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Antibacterial Activity of Essential Oil of *Corymbia citriodora* Leaves against *Escherichia coli* and *Staphylococcus aureus*

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Authors' contributions

This work was carried out in collaboration between both authors. Authors EM and IM designed the study. Author EM collected data and performed the statistical analysis. Authors EM and IM managed the literature searches and wrote the first draft of the manuscript. Author EM addressed subsequent reviewer comments and suggestions for improvement. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To examine the antibacterial activity 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

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activity against both *E. coli* and *S. aureus.* The diameter of zones of inhibition by the leaf extracts of *C. citriodora* was 13.3±2.0 – 35.4±5.1 mm and 12.1±1.9 – 32.7±5.1 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 phytoceutical(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.

Keywords: Corymbia citriodora; antibacterial activity; essential oil; Escherichia coli; Staphylococcus aureus.

1. INTRODUCTION

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

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

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

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction of Essential Oil

C. citriodora mature fresh plant leaves were collected during the flowering stage (July 2013) from Malawi College of Forestry and Wildlife (MCFW) Arboretum, Dedza, Central Malawi. The Arboretum is located at altitude 1600 m 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 Bachir and Benali [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).

2.2 Microorganisms and Antimicrobial Assay

The essential oil of *C. citriodora* leaves, were tested for antibacterial activity against twenty isolates of *E. coli* and sixteen isolates of *S. aureus* microorganisms. *S. aureus* were isolated using the protocols described in the Compendium of Methods for the Microbiological Examination of Foods [17] and *E. coli* using the multiple tube-fermentation technique described by Mehlman et al. [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 Bachir and Benali [19].

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.

3. RESULTS

Summary of the results on the inhibition of bacterial growth of essential oil of *C. citriodora* against the two tested microorganisms are presented in Figs. 1 and 2. The results showed that the essential oil of *C. 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 *E. coli* and *S. aureus*, was significantly increased. At low concentrations, a significant higher inhibitory effect was observed on the growth of microorganisms in comparison with those controls.

Diameter of inhibition zone of the essential oil of *C. citriodora* leaves ranged from 12.1±1.9 mm to 35.4±5.1 mm. The highest zone of inhibition was obtained for *E. coli* (10^{-3} dilution) with 100% concentration of essential oil of *C. citriodora* leaves, while the lowest activity was shown against *S. aureus* (10^{-1} dilution) with 25% concentration of essential oil of *C. citriodora* leaves.

4. DISCUSSION

The study revealed that essential oil of leaves of *C. citriodora* leaves has antimicrobial activity against the microorganisms *S. aureus* and *E. 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 Bachir and Benali [8] who reported an increase of microbial activities with an increasing essential oil of *Eucalyptus globulus* leaves against *S. aureus* and *E. coli*. Bachir and Benali [19] also reported that increasing essential oil of *E. camaldulensis* leaves increased the microbial activities against *E. coli* and *S. aureus*. In addition, Duraipandiyan and Ignacimuthu [21] reported a significant inhibitory effect of the growth of *E. coli* and *S. aureus* from essential oil of *Cassia fistula* leaves. The results reported by Damjanović-Vratnica et al. [22], which show an increase of inhibitory effect of the growth of *E. coli* and *S. aureus* with an increase of essential oil of *E. globulus* leaves from Montenegro, also support the present findings. In contrast, Gonçalves et al. [23] observed no inhibitory effect of the growth of *E. coli* but only in *S. aureus* from essential oil of *Psidium guajava* leaves. Possible explanation for the differences as described by Dorman and Deans [24] state that antibacterial activity depends on the type of essential oil and on the structure of the cell of microorganisms.

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]. In support of this, different studies [28-30] have reported that essential oils that contains high concentration of thymol, linalool, carvacrol, 1, 8-cineole has a significant antibacterial activity. Santurio et al. [28] reported that the essential oil of *Thymus*

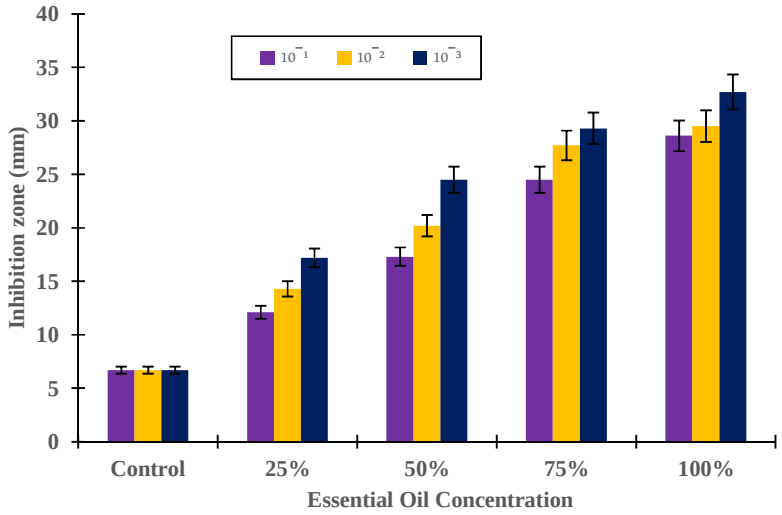

Fig. 1. Antimicrobial activity evaluation of the essential oil of *C. citriodora* leaves against *S. aureus*

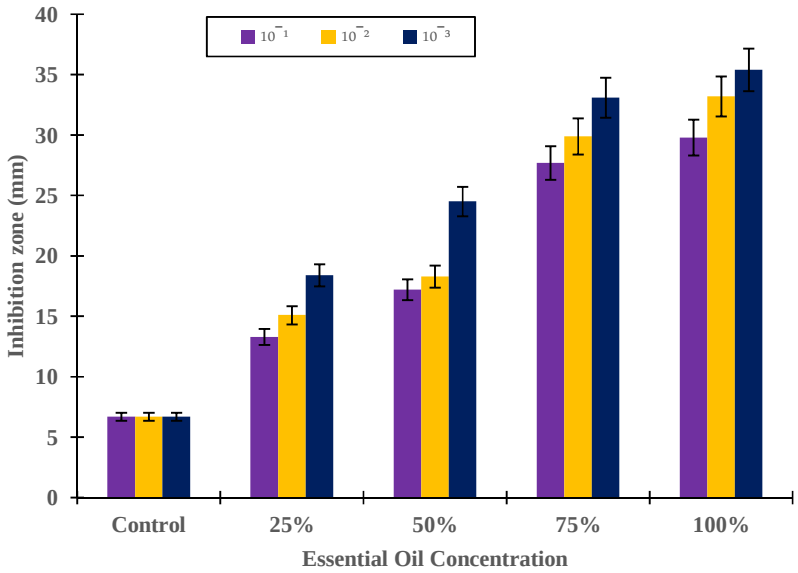

Fig. 2. Antimicrobial activity evaluation of the essential oil of *C. citriodora* leaves against *E. coli*

vulgaris and its major component thymol showed antibacterial activity against *E. coli* strains *In vitro*. Such activity was attributed to high concentration of thymol in the essential oil. An important role of antibacterial activity of essential oil of thyme and oregano against *E. coli* isolated from bovine faeces has also been observed [29]. The phenolic compounds carvacrol and thymol were responsible for the activity of these oils.

Klein et al. [30] also reported a significant antibacterial activity of high content of essential oil components (thymol, linalool, carvacrol, 1, 8-cineole) against *E. coli*.

Other studies [27,31,32] have reported that high sensitive character of some bacteria, to some essential oils, is due to presence of high concentration of α-pinene, *p*-cymene or α-terpineol. Elaissi et al. [27] and Bakkali et al. [31]

reported a high sensitive character of *S. aureus* to essential oils with high content of *p*-cymene. Other researchers reported that this sensitivity of *S. aureus* was due to the single layer wall of the bacteria [33]. *S. aureus* and *E. coli* were resistant to high content of α-pinene [32], while Klein et al. [30] reported a high sensitive character of *E. coli* to high content of essential oil components (α-pinene and α-terpineol). In the present study, a significant antibacterial activity may be attributed to presence of high content of citronellal, citronellol, linalool, or 1,8-cineole in the essential oil of *C. citriodora* leaves [10]. The study, therefore, suggests that characterization and isolation of the active phytoceutical(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.

5. CONCLUSION

The present study has revealed that essential oil of *C. citriodora* leaves have significant antibacterial activity against *E. coli* and *S. aureus*. This confirms traditional medicine use of essential oil of *C. citriodora* leaves as an antibacterial agent. The results of this study, therefore, form a good basis for selection of essential oil of *C. citriodora* leaves for their use as a natural antimicrobial agent for the treatment of several infectious diseases caused by *E. coli* and *S. aureus*, which have developed resistance to antibiotics.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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