BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 513-518 | DOI: 10.14421/biomedich.2025.141.513-518 ISSN 2540-9328 (online)

Phytochemical, Antioxidant, and Antibacterial Activities of Stem Bark Fractions of *Eucalyptus globulus* Against Multidrug-Resistant Bacterial Isolates

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Manuscript received: 25 Maret, 2025. Revision accepted: 23 July, 2025. Published: 01 August, 2025.

Abstract

The growing challenge of antimicrobial resistance (AMR) has intensified the need for alternative therapeutic agents, with medicinal plants offering promising solutions due to their bioactive compounds. This study investigated the antimicrobial and antioxidant properties of *Eucalyptus globulus* bark extracts against multidrug-resistant bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Plant samples were collected from Adamawa State Polytechnic, Nigeria, authenticated (voucher ASP-765), and subjected to reflux extraction using hexane and water. Phytochemical analysis revealed alkaloids, phenols, tannins, glycosides, and terpenoids in both methanol and aqueous extracts, while flavonoids and steroids were absent in aqueous extracts, and methanol extracts lacked saponins. Antibacterial activity was assessed through agar well diffusion and broth dilution assays, demonstrating a concentration-dependent effect. Methanol extracts showed greater efficacy against *E. coli* and *P. aeruginosa* (12.8–13.8 mm inhibition zones), while aqueous extracts were most effective against *S. aureus* (19.3 mm at 100 mg/mL). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 25 to 50 mg/mL, with *E. coli* exhibiting the lowest MIC (25 mg/mL), highlighting the extract's antimicrobial potential. The antioxidant activity was evaluated using phosphomolybdate and ferricyanide assays. The methanol extract exhibited strong free radical scavenging activity, with a total antioxidant capacity (TAC) expressed in ascorbic acid equivalents (AAE), although lower than pure ascorbic acid. Statistical validation (one-way ANOVA, $p < 0.05$) confirmed the significance of the results. These findings support the traditional medicinal use of *E. globulus* and its potential for combating antibiotic-resistant infections and oxidative stress-related conditions. Further studies are recommended to isolate bioactive compounds, determine mechanisms of action, and develop optimized therapeutic formulations for AMR management.

Keywords: *Eucalyptus globulus*; antimicrobial resistance; phytochemicals; antioxidant activity; medicinal plants.

INTRODUCTION

Medicinal plants have played a crucial role in treating various diseases and infections for centuries, owing to their natural antibacterial, antifungal, and antiviral properties (Aladejana *et al.,* 2024). Recently, there has been a growing interest in harnessing plant-derived compounds to combat infectious diseases, cancer, and the increasing challenge of antimicrobial resistance (AMR). The widespread use and misuse of antibiotics, coupled with bacteria's innate ability to develop resistance to synthetic drugs, have contributed to the emergence of multidrug-resistant pathogens (Alara & Alara, 2024). This underscores the urgent need for alternative treatments, with plant-based compounds emerging as promising candidates. Many medicinal plants contain bioactive molecules that could serve as the foundation for developing safer and more effective therapeutic agents (Chaachouay & Zidane, 2024). As researchers explore novel antibacterial solutions, these natural resources are increasingly recognized as valuable contributors to innovative drug development to address the global AMR crisis.

Traditional medicine, deeply rooted in cultural practices and economic realities, remains a cornerstone of healthcare in many parts of the world, particularly in Africa (Eruaga *et al.,* 2024). However, despite their widespread use, many of these plants have not undergone rigorous scientific validation. Establishing a comprehensive database documenting their medicinal properties could facilitate their integration into modern healthcare systems, providing cost-effective and safer alternatives to synthetic drugs (Aruwa & Sabiu, 2024). In Nigeria, plants like *Eucalyptus globulus* (Blue gum) have long been used for their antimicrobial, anti-inflammatory, and wound-healing benefits (Sa'id &

Abdullahi, 2022). As part of their natural defense mechanisms, these compounds exhibit diverse pharmacological properties, including antioxidant, anticancer, and antimicrobial activities, reinforcing their significance in the quest for novel therapeutic solutions (Anwar *et al.,* 2025).

The rise of multidrug-resistant bacterial infections presents a major global health challenge intensified by antimicrobial resistance (AMR) and the persistence of bacterial cells that evade antibiotic treatments (Karnwal *et al.,* 2025). These persistent cells contribute to recurrent infections and treatment failures. AMR, often referred to as a silent pandemic, was associated with approximately 4.95 million deaths in 2024 (Aslam *et al.,* 2024). Six major bacterial pathogens—*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*—were responsible for nearly 75% of these cases (Ntim *et al.,* 2025). Recognized by the World Health Organization (WHO) as priority pathogens, these bacteria highlight the urgent need for new antimicrobial strategies (WHO, 2024). In addition to strengthening surveillance systems and promoting responsible antibiotic use, developing novel antimicrobial compounds is crucial to controlling the escalating AMR crisis.

Among these threats, methicillin-resistant *Staphylococcus aureus* (MRSA) stands out as a particularly difficult-to-treat pathogen (Mandal *et al.,* 2024). This Gram-positive bacterium is responsible for a variety of infections and has developed resistance to key antibiotics, including vancomycin, daptomycin, and linezolid (Rajput *et al.,* 2024). MRSA also can form biofilms and persister cells, further increasing its tolerance to conventional treatments (Kaushik *et al.,* 2024). This growing concern has driven interest in exploring natural antimicrobial agents as potential alternatives to conventional disinfectants. Advancing such innovative approaches is essential to tackling resistant infections and alleviating the global health burden posed by AMR.

Antioxidants play a crucial role in maintaining cellular homeostasis by inhibiting or neutralizing the harmful effects of free radicals in the body (Bajaj *et al.,* 2024). Oxidative stress, resulting from an imbalance between antioxidants and reactive oxygen species (ROS), has been implicated in the pathogenesis of various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Muscolo *et al.,* 2024). *Eucalyptus globulus*, are recognized as rich sources of antioxidants, making them promising candidates for therapeutic applications and dietary supplements.

MATERIALS AND METHODS

Collection, Identification, and Processing of Plant Materials

The bark of *Eucalyptus globulus* was collected within the grounds of Adamawa State Polytechnic, Yola, Nigeria. The samples were verified and registered under the voucher number ASP-765 by the Department of Forestry Technology at the same institution.

Extraction of Crude Extract of *Eucalyptus globulus*

The crude extract was obtained using the reflux extraction method, following the procedure outlined by Ewansiha et al. (2020). Normal hexane and water were used as extraction solvents. A total of 100 g of finely ground, dried plant material was dissolved in 400 ml of the respective solvents. After refluxing, the mixtures were filtered through filter paper to obtain a clear filtrate. The filtrate was then concentrated to a semi-solid form using a Rotary Evaporator and further dried with a water bath to yield the crude extract.

Bioassay Studies

Test Isolates

The multidrug-resistant clinical isolates used in this study were obtained from the microbial culture bank of Modibbo Adama Teaching Hospital in Yola, Nigeria. These isolates included *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Their identities were confirmed using standard biochemical tests, following the classification guidelines outlined in Bergey's Manual of Systematics of Archaea and Bacteria (Abaka *et al.,* 2024).

Preparation of Stock Solution of Extract

The stock solution for each extract was prepared following the method of Habibu et al. (2021), with slight modifications. Specifically, 0.4 g of each extract was dissolved in 2 mL of 20% DMSO to obtain a final concentration of 200 mg/mL. This stock solution was then serially diluted to generate working concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL.

Inoculum Standardization

The direct colony suspension method was utilized, in which 24-hour-old colonies of each test isolate were suspended in 2 mL of sterile normal saline. The turbidity was then adjusted to correspond with the 0.5 McFarland Standard.

Antibacterial Susceptibility Test of the Crude Extract

A sterile cork borer (6 mm in diameter) was used to create wells in the culture medium. Subsequently, 100 µL (0.1 mL) of the extracts at concentrations of 40 mg/mL and 50 mg/mL, along with the positive control (30 µg/mL doxycycline) and the negative/solvent control (dimethyl sulfoxide, DMSO), were introduced into the wells. The plates were left undisturbed on the bench for

approximately 30 minutes to allow proper diffusion of the extracts into the medium. Incubation was carried out at 37°C for 18 to 24 hours. Following incubation, the culture plates were examined for the presence of clear zones around the wells, indicating antibacterial activity. The zone of inhibition (ZOI) was measured in millimeters. All tests were performed in triplicate (Ewansiha, 2020).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) was determined using the tube dilution method, while the Minimum Bactericidal Concentration (MBC) was assessed following the procedure described by Saleh et al. (2024). For MIC testing, serial two-fold dilutions of the plant extracts were prepared in Nutrient broth. An initial 1:1 mixture was made by combining 1 mL of Nutrient broth with 1 mL of the 100 mg/mL extract solution, serving as the reference standard. Subsequent dilutions produced concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL. Each dilution tube was inoculated with 0.1 mL of a standardized microbial suspension and incubated at 37°C for 24 hours. The MIC was identified as the lowest extract concentration that completely inhibited visible bacterial growth.

For MBC determination, aliquots from MIC tubes showing no bacterial growth were transferred onto fresh Nutrient agar plates and incubated for another 24 hours. The MBC was defined as the lowest extract concentration that resulted in no bacterial colony formation on the agar plates.

Antioxidants Activity

Total Antioxidant Capacity

The total antioxidant capacity (TAC) of the extract was evaluated following the method described by Dahiru et al. (2024). A 0.5 mL aliquot of the sample, dissolved in distilled water at a concentration of 300 µg/mL, was combined with 2 mL of phosphomolybdate reagent in a capped tube and incubated at 95°C for 10 minutes. The absorbance of the sample was then measured at 695 nm using a UV-Vis spectrophotometer (Model V1000) against a blank solution consisting of phosphomolybdate reagent and distilled water, which underwent the same treatment as the sample. Additionally, ascorbic acid (AA) at varying concentrations (20–100 µg/mL) was used to generate a calibration curve. The TAC was expressed as ascorbic acid equivalent (AAE) in µg/mL based on triplicate determinations.

Reducing Power Assay

The reducing power of the extract was assessed following the method of Dahiru et al. (2024). (1986). A 0.75 mL aliquot of the extract at varying concentrations was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v). The mixture was then incubated in a water bath at 50°C for 20 minutes. The reaction was halted by adding 0.75 mL of 10% trichloroacetic acid (TCA), followed by centrifugation at 800 g for 10 minutes. A 1.5 mL portion of the supernatant was combined with 1.5 mL of distilled water and 0.1 mL of ferric chloride solution (0.1%, w/v) and allowed to react for 10 minutes. The reducing power of the extract was expressed as an equivalent of ascorbic acid (Dahiru *et al.,* 2024).

Statistical Analysis

Data were presented as the mean ± standard error of the mean (SEM) from three independent experiments. For in vitro antioxidant assays, one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$) was used to compare differences among the various fractions across different antioxidant assays. A probability value of $P < 0.05$ was considered statistically significant.

RESULTS

Table 1. Results of phytochemical screening of methanol, and aqueous extracts of *E. globulus* bark.

S/N	Name of the phytochemical	Presence (+) and absence (-) in different extracts	
		Methanol extract	Aqueous extract
1	Alkaloids	+	+
2	Flavonoids	+	-
3	Phenols	+	+
4	Tannins	+	+
5	Cardiac Glycosides	+	+
6	Steroids	+	-
7	Saponins	-	+
8	Terpenoids	+	+

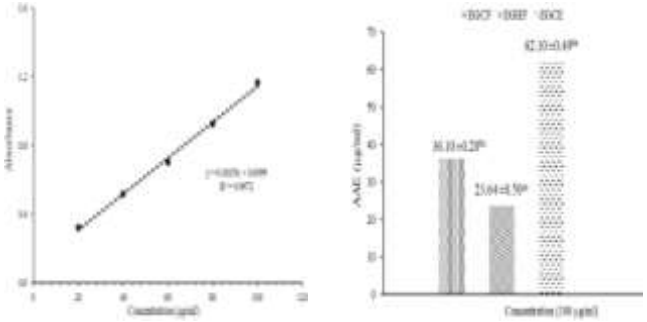

Figure 1. Total antioxidant capacity; a) Ascorbic acid calibration curve and b) AAE total antioxidant capacity. Value with [a] superscript is significantly ($p < 0.05$) lower than EGHF and EGCE. Value with [b]and [c] superscripts is significantly ($p < 0.05$) higher than EGHF and EGCF, respectively while values with superscripts are significantly ($p < 0.05$) lower than EGCE.

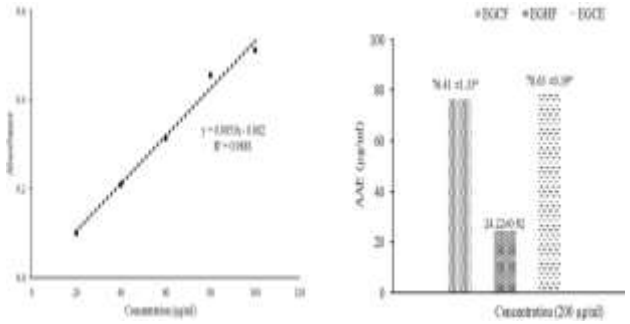

Figure 2 presents the total antioxidant capacity (TAC) Total reducing power; a) Ascorbic acid calibration curve and b) AAE total reducing power. Values with [a] superscript are significantly ($p < 0.05$) higher than EGHF.

Table 2. Zone of inhibition (mm) of the organisms caused by Aqueous and Methanol extracts of *E. globulus.*

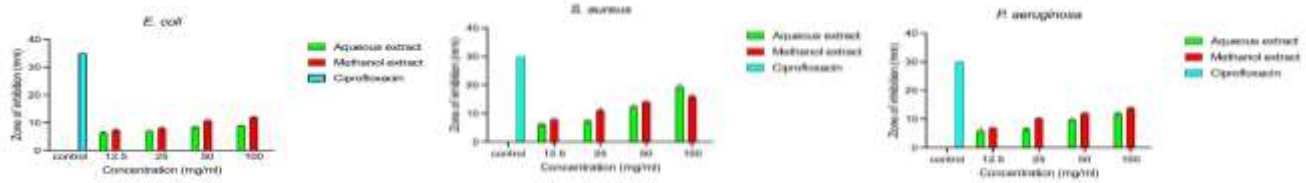

Table 3. Minimum inhibitory concentration and minimum bactericidal concentration of methanol bark extracts of *E. globulus* against test organisms.

Test organism	Incubation Time (h)	Leaf extract concentration (mg/ml					Remark
MIC		**100**	**50**	**25**	**12.5**	**6.25**	
E. coli	24	+	+	–*	–	-	25
S. aureus	24	+	–*	–	–	–	50
P. aeruginosa	24	+	–*	–	–	-	50
MBC							
E. coli	24	+	-	-*	-	-	25
S. aureus	24	+	+	-*	-	-	25
P. aeruginosa	24	+	-*	-	-	-	50

DISCUSSIONS

The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for health maintenance has been broadly observed (Hlatshwayo *et al.,* 2025). Modern-day pharmacopeia, however, contains at least 25% of drugs derived from plants and many other synthetic analogs, built on prototype chemical substances isolated from plants (Rahman *et al.,* 2024). Involvement in medicinal plants as re-budding health assistance has been fueled by the rising charges of prescription drugs in safeguarding personalized health and well-being and the bioprospecting of new plant-derived drugs (Buragohain *et al.,* 2024).

The aqueous and methanol extracts of *E. globulus* bark shared common phytonutrients like alkaloids, phenols, tannins, glycosides, and terpenoids, aligning with Saleh et al. (2024). However, saponins were absent in the methanol extract, while flavonoids and steroids were absent in the aqueous extract. Differences from Ewansiha et al. (2024) may be due to variations in extraction conditions and plant origin.

The antibacterial assay varied greatly in terms of inhibitory potential. Table 2 shows the antibacterial activity of four *E. globulus* doses against *S. aureus, E. coli,* and *P. aeruginosa*. Methanol extract (AE) of *E. globulus* seeds had the highest activity against*, E. coli,* and *P. aeruginosa*, with inhibition zones measuring 12.8 mm and 13.8 mm at the concentration of 100 mg/ml. The aqueous extract (ME) demonstrated marginally higher effectiveness against *S. aureus* showcasing inhibition zone diameters of 19.3 mm at 100 mg/ml. The lowest activity was recorded for the aqueous extract for the three bacteria isolates. The findings indicate that the methanol extract of *E. globulus* inhibited bacterial growth more effectively than the aqueous extract. This result is in tandem with that obtained by Saleh et al. (2024). Isyaka et al. (2024) reported that *E. globulus* leaves exhibit a zone of inhibition against three of the studied species *E. coli*, *S. aureus*, and *S. typhi* at all concentrations. With a

zone of inhibition of 17.7 mm, the extract was most effective against *S. aureus*. Differences in bacterial targets and inhibition zones likely result from variations in plant species, extraction methods, and experimental conditions.

The lowest MIC/MBC recorded in this study was 25 mg/mL and 25 mg/mL against *Escherichia coli* the lower the MIC and MBC, the more potent and effective the antimicrobial agent is against the tested microorganism as reported by Ewansiha et al. (2024).

The findings of this study highlight the significant antioxidant activity of the methanol fraction of *Eucalyptus globulus* stem bark, reinforcing its traditional medicinal applications. The assessment of total antioxidant capacity (TAC) and reducing power confirmed its notable free radical scavenging ability, although it was generally less effective than ascorbic acid, a well-established antioxidant standard. These results indicate that while the methanol fraction exhibits strong antioxidant potential, its efficacy may be enhanced when combined with other bioactive fractions or complementary compounds with synergistic effects.

The potent antioxidant activity observed in the methanol extract is likely due to the presence of lipophilic phytochemicals, such as terpenoids and phenolic compounds, which are efficiently extracted by methanol. These compounds are widely recognized for their free radical scavenging properties and may account for the observed bioactivity. The crude extract also displayed antioxidant activity, though with varying effectiveness across different assays, possibly due to its broader chemical diversity. This mixture of polar and non-polar compounds may enable the crude extract to neutralize a wider range of oxidative species. However, the methanol fraction, being more concentrated in specific active compounds, demonstrated superior antioxidant activity, suggesting that targeted extraction methods may optimize its therapeutic potential.

CONCLUSION

This study reveals that aqueous and methanol extracts from *E. globulus* bark possess notable antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. These findings highlight the potential of *E. globulus* as a valuable natural antibacterial agent for medicinal and therapeutic applications.

Acknowledgments: Special gratitude goes to the Department of Science Laboratory Technology, Adamawa State Polytechnic Yola.

Authors Contributions: For Example, Abdulazeez Mumsiri Abaka & Nazuwa Dominic designed the study. Abdulazeez Mumsiri Abaka, Alex Yeri Emmanuel & Zayyad Dahiru Aliyu carried out data collection and laboratory work. Abdulazeez Mumsiri Abaka, Alex Yeri Emmanuel & Zayyad Dahiru Aliyu wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are competing interests.

Funding: No funding.

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