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Eucalyptus extract and its synergistic effect on bacteria causing urinary tract infections in Diyala province

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ABSTRACT: To assess the impact of E. camaldulensis extract against bacteria that are isolated from urine sample from UTI patient in Baquba city Then mix this plant with antibiotics to see its effect. In addition to examine those pathogens bacteriologically. The sensitivity of the isolates to (13) antibiotics was tested, and the bacterial isolates showed a variation in the rates of their resistance to these antibiotics. The results showed that the antibiotic Imipenem had the most effect on the bacterial isolates under study, while the bacterial isolates showed high resistance to the rest of the antibiotics. The minimum inhibitory concentration (MIC) was determined for 3 antibiotics, namely, Gentamycin, Amikacin and Amoxicillin. These values for the antibiotics ranged between <4-1024, <4-1024 <<512-1024) mcg/ml, respectively. The Ethanolic Extract of Eucalyptus Exhibit highest antibacterial effect on S.areus on (o.62 g/1.5 ml and 0.31g/750ml). Lower respectively antibacterial effect on Proteus and Pseudomonas but no antibacterial effect against Klebsiella. Bacterial isolates were selected on the basis of their most frequent urinary tract infections and their resistance to the effect of antibiotics (K. pneumoniae, P. aeruginosa and S. aureus). The amount of the mixed effect was determined by mixing Eucalyptus with three antibiotics, namely Amikacin and Gentamycin Amoxicillin. The minimum inhibitory concentration of the isolates under study was compared before and after mixing the antibiotics. The effect of mixing was a synergistic effect for all bacterial isolates. On the other hand, the synergistic effect was clear on the gram-positive isolates of S. aureus between eucalyptus and beta-lactams (and Amoxicillin).

Keywords: Ethanolic Extract of Eucalyptus, Eucalyptus, urinary tract infections

1. INTRODUCTION

Urinary tract infections (UTIs) are set of urinary tract inflammations or disorders mainly of microbial cause, i.e., specifically bacterial. Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus spp. and beta-hemolytic streptococci are Gram positive bacteria responsible for UTI, on other hand the Gram-negative ones they are mainly cause that's infections include E. coli, Proteus spp., Pseudomonas aeruginosa, Klebsiella strains, Salmonella spp., Neisseria gonorrhea and other non-Gram bacteria as Mycobacterium tuberculosis, Mycoplasma spp. and Chlamydia spp. [1]. Chemically based antimicrobials have an efficacy to kill pathogenic bacteria cause UTI, while they show dangerous side effects in those patients how complaining of UTI. The researchers suggest a way to minimize the toxicity of chemical antibacterial agents by replacing them by natural source materials having a same role in fighting the infections such as some medically important plants extract [2].

Eucalyptus camaldulensis an important species in Eucalyptus group (medical plant) in Middle East area, especially in Iraq, it has vital role against antihyperglycemic, antioxidant activities and microbial infections by its essential oil [3].

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The researchers describe its effects of bacterial inhibitions on some species as well as Staphylococcus aureus, Klebsiella pneumonia and Serratia marcescens spp. isolated from different clinical and non-clinical samples and they recommended the future researches to investigate its effects on other species [4].

FIGURE 1. - Eucalyptus plants

2. MATERIALS AND METHODS

The study was applied to (50) women and (50) children attending Al-Zahra Hospital for Women and Children, where urine samples were collected from patients with urinary tract infection and according to a questionnaire form presented to them including (patient's name, gender, age, address, specimen type, reception date, reception time and if it pregnant or lactating). After collecting the sample within two hours, it is taken to the laboratory at room temperature with the container lid closed tightly. Urine samples were examined via microscope and samples which have positive results were all diagnosed by culturing on MacConkey agar, blood agar media , manitol salt agar , EMB(eosin methylene blue) and nutrient agar . Catalase test and Urease test also uesded to diagnosis the bacteria . VITEK compact- 2 system was used to identify of bacteria isolates and to detect antibiotic susceptibility test and determination of minimum inhibitory concentrations (MICs) is identified by the dilution method.

Preparation of the Eucalyptus powder:

Collected papers of Eucalyptus trees from Gardens technical Institute Baquba have been Washed with tab water then lifted to dry for one week under the Sun, then molar was used for milling it to get good textures of powder. The extraction done by mixing 200g of powder dry with 600ml of alcohol (ethanol alcohol 96%) in the glass flask, been mix the mixture on magnetic stirrer for 12-hour on temperature 30c. The mixture has been filtered by filter paper and the extract was collected by small containers (cub urine collection). This container put in an incubator 37c for 5 days to get good quantities of powder [5], to prepare of extracts; hot water 100 g E. camaldulensis of powder was mixed with 900 ml of distilled water in a glass flask and heated on the heater with continuous stirring by magnetic stirrer for 24 hours. Later, after the specified time, the mixture was cooled at room temperature and filtered by laboratory filter paper to obtain the plant extract. The extract was then collected in small containers (urine collection containers). The containers were then placed in the incubator at 40 ° C to increase the concentration of the solution for 24 hours.

Testing of essential oil efficacy

Extracts prepared by methods (ethyl alcohol at 96% concentration and hot water extract) were tested for activity on the S. aureus bacteria isolated from the urine of a person with urinary tract infection. Made discs of several circular layers in a diameter of 3.2 mm diameter of filter paper and placed in the oven 180c to avoid contamination and then immersed in the extract To act as antibiotic discs and then placed on the surface of the media during the implantation of bacteria and the next day was the effectiveness of the extract on the growth of bacteria antibiotic sensitivity test. The method [6] was used.

The focus of the test of mixing eucalyptus with antibiotics

This test depends mainly on the combination of different antibiotics and eucalyptus and the experiment of mixing in several proportions by preparing sequential concentrations of the active substance and similar to the concentrations of the antibiotics. The two materials were used by using bacteria that showed resistance to the antibiotics in the current study. The test was conducted using the following method:

Checker Board Assay

In this test, a solid culture medium was used, and concentrations of the active substance were prepared similar to those prepared for the antibiotics, and the mixing ratio between the active substance and the antibiotic was determined. Then the fractional inhibitory concentration values were calculated for the Gykerboard test.

3. RESULTS & DISCUSSION

Current study showed that 16 samples from 100 samples diagnosed as bacterial UTI and this isolated were grouped into two distinct groups: 13 isolates as (81.25 %) were gram negative bacteria and 3 isolates (18.75 %) diagnosed as gram positive bacteria. Isolated for gram negative bacteria diagnoses as Klebsiella pneumoniae (37.5 %), Pseudomonas aeruginosa (31.25 %), Proteuse mirabilis (12.5 %) and isolates for gram positive bacteria diagnoses as Staph. aureus (18.75 %) as shown in (Table 1).

Table 1. - Distribution patients infected with urinary tract infection according to type of isolate

Type	Bacterial Isolates	Total) 16 isolates)	
		No.	%
G – ve bacteria	*Klebsiella pneumoniae*	6	37.5
	Pseudomonas aeruginosa	5	31.25
	Proteuse mirabilis	2	12.5
G + ve bacteria	*Staph. aureus*	3	18.75

The antibiotic susceptibility tests in current study showed that most of isolated as Klebsiella pneumoniae , Pseudomonas aeruginosa and Proteuse mirabilis had a high resistance to most used antibiotics, mainly against ciprofloxacin and gentamicin. The susceptibility against of imipenem and meropenem. In (table 2), the results of an antimicrobial sensitivity test are illustrated.

Table 2. - Antimicrobials susceptibility test of gram negative isolates

Antibiotic (13 isolates)		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Proteuse mirabilis*	
		S(100%)	R(100%)	S(100%)	R(100%)	S(100%)	R(100%)
Ticarcillin	TIC	5(83.3)	1(16.6)	0	5(100)	2(100)	0
Piperacillin	PIP	5(83.3)	1(16.6)	0	5(100)	2(100)	0
Tazobactam	TZP	5(83.3)	1(16.6)	3(60)	2(40)	2(100)	0
Ceftazidime	CAZ	5(83.3)	1(16.6)	3(60)	2(40)	1(50)	1(50)
Ciprofloxacin	CIP	4(66.6)	2(33.3)	3(60)	2(40)	2(100)	0
Cefepime	FED	5(83.3)	1(16.6)	4(80)	1(20)	2(100)	0
Aztreonam	ATM	5(83.3)	1(16.6)	3(60)	2(80)	2(100)	0
Imipenem	IPM	6(100)	0	5(100)	0	2(100)	0
Meropenem	MEM	6(100)	0	5(100)	0	2(100)	0
Momocycline	MNO	6(100)	0	5(100)	0	2(100)	0
Gentamicin	GM	4(66.6)	2(33.3)	2(40)	3(60)	1(50)	1(50)
Pefloxacin	PEF	5(83.3)	1(16.6)	3(60)	2(40)	2(100)	0
Trimethoprim	SXT	5(83.3)	1(16.6)	3(60)	2(40)	2(100)	0
Tobramycin	TM	5(83.3)	1(16.6)	0	5(100)	2(100)	0

The antibiotic susceptibility tests in current study showed that most of isolated S. aureus had a high resistance to most used antibiotics, mainly against oxacillin. The susceptibility against of erythromycin with vancomycin had the highest rates among majority isolates of urine. In (table 3), the results of an antimicrobial sensitivity test are illustrated.

Table 3. - Antimicrobials susceptibility test of gram positive isolates

Antibiotic		*S. aureus* (3 isolates)	
		S(100%)	R(100%)
Benzylpenicillin	P	2(66.6)	1(33.3)
Ciproflaxcin	CIP	3(100)	0
Clindamycin	CM	2(66.6)	1(33.3)
Erythromycin	E	3(100)	0
Fosfomycin	FOS	2(66.6)	1(33.3)
Fusidic acid	FA	2(66.6)	1(33.3)
Gentamycin	GM	2(66.6)	1(33.3)
Imipenem	IMP	3(100)	0
Linezolid	LNZ	3(100)	0
Moxifloxacin	MXF	3(100)	0
Oxacillin	OX1	1(33.3)	2(66.6)
Rifampicin	RA	2(66.6)	1(33.3)
Teicoplanin	TEC	3(100)	0
Tetracycline	TE	2(66.6)	1(33.3)
Tigecycline	TGC	3(100)	0
Trimethoprim/sulfamethoazole	SXT	2(66.6)	1(33.3)
Vancomycin	VA	3(100)	0

The minimum inhibitory concentrations of the most antibiotic-resistant bacteria that resisted more than 9 antibiotics were studied for several considerations, including its high accuracy and the importance of this test in medicine and treatment of bacterial infections. Three types of antibiotics were used: Gentamycin, Amikacin, and Amoxicillin .

The results in Tables (2-4) and (3-4) indicate that the values of the minimum inhibitory concentrations of the minimum inhibitory concentration of Gentamycin was reached (4 -1024) µg/ml for Klebsiella spp. And (256) µg/ml for Pseudomons aerauginosa bacteria, while the MICS value for isolates of Staphylococcus aureus was (512-<1024).

Table 4.- minimum inhibitory concentrations of some antibiotics used against the bacteria of the intestinal family under study (µg/ml)

stopping point	≥64	≥32	≥16
K.pneumoniae $_{62}$	16	64	<1024
K.pneumoniae $_{58}$	8	32	4
K.pneumoniae $_{69}$	16	64	512
K.pneumoniae $_{103}$	64	256	512
K.pneumoniae $_{108}$	16	64	32
K.pneumoniae $_{124}$	4	4	64
Ps.aeruginosa $_{60}$	1024	128	1024
Ps.aeruginosa5	16	32	32
Ps.aeruginosa9	128	64	512

Ps.aeruginosa18	64	64	1024
Ps.aeruginosa7	128	1024	256
p.mirabilis 34	512	1024	1024
p.mirabillis$_{46}$	512	1024	1024

And (128) for Pseudomons aerauginosa. As for the MICS value of isolates of Staphylococcus aureus (128-1024), the reason for the high resistance may be attributed to the production of aminoglycoside-modifying enzymes that work to modify the co-modifying of the amine or hydroxyl group and thus produce antigens with weak linkage to ribosomes, or This may be attributed to the change in the binding site of the ribosome to the antigen [9].

The values of the minimum inhibitory concentrations of Amoxicillin antagonist ranged between (1024-<1024) µg/ml for Klebsiella spp. isolates Pseudomons aerauginosa isolates, between (512-1024) for Staph.aureus (1024).

The high percentage of resistance to penicillin antagonists, which include (amoxicillin and paracillin), is due to medical resistance to one of these mechanisms, which is the destruction of the antagonist by beta-lactamase enzymes or the failure of the antagonist to penetrate and reach the target site (penicillin binding protein PBPs). target, or reduce the affinity of the antagonist's binding to PBPs [10].

Table 5. - minimum inhibitory concentrations of some antibiotics used against staphylococci and micro-coccus under study (µg/ml)

Anti **Isolation**	**AK**	**AMC**	**GN**
stopping point	≥64	≥32	≥16
Staph.aureus$_{25}$*	128	512	512
Staph.aureus$_{86}$	1024	1024	1024
Staph.aureus$_{107}$	256	4	<1024

GN = Gentamycin

isolation number CTX= Cefotaxime

AMC = Clavulanic acid + Amoxicillin

CL= Cephalexin

AK = Amikacin

AX = Amoxicillin

PRL= Piperacillin

CIP = Ciprofloxacin

Table 6. - Bacteria cause UTI in research population

Bacteria	**Alcohol** **(0.62g/1.5ml) 0.62gm**	**Alcohol** **(0.31g/750ml) 0.31gm**
Klebsilla	R	R
Pseudomonase	8mm	8mm
Proteuse	15mm	10mm
S.aureas	21mm	20mm

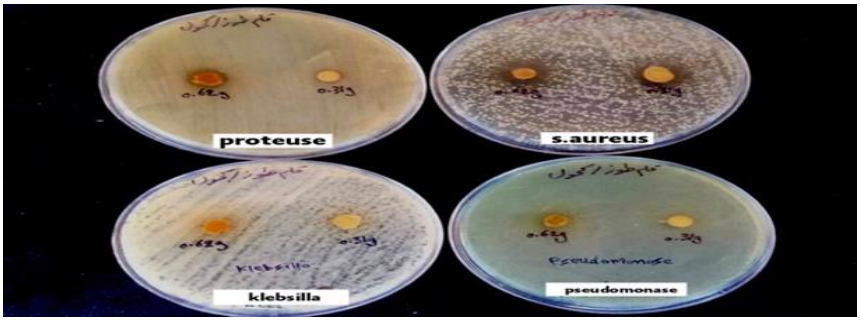

FIGURE 2. - Result of the Effect of Eucalyptus Leaves extract on some types of bacteria and size of inhibition zone

Effect of mixing anti-biotics with eucalyptus on isolates under consideration .The mixture of the active substances (eucalyptuses) and antibiotics was studied using the checkerboard method described by [12] by calculating the inhibitory concentration factor (FIC) for 10 bacterial isolates and they were selected according to their resistance to the antibiotics used in the test. In this study, the antagonists Amikacin, Gentamycin, Cefotaxime, Amoxicillin mixed with the active substance . Mixing the active substance eucalyptuses with antibiotics has been tried, and the best mixing ratio was determined. It was found that the ratio of mixing the eucalyptus/antagonist in a ratio of 1:4 is the best.

The inhibitory concentration of the purified substance (eucalyptus) was also determined. The MIC value was (1024) µg/ml for K. pneumoniae, while the MIC value for Ps. aeruginosa bacteria was (256) µg/ml. On the other hand, the MIC value for the Gram-Staph-positive isolates was also determined S.aureus (64) mcg/ml).

The tables below show a significant decrease in the MIC range of antibiotics after the process of mixing it with eucalyptus than it is in the case of using each antibody alone. The effect of mixing Ciprofloxacin antagonist was, as for the aminoglycoside group antagonists, which included Amikacin and Gentamycin antagonists, the effect of mixing with eucalyptus showed a synergistic effect for all bacterial isolates, on the other hand, the effect of mixing between an antagonist Amoxicillin and (eucalyptus) only showed a synergistic effect, and also in the isolate showed a synergistic effect. on three isolates, while (11) isolates showed an ineffective effect.

Table 7. - Effect of mixing amikacin with eucalyptus on the values of the minimum inhibitory concentrations of the isolates under study

Isolates	MIC . values				
	catechin µg/ml	amoxicillin µg/mL mixture	µg/ml mixture	FIC Index	Effect type
Staph.aureus $_{86}$	1024	128	8	0.069	**Synergistic**
Ps.aeruginosa $_{7}$	512	64	16	0.28	**Synergistic**
K.pneumoniae $_{103}$	64	1024	8	0.132	**Synergistic**

*FIC: Fractional inhibitory Concentration

Table 8. - Effect of mixing anti-gentamicin with eucalyptus compound on the values of minimum inhibitory concentrations of the isolates under study

Isolates	MIC. values				
	catechin µg/ml	amoxicillin µg/mL mixture	µg/ml mixture	FIC Index	Effect type
Staph.aureus $_{86}$	1024	256	128	0.625	**add**
Ps.aeruginosa $_{7}$	512	1024	128	0.375	**synergistic**

Table 9.- Effect of mixing amoxicillin antagonist with eucalyptus compound on the values of minimum inhibitory concentrations of the isolates under stud

Isolates	MIC. values				
	catechin µg/ml	amoxicillin µg/mL mixture	µg/ml mixture	FIC Index	Effect type
Staph.aureus $_{86}$	1024	1024	512	1	**unimpressive**
Ps.aeruginosa $_{7}$	512	1024	512	1.5	**unimpressive**
K.pneumoniae $_{103}$	64	1024	8	0.132	**synergistic**

The immediate treatment of hospital-related injuries must include broad-spectrum antibiotics that work to eliminate the microorganism, but the toxicity of the antibiotic often limits the possibility of increasing the dose for the individual to achieve the concentration that could affect reducing the number of bacteria, so these infections, especially in weak people Immunologically, it requires the use of an effective treatment that includes two antibiotics. These therapeutic mixtures will reduce the toxicity of the antibiotic than if both are used separately, by reducing the dose of one antibody, as well as reducing the risk of the emergence of resistant strains and enabling them to overcome the infection of patients with difficult to treat injuries. [12].

4. DISCUSSION

Based on the results, ethanolic leaf extract of Eucalyptus camaldulensis in study have significant antimicrobial activity of the studied microorganisms. Antimicrobial effect of the extracts was different, depending on the type of bacteria, thus, the gram positive bacterium Staphylococcus aureus is higher sensitivity compare to other and showed zone 21mm in 0.62g concentrations of Eucalyptus camaldulens is leaves ethanolic extracts and showed 20mm zone in 0.31g concentration of ethanolic extract when Proteuse showed 15mm zone in 0.62g concentrationand 10mm zone in 0.31g concentration.

Pseudomonase bacteria also effect but less than other which is 8mm zone in 0.62g concentration and 8mm zone in 0.31g concentration, Klebsilla resisted the extract and un effect. Due to differences in cell structure of bacteria These points are consistent with the results obtained in this study.The results show alcoholic extract concentrations have inhibition effect on growth of Staphylococcus aureus, Proteuse and Pseudomonase. Alcoholic extract of Eucalyptus leaves are able to inhibitory of growth the bacteria.

It was clear from the results related to antibiotic resistance that all bacterial isolates possess resistance to most of the antibiotics used in this study and in varying proportions. Bacteria isolated from urinary tract infections for antibiotics.

Some of the antibiotics in this study showed a relatively high activity against the isolated bacteria, such as the quinolines, Ciprofloxacin and Norfloxacin, and the aminoglycosides Gentamycin and Amikacin. And Trimethoprim + Sulphamethoxazole)

As for the anti-carbapenems, especially the anti-Imipenem, they come first in the treatment of urinary tract infections, as they are considered to be of a wide range of antibiotics in their effect on many types of bacteria that cause UTI and have few side effects [13].

The results of the current study showed that most of the bacterial isolates were resistant to the group of penicillins represented by the antagonists Amoxicillin and Piperacillin. The percentage of their resistance was (77%) and (64%), respectively, of the total isolates. As for the cephalosporins, represented by Cephalexin and Cefotaxime, the percentage of their resistance was (74%). and (62%) of the total isolates, respectively, as these antibiotics inhibit the process of manufacturing the cell wall of bacteria by interfering with the process of manufacturing the peptidoglycan layer. A pathway to break the beta-lactam ring in the group of penicillins and cephalosporins[14].

The results of this study were consistent with what [15] found that most of its isolates were resistant to the penicillin group (Piperacillin and Amoxicillin). As for the anti-aztronam of the modern beta-lactam antibiotics, the resistance rate to it reached (50%) of the total isolates. (aztronam), This may be due to the fact that these antibiotics are sensitive to the beta-lactamase enzymes secreted by P.mirabilis, K.pneumoniae and E.coli, and the rest of the isolates, or to the lack of affinity for the binding of antibiotics to proteins responsible for the durability of the cell wall called Penicillin binding proteins [16]. While the resistance of the antibiotics of the aminoglycoside group, including the anti-

Gentamycin Amikacin, which had a resistance ratio (43%) and (31%), the cause of bacterial resistance to aminoglycosides may be attributed to three mechanisms: modification of the antibody molecule mediated by modified enzymes (Adenylating, Phosphorylating) Acetylating, or mutation Chromosomal, such as a mutation in the gene encoding the target protein in the subunit ribosomal S30, causing the antigen to lose its affinity for binding to the target protein and reduce the permeability of the bacterial cell to the antigen [17].

As for Imipenem, which belongs to the Carbapenems group, the isolates showed great sensitivity to it, as the resistance rate to it was only (4%). The blaOXA–23gene that encodes for resistance to this antigen [18].

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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