



ISSN: 0067-2904

The Biological Activity of *Eucalyptus rostrata* Leaves Extraction against *E.coli* and *Staphylococcus aureus* isolated from Iraqi Patients

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Abstract

The inhibitory effect of *Eucalyptus rostrata* leaves extraction was investigated on multiple-antibiotic-resistant pathogenic bacteria (*E.coli* and *S. aureus*), isolated from Iraqi patients. The minimum inhibitory concentrations in a final concentration of 10 mg/ml. Tow fold dilutions was done from (12.5- 100) mg/ ml to examine the antibacterial effect of different concentrations of the plant extract on both bacteria. The study results revealed that *Eucalyptus rostrata* extract has a potential inhibitory effect on both gram negative and gram positive species. The current study supports the traditional approach of using *Eucalyptus rostrata* leaves extraction in treatment trails against bacterial infections.

Keywords: Antibacterial, Extracts, *Eucalyptus*, *rostrata*

الفعالية الحيوية للمستخلص الكحولي لأوراق نبات اليوكالبتوس ضد بكتيريا الأشرشيا القولونية والعنقوديات الذهبية المعزولة من مرضى عراقيين

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الخلاصة

تم دراسة النشاط المضاد للبكتيريا لمستخلصات أوراق من اوكالبتوس على بكتريا ممرضة مقاومة للعديد من المضادات الحيوية (البكتريا القولونية و المكورات العنقودية الذهبية) بطريقة الانتشار في الأكار. تم عمل مستخلص كحولي بالميثانول لأوراق النبات، تم اختبار الفعالية البيولوجية للتركيز (100 ، 50 ، 25 ، 12.5) ملغرام/ مل. أظهرت نتائج الأختبارات ان للمستخلص فعالية تثبيطية لكلا النوعين من البكتريا وكانت البمكورات العنقودية أكثر حساسية من البكتريا القولونية . تدعم هذه الدراسة الاستخدام التقليدي لليوكالبتوس كعامل مضاد للجراثيم.

Introduction

In the past plants were in treatments of infectious diseases many countries, before the production of new medical drugs. These plants compose of many substances that could be used to heal many bacterial disease, and also, as precursors for effective drugs production [1]. About 50% of the drugs is of plant origin, which have a crucial role in drug development in the clinical drug industry [2]. *Eucalyptus* is one of the ethnomedicinal plants which belong to Myrtaceae family. This plant always used as a treat sore throat and also many bacterial infections that cause respiratory or urinary tract infections. The essential oils of the *Eucalyptus* leaves can be used as a treatment of many lung diseases; also, its volatile oils can be used as expectorants [3]. Most topical treatments which contain

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eucalyptus oil can be used as traditional original medicines in order to cure wounds and also infections caused by fungi. The *Eucalyptus* oil which obtained by steam distillation of leaves has Eucalyptol (1,8-cineole), which is ingredient, responsible for its various bacterial inhibitory effect [4]. The antimicrobial effect of the methanolic extracts also has been reported [5].

The bacterial resistance to the antimicrobial drugs is the reason of the further research to discover safe and effective antibacterial treatments [6]. The discovery of plants that have a biological activity against bacteria is an important goal for phytochemical investigation, showed the presence of tannins, saponins and also glycosides [7]. *Eucalyptus* is a genus of flowering trees that include a distinct group with a multiple-stem mallee growth habit in the myrtle family. It includes the tallest known flowering plant on the Earth. In this study, the *in vitro* antimicrobial activity of *Eucalyptus rostrata* leaves extract, were evaluated against some bacteria.

MATERIALS AND METHODS

Plant Material

Eucalyptus rostrata was collected from the gardens around the biology department of college of Science in Baghdad University in February of 2017 and was kindly identified by botany expert. The leaves were air-dried and a weight of 250 g was used.

Control

The control used for the experiments was dimethyl sulfoxide (DMSO).

The Procedure of Extraction:

The dried leaves powder (250 g) was subjected to alcohol extraction with methanol as the extraction solvent. Then, was homogenized the mixture with glass stick and the mixture was heated for 4 hours with shaking of mixture each 15 min. The mixture was filtered by gauze and put the separated in tubes for centrifugation. the extract was put in plates and incubated in oven for three days. Finally the powder was obtained by scrabbling the surface of plates, which used as an anti bacterial substance [8].

Bacterial isolates:

Two types of bacteria were used in this study were one Gram-negative and Gram positive bacteria, *E.coli*, *Staphylococcus aureus*. They were isolated from Iraqi patients and were identified by using cultural and biochemical tests and according to [9].

Determination of antibacterial activity:

The biological activity of the plant extracts was investigated by agar diffusion method as described by Adeniyi *et al* [1].

Results and Discussion:

The results of the antibacterial effect of the plant leaves determination was obtained by taken the means of inhibition zone on agar (**Table 1**), showed that the methanol extracts represent a good agent that possesses an antimicrobial activity. The methanol extracts revealed variation in degrees of inhibition zone on the tested microorganisms at different concentrations start from 12.5 to 10 mg/ml. The methanol extracts revealed higher activity against *Staphylococcus aureus* than against *E.coli*.

The minimum inhibitory concentration of the plant leaves extract ranged between 12.5 mg/ml (Table-1). *Eucalyptus* plant is known as rich source of bioactive compounds represented by several secondary metabolites, that have a variety of antimicrobial activities. The emergence of microbial resistance to the chemical employed antimicrobial agents increase the importance of *Eucalyptus* plant extracts to be used as antimicrobial agent [7].

Table (1): The antibacterial activity of Eucalyptus methanol extraction against *E.coli* and *S.aureus*.

Concentration $\mu\text{g/ml}$	Mean of Inhibition zone for <i>E.coli</i> (mm)	Mean of Inhibition zone for <i>Staph. aureus</i> (mm)
12.5	16	19
25	19	20
50	21	22
100	24	34
Control	NIZ	NIZ

Control=DMSO , NIZ= No Inhibition Zone.



(A)



(B)

Figure (1): Inhibition zone by agar diffusion method: (A), represent the antimicrobial activity of (100,50,25,12.5) $\mu\text{g/ml}$ of eucalyptus leaf extract against *Staph. aureus*. (B), represent the antimicrobial activity of (100,50,25,12.5) $\mu\text{g/ml}$ of eucalyptus leaf extract against *E.col*.

The results revealed agreement with the many scientific studies recently done, which tested the *Eucalyptus* essential oil in different concentrations against different strains of *Escherichia coli* and *Staphylococcus aureus* [10]. Because of the safety and low cost of the medicinal plants and due to their ability to inhibit bacterial resistance to many types of antibiotics, many plants have been evaluated and reviewed

The biological efficiency [11]. *Eucalyptus* essential oil against the methicillin-resistant *S.aureus* (MRSA) strains is stands out [12,13] In the same study It was found that *Eucalyptus* oil has higher antibacterial activity than chlorhexidine on *S. aureus* gram-positive bacterium and gram-negative bacteria: *E. coli*. In another study, the *Eucalyptus* essential oil of *E.*

shows moderate to high antibacterial activity against many bacteria, yeast. The methanol fraction effect revealed that polar fraction showed strong biological activity against 7 microorganisms while the non-polar fractions have no inhibitory action except *E. coli* bacterial strains. So, the study concludes that the essential oil has a stronger action than those of methanol extracts. The higher antimicrobial activity of the essential oil may be due to the existence of compounds with antimicrobial properties, like, 1,8-cineole (eucalyptol) which has strong antimicrobial properties against many pathogens [13,14]. Other compounds such as, limonene, α -pinene, *P*-cymene, which have relatively strong antimicrobial activities [14,15], are responsible for this activity. The synergistic action of all these chemicals and the essential oils is the reason of the *Eucalyptus* antimicrobial effect. Results showed that the aqueous and alcoholic of eucalyptus leaf extracts have great effect on many infectious organisms [16]. Furthermore, studies indicated that the gram positive bacteria are more sensitive to the essential oils than the gram negative bacteria [17], while the results of our study show the higher sensitivity of the gram negative bacteria.

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