Aromatic or saturated organic compounds are most often obtained through initial ethanol or methanol extraction (18).

From the results, it can be concluded that gram-negative bacteria were resistant to some plant extracts. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (20).

The inconsistent antibacterial activity of some plant extracts may be due to the season of plants were harvest, the type of plant material used (fresh or dry) and the type of solvent used, in addition, the amount of plant constituents responsible for the antibacterial activity (21).

Table(2): Effect of corn silk extracts on bacteria using broth dilution technique.

Extract	Concentration mg/ml	*P. mirabilis*	*K. aerogenes*	*E. coli*
Hot alcohol	50	+ +	+ +	+ +
	100	+ + +	+ + +	+ + +
Cold alcohol	20	+	-	-
	25	+	+	+
Hot water	25	-	-	+
	50	-	-	-
Cold water	25	-	-	-
	50	-	-	+
	100	-	-	+

+ + +High response
+ + Intermediate response
+ Less response
- No response

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