

## Antibacterial Activity of Eucalyptus Extracts on Methicillin Resistance *Staphylococcus aureus*

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**Abstract:** Methicillin-resistant *S. aureus* is a major cause of community and hospital acquired infections. There is an urgent need to develop anti-MRSA agents with novel mechanisms of action. Eucalyptus is a tall and evergreen tree, which is cultivated in many countries. Essential oil of Eucalyptus and their major constituents possess toxicity against a wide range of microbes including bacteria and fungi. In the present study, antibacterial activity of Eucalyptus alcoholic and aquatic extracts were investigated against a battery of clinically important MRSA strains. The alcoholic extract of Eucalyptus leaves was obtained by 95% ethanol and aquatic extract by boiling from the fresh leaves. Different concentrations of extracts were prepared and used for the antibacterial assay by agar well diffusion method. Bacterial DNA extracted by lysostaphin and boiling method was used to detect *mecA* gene from standard and clinical strains using a PCR amplification assay. Result of this study show that alcoholic and aquatic extract of Eucalyptus have antibacterial effects. In *mecA* negative *Staphylococcus aureus* the maximum zone of alcoholic extract was 18 mm at 30  $\mu$ L volume, whereas in *mecA* positive *Staphylococcus aureus* the maximum zone of alcoholic extract was 14 mm at 30  $\mu$ L volume. Among the various components of Eucalyptus oil, 1,8-cineole is the most important and is largely responsible for a variety of its antibacterial properties. According to the results, alcoholic extract of Eucalyptus has higher antibacterial effect than aquatic extract. Due to various chemical components of Eucalyptus more attention must be taken for the clinical application of this plant.

**Key words:** Alcoholic extract, antibacterial effects, Eucalyptus, methicillin-resistan, *mecA*, *Staphylococcus aureus*

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### INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA), which is resulted from the selective pressure of antibiotics currently used has increased relentlessly and well recognized as a global nosocomial problem in recent years. It has now emerged as the predominant and serious pathogenic bacterium, leading to high morbidity and mortality (Barrett, 2005; Berger-Bächi and Rohrer, 2002; Goldstein, 2007). MRSA normally possesses a multidrug-resistant genotype, which causes it resistant to  $\beta$ -lactams, aminoglycosides, fluoroquinolones and macrolides. It is only sensitive to the glycopeptides, i.e., vancomycin and teicoplanin that are considered as the last few effective agents. But, resistant isolate has also been reported. Clinical isolates of MRSA are often encountered, especially, in patients of Intensive Care Units (ICU) admission and of those who are elderly and repeatedly hospitalized. There is an urgent need to develop anti-MRSA agents with novel mechanisms of action (Barrett, 2005; Berger-Bächi and Rohrer, 2002; Enright, 2003; Kalenic *et al.*, 2008).

Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many other countries including India. The genus name Eucalyptus comes from the Greek word Eucalyptus, meaning well-covered and refers to its flowers that, in bud, are covered with a cup-like membrane.

Though, native to Australia, its therapeutic uses have been introduced and integrated into traditional medicine systems, including Chinese, Indian Ayurvedic and Greco-European. It is focused towards local use as an antiseptic (Nair *et al.*, 2008; Ahmad and Beg, 2001; Zgoda and Parter, 2001; Lee *et al.*, 2007). Topical application of Eucalyptus oil is effective against methicillin resistant *S. aureus* infection. Moreover, the antibacterial action of Eucalyptus on local application is also reported.

Eucalyptus species in Australia are adapted to growing in a range of conditions, from rainforests in the tropics to subalpine sites at treeline and from hot arid to moist temperate zones.

The emergence of resistance to conventional antimicrobials is a serious problem that physicians face. This necessitates constant development of newer agents, which can inhibit the growth of or kill resistant organisms.

## MATERIALS AND METHODS

The leaf material was collected in the month of September, 2008. It was identified by Faculty of Biology, Payame Noor University Iran. The alcoholic extract was obtained by 95 ethanol and aquatic extract by boiling from the fresh leaves. Different concentrations of extracts were prepared (10, 20, 30, 40, 50, 60 and 70  $\mu$ L). This was used for the antibacterial assay.

In this study, *Staphylococcus aureus* (MRSA 400) which have a *mecA* gene after culture on Muller Hinton agar, colony PCR with specific primer (5'-CCTAGTAAAGCTCCGGAA-3' and 5'-CTAGTCCATTTCGGTCCA-3') (10) were done for approve the *mecA* gene and then electrophoresis in 1% agarose gele (Su Mi Choi *et al.*, 2003). In addition, colonies PCR were done for *mecA* negative *Staphylococcus aureus* (ATCC 25923).

Antibacterial assay was done by agar well diffusion method. In these method, after production of wells in Muller Hinton agar, different volumes of extracts aloe and extract with different volumes of methicillin inoculated in wells. After inoculation culture media incubated in 37°C for 24 h.

## RESULTS

In this study, after DNA extraction from bacteria by lysostaphyn and Boiling method by use of *mecA* specific primers, PCR were done for *mecA* positive and PCR negative bacteria (Fig. 1). PCR products with these primers have 341 bp.

Result of this study show that alcoholic and aquatic extract of Eucalyptus have a antibacterial effect. According to results, alcoholic extract of Eucalyptus has antibacterial effect higher than aquatic extract (Fig. 2 and 3). In PCR negative *Staphylococcus aureus* the maximum zone of Alcoholic extract was 18 mm at 30  $\mu$ L volume and after 40  $\mu$ L (50, 60 and 70  $\mu$ L) the diameter zone have not any difference. The control negative (distillated water) not produce the antibacterial zone and control positive (alcohol 96%) produce 5 mm zone in both PCR negative and PCR positive *Staphylococcus aureus*.

In PCR positive *Staphylococcus aureus* the maximum zone of Alcoholic extract was 14 mm at 30  $\mu$ L volume and after 40  $\mu$ L (50, 60 and 70  $\mu$ L) the diameter zone was decrease.

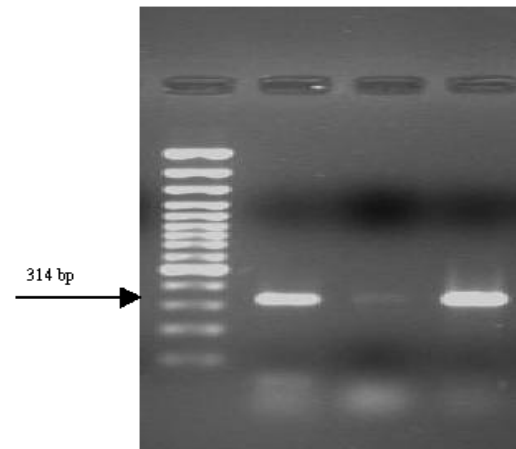


Fig. 1: Gel electrophoresis of DNA fragments amplified by *mecA* specific primers. Lane 1): 100 bp DNA ladder, 2): Control positive, 3): Control negative and 4): Clinical sample with *mecA* gene

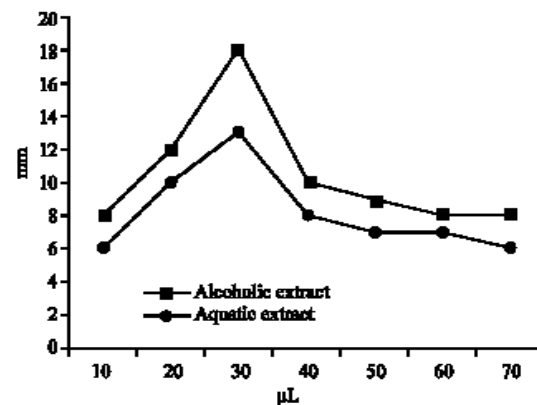


Fig. 2: Zone of inhibition (mm) produced by different concentration of alcoholic and aquatic extract on PCR negative *staphylococcus aureus*

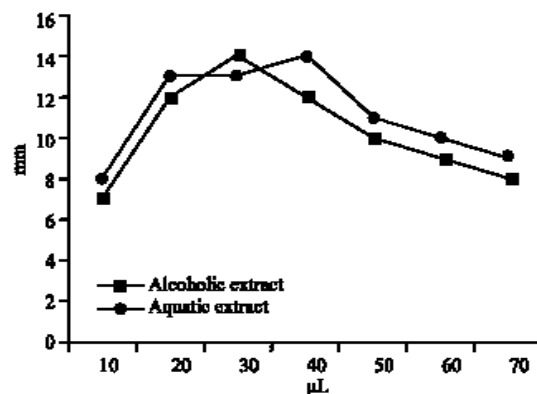


Fig. 3: Zone of inhibition (mm) produced by different concentration of alcoholic and aquatic extract on methicillin resistance *staphylococcus aureus*

## DISCUSSION

Since, multidrug resistance of these microorganisms is a major medical problem, screening of natural products in a search for new antimicrobial agents that would be active against these organisms is the need of the hour. Here in the present study, antibacterial activity of Eucalyptus extracts were checked against a battery of clinically important MRSA strains.

The antibacterial activity of Eucalyptus extracts has been due to the components such as 1, 8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene and aromadendrene (Yang *et al.*, 2004).

The various components of Eucalyptus extracts and essential oil act synergistically (and not additively) to bring the overall antibacterial activity. Among the various components of Eucalyptus oil, 1,8-cineole is the most important one and in fact, a characteristic compound of the genus Eucalyptus and is largely responsible for a variety of its antibacterial and pesticidal properties.

Eucalyptus essential oils and their major constituents possess toxicity against wide range of microbes including bacteria and fungi, both soil-borne and post-harvest pathogens. They have been found to reduce mycelial growth and inhibit spore production and germination (Negahban and Moharramipour, 2007; Liu *et al.*, 2008).

Recently, Lee *et al.* (2007) demonstrated that lemon-scented Eucalyptus oil (at 10 mL 1<sub>1</sub> air) controlled the apple gray mold by 70%. Cermelli *et al.* (2008) screened *E. globulus* oil against 120 isolates of *Streptococcus pyogenes*, 20 isolates of *S. pneumoniae*, 40 isolates of *S. agalactiae*, 20 isolates of *Staphylococcus aureus*, 40 isolates of *Haemophilus influenzae*, 30 isolates of *H. parainfluenzae*, 10 isolates of *Klebsiella pneumoniae*, 10 isolates of *Stenotrophomonas maltophilia* and a strain each of adenovirus and mumps virus and reported that *H. influenzae*, *H. parainfluenzae*, *Stenotrophomonas maltophilia* and *Streptococcus pneumoniae* are very susceptible.

## CONCLUSION

The study concluded that Eucalyptus oil could be used for the control of respiratory tract bacteria.

Studies demonstrated the antibacterial activity of essential oil extracted from *Eucalyptus camaldulensis*, *E. tereticornis*, *E. alba*, *E. citriodora*, *E. deglupta*, *E. globulus*, *E. saligna* and *E. robusta* against *Pseudomonas aeruginosa*. They concluded that

composite essential oils were more effective than the additive activity of their major constituents such as 1, 8-cineole, a-pinene and p-cymene (Cimanga *et al.*, 2002; Delaquis *et al.*, 2002; Jeane Silva *et al.*, 2003).

The results of our study show that alcoholic extract is effect higher than aquatic extract, although both of them have antibacterial effect. The results show that after the definitive concentration the antibacterial effect don't any deference and usually the same. Because of this phenomenon and according the many component t of Eucalyptus much attention that for the usage of this plant.

At the end recommended that the essential oil of Eucalyptus prepared and by GC chromatography, the main effective substance determined.

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