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ANTIBACTERIAL ACTIVITY OF *Eucalyptus globulus* ON URINARY TRACT CLINICAL BACTERIAL ISOLATES

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ABSTRACT

This study evaluates on antibacterial activity of *Eucalyptus globulus* on urinary tract clinical bacterial isolates with a view to determine antibacterial potential of *E. globulus* plant parts. Agar diffusion and phytochemical screening standard methods were adopted. Results showed that five phytochemicals namely: alkaloids, flavonoids, cardiac glycosides, Saponins and tannin were found in both leaf and stem bark of the plant. Antibacterial activity of *E. globulus* demonstrated promising activity of the leaf ethanol extract against *Escherichia coli* with 37mm as inhibition zone at 500mg/ml. The *E. globulus* stem bark ethanol extract showed no activity against *Klebsiella* spp at all the concentrations tested. The minimum inhibitory concentrations (MIC) of the leaf and stem bark were all 60mg/ml against *E. coli* and 60mg/ml; 100mg/ml respectively against *Klebsiella* spp. The Minimum Bactericidal Concentration (MBC) of the leaf and stem bark ethanol extracts of the plant were 60mg/ml and 20mg/ml against *E. coli* respectively *and* 20mg/ml and 60mg/ml respectively against *Klebsiella* spp. The current findings support the use of *E. globulus* leaf and stem bark in the folklore medicine as antibacterial agents.

Keywords: Antibacterial potential, *Eucalyptus globulus,* phytochemical screening, Agar diffusion, Urinary tract

INTRODUCTION

Chemical and biological investigations of ethno medicinal plants with high therapeutic indices and reputation of being curative have furnished the world with many of clinical potent drugs (Khan *et al.*, 2014; Maria *et al.*, 2016). Interestingly, at least 119 compounds derived from 91 plant species are considered as promising drugs, currently in use and that 77% of them were obtained from ethno medicine (Khan *et al.*, 2014). The search for new antibacterial agents has increased with the increase in bacterial infections as well as a result of bacterial resistance, attributed to the overuse of certain agents, such as antibiotics (Nickel, 2005). *Eucalyptus globulus* is an aromatic tree belonging to *Myrtaceae* (Cazarolli *et al.*, 2008). The *Eucalyptus* plant parts are used to control several diseases derived from microbial infections (Bachir and Benali, 2008). The *Eucalyptus* oil (EO) extracted from leaves, fruits, buds and bark of *E. globulus* has been found promising as antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer agent (Maria *et al.*,2016). Several studies showed a moderate antimicrobial activity of EO from *E. globulus* both on Gram-negative (*Salmonella enteritidis, Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecium, Listeria monocytogenes 4b* and *Listeria monocytogenes* EGD-e) (Bachir, 2008). Further studies are necessary to investigate other prime effects of *E. globulus* and its possible medicinal potentials useful in the treatment of a greater number of pathological conditions (Maria *et al.*, 2016). The modern medicine and healthcare services are very expensive and most local communities cannot afford these services (Teodora *et al.*, 2011). Thus, the current study evaluates antibacterial activity of *E. globulus*_leaf and stem bark extracts against urinary tract clinical bacterial isolates.

MATERIALS AND METHODS

Sample collection and processing

The stem bark and fresh leaves of *Eucalyptus globulus* were obtained from Unguwar Boro, Chikun Local Government, Kaduna, Kaduna State. The taxonomic identity of the plant was confirmed using reference voucher specimens in the herbarium of Applied Science Department, Kaduna Polytechnic. Voucher specimen with reference number 010216 was deposited. The stem bark and leaves were air dried at room temperature (28±2^{o}C) for two weeks. The air-dried plant materials were separately pulverized using clean mortar and pestle. The powdered samples were kept in air-tight plastic containers prior to extraction.

Extraction of *Eucalyptus globulus* Plant Materials

Fifty grams (50g) of the pulverized leaves and stem bark of *E. globulus* were weighed using electric weighing balance. These were separately put into conical flasks a (1000 ml) .Five hundred

milliliter (500ml) of ethanol was put into each sample. The preparations were mixed and subsequently covered with cotton wool and aluminum foil. These were kept at room temperature (28±2ºC) for 72 hours with frequent agitation. The percolates were filtered using Whatman's No. 1 filter paper. The filtrates were concentrated using water bath set at 78ºC.

Phytochemical screening of *E. globulus* Leaves and Stem bark extracts

Qualitative phytochemical screening of *E.globulus* leaves and stem bark extracts was carried out using standard protocols (Trease and Evans, 1989; Sofowora, 1999; Harbone, 1998).

Clinical Bacterial Isolates

Clinical isolates of *Escherichia coli* and *Klebsiella* were obtained from Shehu Kangiwa Medical Centre (SKMC), Kaduna Polytechnic. The isolates were confirmed using Gram's staining and biochemical tests (Cheesbrough, 2002).

Bioassay

Preparation of Culture Media

Mueller Hilton agar and nutrient broth were prepared according to the manufacturer's instructions. The media were sterilized by autoclaving at 121^0C for 15minutes.

Preparation of Over Night Broth Cultures

Two to three well grown colonies from each of the confirmed cultures were separately and aseptically introduced into sterile nutrient broth in test tubes. These were incubated at 37^0C for 24hours.

Preparation of 0.5 McFarland Standard

A 1%$^v/_v$ solution of sulphuric acid (H_2SO_4) and 1%$^v/_v$ solution of barium chloride ($BaCl_2.2H_2O$) were used to prepare 0.5 McFarland standard. The standard was kept at room temperature (28±2^{0}C) prior to inocula standardization (Cheesbrough, 2002).

Standardization of Inocula

The previously prepared overnight broth cultures of each bacterial isolate was adjusted to the 0.5 McFarland standard (Cheesbrough, 2002).

Preparation of Varied Concentrations of *E. globulus* Leaves and Stem bark extracts

Stock solutions of *E.globulus* leaves and stem bark extracts were separately prepared by dissolving 1g of each extract in 1ml of Dimethyl sulfoxide (DMSO). From each of the stock solution, 0.1ml was transferred into a sterile bijou bottle and 0.9ml DMSO was added to give 100mg/ml. Subsequently, 0.3ml of each stock solution was separately transferred into another sterile bijou bottle containing 0.7ml DMSO, to give 300mg/ml. Similarly, 0.5ml of each stock solution was transferred into another sterile bijou bottle containing 0.5ml DMSO to give 500mg/ml (Deeni and Hussein, 1991).

Antibacterial Activity of *E. globulus* extracts

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. The sterile Mueller Hilton agar was poured into sterile Petri plates and allowed to solidify. A sterile standard cork-borer (6mm) was used to cut wells on the surface of the agar. Sterile wire loop was used to inoculate standard bacterial inocula radially on the agar pour. A 0.1ml of the different concentrations (100,300 and 500 mg/ml) of the extracts was separately put into dug wells using sterile 1ml syringe. The preparations were incubated at 37^0C for 24 hours. Zones of inhibition were measured in millimeters (Bauer *et al*., 2003).

Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution method was used as described by (Pelzer *et al.,* 1999). Standardized suspension of each clinical bacterial isolate was inoculated into separate series of test tubes, each containing 4 ml nutrient broth. A 1 ml of each varied concentrations (500,300 and 100) mg/ml of each extract was sequentially introduced into the inoculated test tubes. Cotton wool and aluminum foil were used to cover the test tubes. The preparations were incubated at 37ºC for 24 hours. Tubes without turbidity were recorded as MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the plant extract was determined by sub-culturing the test tubes from minimum inhibitory concentration tubes that showed no growth on nutrient agar and incubating for 24 hours at 37^0C. The minimum bactericidal concentration was represented by the plate with the lowest concentration without growth.

RESULTS

TABLE 1: Phytochemical profile of *Eucalyptus globulus* leaf and stem bark ethanol extracts

	Phytochemicals profile				
Extracts	Alkaloid	Flavonoids	Cardiac glycoside	Saponins	Tannins
EEL	+	+	+	+	+
EES	+	+	+	+	+

KEY: EEL= *E. globulus* ethanol leaf extract; EES= *E. globulus* ethanol stem bark extract; + indicates presence of phytochemicals

Table 2: Antibacterial activity of *E. globulus* leaf and stem bark ethanolic extracts against *E. coli* and *Klebsiella* spp.

Zones of inhibitions (mm) of *E. globulus* plant extracts (mg/ml) against the test isolates

	E. coli			*Klebsiella* spp		
Extracts	100	300	500	100	300	500
Leaf	18.5	32.0	37.0	17.0	20.0	23.5
Stem bark	12.5	13.0	16.5	NA	NA	NA

Key: NA means no activity

TABLE 3: MIC and MBC of *E. globulus* leafs and stem bark extract against *E. coli* and *Klebsiella* spp.

TEST	MIC (mg/ml)		MBC (mg/ml)	
ISOLATES	Leaves	Stem Bark	Leaves	Stem Bark
E. coli	60	60	60	20
Klebsiella spp	60	100	20	60

DISCUSSION AND CONCLUSION

Ethno medicinal plants possess antibacterial activity due to vast array of phytocompounds which in the extracted form can be effectively and successfully utilized to treat microbial infections. These plants are easily accessible, have cheaper mode of treatment and show fewer side effects or adverse reactions as compared to modern synthetic drugs (Khan *et al.*, 2014). *E. globulus* which is used in the folklore medicine could probably offers a great reservoir for the discovery of antibacterial drug. This study evaluates antibacterial activity of *E. globulus* leaf and stem bark extracts with a view to determine its antibacterial potentials. The result of this study will go a long way in providing data base for further pharmacological studies as well as for pharmacognosy and drug development. The phytochemical screening of *E. globulus* plant extracts beyond reasonable doubt demonstrated the phytochemical potential in the plant parts. The occurrence of five phytochemicals in the extracts further buttressed the use of *E. globulus* plant parts in the treatment of bacterial infections. The functional groups of some compounds found in most plant materials, typically: alcohol, phenols, terpenes and ketones justified their antimicrobial characteristics (Bachir and Benali, 2008).The presence of these phytochemicals in *E. globulus* indicates a wide range of biological and pharmacological activities *in vitro* (Yamamoto and Gaynor, 2001). Earlier studies reported on anti-inflammatory, antioxidant, anti-microbial, anti-cancer (Cazarolli *et al*., 2008), and anti-diarrheal activities of flavonoids (Schuier *et al*., 2005).

The promising antibacterial activity of *E. globulus* leaf extract against *E .coli*, especially at 500mg/ml supported the use of its leaf in the traditional system of medicine to treat bacterial infections. The leaves of *E. globulus* are used as expectorant, stimulant, antiseptic, carminative, whereas the volatile oil is said to be antimalarial and disinfectant (Khan *et al.*, 2014). Bachir and Benali (2008) reported promising antibacterial activities of essential oils from leaves of two *Eucalyptus* species *(globulus* and *camaldulensis)* with excellent inhibitory effect on *S. aureus* than that of *E. coli*.

The MIC and MBC values especially of *E. globulus* leaf extract against *E. coli* could be useful in providing information on the therapeutic profiles of this plant, as potential candidate for drug development from natural products. Khan *et al.*, (2014) found that essential oil obtained from *E. globulus* and *E. camaldulensis* is effective against *E. coli* and *S. aureus*.

CONCLUSION

Eucalyptus globulus plant parts; especially the leaf had promising antibacterial activity against *E. coli* due to the vast array of phytochemical compounds.

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