Antibacterial Effect of some Herbal plants against Pathogenic Bacteria Isolated from Patients with Urinary Tract Infections (UTI)

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Abstract

The antibacterial activity of ethanol and water extracts of borage officinal's Eucalyptus leaves, and Cinnamon, were investigated in vitro using disk diffusion method. All Extracts were tested against four pathogenic bacteria (**Staphylococcus aureus**, **Klebsiella spp**, **Pseudomonas aregenosia and Proteus spp).** Cinnamon ethanol extract exhibited better antibacterial activity against gram positive and gram negative bacteria then aqueous extract gave the same effect against the same bacteria. Cinnamon ethanol extract gave highest effect against **S. aureus**, then lower effect on **Klebsiella spp** and **Proteus spp,**and no effect against **P. aregenosia.**

borage officinal's ethanol extract gave the highest effect against **S.aureus** ,then very lower effect was shown against **P.aregenosia,** while no effect was observed against **Klebsiella** spp and **Proteus spp** . Eucalyptus leaves ethanol extract gave high effect on **S. aureus**,while low effect was observed against **P. aregenosia** and **Proteus spp,** no effect was shown against **Klebsiella spp.** In conclusion the antibacterial activity of the plant extracts studied showed a better effect on Gram positive bacteria as compare with Gram negative bacteria.

Keywords: Antibiotics, antimicrobial resistance, antibacterial effect , bacterial cell wall herbal plants.

التأثير المضاد للبكتيريا لبعض الأعشاب ضد البكتيريا المعزولة من الأشخاص المصابين بالالتهاب المجاري البولية

تم التحقيق في النشاط المضاد للبكتيريا من مستخلصات الإيثانول والمستخلصات المائية من أوراق الأوكالبتوس لسان الثور والقرفة ، في المختبر باستخدام طريقة نشر القرص. تم اختبار جميع المستخلصات ضد أربعة أنواع من البكتيريا المسببة للأمراض (*Staphylococcus aureus (المكورات العنقودية الذهبية المذهبة)* , *Pseudomonas aregenosia and Proteus spp (Klebsiella spp (الزوائف الزنجارية)*. أظهر مستخلص الإيثانول من القرفة نشاطًا مضادًا للجراثيم بشكل أفضل من المستخلص المائي للقرفة ضد البكتيريا الموجبة والسالبة لصبغة جرام الذي أعطى تأثير ضد البكتيريا نفسها. مستخلص الإيثانول من القرفة اعطى أعلى تأثير ضد المكورات العنقودية الذهبية المذهبة *S. aureus* ثم تأثير أقل على *Klebsiella spp* و *Proteus spp* ، ولم يكن له أي تأثير ضد الزوائف الزنجارية *P. aregenosia* .

أعطى مستخلص الإيثانول من لسان الثور borage officinal أعلى تأثير ضد المكورات العنقودية الذهبية المذهبة *S.aureus* ، ثم ظهر تأثير أقل للغاية ضد *P.aregenosia*، بينما لم يلاحظ أي تأثير ضد *Klebsiella* spp و *Proteus spp*.

أعطى مستخلص الإيثانول من أوراق الأوكالبتوس تأثيرًا كبيرًا على المكورات العنقودية الذهبية المذهبة ، بينما لوحظ تأثير منخفض ضد الزوائف الزنجارية و *Proteus spp* ولم يظهر أي تأثير على *Klebsiella spp*. في الختام ، أظهر النشاط المضاد للبكتيريا في المستخلصات النباتية التي تمت دراستها تأثيرًا أفضل على البكتيريا الموجبة للجرام مقارنةً بالبكتيريا سالبة الجرام.

Introduction

Several pathogens still represent a major public health problem in both developed and developing countries. ***Salmonella spp., Clostridium perfringens, Campylobacter, Vibrio parahaemolyticus, and entero-pathogenic Escherichia coli*** cause over 90% of all cases of food poisoning. The extensive use of antimicrobials has driven increasing resistances among several bacterial species and, as a matter of fact, the efficacy of these inhibition compounds is seriously decreased. In the recent decades emerge of antibiotic resistant pathogens has been a worldwide problem. The undesirable side effects of some of antibiotics made us to search for new sources to combat these problems (1, 2, 3)

The researchers are showing interest towards natural products with bactericidal activity. Several compounds found in plants, which have long been used as agents for food preservation, represent natural alternatives to chemicals for the maintenance or shelf-life extension of food products. Search for new materials to fight against this problem seems necessary (4).

The plant world is the source of many medicines. Recently, researchers have estimated that there are about 400,000 species of plants worldwide, including about a quarter or a third have been used by companies for medicinal purposes .Humans use plants for thousands of years to treat various diseases, in many developing countries; much of the population relies on traditional doctors and their collections of medicinal plants to cure them (5)**.** Herbals can play an important role in conserving biodiversity. These plants are actually very familiar to rural people who are very sensitive to their scarcity and their disappearance. Indeed, medicinal plants play an important role of health care population and represent a significant source of income for many families in the country side and cities (6).

Borage is a large hairy annual herb that is a member of Boraginaceae family. It grows in mostof Europe, in the Mediterranean region, and also in northern parts of Iran. The flowers are bright blue and star-shaped and the fruit consists of four brownish-black nutlets. Borage flourishes in ordinary soil and may be propagated by division of rootstocks and by cuttings of shoots in sandy soil in a cold frame in summer and autumn or from seeds sown in good light soil from mid of March to May (7,8). Borage constituents have been isolated by different investigators; they include gamma-linolenic acid (GLA), alpha-linolenic acid(ALA), delta6-fatty acyl desaturase, delta8-sphingolipid desaturase and due to its content in gamma linolenic acid (GLA), borage is gaining increasing agricultural interest because GLA seems to have anti-inflammatory effects. Borage flower might have an antioxidant effect, and No adverse effects have been found. Although no side effects have been reported, but borage leaves, flowers, and seeds contain small amounts of pyrrolizidine alkaloids that may be hepatotoxic (damaging to the liver) especially at high doses for long periods of time (9).

Benefits of Borage has been used by Iranian folk as a mood enhancer, an anxiolytic, anti inflammatory, anti laxative,an emollients and also it has been use for treatment of infectious diseases ,skin disorders including eczema, seborrheic dermatitis,and neurodermatitis. It is also use for rheumatoid arthritis (RA), stress, diabetes, attention deficit-hyperactivity disorder (ADHD), acute respiratory distress syndrome, alcoholism, and stroke. The flowers and the leaves of borage are used medicinally in France as an antifebrile, anti-depressive, for the treatment of stress and of circulatory heart diseases, for pulmonary complaints, as a poultice for inflammatory swellings, and for a hormone problem called adrenal insufficiency, for "blood purification," to increase urine flow, to prevent inflammation of the lungs, as a sedative, and to promote sweating. Borage is also used to increase breast milk production and to treat bronchitis and colds (10,11,12) .

Cinnamon species are the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. The most important constituents of cinnamon are cinnamaldehyde and transcinnamaldehyde, which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (13,14). Cinnamon bark contains procyanidins and catechins, The components of procyanidins include both procyani-din A-type and B-type linkages These procyanidins extracted from cinnamon and berries also possess antioxidant activities (15,16). Benefits of cinnamon In addition to being used as a spice and flavoring agent, cinnamon is also added to flavor chewing gums due to its mouth refreshing effects and ability to remove bad breath. (17).
Cinnamon can also improve the health of the colon, thereby reducing the risk of colon cancer Cinnamon is a coagulant and prevents bleeding Cinnamon also increases the blood circulation in the uterus and advances tissue regeneration This plant plays a vital role as a spice, but its essential oils and other constituents also have important activities, including antimicrobial, antifungal, antioxidant, and antidiabetic (18,19).

Cinnamon has also been traditionally used as tooth powder and to treat toothaches, dental problems, and oral microbiota. (20). Several antimicrobial activities of cinnamon and its oils have been reported in various studies For example, Matan *et al*.2006 reported the effects of cinnamon oils on different bacteria, fungal, and yeast species (21).

Eucalyptus actually refers to a large genus of flowering trees that has over 700 different species, most of which are located in Australia and New Zealand, although some of the more widespread species can be found throughout Southeast Asia. Due to the diverse uses of eucalyptus, it has been naturalized in various other tropical and temperate regions throughout the world **.**Benefits of Eucalyptus leaves are first Respiratory Health, eucalyptus leaves act as expectorants, helping to remove excess phlegm and mucus from the sinuses and respiratory tracts, eliminating the natural environment for bacteria and other pathogens to multiply and spread. In terms of bronchitis, the common cold, and even flu symptoms. Second is Immune System booster; third is Skin Health

the natural antibacterial properties of eucalyptus make it ideal for protecting skin health as well. Diabetes management and prevention although the exact chemical pathway is unknown, research has shown that brewing eucalyptus leaves into tea can be an effective preventative measure or treatment for diabetes (22).

Aim of study: our study foxing on the detection of antibacterial effects of borage officinal's, Eucalyptus leaves, and Cinnamon.

Material and Method

Preparation of the herbal powder: The herbal plants (borage officinal's, Eucalyptus leaves, and Cinnam) were collected from the local market of Baghdad.

Preparation of extraction: Herbal powder (500 gram) was extracted in 1500 ml of either ethanol or water for 12 hours by mixing on a magnetic stirrer, and filtered by whatman filter paper NO 1. Supernante was collected. Then the filtrates were divided into portions and left to dry at 55 °C. Different weights 0.4g ,0.3g ,and 0.62g of herbal obtain from ethanol extract were mix with 1.5ml of DMSO, then filtered the mixture through Millipore filter 0.22 microfilter and stored as stoke solution at 4C° until use .Then many concentration of this ethanol extraction was prepared (borage officinal's 0.4g/1.5ml and 0.2g/1,5ml),(Eucalyptus leaves 0.62g/1.5ml and 0.31g/ 1,5ml), (Cinnamon 0.3g /4 ml and the concentration of Cinnamon Distilled water extraction 1.87g /4 ml).

Isolation and identification of bacteria:
Twenty four samples of urine were collected from patients with UTI (urinary tract infection) from Ibn Al-Nafees Hospital .All samples were cultured on blood and MacConkey agar, then Gram stain, Urease test , Catalase test and other media like mannitol salt agar, nutrient agar and milk agar was done for further isolation and identification of pathogenic bacteria.

Antibacterial activity of the ethanol and aqueous herbal extracts:
Disk diffusion method on Mueller - Hinton agar was used to search for antibacterial activity.(34)

Turbidity standard: Prepare the turbidity standard by compare the turbidity of bacterial suspension which be cultured on Mueller -Hinton agar with turbidity of machferland tube number 1 (Approx. cell density (1X10^8 CFU/mL) : 3.0). (33) Disc dispenser can be used to apply the discs to the inoculated plate. A maximum of four discs can be placed on a 9-10 cm plate, approximately 15 mm for edge of plate. Each disc should be gently pressed down to ensure even contact with the medium.The plate should be placed in an incubator at 37°c for overnight incubation, the diameter of each zone (inoculating the diameter of the disc) should be measured and recorded in mm. The results should then be interpreted according to the critical diameters. The measurements can be made with a ruler on the under-surface of the plate without opening the lid.

Results and *Discussions*

Isolation and identification of bacteria:
The result of isolation and identification of bacterial isolates were presented in the Table (1) indicated the morphological and some biochemical characteristics of

bacteria isolated from the patients with UTI.

Antibacterial activity

Ever increasing demands from consumers for use of natural agents as additives and food preservatives, and the increased incidence of new and re-emerging infections, has led to a search for new and more effective antimicrobial compounds that have diverse chemical structure and novel mechanism of action. Plants are an invaluable source of pharmaceutical products, because they have an almost infinite ability to synthesize compounds with different antimicrobial activity against various pathogenic and opportunistic microorganisms (23). Each extracts were tested against the four isolates and identified bacteria (*S. aureus, P. aeruginosa, Klebsilla.spp and Proteus.spp)* . The antibacterial activity of the extracts were recorded as the mean diameter of the resulting inhibition zones of growth measured in (millimeters). The antibacterial activity of herbal extracts are summarized in Table (2,3,4 and 5).

In this study all herbal used on *S.aureus* have antibacterial effects ,but some of these herbal used have effects or no effect on gram negative bacteria ,as seen in table (2,3,4and 5),and figure (1,2and 3).

Table (1): the morphological and characteristics of bacteria on different culture media with some biochemical test

No.	Bacteria	Blood agar	MacConky agar	Mannitol-salt agar	Nutrient agar or milk agar	Gram stain	Catalase test	Urease test
6,1,9,	***Klebsiella***	White colony, no hemolysis	Pink mucoid colony	*	White colony	G-ve rod	#	–
22,10,2	***Proteus***	Swarming , no hemolysis	Pale colony N.L.F	*	White colony	G-ve rod	#	+ pink
4,7,20	***P.aregenosia***	White colony with B-hemolysis	Pale colony N.L.F	*	Blue to green color	G-ve rod	#	–
5, 8, 16,19	***S.aureus***	B-hemolysis	*	Mannitol fermenter	White colony	G + grape like clusters	+ bubbles	#

Notes:***** no growth , # not done this tes

Table (2): Diameter of inhibition zone of different plant are reputation against S.aureus

bacteria	*Type of plants*	*Concentration of ethnolic extract or D.W*	*Size of inhibition zone*
S.aureus	borage officinal's	0.4 g /1.5 ml	20 mm
		0.2 g /1.5 ml	16 mm
	Cinnamon	0.3 g /4 ml, D.W 1.87g/4ml	21 mm,16mm
	Eucalyptus leaves	0.62 g /1.5 ml	21 mm
		0.31 g /1.5 ml	20 mm

Fig(1):- Inhibition zone with different types and concentration of plant extracts against S.aureus, which cultured on Mueller -Hinton agar

borage officinal's Eucalyptus leaves Cinnamon

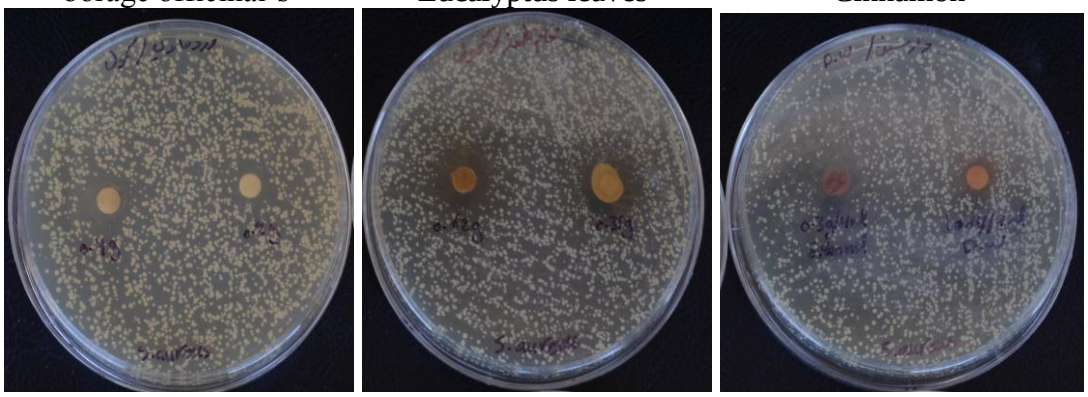


Table (3) : Diameter of inhibition zone with different types and concentration of plant extracts against Proteus.spp

bacteria	*Type of plants*	*Concentration of ethnolic extract or D.W*	*Size of inhibition zone*
proteus	borage officinal's	0.4 g /1.5 ml	R
		0.2 g /1.5 ml	R
	Cinnamon	0.3 g /4 ml, 1.87g/4ml D.W	9 mm, 8 mm
	Eucalyptus leaves	0.62 g /1.5 ml	15mm
		0.31 g /1.5 ml	10 mm

Fig(2):- Inhibition zone with different types and concentration of plant extracts against Proteus spp, which cultured on Mueller -Hinton agar

Eucalyptus leaves Cinnamon borage officinal's

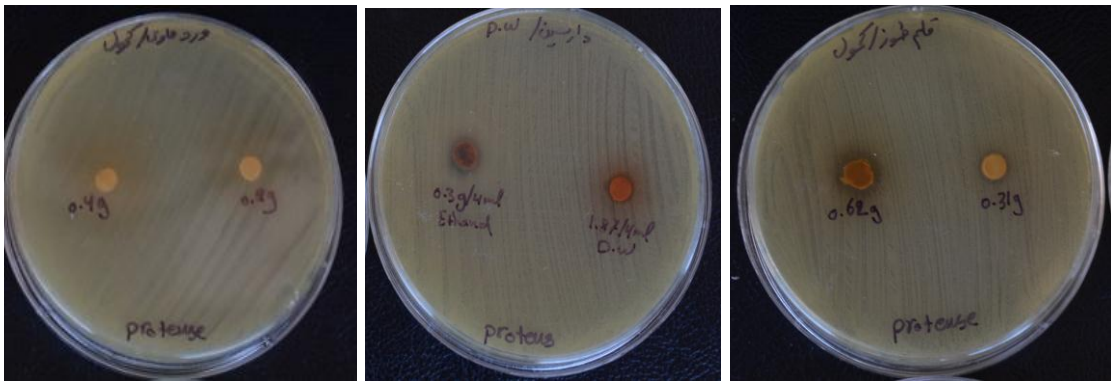


Table (4): Diameter of inhibition zone with different types and concentration of plant extracts against Klebsilla.spp.

bacteria	*Type of plants*	*Concentration of ethnolic extract or D.W*	*Size of inhibition zone*
Klebsilla	borage officinal's	0.4 g /1.5 ml	R
		0.2 g /1.5 ml	R
	Cinnamon	0.3 g /4 ml, (1.87g/4ml D.w)	15 mm, 7mm
	Eucalyptus leaves	0.62 g /1.5 ml	R
		0.31 g /1.5 ml	R

Fig(3):- Inhibition zone with different types and concentration of plant extracts against Klebsilla spp, which cultured on Mueller -Hinton agar

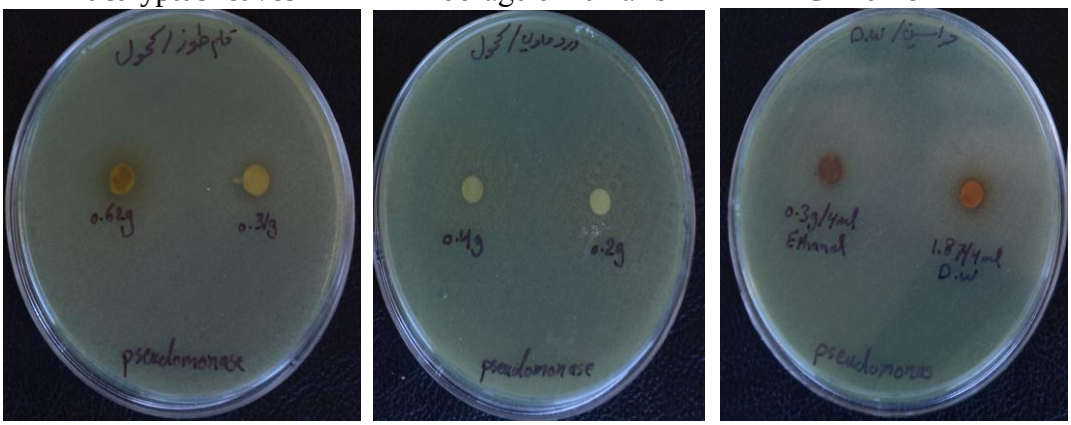
borage officinal's Cinnamon Eucalyptus leaves

Table (5): Diameter of inhibition zone with different types and concentration of plant extracts against P.aeruginosa.

bacteria	***Type of plants***	***Concentration of ethnolic extract***	***Size of inhibition zone***
P. aeruginosa	borage officinal's	0.4 g /1.5 ml	8mm
		0.2 g /1.5ml	R
	Cinnamon	0.3 g /4 ml,(1.87g/4ml DW)	R, R
	Eucalyptus leaves	0.62 g /1.5 ml	8mm
		0.31 g /1.5ml	8mm

Fig(4):- Inhibition zone with different types and concentration of plant extracts against P.aeruginosa, which cultured on Mueller -Hinton agar.

Eucalyptus leaves borage officinal's Cinnamon



The main factors that determine antibacterial activity are type, composition of the extract used, pH and temperature of the environment (27). The pH may effects on the antibacterial activity of extract like *R. sativus* had excellent antibacterial activity at acidic pH, and that increasing the pH of the extracts toward alkaline led to a significant drop in their inhibitory action. It has been reported that antibacterial compounds seemed to be stabilized in cationic forms that may interact with and disrupt the negatively charged bacterial cells (28).

Successful extraction of bioactive compounds from plant material depends on the solvent used in the extraction procedure. the extraction of the plant with the organic solvents methanol, ethyl acetate, and chloroform resulted in much greater antibacterial activity against all the health-damaging bacteria than extraction with water ***(29),*** and this study agree with (syed)as ethanol extract of Cinnamon give batter results on bacteria then Cinnamon water extract on the same bacteria.

The activity of cinnamon is due to the presence of cinnamaldehyde, an aromatic aldehyde that inhibits amino acid decarboxylase activity (*30*), and has been proven to be active against many pathogenic bacteria (***31***). Cinnamon bark is rich in cinnamaldehyde (50.5%), which is highly electro-negative. Such electro-negative compounds interfere in biological processes involving electron

transfer and react with nitrogen-containing components, e.g. proteins and nucleic acids, and therefore inhibit the growth of the microorganisms.
Another study done by Fyfe *et.al*. (32) Demonstrated that cinnamon and clove significantly decreased the production of enterotoxin A and enterotoxin B of *staphylococcus aureus*.

Gram-negative bacteria are in general more resistant to large number of antibiotics and chemotherapeutic agents than are gram-positive bacteria. A survey of recently reported antibiotics of natural origin showed that >90% lacked activity against E.coli, although they were active against gram-positive bacteria (24).
Mechanisms of Antimicrobial Resistance Prevention of accumulation of antimicrobials either by decreasing uptake or increasing efflux of the antimicrobial from the cell ,Changes in outer membrane permeability Drug molecules to a cell can be transferred by diffusion through porins, diffusion through the bilayer and by self-uptake. The porin channels are located in OM(outer membren) of Gram-negative bacteria. The small hydrophilic molecules (β-lactams and quinolones) can cross the OM only through porins. The decrease in number of porin channels, lead to decreased entry of β-lactam antibiotics into the cell, hence resistance .(25,26)

Plasmid Mediated Drug Resistance by Conjugation between the commensal-commensal, commensal-pathogen and pathogen-pathogen are responsible for the development of resistance in bacteria. Acquisition of resistance by transduction is common in gram positive bacteria like Staphylococcus (penicillinase plasmid); where as in gram negative bacteria conjugation is a major mechanism of transfer of drug resistance and can occur in unrelated genera ***(35).***

The cell wall of Gram-positive bacteria is formed of a thick layer of peptidoglycan that protects against osmotic rupture. The basic subunit of the peptidoglycan component is a disaccharide monomer of *N* -acetylglucosamine (NAG) and *N* -acetylmuramic (NAM) pentapeptide. The pentapeptide consists of amino acid residues alternating between L- and D-stereoisomers and terminating in D-alanyl-D-alanine. A stem peptide of variable length and composition is attached to the third amino acid of this pentapeptide. Pentapeptides are then joined with stem peptides to form a cross-link between polysaccharide chains. This reaction is catalyzed by a transpeptidase. This transpeptidation reaction is sensitive to inhibition by β-lactams. The penicillin-sensitive reactions are catalyzed by a family of closely related proteins, penicillin-binding proteins (PBPs). β-Lactam antibiotics produce their lethal effect on bacteria by inactivation of multiple PBPs simultaneously, and thus inhibiting cell wall synthesis. The inhibition of PBPs also leads to disruption of a crucial event probably at the time of cell division. This disturbed morphogenesis is hypothesized to initiate cell death *(25)* .

In conclusion cinnamon, borage officinal's and Eucalyptus leaves were found to have important antibacterial activity against *S. aureus*. In this regard, the use of them as natural preservatives in food products (to prevent food poisoning by *S. aureus*) may be alternative of chemical additives they can be also incorporated into creams, lotion to treat diseased caused by *S. aureus,* and may addition these herbals to sapon to decrees the urinary tract infection**.** The study also

shows that further research on the effects of spices and essential oils on microorganisms can be rewarding to pursue in the search for new broad spectrum antimicrobial agents.

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