OPEN ACCESS
Global Journal of Research in Medical Sciences
ISSN: 2583-3960 (Online)
Volume 05 | Issue 04 | July-Aug. | 2025
Journal homepage: https://gjrpublication.com/gjrms/
Original Research Article

Antibacterial and antioxidant potential of *Eucalyptus globulus* against biofilm-forming bacteria

*Abdulazeez Mumsiri Abaka [1], Musa Hammandama Belel [2], Mohammed Badamasi Dangana [3], Keta Biman Abubakar [4], Fadimatu Adamu Dahiru [5]

[1,2,3,4,5] Science Laboratory Technology Department, School of Science and Technology, Adamawa State Polytechnic, Yola.
[1]Orcid ID: 0000-0002-6160-7426
[2]Orcid ID: 0009-0000-9795-7862
[3]Orcid ID: 0009-0000-4669-2910
[4]Orcid ID: 0009-0007-8372-6964
[5]Orcid ID: 0009-0008-0378-7992

DOI: 10.5281/zenodo.16782642 **Submission Date: 05 July 2025 | Published Date: 09 Aug. 2025**

Corresponding author: Abdulazeez Mumsiri Abaka
Science Laboratory Technology Department, School of Science and Technology, Adamawa State Polytechnic, Yola.
Orcid ID: 0000-0002-6160-7426

Abstract

Eucalyptus globulus, a versatile evergreen native to Africa and Asia, has been globally recognized for its medicinal properties, mainly because of its rich phytochemical content, including eucalyptol, flavonoids, and terpenoids. This study examined the antibacterial and antioxidant potential of E. globulus leaf extracts against biofilm-forming pathogens (Staphylococcus aureus, Escherichia coli, and Salmonella typhi), which are becoming increasingly resistant to standard antibiotics. Leaves were collected, authenticated (voucher ASP-1725), and extracted using reflux extraction with N-hexane and water-based solvents. Phytochemical screening identified alkaloids, flavonoids, phenols, tannins, and terpenoids in the N-hexane extract; meanwhile, the aqueous extract contained phenols, tannins, steroids, and saponins. Antibacterial activity was tested through agar well diffusion and broth dilution methods. Both extracts showed concentration-dependent inhibition, with the aqueous extract being more effective against S. aureus (15.03 ± 0.38 mm at 100 mg/mL) and S. typhi (MIC: 12.5 mg/mL). The N-hexane extract was more effective against E. coli (14.07 ± 0.22 mm at 100 mg/mL), although amoxicillin controls displayed significantly higher activity. Minimum bactericidal concentration (MBC) values ranged from 12.5 to 50 mg/mL, with S. aureus being the most susceptible. Antioxidant tests revealed significant activity, with the aqueous extract exhibiting the highest ascorbic acid equivalent (AAE) capacity (78.63 ± 0.19 µg/mL), which is attributed to its high phenolic and flavonoid levels. These results support traditional uses of E. globulus and suggest its potential as a supplementary antimicrobial agent in the face of rising antibiotic resistance. The study emphasizes the importance of solvent choice in phytochemical extraction, with water-based extracts showing greater effectiveness against Gram-negative bacteria. Further research is needed to isolate active compounds and explore possible synergistic effects with standard antibiotics.

Keywords: *Eucalyptus globulus, antimicrobial resistance, biofilm, phytochemicals, antioxidant, solvent extraction.*

Introduction

Eucalyptus globulus, a versatile evergreen native to Australia and Tasmania, belongs to the Myrtaceae family and has been valued for thousands of years for its wide-ranging therapeutic and practical uses (Malakar, 2024). Family members are rich sources of polyphenols and terpenoids, with eucalyptol or cineol as their primary component (Moges *et al.*, 2024). Now cultivated worldwide, especially in Africa and the Americas, E. globulus displays significant botanical and

chemical diversity (Mendel *et al.,* 2025). It is known by various common names such as "Tasmanian Blue Gum," "Fever Tree," "mkaratusi" (Swahili), and "zaiti" (Hausa), highlighting its cultural importance globally (Ewansiha *et al.,* 2024). The plant's value stems not only from its adaptability and height, which can reach up to 70 meters, but also from its rich phytochemical makeup (Shala *et al.,* 2021). Different parts of the tree, leaves, buds, fruits, and branches, produce essential oils containing biologically active compounds like 1,8-cineole (eucalyptol), α-pinene, spathulenol, and aromadendrene (Shiekh *et al.,* 2025).

More broadly, *E. globulus* exemplifies the profound and enduring role of medicinal plants in human health. From ancient to contemporary healing practices, plants rich in flavonoids, phenols, terpenoids, and alkaloids have treated a variety of conditions (Barman and Biswas, 2025). As science continues to validate these traditional uses, medicinal plants have gained renewed attention in drug discovery and integrative healthcare (Balkrishna *et al.,* 2024). *Eucalyptus globulus* underscores how biodiversity, ancestral knowledge, and modern science intersect, offering sustainable, effective solutions for current and future health challenges (Dean, 2024).

The rapid and widespread emergence of antimicrobial resistance (AMR) poses a critical threat to global public health, primarily driven by the mechanism of antibiotic selective pressure (Salam *et al.,* 2023). This process eliminates susceptible bacteria while allowing resistant strains to survive and multiply, resulting in superinfections that are harder to treat, costlier, and associated with increased hospital stays and mortality (Ahmed *et al.,* 2023). *Staphylococcus aureus* exemplifies the challenge, as it has developed resistance to multiple antibiotics and is implicated in hospital-acquired, community-associated, and veterinary infections (Bale, 2021). Its remarkable ability to form biofilms, structured microbial communities encased in a self-produced matrix, further enhances its resilience (Almatroudi, 2025). Biofilm formation shields bacteria from host defenses and antimicrobial agents, allowing infections to persist and recur, thereby significantly complicating treatment efforts (Sharma *et al.,* 2023).

The growing inefficacy of antibiotics raises the possibility of entering a post-antibiotic era, where once-manageable infections could become life-threatening (Chandrakar and Thakur, 2024). This presents a global security concern, with implications for pandemics and bioterrorism. AMR currently causes an estimated 700,000 deaths annually and could rise to 10 million by 2050 without urgent intervention (Michaud *et al.,* 2024). Developing countries, where infectious diseases are already a major burden, are particularly vulnerable. Biofilms play a crucial role in AMR, serving as both a physical barrier and a genetic exchange platform for resistance traits (Almatroudi, 2025). Composed of polysaccharides, proteins, and other organic materials, biofilms enable bacterial survival under harsh conditions and promote the spread of resistance genes within the microbial community (Singh *et al.,* 2023). Bacterial genera such as *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Pasteurella*, *Bacillus*, and *Salmonella* exploit this strategy, making biofilm-associated infections notoriously difficult to eradicate (Araújo *et al.,* 2024). Alarmingly, biofilms are implicated in over 60% of microbial infections and are responsible for nearly two-thirds of all human bacterial infections (Datta *et al.,* 2024).

Materials and Methods

Collection and Preparation of Plant Samples

Leaves of Eucalyptus globulus were collected from the Adamawa State Polytechnic in Yola. Expert verification in the Department of Science Laboratory confirmed the plant's identity, with a plant scientist officially authenticating the species and assigning a voucher number ASP-1725. After collection, the leaves were examined and prepared for analysis. They were dried under ambient conditions with good ventilation, avoiding direct sunlight to preserve active compounds. Once dried, the leaves were ground with a mortar and pestle and then sieved to produce a fine powder. This powder was kept in airtight, light-resistant glass containers at room temperature until extraction and further tests.

Extraction of Crude Compounds from *Eucalyptus globulus*

Crude extracts were prepared using the reflux extraction method, in line with the protocol described by Abaka et al. (2024). Solvents used included ethanol and distilled water (aqueous phase). A total of 100 grams of the dried plant powder was mixed with 400 mL of each solvent and subjected to reflux extraction. The resultant mixtures were filtered through Whatman filter paper to obtain clear filtrates. The filtrates were then concentrated using a rotary evaporator and further dried on a water bath to yield semi-solid crude extracts.

Preparation of Stock Solutions for Antibacterial Testing

Measured quantities of the crude extracts, 200 mg and 250 mg, were dissolved in 5 mL of 20% dimethyl sulfoxide (DMSO) to prepare working concentrations of 40 mg/mL and 50 mg/mL, respectively, as described by Ewansiha et al. (2024).

Phytochemical Screening

Qualitative analysis was conducted on the crude extracts to determine the presence of various secondary metabolites. The targeted phytochemicals included alkaloids, steroids, tannins, flavonoids, saponins, terpenoids, and phenolic compounds. The standard phytochemical procedures outlined by Abaka et al. (2024) were followed for this analysis.

Source and Isolation of Microorganisms

The biofilm-producing bacterial strains, including *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi,* were obtained from the Department of Microbiology at Modibbo Adama University Teaching Hospital, Yola. Inocula were prepared by transferring 3 to 5 colonies of each bacterial strain into 5 milliliters of nutrient broth. The inoculated broths were then incubated at 35 ºC for 2-3 hours until reaching the logarithmic growth phase. Subsequently, the bacterial suspensions were adjusted to match the 0.5 McFarland Standard for susceptibility testing, following the guidelines outlined by the National Committee for Clinical Laboratory Standards (NCCLS, 1993).

Identification and Confirmation of Microbial Isolates

The collected samples were cultured on freshly prepared nutrient agar and incubated at 37°C for 24 hours. After incubation, the morphology of the bacterial colonies was assessed. Identification was carried out using Gram staining and biochemical assays, following methods outlined by Abaka et al. (2024). The organisms identified included *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus.*

Antibacterial Susceptibility Testing

The isolated bacterial strains were tested for susceptibility to standard antibiotics and confirmed using cultural and biochemical identification techniques as recommended by Nkang (2009). The strains were also assessed for contamination, and pure cultures were stored on nutrient agar slants at 4°C for subsequent use.

Standardization of Inoculum

A loopful of each test organism was transferred from nutrient agar slants into nutrient broth and incubated at 37°C for 24 hours. The microbial suspensions were then adjusted with normal saline to match the turbidity of the 0.5 McFarland standard, representing approximately 1.0×10^8 CFU/mL. This standard was prepared by combining 0.5 mL of 1% H_2SO_4 with 99.5 mL of 1% $BaCl_2$, as described by Ewansiha et al. (2024).

Antibacterial Susceptibility Testing of the Crude Extract

To evaluate the antibacterial activity of the crude extracts, wells were created on the agar plates using a sterile cork borer measuring 6 mm in diameter. Each well was filled with 100 µL of the prepared extract concentrations (40 mg/mL and 50 mg/mL). A positive control containing 30 µg/mL of amoxicillin and a negative control consisting of 20% dimethyl sulfoxide (DMSO) were also included. The plates were left undisturbed on the bench for approximately 30 minutes to facilitate diffusion of the extracts through the agar medium. Subsequently, all plates were incubated at 37°C for 18 to 24 hours. After incubation, zones of inhibition (ZOI), indicating an antibacterial effect, were visually identified as clear areas surrounding the wells. These zones were measured in millimeters (mm) to assess the efficacy of each extract. The entire procedure was replicated three times to ensure accuracy and reproducibility (Ewansiha, 2024).

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

The broth dilution assay, as outlined by Dahiru *et al.* (2024), was used to test the extracts. The extracts were diluted to 10-fold concentrations in the nutrient broth. To each dilution, 0.1 milliliters of standardized bacterial inoculum was added. Negative control tubes devoid of bacterial inoculation were prepared concurrently. The tubes were then aerobically incubated at 37 ºC for 24 h. The MIC was identified as the lowest concentration of the extract that hindered the growth of the test bacterium. To determine the MBC, a loopful from each tube with no visible growth in the MIC assay was transferred onto fresh nutrient agar plates (Oxoid). These plates were then incubated at 37°C for 24 h, followed by observation and recording of any growth.

Antioxidant Assay

Total antioxidant capacity

The antioxidant capacity of the plant extract was assessed following the method outlined by Abdullahi et al. (2024). A 0.1 ml portion of the sample solution, equivalent to 100 mg, was mixed with 1 ml of a reagent solution containing 0.6 M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate. Methanol was used as a substitute for the sample in the blank. The tubes were sealed and heated in a boiling water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of each aqueous solution was measured at 695 nM.

Total reducing Power

The reducing power was assessed following the protocol described by Akinmoladun et al. (2010). Different concentrations of the extract (0.75ml) were mixed with phosphate buffer (0.2M, pH 6.6, 0.75 ml) and potassium hexacyanoferrate (K3Fe (CN)6) (1%, w/v, 0.75 ml). The mixture was then incubated at 50 °C for 20 minutes in a water bath. The reaction was halted by adding trichloroacetic acid (TCA)solution (10%, 0.75 ml) and subsequently centrifuged at 800 g for 10 minutes. The supernatant (1.5 ml) was combined with distilled water (1.5 ml) and ferric chloride solution (0.1%, w/v, 0.1 ml), and left to stand for 10 minutes. The absorbance of the reaction mixture was measured at 700 nm to determine its reducing power. Higher absorbance values indicate greater reducing power, expressed in terms equivalent to ascorbic acid.

Statistical Analysis

Data was expressed as the mean standard error of the mean from three separate observations. For in vitro antioxidant assays one way ANOVA test followed by Tukey's test ($P < 0.05$) was used to analyze the differences among various fractions for different antioxidant assays. A probability of $P < 0.05$ was considered significant.

Results

Table 1. Phytochemical screening results for N-hexane and aqueous extracts of *E. globulus*

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

Key: + Positive -Negative

Table 2. Zones of Inhibition (mm) of *E. globulus* N-hexane Extract

Isolate	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Control (Amox)†
S. aureus	11.67 ± 0.33	8.50 ± 0.21	6.87 ± 0.12	3.87 ± 0.18	0.00 ± 0.00	40.00 (NA)
S. typhi	12.60 ± 0.25	10.20 ± 0.12	7.97 ± 0.12	5.17 ± 0.03	0.00 ± 0.00	38.00 (NA)
E. coli	14.07 ± 0.22	12.10 ± 0.26	9.57 ± 0.12	5.53 ± 0.18	0.00 ± 0.00	35.00 (NA)

Table 3. Zones of Inhibition (mm) of *E. globulus* Aqueous Extract

Isolate	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Control (Amox)†
S. aureus	15.03 ± 0.38	12.37 ± 0.32	8.87 ± 0.50	3.90 ± 0.15	0.00 ± 0.00	40.00 (NA)
S. typhi	10.50 ± 0.35	8.77 ± 0.18	4.80 ± 0.35	2.57 ± 0.09	0.00 ± 0.00	38.00 (NA)
E. coli	13.73 ± 0.46	9.77 ± 0.42	7.23 ± 0.22	3.27 ± 0.41	0.00 ± 0.00	38.00 (NA)

Table 4: Minimum Inhibitory Concentration (MIC) of *E. globulus* extracts against *S. aureus*, *E. coli*, and *S. typhi*

Organisms	Extract	Concentration (mg/ml)					
		100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml
S. aureus	Aqueous	_	_	_	_*	+	+
	N-hexane	_	_*	+	+	+	+
S. typhi	Aqueous	_	_	_	_*	+	+
	N-hexane	_	_	_*	+	+	+
E. coli	Aqueous	_	_	_*	+	+	+
	N-hexane	_	_*	+	+	+	+

Key: * = MIC value + = turbidity - = no turbidity

Table 5. Minimum Bactericidal Concentration of *E. globulus* extracts on test isolates.

Test organisms	Aqueous extract (mg/ml)	N-hexane extract (mg/ml)
S. aureus	25	25
S. typhi	12.5	25
E. coli	25	50

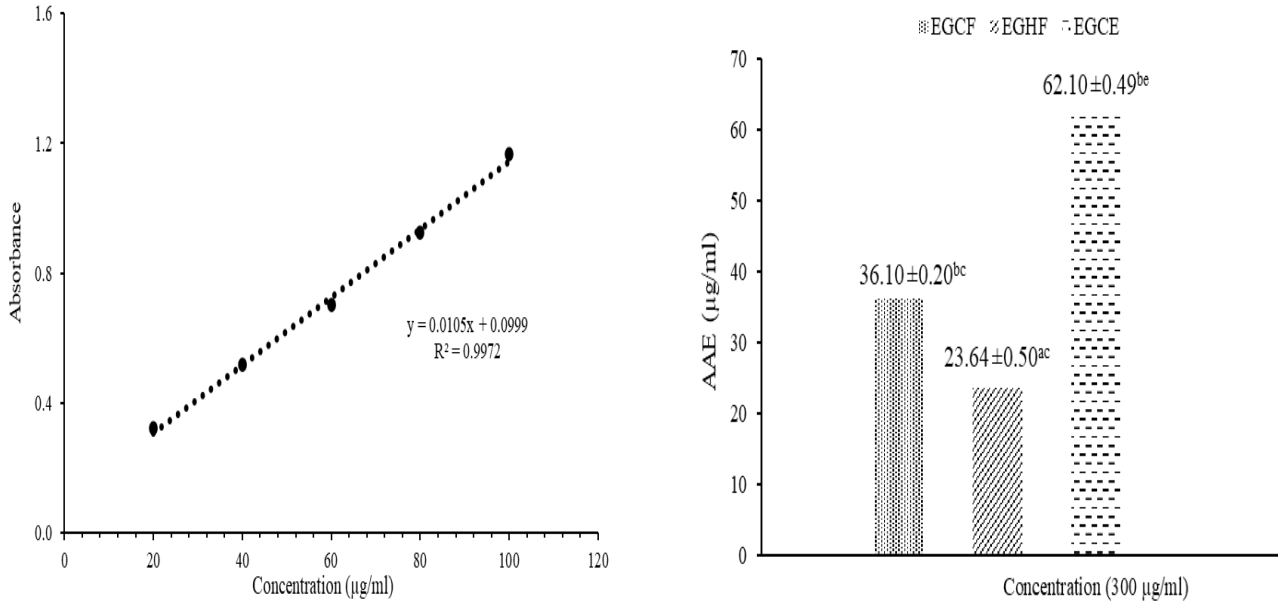

Fig. 1. Total antioxidant capacity of *E. globulus*

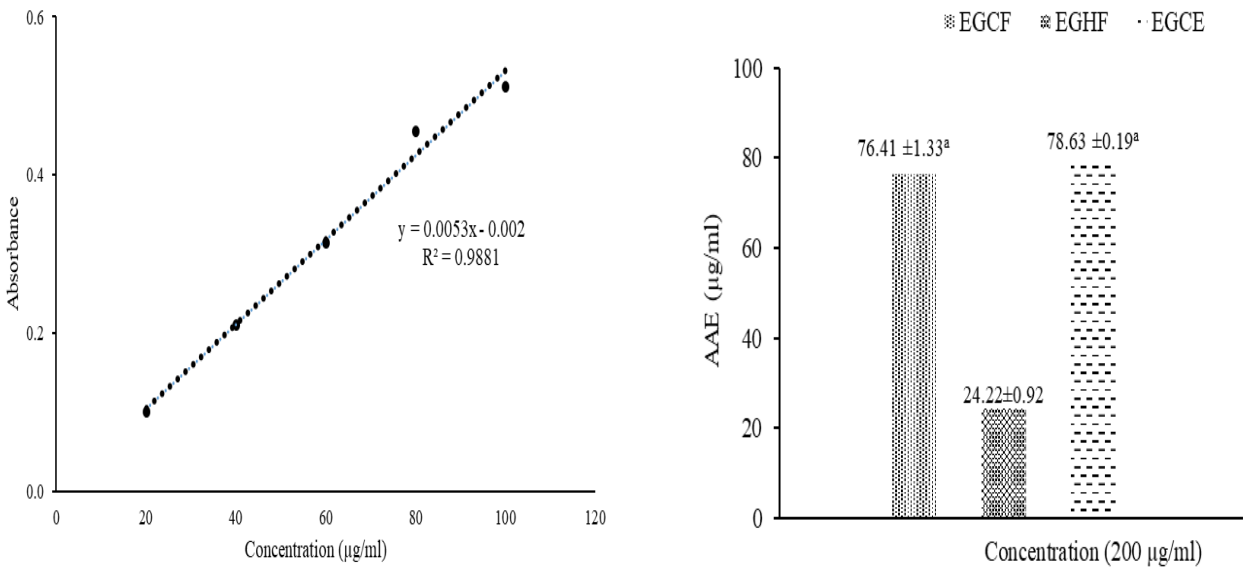

Figure 2. a) The calibration curve for ascorbic acid, and b) the total reducing power of AAE, with values marked by superscripts indicating significantly higher activity ($p < 0.05$) compared to EGHF.

Discussion

Table 1 shows the result of the phytochemical analysis of *Eucalyptus globulus* extracts, revealing that the N-hexane extract contained a wider range of phytochemicals compared to the aqueous extract. Alkaloids, flavonoids, phenols, tannins, cardiac glycosides, steroids, and terpenoids were all present in the N-hexane extract, while only phenols, tannins, steroids, and saponins were detected in the aqueous extract. Notably, saponins were exclusively found in the aqueous extract. In contrast, alkaloids, flavonoids, cardiac glycosides, and terpenoids were absent from it, indicating that solvent polarity plays a significant role in phytochemical extraction.

Phytochemical profiling of *Eucalyptus globulus* extracts demonstrated a distinct solvent-dependent variation in compound composition, with the N-hexane extract containing a broader spectrum of secondary metabolites compared to the aqueous extract. Alkaloids, flavonoids, phenols, tannins, cardiac glycosides, steroids, and terpenoids were present in the N-hexane fraction, whereas only phenols, tannins, steroids, and saponins were identified in the aqueous extract. This pattern suggests that non-polar solvents like N-hexane efficiently extract lipophilic compounds such as alkaloids, flavonoids, cardiac glycosides, and terpenoids. In contrast, polar solvents such as water favor the solubilization of more hydrophilic components like saponins. Similar solvent-selective extraction trends have been reported in Nigerian studies involving medicinal plants, including those by Saber et al. (2024) and Ewansiha et al. (2024), reinforcing the role of solvent polarity in determining phytochemical profiles.

The exclusive presence of saponins in the aqueous extract and the absence of other metabolites such as alkaloids and flavonoids highlight the influence of solvent affinity and phytochemical solubility. Previous research by Bachir and Benali (2012) on *E. globulus* leaves corroborated these findings, revealing that saponins and tannins are preferentially extracted in aqueous solutions, while terpenoids and glycosides are more abundant in organic solvents. Such differences in phytochemical composition may also stem from ecological and environmental factors, including climate, soil type, and maturity of plant material at harvest, which are known to affect secondary metabolite biosynthesis. Understanding these variations is critical in pharmacogenetic applications, as the therapeutic potential and bioactivity of *E. globulus* extracts are closely tied to their constituent phytochemicals and the method of extraction employed.

Table 2 presents the antibacterial activity of *E. globulus* N-hexane extract against *S. aureus*, *S. typhi*, and *E. coli* at various concentrations, compared to amoxicillin as a control. A concentration-dependent inhibitory effect was observed, with *E. coli* showing the highest susceptibility (14.07 ± 0.22 mm at 100 mg/mL), followed by *S. typhi* (12.60 ± 0.25 mm) and *S. aureus* (11.67 ± 0.33 mm). No inhibition was recorded at 6.25 mg/mL for any isolate, while amoxicillin exhibited significantly higher activity against all isolates, indicating the extract's limited but notable antibacterial potential at higher doses.

The findings from Table 2 demonstrate that the N-hexane extract of *Eucalyptus globulus* exhibits a concentration-dependent antibacterial effect against all tested organisms. However, its activity remains modest when compared to amoxicillin. The highest susceptibility was observed in *Escherichia coli*, which contrasts with the typical resistance

patterns seen in Gram-negative bacteria due to their outer membrane barriers (Adegbite and Kolapo, 2021). This suggests that specific non-polar phytoconstituents in the *E. globulus* extract may be able to penetrate or disrupt the bacterial envelope at higher concentrations. *Salmonella typhi* and *Staphylococcus aureus* also showed moderate inhibition, although the effect was reduced relative to *E. coli*, with *S. aureus* demonstrating the least sensitivity. These observations differ slightly from those of Nwinyi et al. (2019), who found *S. aureus* to be highly susceptible to *E. globulus* essential oils, potentially reflecting variations in extraction methods, solvent specificity, or bacterial strain resistance profiles. The absence of activity at 6.25 mg/mL across all isolates highlights the extract's limited potency at low doses and reinforces the importance of concentration in achieving therapeutic relevance. While amoxicillin outperformed the extract significantly, the presence of measurable inhibition zones at higher concentrations suggests that the n-hexane extract of *E. globulus* contains bioactive compounds worthy of further investigation, particularly for use in developing adjunct or alternative antimicrobial therapies in the face of rising antibiotic resistance.

Table 3 shows the result for the antimicrobial activity of aqueous *E. globulus* extract was evaluated against three clinical bacterial isolates, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*, at five concentrations (100, 50, 25, 12.5, and 6.25 mg/mL). The extract exhibited concentration-dependent inhibition across all tested organisms, with the highest zones of inhibition recorded at 100 mg/mL. *S. aureus* showed the highest susceptibility, with a zone of inhibition of 15.03 ± 0.38 mm at 100 mg/mL, followed by *E. coli* (13.73 ± 0.46 mm) and *S. typhi* (10.50 ± 0.35 mm). At 6.25 mg/mL, no detectable inhibition was observed in any isolate, indicating the lower threshold of antimicrobial efficacy for the extract. Comparatively, the standard antibiotic (Amoxicillin) produced significantly higher zones of inhibition, 40 mm for *S. aureus* and 38 mm for both *S. typhi* and *E. coli*.

Table 3 reveals that the aqueous extract of *Eucalyptus globulus* exhibited concentration-dependent antibacterial activity against all three tested clinical isolates: *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*. The extract was most effective at the highest concentration (100 mg/mL), with *S. aureus* showing the greatest susceptibility (15.03 ± 0.38 mm), followed by *E. coli* (13.73 ± 0.46 mm), and *S. typhi* (10.50 ± 0.35 mm). These results suggest that water-soluble phytochemicals, such as flavonoids, tannins, and phenolic compounds, may be responsible for the observed antimicrobial effects—an assertion supported by the work of Eze et al. (2020), who reported strong antibacterial activity from aqueous extracts of *Eucalyptus* species due to their rich polyphenolic profile. The lack of inhibition at 6.25 mg/mL across all isolates indicates a clear threshold below which the extract loses its efficacy, reinforcing the need for adequate dosing in any future therapeutic applications. Interestingly, the Gram-positive *S. aureus* was more susceptible than the Gram-negative strains, a pattern that aligns with previous findings by Oladunmoye and Kehinde (2018), which attributed this to the more accessible peptidoglycan layer of Gram-positive bacteria. Despite these promising findings, the zones of inhibition produced by the aqueous extract were significantly smaller than those of amoxicillin (40 mm for *S. aureus* and 38 mm for both *S. typhi* and *E. coli*), highlighting the extract's relatively moderate potency. Nevertheless, the consistent antibacterial activity at higher concentrations suggests that aqueous *E. globulus* extract holds potential as a complementary or supportive agent in antimicrobial therapy, particularly in light of growing antibiotic resistance and the global push for plant-based alternatives.

Table 4 shows that both the aqueous and N-hexane extracts demonstrated inhibitory activity against *Staphylococcus aureus* at a minimum concentration of 12.5 mg/ml, with the n-hexane extract maintaining its effectiveness even at lower concentrations, indicating greater potency. In the case of *Salmonella typhi*, the aqueous extract was active from 12.5 mg/ml, whereas the n-hexane extract required a higher concentration of 25 mg/ml to exhibit similar inhibition. *Escherichia coli* appeared to be the most resistant among the tested organisms, as inhibition was observed only at 25 mg/ml with the aqueous extract and at 50 mg/ml with the n-hexane extract.

The antibacterial performance of both aqueous and n-hexane extracts of *Eucalyptus globulus* observed in this study underscores the plant's broad-spectrum activity, with *Staphylococcus aureus* being the most susceptible organism. The inhibitory effect of both extracts at 12.5 mg/ml against *S. aureus*, with sustained activity of the n-hexane extract at even lower concentrations, highlights its potent lipophilic constituents, likely terpenoids and essential oils. This observation aligns with the findings of Nwinyi et al. (2019), who reported strong antibacterial activity of *E. globulus* essential oils against *S. aureus*, particularly in non-polar solvents. For *Salmonella typhi*, the superior activity of the aqueous extract from 12.5 mg/ml supports the results of Olorunfemi et al. (2020), who noted that aqueous fractions of *Eucalyptus* species were rich in polar phytochemicals like flavonoids and tannins, which are effective against enteric Gram-negative bacteria. In contrast, *Escherichia coli* displayed the highest resistance, requiring 25 mg/ml and 50 mg/ml of aqueous and n-hexane extracts, respectively, for inhibition. This is consistent with the work of Adegbite and Kolapo (2021), who attributed the reduced susceptibility of *E. coli* to its robust outer membrane, which acts as a permeability barrier, especially against non-polar compounds. These findings support the notion that solvent polarity significantly influences extract potency and that aqueous *E. globulus* extracts may offer greater therapeutic potential against Gram-negative pathogens.

Table 5 shows the result for the Minimum Bactericidal concentration; the aqueous and n-hexane extracts exhibited equal inhibitory activity against *Staphylococcus aureus*, both being effective at a concentration of 25 mg/ml. Against *Salmonella typhi*, the aqueous extract proved more potent, showing inhibition at 12.5 mg/ml compared to 25 mg/ml required by the n-hexane extract. *Escherichia coli* demonstrated the highest resistance, with inhibition observed at 25 mg/ml for the aqueous extract and 50 mg/ml for the n-hexane extract.

The antibacterial effects of *Eucalyptus globulus* extracts observed in this study reveal promising therapeutic potential against common pathogens. Both aqueous and n-hexane extracts showed equal efficacy against *Staphylococcus aureus* at 25 mg/ml, a result consistent with findings by Nwachukwu et al. (2019), who reported similar inhibitory effects of *E. globulus* methanolic and aqueous extracts on Gram-positive organisms due to the plant's high content of phenolic compounds and flavonoids. The superior activity of the aqueous extract against *Salmonella typhi* at 12.5 mg/ml compared to the 25 mg/ml required by the n-hexane extract aligns with the observations of Bello and Gwarzo (2020), who highlighted that water-soluble phytochemicals in *Eucalyptus* species, including tannins and saponins, are more active against enteric Gram-negative bacteria. Meanwhile, the greater resistance shown by *Escherichia coli*, particularly to the n-hexane extract (50 mg/ml), supports the findings of Okoye et al. (2021), who attributed the reduced susceptibility of *E. coli* to its outer membrane barrier, which limits the penetration of non-polar antimicrobial compounds. These findings emphasize the importance of solvent polarity in extraction processes and suggest that aqueous extracts of *E. globulus* may offer more effective antibacterial action, especially against Gram-negative enteric pathogens.

Figure 1 comprises two panels illustrating antioxidant activity assessment. The left panel shows a standard calibration curve of absorbance versus concentration (µg/mL) for ascorbic acid, exhibiting a strong linear relationship ($R^2 = 0.9972$) with the regression equation y=0.0105x+0.0999, indicating reliable quantification. The right panel presents the ascorbic acid equivalent antioxidant capacity (AAE) of three different extracts, EGCF, EGHF, and EGCE, each at a concentration of 300 µg/mL. Among the extracts, EGCE showed the highest antioxidant activity with an AAE of 62.10 ± 0.49 µg/mL, followed by EGCF (36.10 ± 0.20 µg/mL) and EGHF (23.64 ± 0.50 µg/mL), with statistically significant differences indicated by different superscript letters.

Figure 2 consists of two panels illustrating the antioxidant activity of plant extracts. The left panel displays a standard calibration curve for ascorbic acid, showing a strong linear correlation between absorbance and concentration (µg/mL) with the regression equation y = 0.0053x - 0.002. A coefficient of determination $R^2 = 0.9881$ is also provided, indicating high precision in measuring antioxidant capacity. The right panel compares the ascorbic acid equivalent antioxidant capacity (AAE) of three extracts, EGCF, EGHF, and EGCE, each tested at a concentration of 200 µg/mL. EGCF and EGCE exhibited significantly higher antioxidant activity (76.41 ± 1.33 µg/mL and 78.63 ± 0.19 µg/mL, respectively) compared to EGHF (24.22 ± 0.92 µg/mL), although all values share the same superscript "a", suggesting no statistically significant difference among them at the specified level.

References

1. Abaka, A. M., Dahiru, M. M., Abubakar, K. B., Luka, J., Abubakar, A., Abdullahi, T. B., & Barau, S. H. (2024). Phytochemical profile and antibacterial activity of *Nigella sativa* against biofilm-producing bacteria uropathogens. *Biology, Medicine, & Natural Product Chemistry, 13*(1), 141–146.
2. Abaka, A. M., Hamuel, J. D., Abdullahi, T. B., & Abubakar, K. B. (2024). In-ovo antiviral activity of *Hibiscus sabdariffa* against Newcastle disease virus. *Biology, Medicine, & Natural Product Chemistry, 13*(1), 305–310.
3. Abdullahi, T. B., Abaka, A. M., Haruna, A., Luka, J., Abubakar, A., & Ahmed. (2024). Phytochemical profile, antioxidant, and antibacterial activity of *Detarium microcarpum* against gastrointestinal bacteria. *ISRG Journal of Multidisciplinary Studies, 2*(7).
4. Adegbite, A. A., & Kolapo, A. L. (2021). Comparative antibacterial activities of polar and non-polar extracts of selected medicinal plants on *Escherichia coli*. *Nigerian Journal of Basic and Applied Sciences, 29*(1), 12–19.
5. Ahmed, S., Ahmed, M. Z., Rafique, S., Almasoudi, S. E., Shah, M., Jalil, N. A. C., & Ojha, S. C. (2023). Recent approaches for downplaying antibiotic resistance: Molecular mechanisms. *BioMed Research International, 2023*(1), 5250040.
6. Akinmoladun, A. C., Obuotor, E. M., & Farombi, E. O. (2010). Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. *Journal of Medicinal Food, 13*(2), 444–451.
7. Almatroudi, A. (2025). Biofilm resilience: Molecular mechanisms driving antibiotic resistance in clinical contexts. *Biology, 14*(2), 165.
8. Araújo, D., Silva, A. R., Fernandes, R., Serra, P., Barros, M. M., Campos, A. M., ... & Castro, J. (2024). Emerging approaches for mitigating biofilm-formation-associated infections in farm, wild, and companion animals. *Pathogens, 13*(4), 320.
9. Bachir, R. G., & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine, 2*(9), 739–742.

10. Bale, M. I. (2021). Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from blood samples of patients attending selected hospitals in Ilorin, Nigeria (Doctoral dissertation, Kwara State University, Nigeria).
11. Balkrishna, A., Sharma, N., Srivastava, D., Kukreti, A., Srivastava, S., & Arya, V. (2024). Exploring the safety, efficacy, and bioactivity of herbal medicines: Bridging traditional wisdom and modern science in healthcare. *Future Integrative Medicine, 3*(1), 35–49.
12. Barman, P., & Biswas, A. (2025). The contribution of traditionally used medicinal plants to modern medicine: An update on the successful journey of medicinal plants of North East Africa. In *Traditional Medicine in North East Africa: Research on Traditional Healer Preparations and Herbs* (pp. 118–146).
13. Bello, M. B., & Gwarzo, M. Y. (2020). Antibacterial activity of *Eucalyptus camaldulensis* and *Eucalyptus globulus* leaf extracts against selected clinical pathogens. *Bayero Journal of Pure and Applied Sciences, 13*(1), 47–52.
14. Chandrakar, P., & Thakur, S. (2025). Antimicrobial resistance: A silent pandemic threatening global health, economy, and the future of antibiotics. *Economy, and The Future of Antibiotics*.
15. Dahiru, M. M., Abaka, A. M., & Ya'u, I. (2024). Antibacterial potential of *Ximenia americana* L. Olacaceae: Molecular docking, molecular dynamics, and ADMET prediction. *Journal of Pharmacy, 4*(1), 51–67.
16. Datta, S., Nag, S., & Roy, D. N. (2024). Biofilm-producing antibiotic-resistant bacteria in Indian patients: A comprehensive review. *Current Medical Research and Opinion, 40*(3), 403–422.
17. Dean, M. (2024). Exploring ethnobotanical knowledge: Qualitative insights into the therapeutic potential of medicinal plants. *Golden Ratio of Data in Summary, 4*(2), 154–166.
18. Ewansiha, J. U., Fori, A., Dahiru, U., & Ijabani, E. (2024). Antibacterial potentials and phytochemical screening of *Eucalyptus globulus* leaves extract against selected isolates. *Sahel Journal of Life Sciences FUDMA, 2*(3), 98–103.
19. Eze, E. A., Orji, M. U., & Nwachukwu, N. (2020). Comparative antimicrobial activity of aqueous and ethanolic leaf extracts of *Ocimum gratissimum* and *Vernonia amygdalina* on some clinical bacterial isolates. *Journal of Pharmacy and Biological Sciences, 15*(3), 32–38.
20. Malakar, M. (2024). *Eucalyptus Spp.: A Wonder Tree*. In *Advances in Medicinal and Aromatic Plants* (pp. vol 1–185). Apple Academic Press.
21. Mendel, Z., Protasov, A., & Branco, M. (2025). Classical biological control of gall wasps in *Eucalyptus* plantations. In *Biological Control of Insect Pests in Plantation Forests* (pp. 223–274). Cham: Springer Nature Switzerland.
22. Michaud, A., Stawicki, S. P., Izurieta, R., & Iyer-Raniga, U. (2024). Global health security: Contemporary considerations and developments. *BoD–Books on Demand*.
23. Moges, G. W., Manahelohe, G. M., & Asegie, M. A. (2024). Phenolic, flavonoid contents, antioxidant, and antibacterial activity of selected *Eucalyptus* species. *Biology, Medicine, & Natural Product Chemistry, 13*(1), 147–157.
24. Nkang, A. O., Okonko, I. O., Mejeha, O. K., Adewale, O. G., Udeze, A. O., Fowotade, A., ... & Babalola, E. T. (2009). Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. *Journal of Microbiology and Antimicrobials, 1*(2), 019–026.
25. Nwachukwu, E. O., Uzoeto, H. O., & Okereke, H. C. (2019). Comparative antimicrobial activity of ethanol and aqueous leaf extracts of *Eucalyptus globulus* against some clinical isolates. *Nigerian Journal of Microbiology, 33*(2), 4315–4320.
26. Nwinyi, O. C., Chinedu, N. S., & Ajani, O. O. (2019). Evaluation of antibacterial activity of essential oils from *Eucalyptus globulus* on multidrug-resistant *Staphylococcus aureus*. *Nigerian Journal of Natural Products and Medicine, 23*, 45–51.
27. Okoye, N. E., Udeze, A. O., & Ajero, C. M. U. (2021). Phytochemical screening and antibacterial efficacy of *Eucalyptus globulus* leaf extracts on Gram-negative bacteria. *Journal of Medical and Applied Biosciences, 13*(2), 88–95.
28. Oladunmoye, M. K., & Kehinde, F. Y. (2018). Evaluation of antibacterial efficacy of polar and non-polar extracts of some Nigerian medicinal plants. *African Journal of Clinical and Experimental Microbiology, 19*(1), 12–19.
29. Olorunfemi, D. I., Adebayo, A. H., & Fadipe, V. O. (2020). Antibacterial and phytochemical analysis of aqueous and ethanolic extracts of *Eucalyptus camaldulensis* and *Eucalyptus globulus*. *African Journal of Clinical and Experimental Microbiology, 21*(3), 305–312.
30. Saber, F. R., Munekata, P. E., Rizwan, K., El-Nashar, H. A., Fahmy, N. M., Aly, S. H., ... & Lorenzo, J. M. (2024). Family *Myrtaceae*: The treasure hidden in the complex/diverse composition. *Critical Reviews in Food Science and Nutrition, 64*(19), 6737–6755.
31. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023, July). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare, 11*(13), 1946. https://doi.org/10.3390/healthcare11131946
32. Shala, A. Y., & Gururani, M. A. (2021). Phytochemical properties and diverse beneficial roles of *Eucalyptus globulus* Labill.: A review. *Horticulturae, 7*(11), 450.

33. Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023). Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms, 11*(6), 1614.
34. Shiekh, R. A. E., Atwa, A. M., Elgindy, A. M., Mustafa, A. M., Senna, M. M., Alkabbani, M. A., & Ibrahim, K. M. (2025). Therapeutic applications of eucalyptus essential oils. *Inflammopharmacology, 33*(1), 163–182.
35. Singh, B. P., Ghosh, S., & Chauhan, A. (2021). Development, dynamics, and control of antimicrobial-resistant bacterial biofilms: A review. *Environmental Chemistry Letters, 19*(3), 1983–1993.

CITATION
Abaka, A. M., Belel, M. H., Dangana, M. B., Abubakar, K. B., & Dahiru, F. A. (2025). Antibacterial and antioxidant potential of Eucalyptus globulus against biofilm-forming bacteria. In Global Journal of Research in Medical Sciences (Vol. 5, Number 4, pp. 86–95). https://doi.org/10.5281/zenodo.16782642