

Original research Articles**Antibacterial activity of essential oil of central Malawi
Corymbia citriodora leaves against *Escherichia coli*
and *Staphylococcus aureus*****ABSTRACT**

Aim: To examine the antimicrobial activities of essential oil of *Corymbia citriodora* leaves on two clinically significant microorganisms, *Escherichia coli* and *Staphylococcus aureus*.

Place and Duration of the Study: The experiment was conducted in Science Laboratory at Malawi College of Forestry and Wildlife, Dedza, Malawi between July and September 2013.

Methodology: The essential oil of *C. citriodora* leaves were obtained by hydrodistillation method. The inhibitory effects of this essential oil were tested against *E. coli* and *S. aureus* using agar disc diffusion method. The bacterial culture were exposed to five different concentration of essential oils: 25%, 50%, 75%, 100% and control (ethyl alcohol) in a completely randomized design in four replicates.

Results: The results obtained indicated that essential oil of *C. citriodora* leaves has antimicrobial activity against both *E. coli* and *S. aureus*. The diameter of zones of inhibition by the leaf extracts of *C. citriodora* was 13-35 mm and 12-32 mm, respectively, for *E. coli* and *S. aureus*. The results revealed that a more significant inhibition was observed with a higher essential oil concentration. Thus, with increasing essential oil concentration, an inhibitory effect on the growth of *E. coli* and *S. aureus* significantly increased. At low concentrations, a significant higher inhibitory effect was observed on the growth of microorganisms in comparison with those controls.

Conclusion: The results, therefore, suggests that characterization and isolation of the active phytochemical(s) from essential oil of *C. citriodora* leaves may provide a valuable antimicrobial agent for counteracting infectious diseases caused by *E. coli* and *S. aureus*, which have developed resistance to antibiotics.

Key words: *Corymbia citriodora*; Antibacterial activity; Essential oil; *Escherichia coli*; *Staphylococcus aureus*.

30 1. INTRODUCTION

31 Medicinal plants have been used since ancient times, and they have been used for treatment of specific
32 illness [1, 2]. The plants contains active components that are used in the treatment of many human
33 diseases [3]. Several studies reported that some plants contain many components such as alkaloid
34 constituents, butanol, chloroform, ethanol, methanol, peptides, phenols and soluble compounds in water,
35 unsaturated long chain aldehydes, and some essential oils [1, 4 - 6].

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37 *Corymbia* is one of world's essential and most widely planted genera [7]. It is well known as medicinal
38 plants because of their biological and pharmacological properties [8]. One of the most important and
39 represented species in pharmaceutical is *Corymbia citriodora* which is the main furnisher of essential oils
40 [9]. *Corymbia citriodora* belongs to the family Myraceae, mostly found in tropical region and is a native to
41 Australia. The essential oils are in great demand in the market. The oils are used with steam and other
42 preparations as an inhalant to relieve colds and influenza symptoms. Because of the refreshing odour of
43 the oil and its efficiency in killing bacteria, it also finds application as antiseptic. The oil plant is also known
44 for its use in soaps, powders, perfumes, air fresheners, disinfectants and natural insect repellent [10].

45

46 Bacterial infectious diseases are widespread all over the world, and the spread of drug resistant microbial
47 pathogens is one of the most serious threats to successful treatment of infectious disease [1, 8].
48 *Escherichia coli* and *Staphylococcus aureus* are the two opportunistic pathogens that cause severe and
49 life threatening infections in patients [8, 11]. Brief description of these two pathogens are well explained by
50 [8]. Numerous studies have reported increasing resistance rates in these two pathogens to antibiotics [12 –
51 16]. Therefore, it is essential to investigate newer drugs with lesser resistance. Natural products of higher
52 plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [3]. In view
53 of this, a study was conducted to examine the antimicrobial activities of essential oil of *Corymbia citriodora*
54 leaves on two clinically significant microorganisms, *Escherichia coli* and *Staphylococcus aureus*. These
55 results can be useful in finding new antibacterial agents.

56

57 2. MATERIALS AND METHODS

58 2.1 Plant material and extraction of essential oil

59 *Corymbia citriodora* mature fresh plant leaves were collected during the flowering stage (July 2013) from
60 Malawi College of Forestry and Wildlife (MCFW) Arboretum, Dedza, Central Malawi. The Arboretum is

located at altitude 1600m above the sea level, longitude 34°16'E and latitude 14°19'S. The area receives 1200 mm to 1800 mm rainfall per annum, with a mean annual temperature of 14°C. It is situated about 85 km south east of Lilongwe the capital. The plant leaves were identified by the Forestry Research Institute of Malawi (FRIM) staff, then they were taken to MCFW Science laboratory for oil extraction. The plant leaves were subjected to steam distillation using a Clevenger type apparatus as described by [8] to extract the essential oil. The oil was stored in the dark at 4°C until the antimicrobial screening. The amount of oil obtained was 1.4% (v/w).

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69 **2.2 Microorganisms and Antimicrobial assay**

In order to evaluate the activity of essential oil of *Corymbia citriodora* leaves, *Escherichia coli* and *Staphylococcus aureus* microorganisms were used for the experiment. *Staphylococcus aureus* were isolated using the protocols described in the Compendium of Methods for the Microbiological Examination of Foods [17] and *Escherichia coli* using the multiple tube-fermentation technique described by [18]. The bacterial culture were exposed to five different concentration of essential oils: 25%, 50%, 75%, 100% and control (ethyl alcohol) in a completely randomized design in four replicates. Antimicrobial activity was carried out using agar disc-diffusion method described by [19].

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78 **2.3 Statistical analysis**

Data obtained on inhibition zone for different concentration of essential oils were tested for normality and homogeneity with Kolmogorov-Smirnov D and normal probability plot tests using Statistical Analysis of Systems software version 9.2 [20]. After the two criteria were met, the data were subjected to analysis of variance (ANOVA) using PROC GLM procedure of the same Statistical Analysis of Systems software and means were separated with Fischer's least significant difference (LSD) at the 0.05 significance level.

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85 **3. RESULTS**

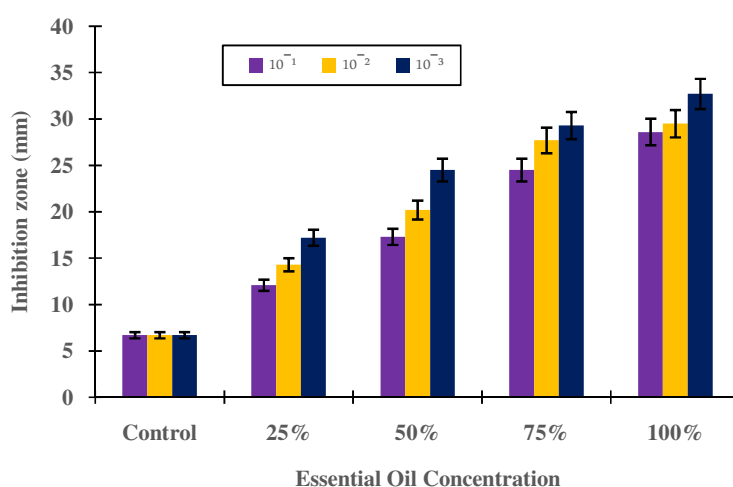
Summary of the results on the microbial growth inhibition by essential oil of *Corymbia citriodora* against the two tested microorganisms are presented in Figures 1 and 2. The results showed that the essential oil of *Corymbia citriodora* leaves has significant antibacterial activity against the two bacterial strains tested. The essential oil showed antibacterial activity with varying magnitude, depending on the size of inoculums and concentration of the essential oil. The higher the concentration of the essential oil the higher the microbial growth inhibition. Thus with increasing essential oil leaves concentration, an inhibitory effect of the growth

of *Escherichia coli* and *Staphylococcus aureus*, was significantly increased. At low concentrations, a significant higher inhibitory effect was observed on the growth of microorganisms in comparison with those controls.

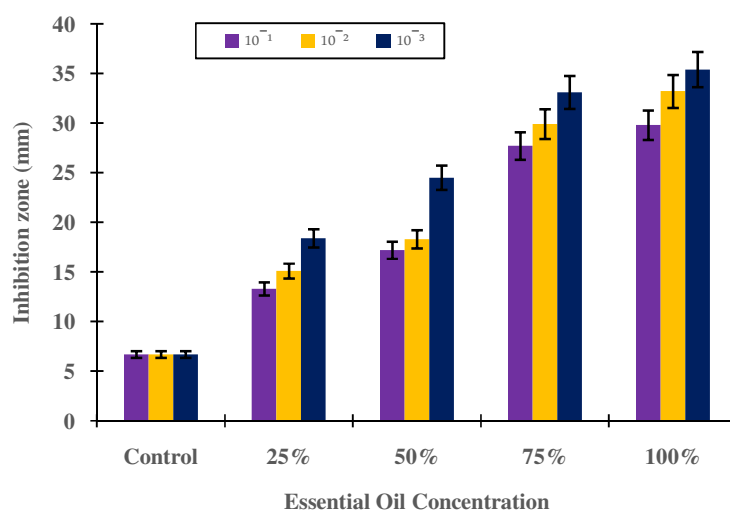
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Diameter of inhibition zone of the essential oil of *Corymbia citriodora* leaves ranged from 12 mm to 35mm. The highest zone of inhibition was obtained for *Escherichia coli* (10^{-3} dilution) with 100% concentration of essential oil of *Corymbia citriodora* leaves, while the lowest activity was shown against *Staphylococcus aureus* (10^{-1} dilution) with 25% concentration of essential oil of *Corymbia citriodora* leaves.

100



101 **Figure 1** Antimicrobial activity evaluation of the essential oil of *Corymbia citriodora* leaves against
 102 *Staphylococcus aureus*
 103



104 **Figure 2** Antimicrobial activity evaluation of the essential oil of *Corymbia citriodora* leaves against
 105 *Escherichia coli*
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4. DISCUSSION

The study revealed that essential oil of leaves of *Corymbia citriodora* leaves has antimicrobial activity against the microorganisms *Staphylococcus aureus* and *Escherichia coli*. The microbial activities increased with an increasing of oil concentration from 25% to 100%. The present findings are in agreement with the results of [8] who reported an increase of microbial activities with an increasing essential oil of *Eucalyptus globulus* leaves against *Staphylococcus aureus* and *Escherichia coli*. Bachir and Benali [19] also reported that increasing essential oil of *Eucalyptus camaldulensis* leaves increased the microbial activities against *Escherichia coli* and *Staphylococcus aureus*. In addition, Duraipandiyar and Ignacimuthu [21] reported a significant inhibitory effect of the growth of *Escherichia coli* and *Staphylococcus aureus* from essential oil of *Cassia fistula* leaves. The results reported by [22], which show an increase of inhibitory effect of the growth of *Escherichia coli* and *Staphylococcus aureus* with an increase of essential oil of *Eucalyptus globulus* leaves from Montenegro, also support the present findings. In contrast, Gonçalves et al. [23] observed no inhibitory effect of the growth of *Escherichia coli* but only in *Staphylococcus aureus* from essential oil of *Psidium guajava* leaves. Possible explanation for the differences as described by [24] state that antibacterial activity depends on the type of essential oil.

Numerous studies have reported that the essential oils of plant species from Myraceae family possess strong antibacterial potential [8, 19, 22, 25 – 27]. The antimicrobial activity of the essential oil of plant species from Myraceae family can be attributed to the presence of different compounds, like 1, 8-cineole, citronellal, citronellol, citronellyl acetate, α -Pinene, p-cymene, β -caryophyllene, β -pinene, eucamalol, limonene and linalool [8]. The study, therefore, suggests that characterization and isolation of the active phytochemical(s) from essential oil of *Corymbia citriodora* leaves may provide a valuable antimicrobial agent for counteracting infectious diseases caused by *Escherichia coli* and *Staphylococcus aureus*, which have developed resistance to antibiotics.

5. CONCLUSION

The present study has revealed that essential oil of *Corymbia citriodora* leaves have significant antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This confirms traditional medicine use of essential oil of *Corymbia citriodora* leaves as an antibacterial agent. The results of this study, therefore, form a good basis for selection of essential oil of *Corymbia citriodora* leaves for their use

as a natural antimicrobial agent for the treatment of several infectious diseases caused by *Escherichia coli* and *Staphylococcus aureus*, which have developed resistance to antibiotics.

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