

The Effect of Aqueous Extract and Essential Oils of the Leaves of *Eucalyptus incrassata* on β - Lactam Resistant *Staphylococcus aureus*

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Abstract

According to the extraction procedure , *Eucalyptus incrassata* leave sample yielded 5% and 2% w/w (Based on dry leaves) of the aqueous extract and essential oils respectively. Disk diffusion method was used to determine the antimicrobial activity of aqueous extract and essential oils of *E . incrassata* leaves against eight isolates of multidrug- resistant of *Staphylococcus aureus* (*S. aureus*) . It was found that aqueous extract and essential oils have variable antimicrobial activity (the inhibition zone diameter ranged from 7 to 14 mm respectively) , while essential oils showed more effect than aqueous extract .

It was noticed that values of Minimal Inhibitory Concentration (MIC) for aqueous extract is different from MIC of essential oils and these values were ranged from 12.5 to 75mg/ml . It was also found that isolates collected from wound infections showed high resistance against aqueous extract and essential oils with MIC ranged from 50-75mg/ml, these variation in the MIC are probably due to some virulence factors which have a unique genetic determinant .

The results indicated that the potential usefulness of the aqueous extract and essential oils of *Eucalyptus incrassata* as an antimicrobial agent can be used in the treatment of multidrug-resistant *S. aureus* infectious .

Key words: *Eucalyptus incrassata* , *Staphylococcus aureus* , β - lactam , aqueous extract , essential oils .

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram positive spherical bacterium that colonizes mainly the nasal passages. It causes a serious infections in human such as pneumonia, mastitis, meningitis ,urinary tract infections, osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wound [1,2,3] . These infections can lead to substantial morbidity and mortality, as well as high healthcare costs[4]. This situation has been exacerbated by the rising incidence of strains that are less susceptible to a variety of antibiotics, making treatment of these infections more difficult [5,6].

Multidrug-resistant *S. aureus* cause major problem in clinical human healthcare . This problem is related to the increasing consumption and misuse of antibiotics [7]. It was reported in many studies that antibiotic abuse in treatment of *S. aureus* infection is the main cause of multidrug-resistant of this bacterium [6,7]. During the past four decades, resistance of *S. aureus* has continued to develop, with decreasing susceptibility to first-line agents such as penicillin, amoxicillin ,oxacillin , and this was followed by rapid spread appearance of methicillin resistant of *S. aureus* (MRSA) strains[4,8] .It was found that frequency resistance of *S. aureus* to β -lactam is increased worldwide, as strains of bacteria that produce β -lactomase have become more common[9,10] . These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy[10,11] .

As a result, for the reasons mentioned in the above , the necessitated need to search for new sources of antimicrobial agents, and among the potential sources of the new agents are the medicinal plants, which have been considered as alternative source for antimicrobial drugs[12]. The medicinal plants are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [13]. One of the most important ethnomedicinal plant is *Eucalyptus camaldulensis* which belongs to the family of Myrtaceae. It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts[3].It was reported in many studies [12,14] that the leaves of the *Eucalyptus camaldulensis* contain essential oil which is used as an antiseptic and have efficiency in killing bacteria. It was also found[14,15] that the phytochemical analysis of the crude extract of the leaves revealed the presence of saponin, saponin glycosides, steroid, cardiac glycoside, tannins, volatile oils, phenols and balsam (gum) , and this extract have the ability to inhibit the growth of *S. aureus* .

The main objective of this study is to examine the effect of the crude extracts of the leaves of *E. camaldulensis* on β - lactam resistant *S. aureus* .

Materials and Method

Collection of plant materials

The leaves of *Eucalyptus incrassate* (*E. increaaaata*) was collected from the garden of College of Education for Pure Science / Ibn AL –Haitham and other region of Baghdad city in April, 2012. The plant was classified at the Department of Biology, College of Education for pure Science / Ibn Al -Haitham . Dry matter percentage was determined in the leaves .

Preparation of plant materials and extract

The green leaves were picked from stem and dried at 40 °C over a period of four days, the dried leaves were milled using an electric grinder and samples were taken to determine the dry matter percentage.

The aqueous solution of leaves was prepared according to [16] and the extraction of essential oils from the leaves was performed by hydrodistillation in a Clevenger type apparatus and according to[17]. A stock of 10% (w/v) concentration for aqueous extract and essential oils were prepared according [16].

Bacterial isolates: Eight local isolates of *S. aureus* were marked (ST1-ST8) as shown in table (1) . All isolates were maintained in screw-caped universal tubes containing nutrient agar(Acumedica,U.S.A.) and store at 4°C.

Antibiotic Susceptibility Testing: Disk diffusion method was used as described by [18] Sterilized filter papers discs (6 mm diameter) with concentration 2.5mg/disc from each of aqueous extract and essential oils were used . Five antibiotic disks were used (table 2) to detect antibiotic susceptibility of eight isolates of *S. aureus* . The plates cultures were observed after 18h of incubation at 35 °C . All tests were performed in duplicate and results recorded as mean values. The zones of growth inhibition were measured in mm and interpreted as sensitive or intermediate or resistant according to[19].

Minimal inhibitory concentration (MIC) test: The MIC of aqueous extract and essential oils against eight resistant of *S.aureus* were determined by an agar dilution method according to[20] .The final concentration for each of aqueous extract and essential oils in the medium were 12.5,25 ,50,75 and 100 mg/ml .The plates cultures were incubated at 35 °C for 18 h and the MIC was defined as the lowest concentration for each of aqueous extract and essential oil inhibiting the visible growth. All determinations were performed in duplicate and results recorded as mean values. Growth control consisting of Muller Hinton Agar was included.

Results and Discussion

A re-test was carried out by using mannitol salt agar as a selective and differential medium for *Staphylococcus aureus* . It was found that all isolates, which were used in this study displayed a positive reaction and that mean that the mannitol positive isolates were *Staphylococcus aureus* [21].Another test was conducted to confirm a susceptibility of isolates under investigation toward five types of antibiotics which belong to β - lactam group .The result showed that all isolates displayed multidrug-resistant toward these types of antibiotics (table 3) .

Extraction procedures yielded 5% and 2% w/w(Based on dry leaves) of the aqueous extract and essential oils respectively, the color of the aqueous extract was brown and have a sticky texture with unique smell.

The antimicrobial activity of the aqueous extract and essential oils was assayed against eight isolates of *S. aureus* . It was found that aqueous extract and essential oils have different antimicrobial activity (the values of inhibition zone range from 7- 14 mm) on multidrug-resistant *S. aureus* isolates (table 4) . These differences in the level of resistance are probably due to several factors, including isolation source, genetic determinants and some virulence factor like biofilm formation [22,23,24].

The results of MIC for aqueous extract and essential oils of *E . incrassata* leaves are listed in table(5). These results revealed that MIC for essential oils was less than aqueous extract which means that all isolates were more sensitive toward essential oils compared with aqueous extract. The variation in antimicrobial activity of aqueous extract and essential oils may be due to several factors including chemical constituents of the extract, drying method of the plant material, and extraction procedures(25,26,27). It was noticed that the isolates ST5 and ST6, which were isolated from wounds infection displayed more resistance to aqueous extract and essential oils of *E . incrassata* leaves compared with other isolates, where the values of MIC for these isolates ranged from 50-75mg/ml, these variations in the MIC are probably due to some virulence factors and unique genetic determinants [22, 24].

In conclusion, the aqueous extract and essential oils obtained from *E . incrassata* leaves have potential as antimicrobial agents in the treatment of infectious multidrug-resistant *S. aureus* . Further investigations of the active components of the plant for the exact mechanism of action and synergistic interaction of plant extracts with some type of antibiotics ,will contribute to the development of new pharmaceuticals.

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Table No. (1) . *Staphylococcus aureus* isolates and Sources

Bacterial Isolates	Sources and Characteristics	Reference
ST1 ST2 ST3 ST4	Isolated from sputum patients. Resistance to penicillin G , Oxacillin, Cephalothin.	Biotechnology Department-College of Science – Baghdad University.
ST5 ST6	Isolated from wounds infection. Resistance to penicillin G, Oxacillin, Cephalothin.	
ST7 ST8	Isolated from gastrointestinal disorder patients. Resistance to penicillin G , Oxacillin, Cephalothin.	

Table No. (2). Types of Antibiotics disks and sources of production

Antibiotic Disk	Symbol	Disk Content	Company
Penicillin G	PG	10	BioAnalyse (Turke)
Ampicillin	AM	10	
Oxacillin	OX	1	
Cephalothin	KF	30	
Cefotaxime	CTX	30	

Table No. (3). Antibiogram results of *S. aureus* isolates

Antibiotic	* Inhibition zone diameter(mm)							
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8
Penicillin G	15	17	14	17	10	12	18	16
Ampicillin	10	8	8	10	7	8	10	10
Oxacillin	7	8	7	7	7	6	9	8
Cephalothin	11	10	11	10	9	8	12	10
Cefatoxime	13	11	11	12	10	8	12	11

* According to [20], the inhibition zone diameter for all isolates revealed that these isolates are resistant toward all these type of antibiotics.

Table No. (4). Disc diffusion test for aqueous solution and essential oils of *E. incrassata* leaves.

Extracts	Inhibition zone diameter(mm)							
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8
Aqueous extract	12	11	12	12	8	7	10	11
Essential oils	14	12	13	14	9	8	11	11

Table No. (5). Minimum inhibitory concentration (MIC) of *S. aureus* for aqueous extract and essential oils of *E. incrassata* leaves.

Extracts	MIC (mg/ml)							
	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8
Aqueous extract	25.00	50.00	25.00	25.00	75.00	75.00	50.00	50.00
Essential oils	12.500	25.00	12.50	12.5	50.00	75.00	25.00	25.00

تأثير المستخلص المائي والزيوت الطيارة لأوراق اليوكالبتوس *Eucalyptus* *incrassate* في المكورات العنقودية الذهبية *Staphylococcus aureus* المتعددة المقاومة لمجموعة مضادات بيتا لاكتام

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الخلاصة

أعتادا على طريقة الاستخلاص ، تم الحصول على 5 ، 2% (على أساس المادة الجافة للأوراق) لكل من المستخلص المائي والزيوت الطيارة من أوراق نبات اليوكالبتوس *Eucalyptus incrassata* . أستعملت طريقة الأقراص المنتشرة للتحري عن الفاعلية الميكروبية للمستخلص المائي والزيوت الطيارة أتجاه ثمانية عزل من المكورات العنقوديات الذهبية ذات المقاومة المتعددة لمجموعة البيتا لاكتام . لقد وجد أن لكل من المستخلص المائي والزيوت الطيارة تأثير جرثومي مختلف (تراوح قطر التثبيط من 7- 14 ملم) ، وقد أظهرت الزيوت الطيارة أكثر فعالية من المستخلص المائي . كما لوحظ تباين قيم التركيز المثبط الأدنى لكل من الزيوت الطيارة والمستخلص المائي ، إذ تراوحت هذه القيم 12.5- 75 ملغم/مليتر لكل منهما على التوالي . كما أظهرت النتائج المقاومة العالية لعزلات البكتريا المعزولة من التهابات الجروح إتجاه كل من الزيوت الطيارة والمستخلص المائي ، وتراوحت قيمة التركيز المثبط الأدنى 50- 75 ملغم /مليتر لكل منهما وعلى التوالي . تشير النتائج أعلاه الى إمكانية الإستفادة من المستخلص المائي والزيوت الطيارة لأوراق اليوكالبتوس لعلاج الأصابات الناتجة من بكتريا المكورات العنقودية الذهبية .

الكلمات المفتاحية : اليوكالبتوس ، المكورات العنقودية الذهبية ، مجموعة البيتا لاكتام ، المستخلص المائي ، الزيوت الطيارة