

PHYTOCHEMICAL, ANTIOXIDANT, AND ANTIMICROBIAL EVALUATION OF *Eucalyptus camaldulensis* Dehnh. LEAF EXTRACT

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ABSTRACT

Rising antimicrobial resistance and infection-associated oxidative stress have renewed interest in plant-derived agents that combine antimicrobial and antioxidant properties. *Eucalyptus camaldulensis* still has limited integrated data linking its phytochemical profile to antioxidant and antimicrobial activities. This study characterised the phytochemical composition of *E. camaldulensis* leaf and evaluated its antioxidant and antimicrobial activities using complementary quantitative assays. Phytochemical screening and quantification were done by standard spot tests and gravimetric assays. Total phenolic, flavonoid and tannin contents were determined spectrophotometrically. Antioxidant activity was assessed by DPPH, FRAP, and Phosphomolybdenum assays. Antimicrobial activity was evaluated against six bacterial species and two fungal isolates by agar-well diffusion and broth dilution, using ciprofloxacin and ketoconazole as standards. Phytochemical screening confirmed the abundance of flavonoids, terpenoids, saponins and alkaloids. Mean TPC, TFC and total tannins were 165.93 ± 0.12 mg GAE/g, 131.13 ± 1.34 mg QE/g and 152.9 ± 1.04 mg TAE/g, respectively. The extract showed moderate DPPH activity ($IC_{50} = 275.2$ μ g/mL), concentration-dependent FRAP increment (~ 6.7 to 37.2 mg AAE/g), with TAC also rising progressively with dose. Antimicrobial testing produced broad-spectrum, dose-dependent inhibition with zones of inhibition ranging from 0–16 mm for bacteria and 0–18 mm for fungi; the strongest activity was against *Proteus mirabilis* and *Fusarium* sp. Bacterial MICs ranged from 6.25–25 mg/mL, with corresponding bactericidal effects at higher concentrations; fungal MIC/MFC values indicated greater tolerance in *Candida* sp. and *Fusarium* sp. The extract demonstrated measurable antioxidant and broad-spectrum antimicrobial activities, although its potency was lower than that of the reference drugs under the assay conditions. Ultimately, the phenolic- and flavonoid-rich profile of *E. camaldulensis* supports its traditional use and justifies further fractionation and compound isolation to identify more potent bioactive constituents for possible drug development.

Keywords: Antimicrobial; Antioxidant; Ethanol; Biological activity; *Eucalyptus camaldulensis*; Secondary metabolites.

INTRODUCTION

Conventional drugs continue to draw heavily on natural sources; approximately 25% of approved pharmaceuticals are plant-derived (Newman and Cragg, 2020). Medicinal plants provide diverse bioactivities: immunomodulatory, anti-inflammatory, antimicrobial and antioxidant, which position them as attractive alternatives or complements to conventional human and veterinary therapeutics (Skoufos *et al.*, 2020). The agri-food and pharmaceutical sectors also exploit plant phytochemicals for value-added products (Ivanova *et al.*, 2024). Bioactive diversity arises from the distribution of secondary metabolites across plant organs, producing broad pharmacological effects that include neuroprotective and anticancer actions (Al-Majmaie *et al.*, 2025; Sharma *et al.*, 2025). Plants serve as important sources of enzymatic and non-enzymatic antioxidants that mitigate oxidative

stress, and synergistic interactions among the metabolites further modulate biological activity (Oyeyinka and Afolayan, 2020; Zhang *et al.*, 2022).

Eucalyptus camaldulensis Dehnh. (River Red Gum; Myrtaceae) is a fast-growing, drought-tolerant tree native to riverine and floodplain habitats and widely cultivated across tropical and temperate regions for timber, pulp, fuelwood, shade, windbreaks, and ecological rehabilitation (Doran and Turnbull, 1997; McDonald *et al.*, 2009). Its broad adaptability and economic value have made it one of the most extensively planted *Eucalyptus* species, particularly in arid and semi-arid environments where it is also used for land reclamation and erosion control (Doran and Turnbull, 1997; McDonald *et al.*, 2009). Ethnopharmacological investigations report anti-inflammatory, antioxidant and antimicrobial properties for *E. camaldulensis* preparations (Reda *et*

al., 2017; Alghamdi and Ababutain, 2019; Onukogu *et al.*, 2019; Usman and Ali, 2025). However, the bibliographic pattern of these studies in the last decade and a half shows major domicile in the middle belt/northern Nigeria and North African/Middle Eastern contexts, and there is comparatively little experimental data from western Nigeria, a gap that limits understanding of regional chemotype variation and local ethnopharmacological relevance.

Given the dual challenges of antimicrobial resistance and infection-associated oxidative stress, integrated profiling that links phytochemistry to antioxidant and antimicrobial function is essential for prioritising lead extracts or compounds for further development. This study, therefore, aims to characterise the phytochemical composition of the ethanol leaf extract of *E. camaldulensis*, evaluate antioxidant capacity and assess antimicrobial activity against six clinically relevant bacteria and two fungi.

MATERIALS AND METHODS

Collection and Preservation of Organisms

Clinical pathogenic bacterial isolates which include *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, as well as fungal isolates: *Fusarium* sp. and *Candida albicans* were utilised for this study. They were obtained from the University of Ibadan Microbiology Laboratory, Oyo State, Nigeria. Pure bacterial isolate cultures were obtained by culturing on selective media (Mueller-Hinton agar and Potato Dextrose Agar for bacterial and fungi respectively) and incubating at 37 °C for 24 hours. They were then inoculated on slants of nutrient agar at an incubation temperature of 37 °C for 24 hours. The pure cultures were kept as stock cultures in the refrigerator (4 °C), for further use.

Plant Sample Collection

Leaves of *E. camaldulensis* were collected at the Forestry Research Institute of Nigeria, Ibadan, identified and authenticated by a taxonomist expert at the Institute. The collected samples were meticulously washed with distilled water and air-dried. The dried leaf samples were pulverised into fine, homogeneous powder and stored in air-tight bottles.

Plant Extract Preparation

Pulverised leaf samples of *E. camaldulensis* (500 g) were extracted in 2.5 L of ethanol for 72 hours with constant shaking using a mechanical shaker (Flask Shaker, Surgifield Medical, England), then filtered (Whatman No. 1) into a sterilised conical flask. The obtained filtrates were concentrated using a rotary evaporator (Hei-Vap Core Rotavap, New Jersey, USA) at 40 °C, leaving the crude extract as residue. This residue was measured on a weighing scale and kept refrigerated before use. Serial dilutions of the ethanol extract were prepared in a sterile McCartney bottle to obtain the working concentrations used for the antioxidant and antimicrobial assays.

Qualitative Phytochemical Screening

Phytochemical screening was carried out to test for phytochemical constituents in *E. camaldulensis* leaf extract. The ethanol leaf extracts were subjected to chemical tests to detect profiles of phytoconstituents using standard assay procedures (Sofowora, 1993; Harborne, 1998; Trease and Evans, 2002). These analyses were conducted to ascertain the presence of secondary metabolite compounds such as flavonoids, alkaloids, cardiac glycosides, saponins, tannins, anthraquinones, steroids and terpenoids.

Quantitative Phytochemical Analysis

Quantification of total tannins, flavonoids and phenolics was performed using external calibration with their respective reference standards. For each assay, serial dilutions of the appropriate standard were prepared and analysed under identical experimental conditions as the samples. Calibration curves were generated by plotting absorbance against standard concentration, and linear regression equations obtained within the linear range were used to interpolate the concentrations of the phytochemicals in the extracts.

Determination of Total Tannin Content

Tannin content was quantified using an adaptation from Amorim *et al.* (2008). For each assay, 0.1 mL of *E. camaldulensis* extract was mixed with 7.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 35% (w/v) sodium carbonate. The volume was adjusted with distilled water up to 10 mL, then the mixture was vortexed and left at room temperature for 30 minutes. The absorbance was recorded at 725 nm using a

double-beam UV/Visible Spectrophotometer (Analytik Jena, Germany). A reagent blank (i.e. water for extract) was run concurrently. Tannic acid standards were prepared and measured under similar conditions, to generate a calibration, in the form of a linear equation ($y = 0.3873x - 0.7006$; $R^2 = 0.99$). The absorbances of the samples were interpolated on the linear curve to determine total tannin content, expressed in mg/g tannic acid equivalent plant extract.

Determination of Total Flavonoid Content

This was determined using the Aluminium Chloride Colorimetry assay (Zhishen *et al.*, 1999). Briefly, 4 mL of each *E. camaldulensis* extract dilution was mixed with 0.3 mL of 5% (w/v) sodium nitrite (NaNO_2), and incubated at room temperature for five minutes. Then, 0.3 mL of 10% (w/v) aluminium chloride (AlCl_3) solution was added, and the mixture was left for a further six minutes to generate the formation of the complex. Then, 2 mL of 1 M sodium hydroxide (NaOH) was introduced, and the reaction volume was made up to 10 mL with distilled water. After vortexing for one minute, the absorbance was measured at 510 nm against a reagent blank (containing all reagents without extract). A quercetin standard curve (5-100 $\mu\text{g/mL}$) was run concurrently, yielding the linear curve $y = 0.0242x - 0.0894$; ($R^2 = 0.98$). The absorbances of the samples were interpolated on the linear curve to estimate the total flavonoid content, as expressed in mg/g QE/g dry extract).

Determination of Total Phenolic Content

Total phenolic content was determined using Folin-Ciocalteu colorimetry assay (Kulkarni *et al.*, 2015). Each *E. camaldulensis* extract dilution (0.1-1.0 mg of extract within linearity range) was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent, and left to react for five minutes. Afterwards, 1.2 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added. This mixture was made up to 10 mL final volume, with distilled water, placed in the dark at room temperature for 30 minutes, and the absorbance was read at 765 nm against a blank (water instead of extract). A gallic acid standard curve (0-100 $\mu\text{g/mL}$) was generated in concurrence, yielding the linearized equation: $y = 0.4967x - 0.586$ ($R^2 = 0.96$). The absorbances of the samples were then interpolated on the linear curve to evaluate phenolic concentration, as expressed in mg GAE/g extract.

Antioxidant Assaying of Plant Extracts DPPH Radical Scavenging Activity

The free-radical scavenging activity of *E. camaldulensis* ethanol leaf extract was evaluated with the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, using ascorbic acid as a positive control. A 0.01 mM DPPH solution was prepared in methanol and shielded from light. In an amber-coloured flask, 100 μL of each sample (dissolved at 0.01 mM) or ascorbic acid standard was added to 2.0 mL of DPPH solution. The mixture was left to stand at room temperature, for five minutes, after which the decrease in absorbance was read at 517 nm against a pure DPPH blank (without sample). Then, radical scavenging activity was calculated thus:

$$\% \text{ Inhibition} = \frac{(A_0 - A_T)}{A_T} \times 100 \quad [1]$$

Where, A_0 = absorbance of DPPH, and A_T = absorbance of DPPH + extract or ascorbic acid. (Gulcin and Alwasel, 2023).

Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power of *E. camaldulensis* ethanol leaf extract was measured as ascorbic acid equivalent. The FRAP reagent was freshly prepared by warming the combination of 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 25 mL of 0.3 M acetate buffer (pH 3.6), to 37 °C. For each assay, 30 μL of ethanol extract (at a known concentration) was mixed with 90 μL of distilled water and 900 μL of freshly prepared FRAP reagent, giving a total reaction volume of 1.02 mL. The reaction mixture was incubated at 37 °C for 30 min, after which the absorbance was measured at 593 nm against a reagent blank containing 30 μL distilled water, 90 μL distilled water and 900 μL FRAP reagent. An ascorbic acid calibration curve was prepared in parallel by replacing the extract with 30 μL of ascorbic acid standard (0.5–100 $\mu\text{g/mL}$), while maintaining the same reaction volumes and incubation conditions. Blank-corrected absorbances of ascorbic acid were plotted against ascorbic acid concentration; the ascorbic-acid equivalent was calculated by interpolation on the ascorbic-acid calibration curve: $y = 0.0202x + 0.1981$; $R^2 = 0.9906$ (Benzie and Strain, 1996).

Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) of the ethanol leaf extract of *E. camaldulensis* was evaluated using

the Phosphomolybdenum methodology (Prieto *et al.*, 1999). 0.5 mL aliquots of extract at known concentrations were mixed with 3 mL of fresh preparation of phosphomolybdenum reagent (sulfuric acid, ammonium molybdate, and sodium phosphate). The reaction mixtures were incubated at 95 °C for 90 minutes, enabling the reduction of Mo(VI) to Mo(V), by any extract antioxidant, forming a green complex. After cooling to room temperature, absorbance was then measured at 695 nm against a reagent blank. The ascorbic acid standards (1-75 µg/mL) were run concurrently to generate a linear calibration curve. The blank-corrected absorbance of each ethanol extract was interpolated to obtain ascorbic acid equivalents (AAE). The ascorbic-acid equivalent was calculated by interpolation on the ascorbic-acid calibration curve: $y = 0.1725x - 0.1067$; $R^2 = 0.9984$. Total antioxidant capacity values were expressed as mg AAE/g of extract. All samples and standards were analysed in triplicate.

Antimicrobial Activity

Agar Diffusion Testing

The antimicrobial activity of ethanol leaf extracts of *E. camaldulensis* was assessed using the agar well diffusion method, following slightly modified standard protocols (Veeraswamy *et al.*, 2022; Irshad *et al.*, 2025). The test microbes include six bacterial isolates, *S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, as well as two species of fungi, *Fusarium* sp. and *C. albicans*.

The dried ethanol extract was reconstituted in 10% (v/v) ethanol prepared with sterile distilled water to obtain a stock solution, from which working concentrations of 100, 50 and 25 mg/mL were prepared by serial dilution. Bacterial inocula were standardised by adjusting the turbidity of freshly prepared cultures to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Similarly, the *Candida albicans* yeast suspension was adjusted to the 0.5 McFarland standard (approximately 1.0×10^6 cells/mL), while the *Fusarium* spp. spore suspension was standardised to approximately 1.0×10^4 spores/mL prior to inoculation. The standardised bacterial and fungal suspensions were uniformly distributed over the surfaces of sterile Mueller–Hinton agar and Potato Dextrose Agar (PDA) plates, respectively, using a sterile spreader to obtain confluent growth. Wells of 8 mm diameter were aseptically bored into the inoculated agar plates using a sterile cork borer, after which

100 µL of each extract concentration (100, 50 and 25 mg/mL) was carefully dispensed into the respective wells. Ciprofloxacin (10 µg/disc) and ketoconazole (1% w/v; equivalent to 10 mg/mL) served as the positive controls for the bacterial and fungal assays, respectively. The bacterial plates were incubated at 37 °C for 24 h, whereas the fungal plates were incubated at 28 °C for 48 h. Following incubation, the diameters of the inhibition zones were measured in millimetres using a transparent ruler. All assays were performed in triplicate, and the average values were calculated and reported.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of ethanol leaf extract of *E. camaldulensis* was determined using the broth dilution method. The standardised bacterial and fungal inocula described above were used for the broth dilution assay. The bacterial suspensions were inoculated into Mueller–Hinton Broth and the fungal suspensions into Potato Dextrose Broth, containing graded concentrations of extract (100-6.25 mg/mL). Tubes for bacteria were incubated at 37 °C for 24 hours, and at 28 °C for 48 hours in the case of fungi. Minimum Inhibitory Concentration was determined as the lowest concentration with no visible microbial growth (European Society of Clinical Microbiology and Infectious Diseases, 2003).

Determination of Minimum Bactericidal/Fungicidal Concentration

Following the minimum inhibitory concentration assay, samples from test tubes that showed no visible microbial growth were sub-cultured onto Mueller-Hinton agar (MHA) for the bacterial isolates and Potato Dextrose Agar (PDA) for the fungal isolates, using a sterile inoculating loop. These plates were incubated at 37 °C for 18-24 hours. The minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), was determined as the lowest concentration of the ethanol leaf extract, where no microbial colony growth was observed on the agar surface. This indicates complete bactericidal or fungicidal activity (Espinell-Ingroff, 2001; Owuama, 2017).

Data Analysis

Experimental testing was carried out in triplicate and results presented as Mean $\pm$ Standard deviation.

Ethical Considerations

No ethical considerations were required for this study.

RESULTS AND DISCUSSION

The phytochemical screening of the ethanol leaf extract of *E. camaldulensis* (Table 1) confirmed the presence of multiple classes of secondary metabolites. Several constituents showed strong reactions, whereas others were detected at moderate levels. These findings establish the chemical composition profile of the extract and provide the basis for subsequent quantitative and bioactivity evaluations.

Table 1. Results of phytochemical screening of *E. camaldulensis*

Phytochemical	Results
Saponins	++ve
Tannins	++ve
Flavonoids	++ve
Cardiac glycosides	+ve
Anthraquinones	+ve
Terpenoids	++ve
Steroids	+ve
Alkaloids	++ve

Note: +ve: present; ++ve: abundant; -ve: absent

Figure 1 presents the gravimetric quantification of alkaloids, saponins and terpenoids in the ethanol leaf extract of *E. camaldulensis*. Table 2 reports the spectrophotometric determinations of total phenolic, total flavonoid, and tannin contents,

expressed as gallic-acid, quercetin equivalents, and tannic acid, respectively. These quantitative data provide the analytical baseline for the antioxidant and antimicrobial evaluations that follow.

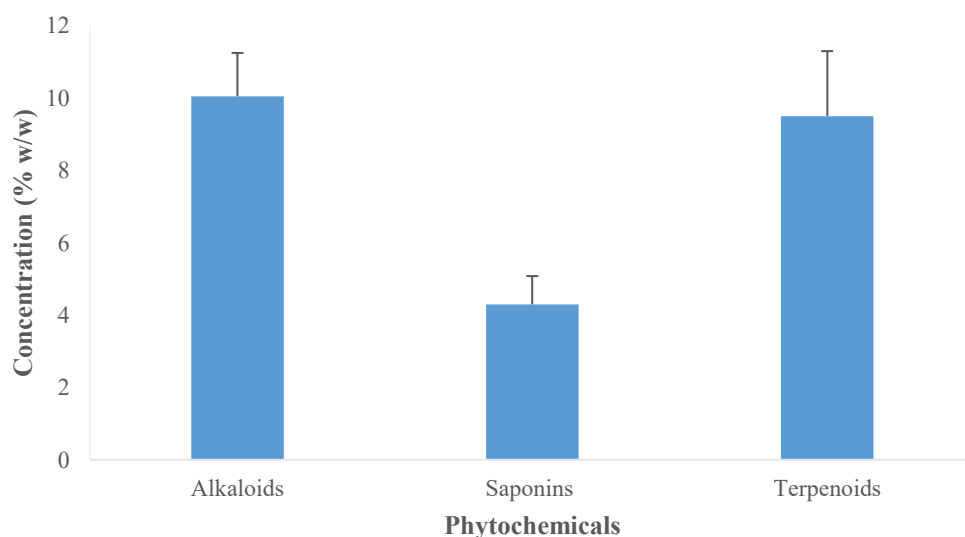


Figure 1. Quantitative levels of alkaloids, saponins, and terpenoids in ethanol leaf extract of *E. camaldulensis* (Bars represent mean concentration, error bars denote standard deviation).

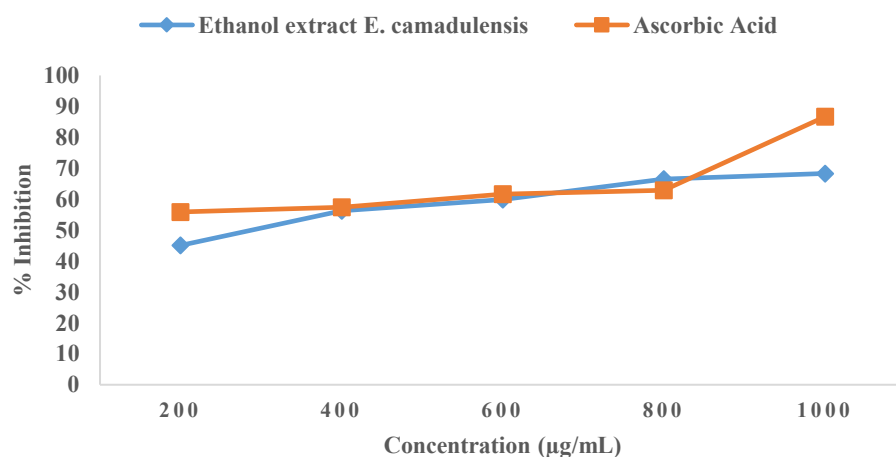
Table 2. Concentration of total phenolics, total flavonoids, and total tannin in ethanol leaf extract of *E. camaldulensis*.

Phytochemicals	Concentration
Total Phenolic Content	165.93 $\pm$ 0.12 mg GAE/g
Total Flavonoid Content	131.13 $\pm$ 1.34 mg QE/g
Total Tannin	152.9 $\pm$ 1.04 mg TAE/g

The result of the DPPH scavenging activity is presented in Figure 2. The *E. camaldulensis* ethanol leaf extract produced a clear, concentration-dependent increase in DPPH radical scavenging. The extract's IC₅₀ was estimated to be 275.2 μ g/mL from the linear regression curve: $y = 0.0284x + 42.184$, and $R^2 = 0.932$. Because ascorbic acid achieved >50% DPPH inhibition at the lowest concentration tested, its IC₅₀ was below

the experimental range and was therefore reported as < lowest tested concentration rather than as the negative value generated by linear extrapolation. Thus, ascorbic acid showed an IC₅₀ that is less than 200 μ g/mL, the lowest test concentration from the linear regression curve: $y = 0.0127x + 53.13$, $R^2 = 0.955$, suggesting that the extract may have moderate antioxidant potency relative to the reference standard.

DPPH Scavenging Activity

**Figure 2.** DPPH scavenging activity of ethanol leaf extract of *E. camaldulensis* against standard ascorbic acid.

Both the FRAP and TAC showed clear, concentration-dependent increases in absorbance, which corresponded to progressive increases in ascorbic acid equivalents in the *E. camaldulensis* ethanol leaf extract (Figures 3 and 4). The FRAP AAE increased markedly across the tested

concentration range, rising from approximately 6.7 mg AAE/g at the lowest concentration to 37.2 mg AAE/g at the highest concentration. The TAC AAE followed a parallel upward trend, attaining its highest ascorbic-acid equivalent at the highest concentration tested.

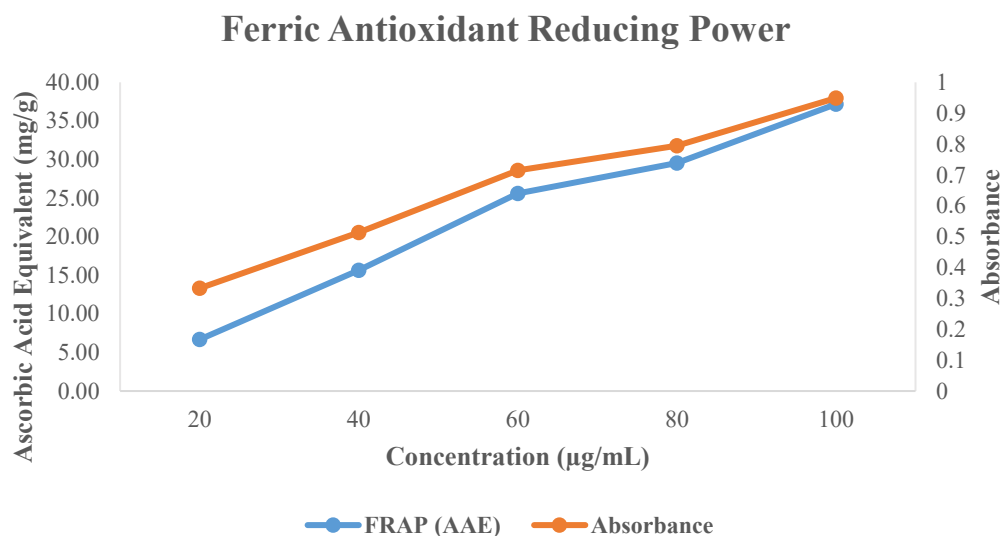


Figure 3. Ferric reducing antioxidant power of ethanol leaf extract of *E. camaldulensis*

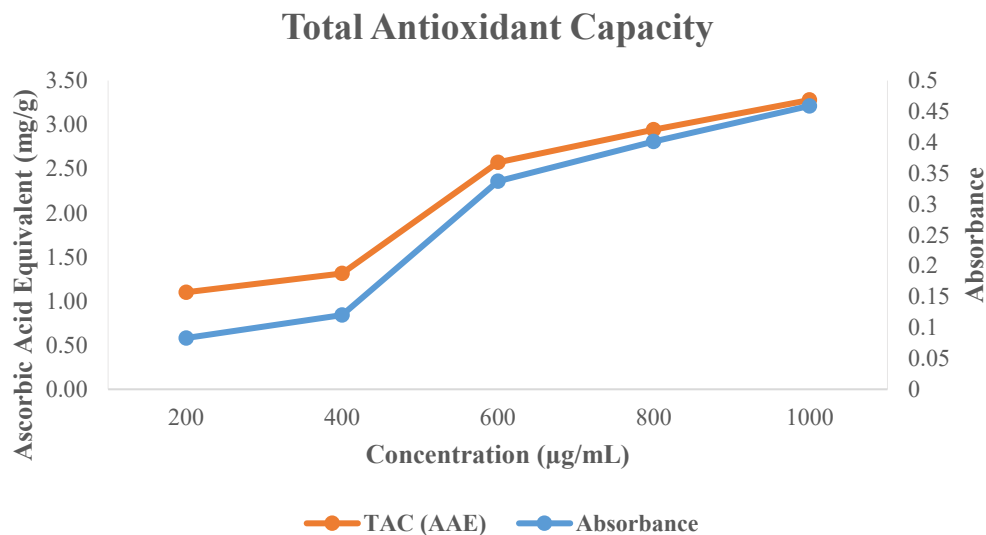


Figure 4. Total antioxidant capacity of ethanol leaf extract of *E. camaldulensis*.

The ethanol leaf extract demonstrated measurable antibacterial activity across the test organisms (Figure 5), with inhibition generally increasing as concentration increased (Figure 6a). At the highest dose, susceptibility was most pronounced in *P. mirabilis* and *K. pneumoniae*. At the same time, *S. typhi* and *P. aeruginosa* showed comparatively limited

sensitivity, particularly at lower concentrations where activity declined or was absent. *S. aureus* and *E. coli* displayed moderate but consistent responses across the gradient. The reference antibiotic ciprofloxacin applied at a dose of 10 µg expectedly produced larger inhibition diameters than the extract for all isolates.

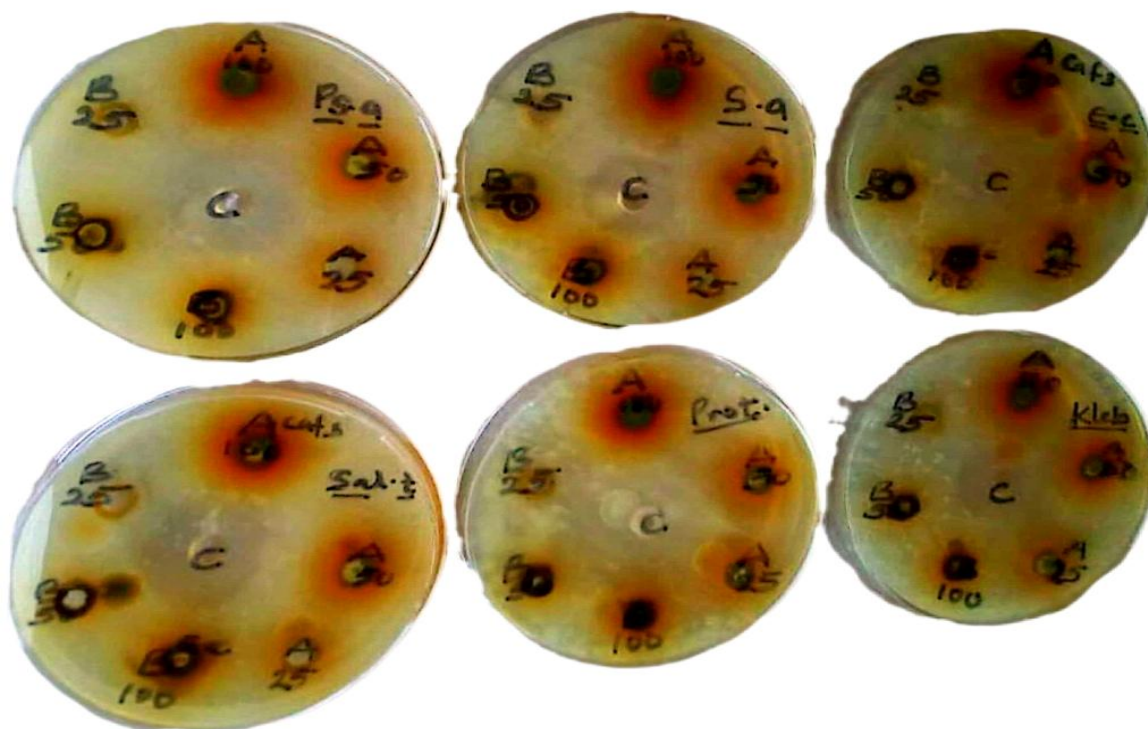


Figure 5. Representative agar well diffusion plates showing the antimicrobial activity of the ethanol leaf extract of *E. camaldulensis* against selected isolates.

A similar concentration-dependent pattern was evident for the fungi (Figure 6b). Activity was clearly expressed at the upper concentrations, with *Fusarium* exhibiting greater susceptibility than *C.*

albicans. Antifungal effects diminished markedly at the lowest concentration tested. As observed with the bacteria, the standard antifungal agent showed stronger inhibition than the plant extract.

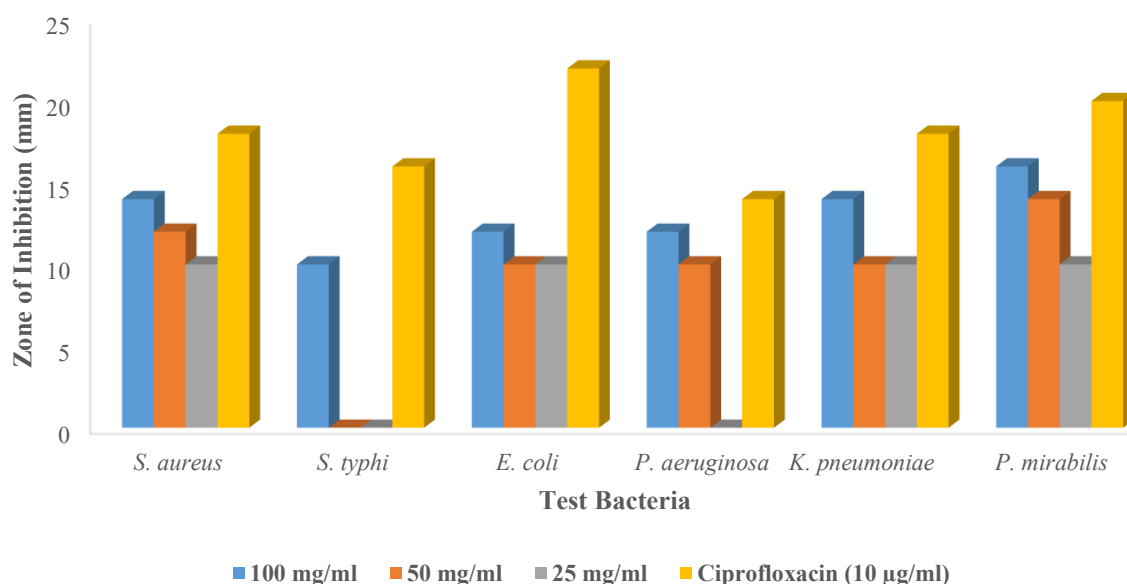


Figure 6a. Antibacterial activity of ethanol leaf extract of *E. camaldulensis* against selected bacteria.

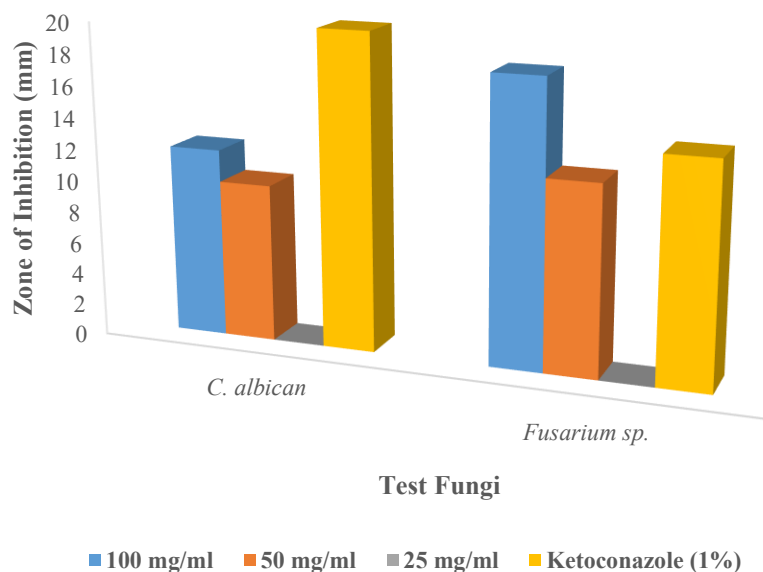


Figure 6b. Antifungal activity of ethanol leaf extract of *E. camaldulensis* against selected fungi.

Broth dilution results further defined the inhibitory and minimum bactericidal/fungicidal concentrations of the ethanol leaf extract (Table 3). Growth suppression occurred at comparatively low concentrations for some organisms, with the lowest MIC observed for *Fusarium sp.*, followed by *K. pneumoniae* and *P. mirabilis*. In contrast, relatively higher concentrations were required to inhibit *S. typhi*, *E. coli*, *P. aeruginosa*, and *C. albicans*. The bactericidal effects of the extract were typically achieved at concentrations equal to or exceeding the MIC values, and in most cases, a higher exposure was necessary to obtain complete lethality. For the fungi, fungicidal action appeared weaker overall; *C. albicans* required a substantial increase above the inhibitory level, while *Fusarium sp.* displayed persistence even toward the upper

limit of the concentration range examined. When benchmarked against the reference antimicrobials, ciprofloxacin and ketoconazole demonstrated markedly greater potency. Ciprofloxacin produced antibacterial inhibition within a MIC range of 0.3125–2.5 µg/mL, while bactericidal activity occurred between 0.625 and 12.5 µg/mL depending on the organism. For the fungi, ketoconazole achieved inhibitory endpoints at 0.12562–2.5% and fungicidal activity at approximately 2.5–5%. These values are substantially lower than those required for the plant extract, emphasizing the comparatively higher intrinsic efficacy of the standard agents despite the extract's observable broad-spectrum action.

Table 3. Minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of test isolates.

Organisms	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
<i>S. aureus</i>	12.5	25	NA
<i>S. typhi</i>	25	25	NA
<i>E. coli</i>	25	25	NA
<i>P. aeruginosa</i>	25	25	NA
<i>K. pneumoniae</i>	6.25	25	NA
<i>P. mirabilis</i>	6.25	25	NA
<i>C. albicans</i>	25	NA	50
<i>Fusarium sp.</i>	3.125	NA	>50

NA – not applicable

The present study reports the phytochemical composition, antioxidant potential, and antimicrobial efficacy of the ethanol leaf extract from *E. camaldulensis* collected in Ibadan, Nigeria. The qualitative screening revealed the presence of all tested phytochemicals in the ethanol leaf extract, with varying proportions (Table 1). This outcome aligns with numerous studies on *E. camaldulensis* leaves and barks in Nigeria, where these secondary metabolites are commonly reported in various solvent extracts/fractions, highlighting their role in plant defence and pharmacological applications (Chuku *et al.*, 2016; Onukogu *et al.*, 2019; Abdulmumin *et al.*, 2021; Adawaraa *et al.*, 2021). Across different geographical regions, Shayoub *et al.* (2015) identified tannins, sterols, triterpenoids, saponins, flavonoids and phenolic compounds in Sudanese *E. camaldulensis* leaf extracts using similar qualitative assays. Likewise, Brigitte *et al.* (2019) reported the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids and steroids in *E. camaldulensis* collected from Burkina Faso. In a subsequent study from the same country, Rosendal *et al.* (2020) attributed variations in the abundance of these phytochemicals to geographical location and environmental conditions. Similarly, Alghamdi and Ababutain (2019) reported abundant tannins, flavonoids and saponins in the ethanol extracts of the bark and leaves of *E. camaldulensis* harvested in Saudi Arabia. The abundance of flavonoids, tannins, and other key phytochemicals in the current study is consistent with reports from Nigeria, where ethanol extracts yielded comparable results, likely due to the solvent's polarity favouring polar polyphenolics (Shagal *et al.*, 2012; Chuku *et al.*, 2016; Anigboro *et al.*, 2020; Obi and Nwakego, 2025).

The values obtained from the quantitative analysis of alkaloids, saponins and terpenoids vary from those reported in some studies using different solvents or locations. For example, Sani *et al.* (2014) found lower alkaloid and comparable saponin levels in aqueous and ethanol extracts from Nigerian *E. camaldulensis* leaves, suggesting that ethanol's intermediate polarity enhances extraction efficiency for these compounds. Similarly, Deshi *et al.* (2023) reported lower alkaloid contents and slightly higher saponin levels in the methanol leaf extract of *E. camaldulensis* collected from Jos, Nigeria. The relatively

comparable saponin contents, despite differences in alkaloid yield, may reflect the influence of extraction solvent and regional growing conditions on the recovery of these phytochemicals.

In contrast, Soumana *et al.* (2024) reported alkaloid, saponin and terpenoid contents of 0.729%, 1.37% and 0.69%, respectively, in *E. camaldulensis*. Likewise, Onukogu *et al.* (2019) reported lower saponin (0.313%) and alkaloid (0.019%) contents than those obtained in the present study. These comparatively lower phytochemical yields may reflect differences in geographical origin, environmental conditions, and extraction protocols, which are known to influence the biosynthesis and recovery of secondary metabolites. The values obtained in the spectroscopic analysis for TPC and TFC are broadly consistent with published reports from other regions. Huang *et al.* (2022) reported a TPC of 111 mg GAE/g in the lipophilic fraction of *E. camaldulensis* collected in Thailand, which is comparable to the value obtained in the present study. Rosendal *et al.* (2020) similarly reported TPC values ranging from 68 to 129 mg GAE/g in ethanol extracts and 171 to 266 mg GAE/g in aqueous extracts of *E. camaldulensis* collected from different locations in Burkina Faso. The highest TPC values were recorded in samples from semi-arid regions, a pattern attributed to environmental influences on phenolic accumulation. In contrast, Suganya *et al.* (2024) reported substantially lower TPC values (5.6–7.8 mg GAE/g) for aqueous leaf extracts from India, which may reflect differences in extraction solvent and methodology.

With respect to flavonoids, the TFC in this study is higher than values reported for the lipophilic fraction in Thailand and for different Burkina Faso locations (Rosendal *et al.*, 2020; Huang *et al.*, 2022). Conversely, higher levels have been reported for ethanol extracts of the plant (11.10 mg GAE/mg for TPC and 15.05 mg QE/mg for TFC) (Nwabor *et al.*, 2019), underlining the large inter-study variability that can arise from differences in solvent, fractionation, plant provenance and analytical protocol.

Differences in phytochemical profiles reported across studies may reflect variations in extraction solvent, extraction protocol, plant provenance and environmental conditions. Geographical variations, such as soil composition and

precipitation in Nigeria compared with drier regions, may enhance phenolic accumulation as a stress response, explaining the substantial yields in this study (Rosendal *et al.*, 2020). Thus, location-specific factors revealed differences in the phytochemical profile, mirroring potential influences on Southwest Nigeria's samples. These findings suggest that *E. camaldulensis* from tropical African regions, such as Nigeria, may offer greater phytochemical richness for pharmaceutical development.

The ethanol leaf extract demonstrated moderate antioxidant capacity across DPPH, FRAP, and TAC assays. In the DPPH assay, a concentration-dependent scavenging was observed, with an IC_{50} of 275.2 $\mu\text{g/mL}$, compared to ascorbic acid's $<200 \mu\text{g/mL}$. This indicates moderate potency, potentially linked to the high TPC and TFC, as phenolics donate electrons to neutralise radicals. Comparable IC_{50} values (244.98 $\mu\text{g/mL}$) were reported for methanol extracts from Nigerian *E. camaldulensis*, suggesting solvent similarity in efficacy (Onukogu *et al.*, 2019). However, stronger activity (IC_{50} 31.46 $\mu\text{g/mL}$) was noted in lipophilic fractions from Thai samples, possibly due to enriched oxygenated terpenes absent in bulk ethanol extracts (Huang *et al.*, 2022). In the same vein, the aqueous and ethanol extracts of the plant from different regions of Burkina Faso exhibited higher antioxidant activity (EC_{50} 8.30-60.30 $\mu\text{g/mL}$) than observed in this study, attributable to the hydrodistillation and subsequent ethanol extraction protocol, enhancing bioactive yield (Rosendal *et al.*, 2020), likewise, the results reported by Suganya *et al.* (2024) for aqueous extracts of the plants in India, Usman and Ali (2025) for methanol extracts of the plant in northern Nigeria, and Ashraf *et al.* (2015) for methanol extract of the plant from Pakistan also demonstrated stronger antioxidant activity.

The FRAP assay produced a clear, concentration-dependent increase in reducing power, rising from 6.7 to 37.2 mg AAE/g, and TAC showed a parallel upward trend. These patterns agree with previous reports of dose-dependent ferric-reducing responses in *E. camaldulensis* extracts (Onukogu *et al.*, 2019; Huang *et al.*, 2022; Suganya *et al.*, 2024). Direct comparisons for TAC are more difficult because many prior studies do not report phosphomolybdenum (TAC) data for this species, limiting the available benchmarks. The moderate

overall antioxidant activity observed, which is stronger than many crude preparations but weaker than optimized fractions or pure standards, likely reflects a combination of biological and methodological factors. Environmental and regional variation (soil, climate, phenological stage) influence secondary-metabolite biosynthesis and can produce higher phenolic loads in semi-arid chemotypes compared with tropical populations (Rosendal *et al.*, 2020). Methodological factors are equally important; solvent polarity and extraction procedure determine which compound classes are recovered (polar solvents favour water-soluble phenolics; non-polar methods concentrate lipophilic terpenes) (Awotedu *et al.*, 2020), and assay conditions (calibration standard, blanking, incubation time) affect numerical outcomes. Taken together, the antioxidant findings indicate that the *E. camaldulensis* leaf extract contains appreciable electron-donating phytochemicals, as evidenced by its concentration-dependent radical-scavenging activity (DPPH), ferric ion-reducing capacity (FRAP), increasing total antioxidant capacity (TAC), and relatively high phenolic, flavonoid and tannin contents, which collectively highlight its redox activity, while highlighting the need for standardized extraction and reporting to enable more precise cross-study comparisons (Onukogu *et al.*, 2019; Huang *et al.*, 2022; Suganya *et al.*, 2024).

The extract exhibited concentration-dependent antimicrobial effects against six bacterial isolates (*S. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*) and two fungi (*C. albicans*, *Fusarium* sp.), with zones of inhibition increasing at higher doses. Pronounced susceptibility was noted for *P. mirabilis* and *K. pneumoniae*, moderate for *S. aureus* and *E. coli*, and limited for *S. typhi* and *P. aeruginosa*. MIC values ranged from 3.125 to 25 mg/mL, with MBC/MFC typically equal or higher, indicating bactericidal/fungicidal action at elevated concentrations. Compared to ciprofloxacin and ketoconazole, the extract was less potent, supporting its role as a complementary agent.

These findings corroborate extensive reviews showing *E. camaldulensis* extracts are active against Gram-positive and Gram-negative bacteria (MIC 0.08 $\mu\text{g/mL}$ -200 mg/mL) and fungi (0.125-1.0% for essential oils) (Sabo and Knezevic, 2019). Methanol extracts from Nigerian middle belt samples yielded comparable zones of inhibition

range with MICs ranging between 7.5–60 µg/mL against similar pathogens, with stronger effects on Gram-negatives like *K. pneumoniae*, mirroring this study's trend (Onukogu *et al.*, 2019). Similarly, another study on the ethanol extracts of the *E. camaldulensis* from another middle belt region in Nigeria found zones of inhibition at the highest test concentration in the range consistent with findings from this study; however, with MIC in the range of 100–200 mg/mL and no bactericidal activity observed at all test concentrations (Chuku *et al.*, 2016). However, lower MICs (0.391–25 µg/mL) in Saudi Arabia bark and leaf extracts suggest solvent and plant part differences enhance efficacy (Alghamdi and Ababutain, 2019). The extract exhibited antimicrobial activity across a zone of inhibition range of 0–16 mm for bacteria and 0–18 mm for fungi. Fungal activity was stronger against *Fusarium* sp. (MIC 3.125 mg/mL) than *C. albicans* (25 mg/mL). Chuku *et al.* (2016) reported a higher MIC of 50 mg/mL against *C. albicans*. Such differences may reflect variation in the solvents and extraction approaches used in other studies, as well as differences in plant provenance and fungal susceptibility *et al.* (Chuku *et al.*, 2016; Ghareeb *et al.*, 2018). In Nigeria, similar ethanol extracts inhibited *S. aureus* and *E. coli* at 25–50 mg/mL, but MBC ratios (1–2) indicate bactericidal consistency (Chuku *et al.*, 2016; Onukogu *et al.*, 2019). The broad-spectrum activity observed in this study may support further investigation of *E. camaldulensis* as a source of antimicrobial leads, including studies on resistant strains and potential adjunctive use with conventional antibiotics, in line with previous combined studies (Sabo and Knezevic, 2019).

In summary, the ethanol leaf extract from Nigerian *E. camaldulensis* exhibits a substantial phytochemical profile, moderate antioxidant capacity, and concentration-dependent antimicrobial effects, comparable to reports from other studies. Discrepancies highlight the need for standardized extraction protocols to harness its full therapeutic potential.

CONCLUSIONS

The screening and bioassay results indicate that *E. camaldulensis* ethanol leaf extract contains several classes of secondary metabolites and exhibits appreciable *in vitro* antioxidant and antimicrobial activities against the test organisms. Although the crude extract generally showed lower antimicrobial

potency than the reference drugs, it demonstrated comparable or superior activity against *Fusarium* sp. at the highest concentration tested, highlighting its potential as a source of bioactive antifungal constituents. These findings support the ethnopharmacological relevance of *E. camaldulensis* and justify further bioassay-guided fractionation, compound isolation, and mechanistic studies. Further work should focus on bioassay-guided fractionation and isolation of the active constituents, followed by re-evaluation of the purified fractions and compounds for antimicrobial potency, along with studies designed to elucidate their possible modes of action, such as membrane disruption or enzyme inhibition, and to assess any interaction with conventional antibiotics. Parallel studies should assess *in vivo* efficacy and safety, including acute toxicity, pharmacokinetics and simple infection models, to bridge *in vitro* activity and translational potential. Finally, extraction and reporting protocols should be standardised (solvent composition, solvent/solid ratio, temperature, harvest/processing metadata and chemotype information) to improve reproducibility, enable meaningful cross-study comparisons, and support development of standardised extract formulations for further preclinical evaluation.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest may influence the writing of this scientific manuscript.

AUTHORS' CONTRIBUTIONS

Sowunmi L.I.: Conceptualisation, Methodology, Investigation, Formal analysis, Review and Editing, and Supervision. Ogunbamowo P.O.: Conceptualisation, Methodology, Formal Analysis, Data Curation, Visualisation, Original Drafting, Review and Editing. Babalola Y.O.: Conceptualisation, Methodology, Investigation, Formal Analysis, Review and Editing. Oyeyinka B.O.: Methodology, Investigation, Formal analysis, Original Drafting, Review and Editing. Emmanuel I.B.: Methodology, Investigation, Formal Analysis,

Review and Editing. Afolabi O.: Methodology, Investigation, Formal Analysis, Review and Editing. All authors reviewed the article, discussion of results, and approved the final version for submission and publication.

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