ANTIBACTERIAL EVALUATION AND PHYTOCHEMICAL ANALYSIS OF *Eucalyptus globules* AGAINST ENTERIC BACTERIAL PATHOGENS IN KANO STATE, NIGERIA

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ABSTRACT: The present study was conducted to evaluate the antibacterial activity of ethanolic and aqueous leaf-extracts of *Eucalyptus globules*. Evaluation of antibacterial activity and phytochemical analysis of aqueous and ethanol crude extracts of *E. globules* that was selected based on ethno-medicinal information on its traditional use was tested for treatment of enteric disease in Kano State. The study has been carried out in September, 2017 at Bayero University Kano. The leaves of the plant were extracted following standard methods (Soaking extraction method and agar-well diffusion) to screen for potential antimicrobial substance, in which ethanolic extract was found to possess high contents of bioactive metabolites compared to aqueous extract. The crude extracts of the plant were also tested against standard reference strains including *Salmonella typhi ATCC 29212, Escherichia coli ATCC 11229, Staphylococcus aureus ATCC 6538* and *Pseudomonas aeruginosa ATCC 15442* at different concentrations, with penicillin (30μg) being used as positive control. Both extracts showed antibacterial activity with minimum inhibitory concentration (MIC) values in the range of 3.13 to 6.25 mg/mL against *S. aureus, E. coli, S. typhi* and *P. aeruginosa,* respectively with ethanolic extract being more potent. However, both extracts were found inactive against *P. aeruginosa* at 50 mg/mL using disc diffusion test. In general, extracts of *Eucalyptus globules* exhibited the highest potency against *S. aureus* and *E. coli,* and then followed by *S. typhi* and *P. aeroginosa*. Thus, this study confirmed the alternative source of medicine for pathogenic enteric bacteria tested and this may be attributed to the presence of bioactive components of the leaves.

Keywords: *Eucalyptus globules*; Crude extracts; Antimicrobial activity; Enteric bacteria; Phytochemicals.

1.0 INTODUCTION

Since the first agricultural settlement, mankind exploited plants such as forth grass, herbs and fruit yielding trees for their medicinal properties. Nowadays, following the discovery of different types of medicinal plants and development of their therapeutic potentials, the practice of traditional medicines is well acknowledged and established as a profession (Assareh *et al.,* 2010). Plants have not only nutritional value but also, have medicinal and ritual or magical values in the eyes of the local community people (Abbink, 1995).

Traditional medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (Philip *et al.,* 2009; Dery *et al.,* 1999). In world history of the medieval times, the heading researchers in medicine, such as Al-Razi Abubakar and Ali Hussein Ibn Sina, have aggregated and composed such a variety of books of medication. In Africa landmasses, countries especially social orders of Egypt passed on their ethnomedicinal information to a significant number of the African countries as writing where to date, the reported records are still been passed on to eras (Bala, 2006).

The World Health Organization also defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal products (WHO, 2001). Phytochemicals often referred to as "secondary metabolities" chemical compounds formed during the plant normal metabolic processes, they were first described at the beginning of the 19th century (Cordell, 1995). The most important of these bioactive compounds of plants are alkaloids, flavanoids, quinones, phenolic compounds, saponins, tannins, coumarins, glycosides, gums, polysaccharides, terpenes and other chemical compounds (Leon *et al.,* 2001; Shariff *et al.,* 2001; Edeoga *et al.,* 2005; Okwu, 2004; Al-Zubaydi *et al.,* 2009). Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects.

Of the tropical and sub-tropical plants, Eucalyptus is one of the medicinal plants which belongs to the order myrtles and Myrtaceae and a large genus of aromatic trees indigenous to Australia, Tasmania and the neighboring Island, and now extensively cultivated in many other countries including Nigeria and other African countries (Adeniyi and Ayepola, 2008; Cakir *et al.,* 2003). The commonest species of eucalyptus are; *Eucalyptus camaldulensis, Eucalyptus polybractea, Eucalyptus smithii* and *Eucalyptus globules* (Gruenwald *et al.,* 1998). Eucalyptus oils are readily steam distilled from the leaves and can be used for cleaning, deodorizing and in very small quantities in food supplements, especially sweets, cough drops and decongestants (Garcia *et al.,* 2004). The oil may also provide antiseptic properties (Gupta *et al.,* 2012). Some

species of Eucalyptus such as globulus, maculate and viminalis with inhibition effect on some Gram-positive bacteria and fungal species have been reported. Thus, possess antimicrobial properties (Kafaru, 1994; Igbe *et al.,* 2010; Indraya *et al.,* 2007; Takahashi *et al.,* 2004).

Antimicrobial agents are substances that interfere with the growth and metabolism of microbes. In common usage, the term denotes inhibition of growth and with reference to specific groups of organisms, terms as antibacterial, antifungal, antiviral and ant protozoa are frequently employed. Antimicrobial agents may either kill microorganisms or inhibit their growth (Elaissi *et al.,* 2012). The increasing resistance of most synthetically derived antimicrobials is of utmost concern. The search for suitable medicinal plants with potent active principles against microbes becomes imperative. Since the antimicrobial properties of eucalyptus plants have been established from previous research works, this study was aimed at investigating the phytochemical composition and evaluating the antimicrobial activity of aqueous and ethanolic leaf-extracts of *E. globules* against enteric pathogenic isolates.

2.0 MATERIAL AND METHODS

2.1 Collection and Preparation of the plant material

This research study was conducted in October 2017 at Faculty of Science, Bayero University Kano, Nigeria The leaves of *E. globules* were collected from School of Technology, Kano State Polytechnic, Nassarawa Local Government area of Kano State and authenticated at the Herbarium of the Department of Biological Sciences, Bayero University Kano located at Gwale, Kano city. The young leaves were excised, washed and shed-dried at room temperature for 4-5 days. The dried leaves were then taken into the laboratory for crushing and grinding into powder using a grinding machine for extraction and further analysis. The antimicrobial evaluation was carried out in Microbiology Department and the phytochemical analysis was made in Chemistry Department, analytical chemistry laboratory of Bayero University, Kano.

2.2 Plant Extraction

The sample powder of the eucalyptus plant was measure 100g each of air dried and coarsely powder plant sample was extracted successively with 500ml of ethanol using a soxhlet apparatus, and the same process was used for water extract using evaporator for 48h (V V Katikala *et al.,* 2009). The extract took 2-3 days after complete solvent evaporation. For water extraction, after four days, the extract was filtered through filter paper No. 1 and allowed to dry in a water bath at 60 °C for solvent elimination. The extracts obtained were both weighed and the percentage yields calculated. Finally, both extracts were then dissolved in 10% dimethyl l sulphoxide (DMSO) for final stalk concentration of 400 mg/mL and stored capped bottles for further used.

2.3 Qualitative Phytochemical Analysis

Phytochemical screening for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids, anthraquinones and coumarins was undertaken using standard qualitative methods as described by (Fadeyi *et al.*, 1989; Trease and Evans, 1989; Harborne, 1992; Sofowora, 1993; Finar, 2003; Parekh *et al.*, 2006).

2.4 Bacterial organism and Culture media

The test organisms used for the antibacterial evaluation were *Escherichia coli ATCC 11229*, *Staphylococcus aureus ATCC 6538 Salmonella typhi ATCC 29212*, and *Pseudomonas aeruginosa ATCC 1544.* They were all obtained as stock pure culture of American Type Culture Collection (ATCC) strains from Aminu Kano Teaching Hospital, Bayero University Kano (AKTH-BUK). The types of media used for antimicrobial evaluation were Nutrient Agar (Science Company, UK) and Nutrient broth which were all prepared according to the manufacturers' specifications.

2.5 Standardization of Bacterial stock culture

The culture stock of each test organism was sub-cultured onto new fresh nutrient agar plates for 24 hours at 37^{o}C to get the active strains accordingly. The organisms contained in suspended broth media were shaken for some minutes prior to incubation for 24 hours at 37°C to get the most active strains needed for minimum inhibitory concentration (MIC). The turbidity was also compared with that of McFarland standard turbidity.

2.6 Bioassay procedure for Antibacterial Evaluation

Agar disk diffusion method was used to evaluate the antibacterial activities of extracts of medicinal plants according to (Trease and Evans, 1989). The 24 hours plate cultures of 0.5 Mc Farland standard (1 to 2 x 10^{8} CFU ml−1) bacterial suspensions were uniformly spread on Nutrient agar plates to form lawn cultures. The ethanol and aqueous crude extracts were dissolved in 10% dimethyl l sulphoxide (DMSO). The stock solutions were prepared at amount of 400 mg/ml for each solvent extract. The blotting paper discs (6 mm diameter) were soaked in various dilute solvent extracts, and dried for 5 minutes to avoid over-flow of extracts in the test media. Antibacterial activity of potential plant extract against bacterial pathogens by disc diffusion technique were identified after incubation for 24 h. at 37°C, and the results were obtained by measuring the zones of inhibition of growth in mm. Standard antibiotic discs (Penicillin 30 μg) were used as positive control.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

The MICs of both extracts were determined by broth dilution technique in (mg/mL) using 96-well microplate (Karakoca *et al.*, 2013) where the stocks of 50 mg/ml of the extracts were resuspended in 10% DMSO to produce two-fold dilutions to obtain six different concentrations (50, 25, 12.5, 6.25, 3.12 and 1.56 mg/mL). Each dilution is then seeded with bacteria suspension ($1x10^3$ cfu/ml) and incubated for 24h at 37^oC. After incubation, the growths of the bacterial isolates in the test tubes were then observed as turbidity using spectrophotometer at 600 nm. The least concentration where no turbidity was observed is then determined and noted as the MIC value. All samples were tested in duplicates. Penicillin antibiotic standard was used as positive control.

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Table 1 below shows results of phytochemical screening of the plant extract for the following compounds such as glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids, anthraquinones and coumarins. Based on the results obtained, all secondary metabolites were present in ethanolic extract except for coumarins that shows negative (-) in ethanol. Whereas in the case of aqueous extract, three active metabolites were found absent (saponin, alkaloid and coumarins).

Table 1: Results of `Photochemical Screening of *Eucalyptus globules*

S/N	Compound Tested	Ethanolic extract	Aqueous extract
1.	Saponin	+	-
2.	Tanin	+++	++
3.	Glycosides	++	+
4.	Terpenoids	++	+
5.	Alkaloid	+	-
6.	Flavonoid	++	++
7.	Anthraquinone	++	+
9.	coumarins	-	-

Note; + = presence, ++ = moderately present, +++ = highly present**,** - = absent

3.2 Antibacterial Activity of *Eucalyptus globules* Leaf-extracts

Crude ethanolic and water extracts of *E. globules* were examined for susceptibility at four different concentrations; 50mg, 100mg, 200mg and 400mg/mL of DMSO using disc diffusion

method. Based on evaluation of antibacterial activity of both extracts, a strong activity was observed against the tested isolates; *E. coli, S. aureus, S. typhi* and *P. aeroginosa* (see Table 2). The most susceptible organisms for ethanolic extract were *S. aureus* and *E. coli*, and then followed by *S. typhi* and *P. aeroginosa*. In the case of water extract, it was observed that all the tested isolates were susceptible at all concentrations of the extract except for *P. aeroginosa* where no zone of inhibition was clearly shown at 50 mg (Table 2). Therefore, *S. aureus* as Gram-positive bacterium was found more sensitive to both extracts of *E. globules* than Gram-negative bacteria (*E. coli, S. typhi* and *P. aeroginosa).*

Table 2: Shows Antibacterial Sensitivity Test of *Eucalyptus globules* Leaf-extracts Against Some Bacterial Isolates

S/N	Isolate	Concentration of the extracts in (mg/mL)/mm Zone Inhibition								
		Ethanolic Extract				Aqueous Extract				Penicillin Used
		400	200	100	50	400	200	100	50	(30 μg)
1	*Eschericachia coli*	17	15	13	9	15	13	10	7	29
2	*Staphylococcus aureus*	19	16	13	10	16	13	11	8	27
3	*Salmonella typhi*	15	12	10	7	12	10	8	7	26
4	*Pseudomonas aeruginosa*	13	10	8	-	10	9	7	-	24

Note; Diameter **< 8.0mm** = low sensitivity, **> 8.0mm** = high sensitivity, **Penicillin agent** serves as standard positive control.

The highest inhibition zone for *S. aureus, E. coli, S. typhi* and *P. aeruginosa* were observed with the highest concentration of both extracts attained from percolation procedure as shown in table 2. This means that antibacterial sensitivity testing at highest concentration (400 mg/mL) for both extracts exhibited the strongest antibacterial activity against all the isolates.

The standard antibiotic agent used for the study; penicillin, inhibited the growth of all the four bacterial strains signifying that the bacteria are not resistant to penicillin antibiotic. It has shown the highest inhibition zones against *E. coli* and *S. aureus*, with diameter of 29mm and 27mm, followed by *S. typhi* (26mm), respectively. The lowest was observed in *P. aeruginosa* with diameter of 24mm.

3.3 The Minimum Inhibitory Concentration (MIC) of *Eucalyptus globules*

Results of MIC were evaluated as the lowest concentration of the extracts at which no visible macroscopic growth or turbidity was observed on the bottom of the test tubes. Therefore, the extraction of *E. globules* using ethanol exhibited the most remarkable antimicrobial activities compared to water extract with MICs of 3.13mg/mL for *S. aureus*, *E. coli* and *S. typhi*. For *P.*

aeruginosa, the MIC was 6.25 mg/mL (Table 3). Whereas, water extract shows 3.13 mg/mL for *S. aureus* and *E. coli*, and 6.25mg/ml for *S. typhi* and *P. aeruginosa* (Table 3).

TABLE 3: Shows Minimum Inhibitory Concentration in (mg/mL) for ethanol and aqueous leaf extracts of *Eucalyptus globules*

Extract Used	**Bacterial Isolate used for the Study**			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeriginosa*
95% Ethanolic Extract	3.13	3.13	3.13	6.25
Aqueous Extract	3.13	3.13	6.25	6.25
Penicillin Agent (μg)	1.56	1.56	1.56	1.56

Note: the lower the **MIC value** the higher the activity of the extract thus, ethanolic extract had better activity against *S. aureus*, *E. coli* and *S. typhi* (3.13 mg/mL) compared to aqueous extract in which the MIC values for *E. coli* and *S. aureus* were 3.13 mg/ml and for *S. typhi* and *P. aeruginosa* were 6.25 mg/ml.

Discussion

The central object of the present study was to evaluate the antibacterial activity of ethanol and aqueous leaf-extracts of *Eucalyptus globules* assayed on the growth of four enteric pathogenic bacteria; representing one Gram-positive and three Gram-negative bacteria by disc diffusion technique. Minimum inhibitory concentration (MIC) and qualitative phytochemical screening were conducted for the wide spectrum highly active antibacterial extracts.

The results obtained in the present study elucidated clearly that; Gram-positive bacterium (*S. aureus*) was more sensitive towards extracts of *E. globules* tested than Gram-negative bacteria (*E. coli, S. typhi* and *P. aeroginosa).* These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances including antimicrobial agents (Burt, 2004). This is similar to have been reported previously by several researchers (Youssef, 1995; Lopez *et al.*, 2005). Various literatures have documented that Gram-negative bacteria were not susceptible to plant extracts when compared to Gram-positive bacteria and this has been attributed to the external lipopolysaccharide (LPS) wall that surrounds the peptidoglycan cell wall of the former (Youssef, 1995; Nostro *et al.*, 2000; Ojala *et al.*, 2000; Lopez *et al.*, 2005).

Moreover, the variety of zones of inhibition appeared on the culture plates implies the changeable degree of efficacy and different phytochemical components of the plant herb on the target bacteria (Saad *et al.*, 2014). The bacterial isolates tested, were all sensitive to both extracts of *E. globulus*; *E. coli, S. aureus, S. typhi* and *P. aeruginosa*, with *S. aureus* being the most susceptible in both extracts (table 2). However, *P. aeruginosa* was completely non-susceptible at 50 mg/ml aqueous extract and this may be influenced by the complexity of its membrane surface as gram negative bacterium, or may be due to effect of low dosage or solvents used during extraction (Akpulu *et al.,* 1994; Teh, 1996).

Qualitative analysis was carried out for screening the presence of major phytochemical components; glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins in *E. globules* extracts. Therefore, the antimicrobial activity exhibited by *E. globules* extracts may be attributed to the presence of these active metabolites in the plant. This agrees with the findings which reported to have said, there is a strong association between the secondary active metabolites and the antibacterial activity of both methanolic and ethanolic extracts of eucalyptus plants (Lewis and Ausubel, 2006; Darogha, 2009; Egwaikhide *et al.,* 2009; Penecilla and Magno, 2011). Thus, plants are rich in a wide variety of secondary metabolites which have been found in-vitro to have antimicrobial properties.

The results of the antimicrobial test obtained have confirmed that the extract obtained from percolation of using ethanol was observed to be more effective than the extract obtained from aqueous solvent. This is likely in light of the fact that, the type of solvent used in the extraction procedure influenced the solubility of the active component of the leaves (Saad *et al.,* 2014; Shanmugam *et al.,* 2014). Thus, ethanol had a high power to extract the active antibacterial compounds in the plant which revealed higher activity with higher zones of inhibition in comparison with aqueous solvent. Moreover, the use of either methanol, ethanol, n-hexane, chloroform, or ether may result in high solubility increase of plant material components in contrast to absolute water solvent alone (Amita and Shalini, 2014). Thus, ethanol may possibly aid in extraction of novel bioactive compounds in plant materials more than aqueous solvent because of polarity difference exhibiting between the solvents (Sultana *et al.,* 2009). Therefore, the yields of extract and consequential antibacterial activities of plant materials are largely dependent on the nature of extraction solvent, due to the presence of different bioactive compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent.

4.0 Conclusion, Recommendations and Future work

Based on the results obtained in this study, all the leaf-extracts of *Eucalyptus globules* have shown to contain different contents of bioactive compounds after phytochemical screening. 95% ethanol is indicated to be a better solvent for bioactive components extraction compared to absolute water. Thus, 95% ethanol was observed to be a better choice for bioactive metabolites

extraction. Also, it has been found that both extracts of *E. globules* leaves possess high potential natural antibacterial activity as they inhibited the growth of enteric bacterial pathogens more effectively (*E. coli, S. aureus, S. typhi* and *P. Aeruginosa*), with ethanolic extract being more potent. In this manner, utilizing *E. globules* leaves may have advantageous well-being impact when used as natural antimicrobials for bacterial disease and infections.

Further demonstration, fractionation and characterization of the activity of *E. globules* should be carried-out to detect possibly the viricidal, nemato-cidal and anti-parasitic activities of the plant. Further suggestion is also made to investigate on the secrets of ethno medicinal herbs flora of Nigeria that have earned creditability in the battle-field of medicine. Moreover, further research on scientific proofs on *safety and toxicity* of the plant are guaranteed. Finally, more efforts on research should be made to increase elucidation of bioactive components of *E. globules* with view to production in a large scale to be used for extraction of useful products in pharmaceutical industries.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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