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Antibacterial activity of *Eucalyptus rostrata* leaves extract against *Staphylococcus* species

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ABSTRACT

Background: *Staphylococcus* species develop resistance to various antibiotics through different mechanisms, making them difficult to treat with standard therapies. Therefore, using alternative strategies to treat *Staphylococcus* species is urgent such as utilizing certain plant extract that may be promising branch in microbial therapy. **Methods:** *Eucalyptus rostrata* was collected from the farm around Al-Shomali hospital, Iraq in March 2023 and was kindly identified by botany expert. The leaves were air-dried and a weight of 10 g was used for liquid extraction. Gram positive bacteria *Staphylococcus* species were isolated from Iraqi patients from different body sites. The antibiotic susceptibility test was done by the standard disk diffusion method on Mueller-Hinton agar (MHA) as well as agar diffusion method. **Results:** The total of 60 *staphylococcus* species isolated clinically from different body sites divided to 30 *S. aureus* and 30 coagulase negative staph species (CoNS). Eucalyptus extracts (10g per 100 ml) gave 65% sensitivity for all isolates. The activity of eucalyptus is more than amoxicillin, ciprofloxacin, and ceftriaxone ($P < 0.001$). The activity of eucalyptus is equal in activity to both amikacin, and ciprofloxacin ($P > 0.05$). There are no significant differences between the sensitivity of eucalyptus in *S. aureus* 21(70%) and coagulase negative staph 18(60%) ($P = 0.58$). **Conclusions:** Eucalyptus *rostrata* extracts gave 65% sensitivity far all isolates. Its activity is more than amoxicillin, ciprofloxacin, and ceftriaxone ($P < 0.001$). The activity of eucalyptus is equal to both amikacin, and ciprofloxacin ($P > 0.05$).

Introduction

The genus Eucalyptus (Myrtaceae) is native to Australia, and this tree is mostly cultivated for paper, pharmaceutical and cosmetics industries. Several species of Eucalyptus are also used in traditional medicine as antiseptics, and against infections of the upper respiratory tract, such as the common cold, influenza and sinus congestion. Essential oil from these species has a therapeutic application in treatment of pulmonary infections by inhalation, and the monoterpene extracted

from *Eucalyptus citriodora*, *Eucalyptus globulus* and *Eucalyptus teretecorni* exhibit antibacterial activity [1].

Two major peaks on the chromatogram of eucalyptus B were identified as gallic acid and ellagic acid, indicative of degradation products from hydrolyzable tannins in the leaves. Considering the evaluation of antioxidant activity by radical scavenging ability, a standardization of eucalyptus

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leaf extract, including the antioxidative polyphenol, oenothain B, is proposed [2].

In the past, plants were used in treatments of infectious diseases in 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. About 50% of the drugs is of plant origin, which has a crucial role in drug development in the clinical drug industry. Eucalyptus is one of the ethnomedicinal plants which belong to Myrtaceae family. This plant always used to 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, the volatile oils can be used as expectorants [3].

Eucalyptus, a native genus from Australia, belongs to Myrtaceae family and comprises about 900 species and subspecies; it is one of the world's most important and most widely planted genera. It has been introduced worldwide, including in Tunisia and mainly cultivated for its timber, pulp and essential oils that present medicinal properties and therapeutic uses. In recent decades, the essential oils and their components of plants have been of great interest as they have been the sources of natural products. The value of Eucalyptus oil for medicinal purposes is based largely on the content of a particular oil constituent: 1,8-cineole (cineole or eucalyptol). Hot water extracts of dried leaves of Eucalyptus citriodora are traditionally used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion [4].

The phytochemical components of the leaves of *E. camaldulensis* have been described, they contain amongst other compounds essential oils particularly cineol, cuminal, phellandrene, aromadendral, valerylaldehyde, geraniol, cymene, catechol, tannins, terpenes and isoprenoids, phenolics, cardiac glycosides, sterols, saponins and flavonoids[5].

One of the most common isolates in hospitals is staphylococcus spp, and many of its strains have developed antibiotic resistance. Staphylococcus spp continue to exist in hospitals despite significant efforts to eradicate them, where they can infect those who are most susceptible to illness [6-9].

The aim of the current study was to evaluate the antibacterial activity of *Eucalyptus rostrata*

extract against clinical isolated staphylococcus species.

Material and methods

The procedure of extraction

Eucalyptus rostrata was collected from the farm around Al-Shomali hospital in March of 2023 and was kindly identified by botany expert. The leaves were air-dried and a weight of 10 g was used. The dried leaves powder (10 g) was subjected to 100ml distal water. Then, was homogenized the mixture with glass stick and the mixture was heated by use autoclave 120 degree for 20min. The mixture was filtered by filter paper and put in tubes. Finally, the liquid extract was obtained by autoclaving water and leave mixture, which used as an anti-bacterial substance.

Bacterial isolates

The types of bacteria were used in this study were Gram positive bacteria (*Staphylococcus*. spp.) they were isolated from Iraqi patients in Al-Shomali hospital in Babylon city, Iraq and were identified by using cultural and biochemical tests. A total of 60 *Staphylococcus* species have been isolated.

The antibiotic susceptibility test

Antibiotic sensitivity was done by the standard disk diffusion method on Mueller-Hinton agar (MHA) using commercial disks. Turbidity standard protocol was followed in order to have homogenized bacterial inoculum suspension. The following antibiotic discs were used for the disc diffusion tests to compare leave extract with these antibiotics: amoxicillin (AML, 25µg), Augmentin (AUG, 25µg), Amikacin (AK, 30 µg), Ciprofloxacin (CIP, 5µg), and ceftriaxone (CRO, 30 µg). Concentration of homogenous leave extract that used in this study is 10%. By well diffusion method, we put 50µL from the solution extract in wells (5mm) on Mueller-Hinton agar for 24hrs.

Data analysis:

All the statistical analysis was done by using SPSS 26 software and Excel app. For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Chi-square was used for non-parametric variables. P value < 0.05 was taken into account to denote statistical significance additionally.

Data was analyzed using percentage and frequencies.

Results

The total of 60 *staphylococcus* species isolated clinically from different body sites divided to

30 *S. aureus* and 30 coagulase negative *staphylococcus* species.

The antibiotic sensitivity and resistance showed that amikacin had high rate of sensitivity 80% followed by augmentin 78.3%. Ciprofloxacin revealed low level of sensitivity 38.3%. Amoxicillin and ceftriaxone had very low sensitivity 10% for each one. Eucalyptus extracts gave 65% sensitivity for all isolates (Figure 1).

Table 1 showed that the activity of eucalyptus is more than amoxicillin, ciprofloxacin, and ceftriaxone significantly (P< 0.001). The activity of eucalyptus is superior to both amikacin, and ciprofloxacin (P> 0.05).

There were no significant differences between the sensitivity of eucalyptus in *S. aureus* 21(70%) and coagulase negative staph 18(60%) (P= 0.58) (Table 2).

Figure 1. Antimicrobial activity of different agents

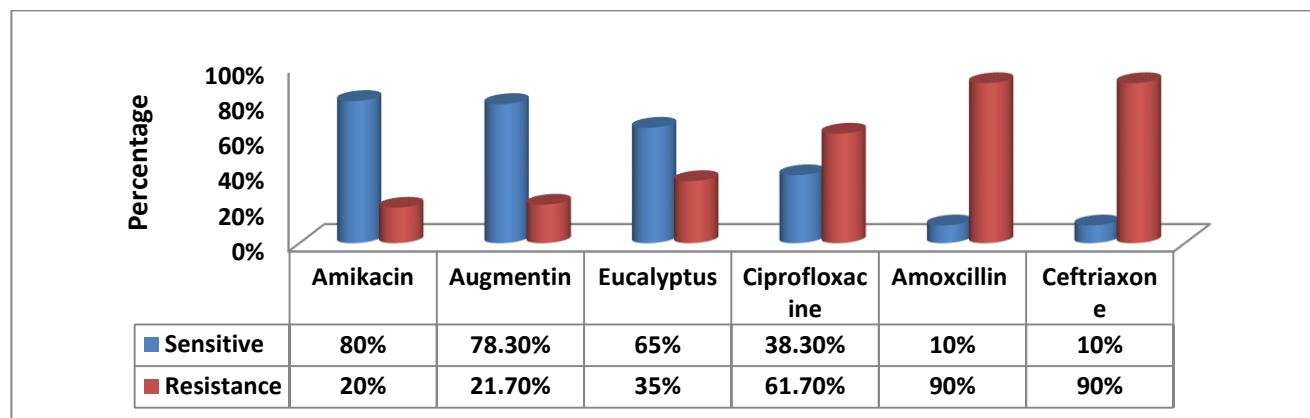


Table 1. Comparison antibiotic activity between eucalyptus and other antibiotics

Antibiotics	Susceptibility N (%)		P value
	Sensitive	Resistance	
Eucalyptus	39 (65)	21 (35)	< 0.001
Amoxicillin	6 (10)	54 (90)	
Eucalyptus	39 (65)	21 (35)	0.15
Augmentin	47 (78.3)	13 (21.7)	
Eucalyptus	39 (65)	21 (35)	0.10
Amikacin	48 (80)	12 (20)	
Eucalyptus	39 (65)	21 (35)	0.006
Ciprofloxacin	23 (38.3)	37 (61.7)	
Eucalyptus	39 (65)	21 (35)	< 0.001
Ceftriaxone	6 (10)	54 (90)	

Table 2. Comparison sensitivity of eucalyptus between *S. aureus* and CoNS

Eucalyptus	Groups N (%)		P value
	<i>S. aureus</i>	Coagulase –ve staph	
Sensitive	21 (70)	18 (60)	0.58
resistance	9 (30)	12 (40)	

Discussion

Antibiotics are essential in modern medicine and are used to treat various infectious diseases caused by bacteria [10-11]. However, the emergence of antibiotic-resistant bacteria has become a global public health threat, leading to increased morbidity, mortality, and healthcare costs. Therefore, it is crucial to use alternative ways to develop modern strategies to fight these bacteria as developing antibiotics from plant extract [12-15].

In this study, a total of 60 *staphylococcus* species were tested, 30 isolates of *S. aureus* and 30 isolates were coagulase negative *staphylococcus* (CoNS). The antibacterial effect of the Eucalyptus leaves extract was determined by measure the diameter of inhibition zone by agar diffusion method.

In the current study, the Eucalyptus extracts gave 65% sensitivity far all isolates.

This finding agreed with many scientific studies. Activity of *Eucalyptus rostrata* leaves extraction against *E. coli* and *Staphylococcus aureus* revealed higher activity against *Staphylococcus aureus* (34mm) at concentration 100 than against *E. coli* (24mm) [3].

In another study, the results obtained showed that essential oil of the leaves of *E. globulus* has antimicrobial activity against gram negative bacteria (*E. coli*) as well as gram positive bacteria (*S. aureus*) [16], other studies also mentioned that *Staphylococcus aureus* showed 4cm sensitivity of leave extract, the aqueous extract showed a good inhibition zone [17-18].

The current study revealed the activity of eucalyptus is more than amoxicillin, ciprofloxacin, and ceftriaxone significantly ($P < 0.001$). The activity of eucalyptus is superior to both amikacin, and ciprofloxacin ($P > 0.05$).

Another study found that an extract concentration of 50 mg/ml inhibits the growths of all the pathogenic bacteria were arrested, though to varying degrees. The least activity in terms of zones of growth inhibition was shown by aqueous extract against *E. coli* (7 mm), *K. pneumoniae* (9 mm), *P. mirabilis* (13 mm), *S. typhi* (12 mm) and *S. aureus* (12 mm) while the highest was demonstrated by the acetone extract, with a recorded zone diameter for *E. coli* (12 mm), *K. pneumoniae* (13 mm), *S typhi* (14 mm), *P. mirabilis* (15 mm) and *S. aureus* (14 mm) on Muller agar [5].

Another study showed 2000 µg/ml concentration of both aqueous and alcoholic extracts was quite effective on *Streptococcus pyogenes* and *Staphylococcus epidermidis* and prevented their growth over the medium. The inhibition zone in well diffusion agar was 17.90 ± 0.28 mm [19-20].

In this study, there were no significant differences between the sensitivity of eucalyptus in *S. aureus* 21(70%) and coagulase negative staph 18(60%) ($P = 0.58$). This finding partially agreed with another study that found the maximum zone of inhibition of purified extract of *E. camaldulensis* was 2.9 cm for *S. aureus*. The antibacterial activity of the leaf extracts of *E. camaldulensis* can be attributed to the action of the phytochemical compounds. There was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram-positive bacteria despite the differences in their cell wall components [21]. While some studies mentioned there are some differences in antibiotic sensitivity among Gram positive and negative bacteria [22-24].

Another research found that the diameter of inhibition zone of essential oils of Eucalyptus species varied from 10 to 29 mm. The largest zone of inhibition was obtained for *Bacillus cereus* (*E. astrengens*) and the lowest for *Staphylococcus aureus* (*E. cinerea*). The essential oils from *E. maideni*, *E. astrengens*, *E. cinerea* (arboretum of Bizerte), *E. bicostata* (arboretum of Aindraham) showed the highest antibacterial activity against *Listeria ivanovii* and *Bacillus cereus* [4].

Conclusions

Eucalyptus extracts gave 65% sensitivity far all isolates. Its activity is more than amoxicillin, ciprofloxacin, and ceftriaxone ($P < 0.001$). The activity of eucalyptus is equal in activity to both amikacin, and ciprofloxacin ($P > 0.05$).

Conflicts of interest

None.

Financial disclosure

None.

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