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# Study of the Effect of Carob *(Ceratonia siliqua L.)* Extract Activity as Antibiotic from UTI

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***Ceratonia siliqua L, E. Coli*,Antibacterial Activity, Ampicillin, Gentamicin, Amikacin, Clindamycin Drugs, Agar Diffusion Method.**
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Abstract

*Escherichia coli* bacterial cells have been collected and selected from(30) patients (most found strain) in urine samples 25 (83.3 %) suffering from infection of urinary tract laid down in Hashimiyah teaching hospital, Babylon during a period from November 2016 to February 2017.

The isolated strain diagnosis is confirmed with Vitek 2 system apparatus which perform to identify species level of *Escherichia coli* isolates .To evaluate the antimicrobial action of the ethanol extract of Carob (*Ceratoniasiliqua L.*) pods only as well as in mixture with certain drugs (64 µg /ml ampicillin, 32 µg /ml gentamicin, 128 µg /ml amikacin, 8 µg /ml clindamycin.) as the wide usage antibiotics in the treatment of UTI bacterial infections which has led to the emergence and spread of resistant strains. Many studies show that the efficacy of antimicrobials can be improved by combining them with crude plant extracts. The antimicrobial activity of the ethanol extract of pods of Carob (*Ceratonia siliqua L.)* alone as well as in mixture with some standard antimicrobials has been evaluated using well diffusion method which demonstrates an in-vitro antibacterial activity of the tested extracts against *E. Coli* bacteria. A combination of the tested extracts( concentration 100%,50%) with antibacterial has increased the activity of the tested antimicrobials. The results reveal the importance of Carob plant extracts when associated with antibiotics to regulator resistance *E. Coli* bacteria developed as a danger to human health.

## Introduction

The bacterial resistance to the known antibacterial agents had become a serious global problem for instance, bacterial infections are liable for 90% of infections located in health care services offerings and 70% of the bacterial infections had been proof against at minimum one antibiotic (1)

Resistance mechanisms may additionally consist of the manufacturing of drug inactivating enzymes, efflux pumps and target-site or outer membrane modifications. Resistance to multiple drugs is typically the result of the mixture of various mechanisms in single isolate or the action of of a single potent resistance mechanisms (14). As a result, new antibacterial agents or combinations are desperately wanted. Ceratonia siliqua L. is a leguminous evergreen tree which is native to the mediterranean location. It belongs to family fabaceae and to the caesalpinioideae sub-family (15). It is far referred to as carob, algarroba, locust bean, locust tree, st. john's bread and in arabic is kharroub (16,17).The primary phytochemicals detected in ceratonia siliqua L. are polyphenols which include condensed and hydrolysable tannins, phenolic

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acids, flavonoids, and flavonoidal glycosids suggesting a capacity antibacterial and cytotoxic activities (18). Carob pods are a conventional a part of the weight loss program inside the Mediterranean place and carob sauce is a general thirst-quencher in many countries including Egypt (19). The greatest communal bacterial agent concerned in causation of UTIs is *Escherichia Coli* (20).Because of this ,the aim of present study is to investigate the bacterial growth inhibitory effect of (*Ceratonia siliqua L*). plant extracts alone and in combination with some traditional antimicrobial drugs (64 µg /ml ampicillin, 32 µg /ml gentamicin, 128 µg /ml amikacin, 8 µg /ml clindamycin) in order to enhance the potential antimicrobial activity of these antibiotics against *Escherichia coli* isolates as most found bacteria in UTI using carob aqueous extract.

**Materials and Methods:**

Bacterial strain:

Bacterial cells of *Escherichia Coli* have been collected and selected from 30 patients (most found strain) in urine samples 25 (83.3 %) suffering from infection of urinary tract whom Fallen asleep in Hashimiyah teaching hospital, Babylon during a period from November two thousand sixty to February two thousand seventy.

Isolation and diagnosis of bacterial strain:

A- After bacterial detection from urine samples, the most popular bacteria that cause urinary tract infection are diagnosis by culturing on suitable bacterial media which reveals in table (1)

**Table (1):Characteristic of *Escherichia Coli* on different culture media**

| | Blood agar base media | MacConkey media | Eosin Methylene Blue media |
|---|---|---|---|
| *Escherichia Coli* | Cells appear as small size, Gram negative reactions with partial hemolysis ability | cells as small, pink colour with lactose fermented ability. | colonies appear with green metallic sheen |

B-the diagnosis is confirmed by Vitek $_2$ system which performed to identify species level for E. coli isolates.

Carob Extract Preparation:

This is accomplished by using maceration approach in which pods of carob have been shaded dried and beaten first by means of hand then via mechanical blender to offer finely grounded powder.500gm of powder become macerated with 80% ethanol solution for 6 hrs. with continuous stirring by magnetic stirrer. After filtration the extract with Millipore 0.45 filter paper ,it has changed into dried and give dark crimson, gummy residue. Residual extract becomes dissolved in water then fractionated by using separated funnel using organic solvent (ethyl acetate 3%) to boom polaries. The extract has turned into lyophilized and stored for similarly use (21).

Antibacterial Activity: -

The antimicrobial actions of *C. siliqua* extract have been evaluated by means of agar-well diffusion assay (22). Microorganism (0.5 ml) of 1 $\times 10^6$ CFU/ml(0.5Mcfarland turbidity) are put in pure petri dishes then twenty mil$_s$ of heated and cooled (45°C) of Muller Hinton media is supplementary to wholle petri dishes . The prepared plats were rotated slowly to make certain uniform distribution of the microorganisms after which they are allowed to solidify on a flat surface. after solidification,

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3 equidistant and circular wells of 10 mm diameter are cautiously punched by the usage of a sterile cork borer. Each sample (5mg/ml) is turned into implemented as triplicate. For prediffusion of the extract to occur; the plates are allowed to stand for one hour then incubated in a single day at 37°c. Finally the plates are observed and zones of inhibition are documented (2).

The Minimum Inhibitory Concentration (MIC) of Bacterial- Minimum inhibitory concentrations (MIC) have been applied by way of broth dilution technique in culture tubes with a few modification. In the tube dilution assay, the extract to start with organized at 50 mg/ml then standard bacterial suspension and specific concentration of extract (100%,75%,50%,25%) had been add to the tubes containing 1.9 ml muller-hinton broth.

Inoculation with (0.1) ml of suspension containing $10^7$ CFU/ml of bacterium were applied for each tubes and incubated at 37c° for twenty-four hours. Tubes are observed for noticeable growth or absence growth in each dilution of tested bacteria. Turbidity indicates a growth of bacteria and MIC which the lowermost concentrations where no growth is visually observed (3).

Determination of the Minimum Inhibitory Concentration (MIC) of Bacterial-Extract-Antibiotics solution:

The initial attention of used antibiotics on this search, 64 µg /ml for ampicillin - 32µg /ml of gentamicin, 128 µg /ml of Amikacin, finally 8µ g /ml for clindamycin as the wide usage antibiotics in the treatment of UTI bacterial infections .Same volumes of the combination (E. coli and extract) combination and antibiotics have been prepared after which diluted in moller- hinton broth to the equal dilution ratio as above. (4). Incubation for overnight at 37°c, is performed for each tubes then examined for the seen growth or absence boom(turbidity) has been determined.

**Result and Discussion**

Antibacterial activity: -

The end result of the conducted experiment the usage of water carob pods extract with disc diffusion approach with specific concentration against *E.coli;* reveal that maximum antibacterial activity become in (100%, and 50)% which exhibited the largest inhibition (diameter of the inhibition region > 25 mm)towards *E. coli* as show in figure:(1):

Figure (1) Antimicrobial action of carob extract pods on (Muller Hinton) media

Antimicrobial action of most common drugs against *E. coli* in UTI were determined also by diffusion method. The drugs were(64µg /ml for ampicillin,32µg /ml for gentamicin,128µg /ml for amikacin, and 8µg /ml for clindamycin.) all of these drugs were effective against expect clindamycin where the isolated strain of *E.coli* exhibit an resistance ,the inhibition zone

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diameters was (8.2mm,7. 8mm,5.5mm,0mm) as shown blow in the figure (2):

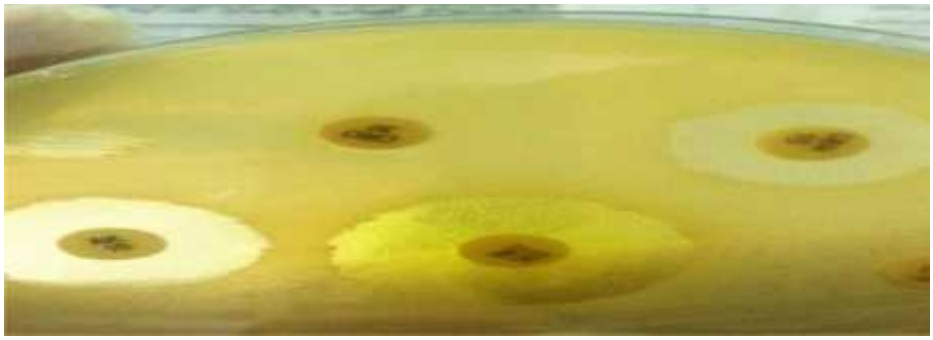

figure (2): Antibiotic Activity of Tested Drugs Against *E. coli* .

After incubation period (24hrs), Minimum Inhibitory Concentration was determined by observing turbidity of tested tubes by naked eye. Turbidity refer to growth of bacteria. MIC is determined as the lowermost concentration of antimicrobial that will prevent noticeable growth of microorganisms after incubation period for overnight . (5) .In this study, MIC values of carob extracts are compared in comparison with some commonly antibiotics used in the current study. Table (2,3,4) and figure (3,4)

**Table (2): MIC of The Tested Extracts Against *E.coli*:**

| Tested bacteria | MIC of Bacteria-Carob- Extract solution |
|---|---|
| *E.coli* | 4 |

**Table (3): The MIC of The Used Antibiotics Against *E.coli*:**

| Tested Bacteria | MIC of bacteria-antibiotics | | |
|---|---|---|---|
| *E.coli* | Ampincillin | Gentamicin | Amikacin |
| | 6 | 8 | 5 |

**Table (4): The MIC of Combinations of Carob-Antibiotics Against *E.coli*:**

| Tested bacteria | MIC of Carob –Antibiotics | | |
|---|---|---|---|
| *E.coli* | Ampicillin | Gentamicin | Amikacin |
| | 5 | 4 | 4 |

In table (2,3,4): illustrates the values MIC for each extract and antibiotic separately as well as the values MIC of extract and antibiotic together respectively. The current results shows synergistic outcome for the two component (tested antibiotics and pods extract)

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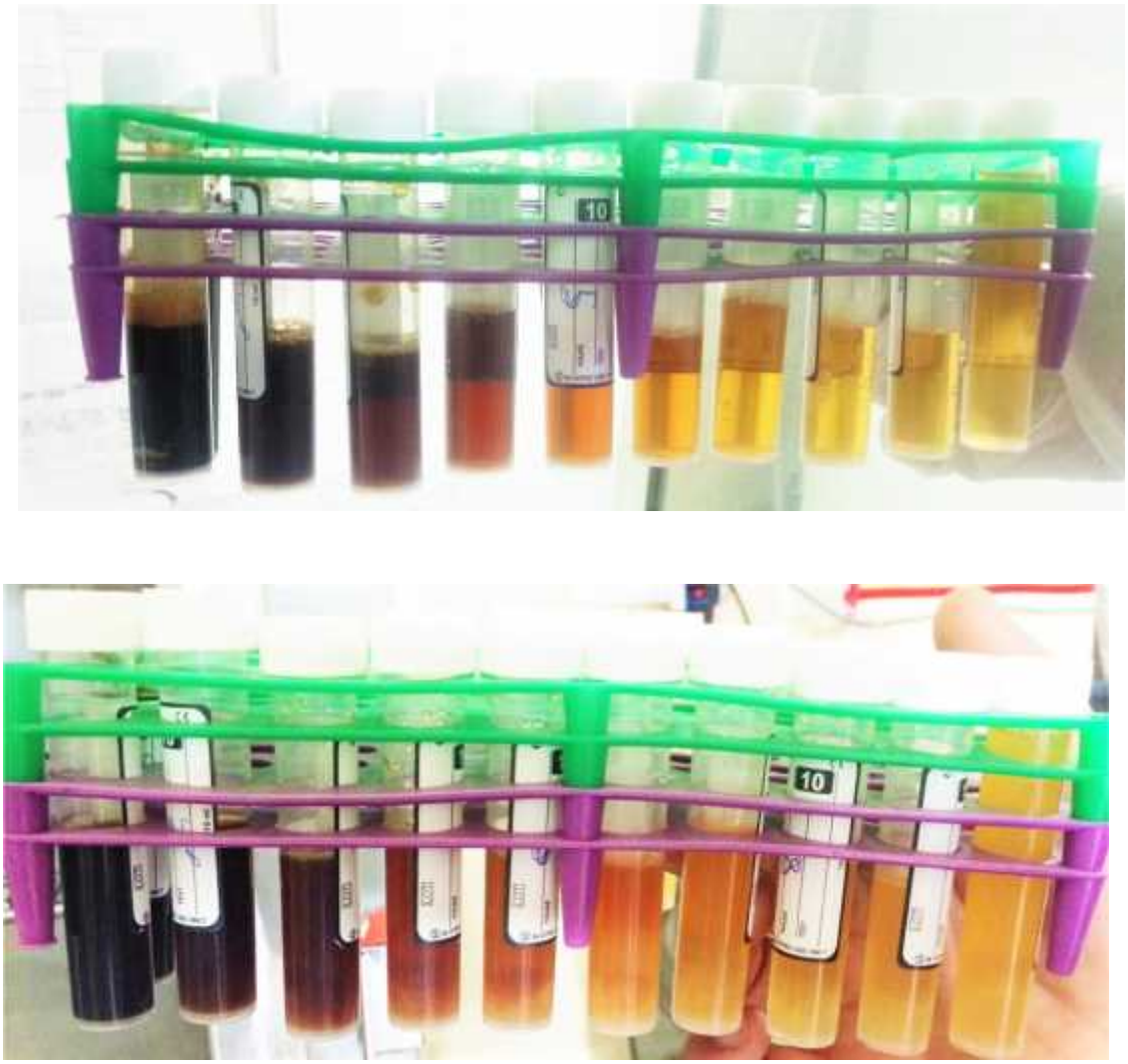

Figure (4):MIC of Antibiotic and bacterial_ extract.

Currently, because of the dramatic failures of synthetic antibiotics to overcome the developing resistant pathogens, medicinal plants emerge as alternative source for new accepted antibacterial agents(6). It is known that phytochemical compounds of medicinal plants like flavonoids, alkaloids, phenols, glycosides, sterols, saponins have curative properties (7). The strong antibacterial activity of *Ceratonia siliqua* L. preparations and synergistic effect within antibiotics may be related to its gradient of flavonoids and tannins. Flavonoids as well as phenolic compounds are present in different quantities in most vascular plants (8).They are a subject of medical research, have pharmacological benefits, including antioxidant, anti-inflammatory, ant allergic, hepatoprotective, antiviral, antimicrobial and ant carcinogenic activities(9)(10) . Quercetin, apigenin and (-)-epigallocatechin are recorded as inhibitors for DNA and RNA synthesis. In other hands , flavanones, quercetin as well as catechins are described to pose inhibitory action on cytoplasmic membrane function. This could illustrate the synergism exhibited by *Ceratonia siliqua* L. extracts and fractions with antibiotics (11) rutin,.Naringin as well as apigenin are Carob flavonoids has stated antimicrobial action(12). It was found also that

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*Ceratonia siliqua* shows an increase in the antimicrobial activity of the tested antimicrobials against the tested microorganisms as the zones of inhibition in antibiotic/plant extract plates are in the range of 1-39 mm wider than the zones of inhibition in the control plates (containing antibiotics without the plant extract) depending on the species of bacteria which in agreement with the results obtained by Bijen and Tuba and Ben Hsouna *et al*., who have reported that methanol extract of *Ceratonia siliqua* shows strong action on, , *Escherichia coli* (13).

CONCLUSION

The results show that plant extracts increase the therapeutic activity of the tested antimicrobials against the tested *E. Coli.*

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## دراسة تأثير المستخلص الكحولي المعزولة لنبات (Ceratonia siliqua) كمضاد حيوي ضد بكتريا الاشريكيا القولونية من اخماج الجهاز البولي

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**الخلاصة**

تم عزل وتشخيص (30) عينة من بكتريا الاشريكيا القولونية من مرضى راقدين في مستشفى الهاشمية التعليمي والذين يعانون من اخماج في الجهاز البولي للفترة من تشرين الثاني 2016 الى شباط 2017 .تم عزل وتشخيص البكتريا اعتمادا الصفات المظهرية بعد زراعتها على الاوساط المناسبة وتم تأكيد هذا التشخيص باستعمال نظام الفايتك 2 الذي يقوم بالتشخيص على مستوى النوع لبكتريا الاشريكيا القولونية المعزولة.

قد تم تحديد الفعالية المضادة للمستخلص الكحولي لبذور الخروب ضد بكتريا الاشريكيا القولونية المعزولة لوحده و كذلك عند دمجه مع مضادات حيوية قياسية مستعملة لمعالجة اخماج الجهاز البولي شملت (الأمبيسلين64 ملغم /مل ، جنتاميسين 32 ملغم \مل ،أميكاسين 128 ملغم\مل ، الكليندامايسين 8 ملغم \مل ). كذلك تمت المقارنة بينهما على اساس اقل تركيز مثبط لنمو بكتريا الاشريكيا القولونيه. وأظهرت هذه الدراسة أن فعالية المضادات الحيوية المذكورة يمكن تحسينها من خلال دمجها مع المستخلص النباتي الخام (تركيز 100%)والتركيز(50%).و كشفت الدراسة ايضا عن أهمية مستخلص الخروب عندما يرتبط مع المضادات الحيوية للسيطرة على البكتيريا المقاومة التي يمكن أن تصبح خطرا على صحة الإنسان .

الكلمات المفتاحية : Ceratonia siliqua L ، بكتريا القولون ، مضاد حيوي ، امبسيلين ، جنتاميسين ، اميكاسين ، كليندامايسين ، طريقة انتشار الاكار.