



The antibacterial activity of the leaf and stem bark extract of *Eucalyptus citriodora* (Hook)

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Abstract: The antibacterial activities of the ethanol extracts of the leaves and stem-barks of *Eucalyptus citriodora* were investigated. The percentage yield of the stem bark and leaves were 43.70% and 8.36% respectively. The phytochemical screening of the plant parts revealed the presence of saponins, tannin, cardiac glycoside, triterpene and phenol. The extracts were tested separately on clinical samples of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* using the agar diffusion and the tube broth dilution methods. The leaves and stem bark extracts of *E. citriodora* exhibited antibacterial activities against the test organisms at all range of concentrations. The Minimum Inhibitory Concentration of the leaf extract ranged between 25 and 50 mg/ml whereas that of the stem barks extract ranged between 25 and 100mg/ml. In this study, ethanolic extracts of leaves and stem bark of *E. citriodora* showed antibacterial activities against the test organisms, thus suggesting their potentials as sources of antibacterial agents.

Keywords: Concentration, *Escherichia coli*, minimum inhibitory, phytochemicals, *Salmonella typhi*.

Introduction

Eucalyptus citriodora is a large, handsome evergreen tree, 24-40 (max.50) m in height; tall, straight trunk, 60-130 cm in diameter; open, grace fulcrown of drooping foliage; bark smooth, white, powdery, sometimes pink, red or blue-grey, on large trunks dark or grey and shaggy (Orwa *et al.*, 2009). The commercially important eucalyptus oil distilled from the leaves of this and other *Eucalyptus* species has medicinal and industrial uses. The oils are used with steam and other preparations as an inhalant to relieve colds and influenza symptoms. Because of the refreshing aroma of the oil and its efficiency in killing bacteria, it also finds its application as antiseptic (Orwa *et al.*, 2009). The Myrtaceae family represents an important source of essential oils with diverse biological activities including bacteriostatic, fungistatic and anti-inflammatory effects. Various Myrtaceae species possess strong antimicrobial potential and their volatile oils are used as antimicrobial and antifungal agents in creams, soaps and toothpastes (Lis-Balchin *et al.*, 2000). Several species of *Eucalyptus* are used in folk medicine as an antiseptic and against infections of the upper

respiratory tract, such as cold, influenza and sinus congestion (Luqman *et al.*, 2008). The essential oil of this plant has been of great importance in combating multidrug resistant bacteria and has been reported by several authors (Luqman *et al.*, 2008; Nair *et al.*, 2008). It has also been reported that different concentrations of the oil of *E. citriodora* showed antibacterial activity against number of gram positive bacteria (Elaissi, 2011).

Bacterial infections continue to cause significant morbidity and mortality worldwide, often caused by treatment failure or treatment option restrictions because of the prevalence of antibiotic-resistant isolates (Ruddock *et al.*, 2005). Resistance to antimicrobial agents may arise through many factors including inappropriate or inadequate antibiotic therapy, selective pressure, and a high prevalence of disease coupled with lack of capacity to initiate surveillance or prevention programs (Sefton, 2002; Tapsell, 2002; Workowski *et al.*, 2008).

The oil of this plant has been over exploited in the quest for finding lasting solution to the prevalence of

multidrug resistant bacteria. Thus it was thought desirably to look into the effects of the leaves and the stem bark of this plant so as to provide scientific insight to the claim of indigenous people with respect to the use of this plant as antibacterial remedy.

Materials and Methods

Collection and preparation of plant material

Eucalyptus citriodora leaves and stem bark were collected in March, 2013 at the motion ground, University of Ilorin and authenticated at the Department of Plant Biology, University of Ilorin. A voucher specimen was deposited at the Herbarium for reference purposes. The leaves and bark were washed thoroughly with water to remove dirt, dried at room temperature for 3 weeks, after which they were ground using mortar and pestle before use for this study. The powdered samples were kept in sterile bottles for further study.

Extraction procedure

Extraction was done using modified method of Gbadamosi and Oyedele (2012). 200g each of the powdered leaves and stem bark were weighed and soaked in 1500ml of 70% ethanol for 10 days using the cold extraction method after which it was decanted and filtered. The solvent was recovered using a rotary evaporator leaving only the crude plant extract of the stem bark and leaf, the plant extract was evaporated to dryness using hot water bath to make sure no solvent was left in the plant extract. Plant extracts were stored in the refrigerator at 4°C prior to further use.

Phytochemical Screening

The ethanolic extracts of the leaves and stem were screened for the presence of secondary metabolites. The tests were carried out using standard procedures (Tiwari *et al.*, 2011).

Collection and maintenance of test organisms

The test organisms that were used were all human pathogenic organisms from clinical origin. These isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella paratyphi*. Test organisms for the assay were obtained from the Microbiology and Parasitology Laboratory University of Ilorin Teaching hospital (UITH) Oke-oyi, Ilorin, Kwara state. The organisms were collected on sterile agar slants and incubated at 37°C for 48h. They were then kept as stock cultures in the refrigerator at 4°C. The organisms were authenticated at the Department of Microbiology, University of Ilorin.

Standardization of inoculum

A loop of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* were picked respectively into 10 milliliters of sterile Nutrient Broth and incubated at 37°C for 18 hours. After 18 hours, turbidity produced was adjusted to match 0.5

McFarland standard which has corresponding bacteria suspension of 1.5×10^8 cfu/ml (Albert *et al.*, 1991).

Tests for antibacterial Activity of the extracts

The agar well method of the agar diffusion technique was used to determine the antibacterial activity of the plant extracts. Sterile Mueller Hinton Agar plates were prepared. A cotton swab dipped in 18 hours old standardized culture broth of the organisms was used to streak the surface of the agar uniformly. A sterile 10mm cork borer was used to punch 5 wells in the inoculated agar and the agar was then removed. About 100µl of molten agar was dropped into the holes to seal the hole in order to prevent leakage of the plant extract, four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml while the 5th well contained a standard antibiotic (streptomycin). 100 µl of each extract was added to the well using a micropipette and three plates were prepared for each extract, a plate per test organism after which three replicates were done. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in millimeter.

Determination of minimum inhibitory concentration (MIC)

For determination of minimum inhibitory concentration (MIC), the broth dilution method of Albert *et al.* (1991) was used.

Results and Discussion

The percentage yield of the plant extracts revealed that the stem bark had the highest yield of 43.70% while the leaf yielded 8.36% (Table 1). The yield may be due to the fibrous nature of the stem bark which enhanced its solubility. It may also be due to the permanency of the stem barks, the leaves are temporal.

Table 1: Percentage yield of the ethanolic extracts of leaves and stem barks of *Eucalyptus citriodora*

Plant parts	Weight of Sample (g)	Vol. of solvent (ml)	Percentage yield (%)
Leaves	200	1500	8.36
Stem bark	200	1500	43.70

Table 2: Phytochemical screening of the leaf and stem bark extracts of *E. citriodora*

Phytochemical component	Leaf extract	Stem bark extract
Saponins	+	+
Triterpenes (sterol)	+	+
Phenols	+	+
Tannins	+	+
Alkaloids	+	+
Cardiac Glycosides	+	+

Note: + = Present; -- = Absent

The results of phytochemical screening carried out revealed the presence of essential phytochemicals such as cardiac glycosides, triterpenes, phenols, tannins and saponins, alkaloid was absent in the samples (Table 2). Ahmad *et al.* (1998) have earlier reported the presence of these phytochemical components in members of the family Myrtaceae. Also Babayi *et al.* (2004) had reported that the phytochemical analysis of the crude extract of *Eucalyptus* species revealed the presence of saponins, phenols, tannins, steroid and cardiac glycosides. These compounds are found to be biologically active. One of the molecular actions of tannins is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as covalent bond formation resulting in the inhibition of cell protein synthesis of the organisms (Stern *et al.*, 1996). Herbs that have tannins as their major components are astringent in nature and are used for treating intestinal disorders (Dharmananda,

2003). Saponins have long been known to have strong biological activity; they can bind with cholesterol and thus interfere with cell growth and division of the organisms (Pamar and Rawat, 2012). Phenols are known to have antimicrobial activity possibly due to enzyme inhibition in the oxidized forms or through more interactions with non-specific proteins (Mulla *et al.*, 2010). Thus, the antibacterial activity of the extracts on the test organisms may be due to the presence of the aforementioned phytochemical components.

The antibacterial screening of the leaves and the stem barks of the plant extracts revealed that at concentration of 200mg/ml, there was a large diameter zone of inhibition and at concentration of 25mg/ml there was a small diameter zone of inhibition (Table 3).

Table 3: Antibacterial activity of crude ethanolic extract of *Eucalyptus citriodora*

Diameter of zone of inhibition (mm) $\pm$ S.E.M

Test organisms	Plant parts	Concentration of extracts (mg/ml)				Control (Streptomycin)
		200	100	50	25	
<i>E. coli</i>	Leaves	11.5 $\pm$ 0.00 ^a	9.50 $\pm$ 0.25 ^a	6.25 $\pm$ 0.25 ^a	5.50 $\pm$ 0.50 ^a	8.50 $\pm$ 0.50 ^a
	Stem-bark	9.00 $\pm$ 2.00 ^b	6.50 $\pm$ 1.50 ^b	5.25 $\pm$ 0.75 ^b	2.75 $\pm$ 0.25 ^b	10.50 $\pm$ 0.50 ^b
<i>Paeruginosa</i>	Leaves	10.50 $\pm$ 0.50 ^a	8.50 $\pm$ 1.50 ^a	6.25 $\pm$ 0.25 ^a	4.25 $\pm$ 1.75 ^a	11.75 $\pm$ 4.25 ^a
	Stem-bark	9.00 $\pm$ 0.50 ^b	5.25 $\pm$ 0.25 ^b	3.75 $\pm$ 0.75 ^b	2.25 $\pm$ 0.25 ^b	11.50 $\pm$ 0.75 ^b
<i>S.paratyphi</i>	Leaves	12.2 $\pm$ 0.25 ^a	9.25 $\pm$ 0.75 ^a	6.85 $\pm$ 1.65 ^a	5.25 $\pm$ 0.25 ^a	10.00 $\pm$ 2.00 ^a
	Stem-bark	9.25 $\pm$ 0.25 ^b	4.00 $\pm$ 0.50 ^b	3.50 $\pm$ 0.50 ^b	2.50 $\pm$ 0.50 ^b	9.75 $\pm$ 0.25 ^b

Legend: Values are means of two replicates $\pm$ standard error of mean

Values with the same superscript along the same column are not significantly different at $P \leq 0.05$

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The ethanolic leaf extracts of *Eucalyptus citriodora* at concentration of 50mg/ml exhibited antibacterial activities against *Pseudomonas aeruginosa* and *Salmonella paratyphi* while at concentration of 25mg/ml it showed antibacterial activity against *Escherichia coli* (Table 3).

The ethanolic stem bark extract of test plant at 50mg/ml exhibited antibacterial activities against *Escherichia coli* while at 100mg/ml it showed activity against *Pseudomonas aeruginosa* also at 25mg/ml it exhibited antibacterial activities against *Salmonella paratyphi* (Table 3).

The crude ethanolic extracts of leaf and stem bark of *Eucalyptus citriodora* inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella paratyphi*. They showed varying degrees of activity based on the concentration, extract and test organism used. This research work has shown that the plant extracts exhibited concentration dependent antibacterial activity. The leaf extract showed higher activity against *Salmonella paratyphi* based on the diameter of zone of inhibition compared with other test organisms, the same was also observed for the stem bark extract. This observation agreed with the work of Akin-Osanaiye et al. (2007) on the antimicrobial activity of *Eucalyptus citriodora*. This also agreed with the work of Gero et al. (2012) where the oil of *Eucalyptus citriodora* showed high antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Candida albicans* compared with the oil of *Eucalyptus camaldulensis*.

The Minimum Inhibitory Concentration (MIC) of the leaves and stem bark extracts was also recorded (Table 4). For the leaves, the MIC for *Pseudomonas aeruginosa* and *Salmonella paratyphi* was 50 mg/ml, while that of *Escherichia coli* was 25mg/ml. For the stem extracts, the MIC for *Pseudomonas aeruginosa* was 100mg/ml, *Salmonella paratyphi* was 25 mg/ml, while that of *Escherichia coli* was 50mg/ml (Table 4).

Table 4: The Minimum Inhibitory Concentration (MIC)

S/N	Test organism	MIC of leaf extract (mg/ml)	MIC of stem bark extract (mg/ml)
1	<i>Pseudomonas aeruginosa</i>	50	100
2	<i>Escherichia coli</i>	25	50
3	<i>Salmonella paratyphi</i>	50	25

These results suggest that the leaf extract is more potent against *Escherichia coli* while higher concentrations will be needed for *Pseudomonas aeruginosa* and *Salmonella paratyphi*. The stem is more potent against *Salmonella paratyphi* and higher concentrations will be needed for *Pseudomonas aeruginosa* and *Escherichia coli*. In the study carried out by Nair et al. (2008), it was revealed that *Eucalyptus citriodora* oil was generally active against all bacterial strains studied except *P. aeruginosa* which was the most resistant bacterial strain studied.

In conclusion, ethanolic extract of leaves and stem barks of *E. citriodora* have shown potential as antibacterial agents against pathogens and could improve treatment and eradication of bacterial strain. There is the need for researchers to isolate and characterize the bio-active principle of this plant so as to increase candidate drugs for microbial infections.

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