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°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±2°C) 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 78°C.

**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; Harborne, 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°C for 15 minutes.

**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°C for 24 hours.

**Preparation of 0.5 McFarland Standard**

A 1%*v*/v solution of sulphuric acid (H2SO4) and 1%*v*/v solution of barium chloride (BaCl2.2H2O) were used to prepare 0.5 McFarland standard. The standard was kept at room temperature (28±2°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 100 mg/ml. Subsequently, 0.3ml of each stock solution was separately transferred into another sterile bijou bottle containing 0.7ml DMSO, to give 300 mg/ml. Similarly, 0.5ml of each stock solution was transferred into another sterile bijou bottle containing 0.5ml DMSO to give 500 mg/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°C 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 37°C 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°C. 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**

<table>
<thead>
<tr>
<th>Phytochemicals profile</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Extracts</td>
<td>Alkaloid</td>
<td>Flavonoids</td>
<td>Cardiac glycoside</td>
<td>Saponins</td>
<td>Tannins</td>
</tr>
<tr>
<td>EEL</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EES</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

KEY: EEL = *E. globulus* ethanol leaf extract; EES = *E. globulus* ethanol stem bark extract; + indicates presence of phytochemicals
ANTIBACTERIAL ACTIVITY OF ..... Ado, Chonoko and Badaru

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E. coli</th>
<th>Klebsiella spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>Leaf</td>
<td>18.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Stem bark</td>
<td>12.5</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Key: NA means no activity

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

<table>
<thead>
<tr>
<th>TEST</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stem Bark</td>
</tr>
<tr>
<td>E. coli</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

Ethno medicinal plants possess antibacterial activity due to vast array of phytochemicals 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 500 mg/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.

REFERENCES


