

## Comparative Antibacterial Effects of Ethanolic Leaf Extracts of *Gambeya albida* and *Newbouldia laevis* on Selected Bacterial Isolates

Ogechukwu C. Dozie-Nwakile<sup>1</sup>, Calistus D. Nwakile<sup>2</sup>, Ebere A. Onyebueke<sup>1</sup>, Ferdinand C. Onwuka<sup>1</sup>, Oluchi M. Okeh<sup>1</sup>, Iniekong P. Udoh<sup>1</sup>, Aghatise K. Erhamwonyi<sup>3</sup>, Rebecca C. Edu<sup>1</sup>, Seto T. Aladenika<sup>1\*</sup>

<sup>1</sup>Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria; <sup>2</sup>Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria; <sup>3</sup>Department of Medical Laboratory Science, Igbinedion University, Okada, Edo State, Nigeria

\*Correspondence: [seto.aladenika@unn.edu.ng](mailto:seto.aladenika@unn.edu.ng); Tel: +2347031636363

Received 21 May, 2026

Revised 21 June, 2026

Accepted 10 July, 2026

Published 13 July, 2026

Copyright © 2026 the authors. This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. ISSN (online: 3093-0715; print: 3093-0723)

<https://doi.org/10.59493/advpbl/2026.1.4>

### Abstract

*Gambeya albida* and *Newbouldia laevis* are West African medicinal plants traditionally used to treat infectious diseases. This study comparatively evaluated the phytochemical profiles and antibacterial activity of ethanolic leaf extracts of both plants against five clinically sourced bacterial isolates (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas aeruginosa*). Quantitative phytochemical screening was performed on both extracts, while antibacterial activity was determined by agar well diffusion, with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determined by broth microdilution. Alkaloids, flavonoids, tannins, and saponins, were present in both plants at varying concentrations. *N. laevis* had higher steroids (44.61 mg/g) than alkaloids (12.66 mg/g) content, while *G. albida* had higher flavonoid (13.7 mg/100g) and tannins (2.56 g/100g) levels. *G. albida* extract produced the largest inhibition zone diameter (IZD) against *E. coli* (IZD = 18.5 mm at 100 mg/mL; MIC = 6.25 mg/mL; MBC = 12.5 mg/mL) and moderate activity against *S. aureus* (IZD = 10 mm; MIC = 50 mg/mL; MBC = 100 mg/mL). *N. laevis* extract inhibited only *E. coli* (IZD = 10 mm; MIC = 50 mg/mL; MBC = 100 mg/mL). Neither extract inhibited *Proteus* spp., *P. aeruginosa*, or *Klebsiella* spp. The difference in antibacterial activity against *E. coli* between the two extracts was statistically significant ( $p = 0.0002$  at 100 mg/mL;  $p = 0.0010$  at 50 mg/mL). These findings validate the traditional use of *G. albida* in managing bacterial infections and support its potential as a complementary antimicrobial agent.

**Keywords:** Antibacterial activity, Antimicrobial resistance, Bacterial infection, Inhibition zone diameter, Medicinal plants, Phytochemical analysis

### Introduction

Medicinal plants have historically been key sources of bioactive compounds with therapeutic value, especially for treating infectious diseases caused by pathogenic bacteria. Rising antimicrobial resistance (AMR) worldwide has spurred more research into plant extracts as a potential substitute or complements to conventional antibacterial drugs [1]. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas aeruginosa*—are among the most clinically significant pathogens. They frequently cause community and hospital infections and are showing growing resistance to conventional antibiotics [2].

*Escherichia coli* is a leading cause of urinary tract infections (UTIs) and gastroenteritis worldwide [3,4], and multidrug-resistant strains have increasingly been isolated from both clinical and environmental sources within Nigerian institutional settings [5] *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), is associated with skin infections, wound sepsis, pneumonia, and invasive disease [6]. *Klebsiella* spp. are opportunistic pathogens implicated in hospital-acquired pneumonia, bloodstream infections, and sepsis, with high morbidity particularly in intensive care settings [7]. *Proteus* spp. are frequently associated with complicated and catheter-related urinary tract infection, and they possess natural resistance to several antimicrobial drugs [8]. *Pseudomonas aeruginosa* is a highly

adaptable opportunistic pathogen known for forming biofilms and using multiple drug resistance mechanisms, which make infections hard to treat.

Native to West and Central Africa, *Gambeya albida*, formerly known as *Chrysophyllum albidum* (Udala; African star apple), is a tropical evergreen in the *Sapotaceae* family. The plant's leaves are a good source of tannins, flavonoids, saponins, alkaloids, and phenolic compounds, which contribute to significant activity against a range of bacterial pathogens [10, 11]. Ethanolic extracts from the leaves have shown the ability to inhibit bacteria like *E. coli* and *S. aureus*, which supports the plant's traditional medicinal use [12, 13]. The *Bignoniaceae* species *Newbouldia laevis* also called Ogilisi or boundary tree, has a long history of use in West African traditional medicine for conditions including epilepsy, malaria, diarrhoea, and infectious diseases. Phytochemical analysis has identified alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids in the plant [14]. Ethanolic leaf extracts have displayed inhibitory activity against organisms including *E. coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*, although reported minimum inhibitory concentrations are relatively high, indicating moderate antibacterial potency [1].

Previous studies have looked at *G. albida* and *N. laevis* separately, but none, to the best of our knowledge, from the Nigerian clinical setting has directly compared their ethanolic leaf extracts against the same set of clinical bacterial isolates. No work has assessed their MIC and MBC values under identical conditions and linked differences in activity to their phytochemical profiles. By using isolates from patients at a Nigerian tertiary hospital, this head-to-head study fills that gap in the regional ethnopharmacology literature and generates evidence relevant to the local burden of antimicrobial resistance. The aim was to compare the antibacterial activity of ethanolic leaf extracts from *G. albida* and *N. laevis* against selected clinical bacterial isolates, determine their phytochemical constituents and MIC/MBC values, and evaluate their potential as complementary antimicrobial agents in Nigeria.

## Materials and Methods

### Plant material collection and authentication

Fresh leaves of *Gambeya albida* (Udala) and *Newbouldia laevis* (Ogilisi) were collected from identified trees in Maryland, Enugu State, Nigeria (6.41836° N, 7.51923° E). Voucher specimens of *Gambeya albida* (UNN/13201) and *Newbouldia laevis* (UNN/13202) were deposited as herbarium sheets at the University of Nigeria Herbarium (UNN), authenticated by Felix I. Nwafor (Curator/Plant Taxonomist), on January 28, 2026.

### Sample processing and extract preparation

The harvested leaves were washed thoroughly with distilled water to remove surface contaminants and subsequently air-dried under shade at room temperature to preserve heat-sensitive phytochemical components. After drying, the leaves were ground into a fine powder using Binatone blender with a dry mill attachment (model BLG-452; Binatone Electrical Limited, Hong Kong, China) and stored in airtight containers until extraction.

Two hundred grams (200 g) of powdered leaf material from each plant was soaked separately in 1,000 mL of 95% ethanol for 24 hours with occasional stirring. Each mixture was subsequently filtered using Whatman No. 1 filter paper, and the filtrates were allowed to evaporate at room temperature to yield

crude extract residues. The residues were collected, weighed, transferred into sterile glass bottles, and stored at 4°C until use.

### Phytochemical analysis

Quantitative phytochemical analysis was performed on the crude extracts to estimate the concentrations of alkaloids, flavonoids, tannins, saponins, phenolic compounds, steroids, and terpenoids, following established standard methods [15, 16].

### Preparation of stock solutions

A stock solution of each crude extract was prepared by dissolving 1.0 g in 10 mL of dimethyl sulfoxide (DMSO) (DMSO; Cat. No. 00124, CAS 67-68-5; Loba Chemie Pvt. Ltd., Mumbai, India) to obtain a concentration of 100 mg/mL. Serial two-fold dilutions were prepared from each stock solution to give working concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL. 10% DMSO was also used as negative control.

### Bacterial isolates and identification

Five bacterial isolates from clinical samples, obtained from the Medical Microbiology Unit, University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, were used in this study: *Staphylococcus aureus*, *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*. All isolates were identified using standard microbiological procedures as described by Cheesbrough [17]. Colonial morphology on MacConkey agar and blood agar was recorded, followed by Gram staining. Biochemical characterization was performed using a battery of conventional tests including catalase, coagulase, oxidase, indole production, methyl red, Voges-Proskauer, citrate utilization, urease activity, and triple sugar iron (TSI) agar reactions, as appropriate for each organism.

### Antibacterial assay

Evaluation of antibacterial activity was done using the agar well diffusion method. Bacterial isolates were seeded on nutrient agar (TM 341; TM Media, Titan Biotech Ltd., Bhiwadi, India) and incubated at 37°C for 24 hours. A few isolated colonies were suspended in sterile normal saline and adjusted to a turbidity equivalent to the 0.5 McFarland standard ( $\sim 1.5 \times 10^8$  CFU/mL). The bacterial suspension was evenly spread over the surface of Mueller–Hinton agar (TM 339; TM Media, Titan Biotech Ltd., Bhiwadi, India) plates using sterile cotton swabs. Wells of 5 mm diameter were aseptically bored into the agar using a sterile cork borer, and 100  $\mu$ L of each extract concentration (100, 50, 25, 12.5, 6.25, and 3.125 mg/mL) was dispensed into the appropriately labelled wells. A 5 mm diameter disc of ciprofloxacin (5  $\mu$ g) was used as the positive control. Plates were pre-diffused at room temperature for 1 hour before incubation at 37°C for 24 hours. After incubation, inhibition zone diameter (IZD) around the wells was measured in millimetres using a calibrated ruler. An IZD  $\leq$  5 mm (equivalent to the well diameter) was recorded as no detectable inhibitory activity.

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was determined using the broth microdilution method. Two-fold serial dilutions of each extract were prepared in Mueller–Hinton broth (TM 325; TM Media, Titan Biotech Ltd., Bhiwadi, India) in 96-well microplates over a concentration range of 3.125–100 mg/mL. Bacterial suspensions (0.5 McFarland) were added to each well and incubated at 37°C for

24 hours. The lowest concentration yielding no visible turbidity was recorded as the MIC. To determine the MBC, aliquots from MIC wells and above were sub-cultured onto nutrient agar and incubated at 37°C for 24 hours; the lowest concentration yielding no bacterial growth was recorded as the MBC.

### Statistical analysis

All quantitative data were expressed as mean  $\pm$  standard deviation (SD) and analyzed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Differences in IZD across the six extract concentrations were analyzed using one-way analysis of variance (ANOVA), with Tukey's HSD test for post hoc pairwise comparisons. An independent-samples t-test compared IZD between the two extract against *E. coli* across all concentrations. A p-value of less than 0.05 was considered significant.

## Results

### Phytochemical composition of leaf extracts

Quantitative phytochemical analysis of the ethanolic leaf extracts from both plants revealed the presence of several bioactive constituents. Results for *G. albida* are shown in Table 1 and for *N. laevis* in Table 2. Both plants contained alkaloids, flavonoids, tannins, and saponins, though the concentrations varies considerably. *N. laevis* was markedly richer in steroids (44.61 mg/g) and alkaloids (12.66 mg/g), while *G. albida* had notably higher flavonoid content (13.7 mg/100g) and tannins (2.56 g/100g). These phytochemical differences likely underlie the differential antibacterial activity observed between the two plants.

### Antibacterial activity of *Gambeya albida* ethanolic extract

The ethanolic leaf extract of *G. albida* showed concentration-dependent antibacterial activity against the test isolates, as shown in Table 3. Highest inhibitory effect was observed against *E. coli*, with IZD values ranging from 18.5 mm at 100 mg/mL to 6.5 mm at 6.25 mg/mL, with no detectable inhibition at concentrations below 6.25 mg/mL. Moderate inhibitory activity was recorded against *S. aureus* (IZD = 10 mm

at 100 mg/mL; 7.5 mm at 50 mg/mL). No measurable inhibitory activity (IZD > 5 mm) was observed against *Proteus* spp., *Pseudomonas aeruginosa*, or *Klebsiella* spp. at any concentration tested.

### Antibacterial activity of *Newbouldia laevis* ethanolic extract

The ethanolic leaf extract of *N. laevis* demonstrated limited antibacterial activity (Table 4). Inhibitory effects were observed only against *E. coli* (IZD = 10 mm at 100 mg/mL; 8 mm at 50 mg/mL), with no measurable inhibition of the remaining four isolates at any concentration tested.

### MIC and MBC of *Gambeya albida* extract

MIC and MBC values were determined only for isolates that demonstrated detectable zones of inhibition in the agar well diffusion assay as presented in Table 5. The *G. albida* extract exhibited bacteriostatic activity (MIC = 50 mg/mL) and bactericidal activity (MBC = 100 mg/mL) against *S. aureus*. Against *E. coli*, markedly lower values were obtained (MIC = 6.25 mg/mL; MBC = 12.5 mg/mL), indicating potent bacteriostatic and bactericidal efficacy at relatively low concentrations. MIC and MBC were not determined for *Proteus* spp., *P. aeruginosa*, and *Klebsiella* spp., as no inhibitory activity was recorded against these organisms in the primary diffusion assay.

### MIC and MBC of *Newbouldia laevis* extract

For *N. laevis* extract, MIC and MBC were determined only for *E. coli*, the sole isolate that exhibited a detectable zone of inhibition in the diffusion assay (MIC = 50 mg/mL; MBC = 100 mg/mL). MIC and MBC testing were not performed for *S. aureus*, *Proteus* spp., *P. aeruginosa*, and *Klebsiella* spp., as none of these organisms showed inhibitory activity against *N. laevis* extract in the primary assay (Table 6).

### Comparative antibacterial activity against *Escherichia coli*

*Gambeya albida* extract consistently produced significantly larger zones of inhibition against *E. coli* than *N. laevis* extract at all tested concentrations (Table 7). This difference was statistically significant (independent-samples t-test: p = 0.031).

**Table 1. Quantitative phytochemical composition of the ethanolic leaf extract of *Gambeya albida***

| Phytochemical Component | Concentration |
|-------------------------|---------------|
| Alkaloids               | 3.44 mg/100g  |
| Saponins                | 3.97 mg/100g  |
| Flavonoids              | 13.7 mg/100g  |
| Phenolic compounds      | 2.65 mg/100g  |
| Tannins                 | 2.56 g/100g   |

**Table 2. Quantitative phytochemical composition of the ethanolic leaf extract of *Newbouldia laevis***

| Phytochemical Component | Concentration |
|-------------------------|---------------|
| Alkaloids               | 12.66 mg/g    |
| Flavonoids              | 17.42 mg/g    |
| Tannins                 | 0.91 mg/g     |

|            |            |
|------------|------------|
| Saponins   | 4.22 mg/g  |
| Steroids   | 44.61 mg/g |
| Terpenoids | 10.76 mg/g |

**Table 3.** Mean  $\pm$  standard deviation (SD) of inhibition zone diameter (IZD, mm) of *G. albida* ethanolic leaf extract against selected bacterial isolates

| Isolate                | 100 mg/ml       | 50 mg/ml        | 25 mg/ml        | 12.5 mg/ml     | 6.25 mg/ml     | 3.125 mg/ml | CIP†            |
|------------------------|-----------------|-----------------|-----------------|----------------|----------------|-------------|-----------------|
| <i>S. aureus</i>       | 10.0 $\pm$ 1.00 | 7.5 $\pm$ 0.50  | ND*             | ND*            | ND*            | ND*         | 39.5 $\pm$ 0.50 |
| <i>E. coli</i>         | 18.5 $\pm$ 0.50 | 15.0 $\pm$ 1.00 | 10.0 $\pm$ 1.00 | 8.0 $\pm$ 1.00 | 6.5 $\pm$ 0.50 | ND*         | 57.0 $\pm$ 1.00 |
| <i>Proteus</i> spp.    | ND*             | ND*             | ND*             | ND*            | ND*            | ND*         | 42.5 $\pm$ 0.50 |
| <i>P. aeruginosa</i>   | ND*             | ND*             | ND*             | ND*            | ND*            | ND*         | 36.5 $\pm$ 0.50 |
| <i>Klebsiella</i> spp. | ND*             | ND*             | ND*             | ND*            | ND*            | ND*         | 47.3 $\pm$ 0.58 |

Concentration values in mg/ml. CIP = Ciprofloxacin disc (5  $\mu$ g), positive control. \*ND = No detectable inhibition (IZD  $\leq$  5 mm, equivalent to well diameter).

**Table 4.** Mean  $\pm$  standard deviation (SD) of inhibition zone diameter (IZD, mm) of *N. laevis* ethanolic leaf extract against selected bacterial isolates

| Isolate                | 100 mg/ml       | 50 mg/ml       | 25 mg/ml | 12.5 mg/ml | 6.25 mg/ml | 3.125 mg/ml | CIP†            |
|------------------------|-----------------|----------------|----------|------------|------------|-------------|-----------------|
| <i>S. aureus</i>       | ND*             | ND*            | ND*      | ND*        | ND*        | ND*         | 39.5 $\pm$ 0.50 |
| <i>E. coli</i>         | 10.0 $\pm$ 1.00 | 8.0 $\pm$ 1.00 | ND*      | ND*        | ND*        | ND*         | 57.0 $\pm$ 1.00 |
| <i>Proteus</i> spp.    | ND*             | ND*            | ND*      | ND*        | ND*        | ND*         | 42.5 $\pm$ 0.50 |
| <i>P. aeruginosa</i>   | ND*             | ND*            | ND*      | ND*        | ND*        | ND*         | 36.5 $\pm$ 0.50 |
| <i>Klebsiella</i> spp. | ND*             | ND*            | ND*      | ND*        | ND*        | ND*         | 47.3 $\pm$ 0.58 |

Concentration values in mg/mL. †CIP = Ciprofloxacin disc (5  $\mu$ g), positive control. \*ND = No detectable inhibition (IZD  $\leq$  5 mm, equivalent to well diameter).

**Table 5.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *G. albida* ethanolic leaf extract

| Isolate                | MIC (mg/mL) | MBC (mg/mL) |
|------------------------|-------------|-------------|
| <i>S. aureus</i>       | 50          | 100         |
| <i>E. coli</i>         | 6.25        | 12.5        |
| <i>Proteus</i> spp.    | ND†         | ND†         |
| <i>P. aeruginosa</i>   | ND†         | ND†         |
| <i>Klebsiella</i> spp. | ND†         | ND†         |

†ND = Not determined; MIC/MBC testing was not performed for organisms showing no inhibition zone diameter (IZD  $\leq$  5 mm) in the agar well diffusion assay. MIC and MBC values were consistent across all three replicates.

**Table 6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *N. laevis* ethanolic leaf extract**

| Isolate                | MIC (mg/mL) | MBC (mg/mL) |
|------------------------|-------------|-------------|
| <i>S. aureus</i>       | ND†         | ND†         |
| <i>E. coli</i>         | 50          | 100         |
| <i>Proteus</i> spp.    | ND†         | ND†         |
| <i>P. aeruginosa</i>   | ND†         | ND†         |
| <i>Klebsiella</i> spp. | ND†         | ND†         |

†ND = Not determined; MIC/MBC testing was not performed for organisms showing no inhibition zone diameter (IZD ≤ 5 mm) in the agar well diffusion assay. MIC and MBC values were consistent across all three replicates.

**Table 7. Comparative inhibition zone diameter (IZD, mean ± SD, mm) of *Gambeya albida* and *Newbouldia laevis* ethanolic extracts against *Escherichia coli***

| Concentration (mg/mL) | <i>Gambeya albida</i> IZD (mm) | <i>Newbouldia laevis</i> IZD (mm) |
|-----------------------|--------------------------------|-----------------------------------|
| 100                   | 18.5 ± 0.50                    | 10.0 ± 1.00                       |
| 50                    | 15.0 ± 1.00                    | 8.0 ± 1.00                        |
| 25                    | 10.0 ± 1.00                    | ND*                               |
| 12.5                  | 8.0 ± 1.00                     | ND*                               |
| 6.25                  | 6.5 ± 0.50                     | ND*                               |
| 3.125                 | ND*                            | ND*                               |
| CIP† (5 µg disc)      | 57.0 ± 1.00                    | 57.0 ± 1.00                       |

†CIP = Ciprofloxacin disc (5 µg), positive control. \*ND = No detectable inhibition. Independent-samples t-test:  $p = 0.0002$  at 100 mg/mL;  $p = 0.0010$  at 50 mg/mL. Statistical comparison was not applicable at concentrations below 50 mg/mL as *N. laevis* showed no detectable inhibitory activity.

## Discussion

This study provides, to the best of our knowledge, the first direct comparison of the antibacterial activity of ethanolic leaf extracts of *Gambeya albida* and *Newbouldia laevis* against a panel of clinically sourced bacterial isolates from a Nigerian tertiary-care setting, with concurrent phytochemical profiling and MIC/MBC determination under identical experimental conditions. Both extracts showed concentration-dependent antibacterial effects, and the differences in inhibition patterns between them can be reasonably explained by their distinct phytochemical constituents.

The phytochemical screening of this *G. albida* revealed a relatively high flavonoid content and tannin concentration, along with alkaloids and saponins; this was similar to results of a previous study [18]. Flavonoids are known to exert antibacterial effects through disruption of cell membrane integrity, inhibition of nucleic acid synthesis, and suppression of efflux pump activity; tannins act by forming irreversible complexes with bacterial enzymes and cell wall proteins; alkaloids interfere with DNA intercalation; and saponins increase membrane permeability through complex formation with lipid bilayer components [19]. The leaf extract of *G. albida*

demonstrated the strongest antibacterial activity, particularly against *E. coli* and *S. aureus*. The pronounced susceptibility of *E. coli* and *S. aureus* observed in this study aligns with recent findings by Ujam *et al.* (2024) [20]. The combined presence of these phytochemicals likely accounts for the pronounced inhibitory activity observed against *E. coli*. The relatively lower activity against *S. aureus* compared to *E. coli* may reflect differences in cell wall composition: Gram-negative organisms such as *E. coli* possess an outer lipopolysaccharide membrane that may be more susceptible to membrane-disrupting polyphenolic compounds, while the thick peptidoglycan layer of Gram-positive organisms like *S. aureus* may confer some degree of protection against certain phytochemical classes [21]. Flavonoids and tannins, the phytochemical classes most likely to be strongly associated with the antibacterial activity observed in this study, have also been reported to exert other significant bioactivities—including cytotoxic and genotoxic effects—in extracts of other Nigerian plants [22].

In contrast, *N. laevis* extract exhibited comparatively weaker antibacterial activity, with measurable inhibition limited to *E. coli* at the two highest concentrations (IZD = 10 mm at 100 mg/mL; 8 mm at 50 mg/mL). Despite its high steroid (44.61 mg/g) and alkaloid (12.66 mg/g) content, the notably low tannin concentration (0.91 mg/g) compared to *G. albida* (2.56 g/100g) may partly explain its limited spectrum of activity, given

the well-established role of tannins in broad-spectrum antibacterial action through protein precipitation and cell wall disruption [19]. These findings are consistent with earlier reports of only mild to moderate antibacterial activity of *N. laevis* leaf extracts [1] and suggest that, while the plant contains potentially bioactive phytochemicals, their concentrations or synergistic interactions in the crude ethanolic extract may be insufficient for broad-spectrum inhibition.

MIC and MBC were determined only for isolates that demonstrated inhibitory activity in the primary agar well diffusion assay, as it is scientifically unjustifiable to establish minimum inhibitory concentrations for organisms against which no inhibition was recorded. For *G. albida* extract, bacteriostatic activity was recorded at MIC = 6.25 mg/mL and bactericidal activity at MBC = 12.5 mg/mL against *E. coli*, indicating meaningful efficacy at relatively low concentrations. Against *S. aureus*, MIC and MBC values of 50 and 100 mg/mL reflect moderate activity. The absence of inhibitory activity against *Proteus* spp., *P. aeruginosa*, and *Klebsiella* spp. by both extracts is consistent with the well-recognised intrinsic resistance mechanisms of these Gram-negative organisms, including reduced outer membrane permeability, constitutive efflux pump overexpression, and enzymatic degradation of antimicrobial compounds [8, 9].

For *N. laevis* extract, MIC and MBC values were determined only for *E. coli* (MIC = 50 mg/mL; MBC = 100 mg/mL), which are notably higher than the corresponding values for *G. albida* (MIC = 6.25 mg/mL; MBC = 12.5 mg/mL). This eight-fold difference in MIC further underscores the superior bacteriostatic potency of *G. albida* extract. One-way ANOVA confirmed a statistically significant concentration-dependent increase in IZD for both extracts against susceptible organisms ( $p < 0.05$ ), and the independent-samples *t*-test confirmed that *G. albida* produced significantly larger inhibition zones against *E. coli* than *N. laevis* at all tested concentrations ( $p = 0.031$ ).

Although ciprofloxacin produced markedly larger zones of inhibition than either plant extract, this comparison should be interpreted in context: ciprofloxacin is a synthetically optimised fluoroquinolone, whereas the plant extracts tested here are crude, unfractionated preparations. The observation that a crude ethanolic extract of *G. albida* achieved a MIC of 6.25 mg/mL against *E. coli*—a clinically relevant uropathogen—is promising and warrants further investigation. The progressive increase in antibiotic resistance among Nigerian clinical isolates, particularly in settings with limited diagnostic infrastructure and access to second-line antibiotics, lends additional relevance to findings of this nature [2]. Bioassay-guided fractionation to isolate active constituents, elucidation of mechanisms of action, cytotoxicity profiling, and in vivo pharmacological evaluation will be necessary steps before any therapeutic application can be considered.

## Conclusion

This study demonstrates, to the best of our knowledge for the first time, that the ethanolic leaf extract of *Gambeya albida* exhibits greater antibacterial activity than that of *Newbouldia laevis* against Nigerian clinical isolates evaluated under identical experimental conditions. Both extracts exhibited concentration-dependent activity, with *G. albida* inhibiting both *Escherichia coli* and *Staphylococcus aureus*, and *N. laevis* inhibiting only *E. coli*. The superior activity of *G. albida* is consistent with its higher flavonoid and tannin content. Neither extract inhibited *Proteus*

spp., *Pseudomonas aeruginosa*, or *Klebsiella* spp. at the tested concentrations, suggesting limited efficacy of these extracts against these Gram-negative organisms under the conditions tested. These findings validate the traditional use of *G. albida* in infectious disease management and establish a phytochemical basis for its differential activity. Future studies should focus on bioassay-guided fractionation, mechanism-of-action studies, cytotoxicity profiling, and in vivo evaluation of the most active fractions.

## Declarations

### Conflict of interest

The authors declare no conflict of interest.

### Authors' contributions

OCD, RCE: research concept and design; OMO, FCO: laboratory analyses; CDN, STA: Data analysis and interpretation; OCD, STA, AKE: writing the article. EAO and IPU read and approved the final manuscript.

### Funding

This work did not receive any external funding.

### Ethical approval

Ethical approval was not required for this study as it involved no human participants and no experimental animals. Bacterial isolates were obtained from routine clinical specimens collected during standard microbiological practice.

## References

1. Nwachukwu MI, Duru MKC, Nwachukwu IO, Obasi CC, Uzoechi AU, Ezenwa CM, Anumodu CK, 2017. In-vitro phytochemical characterization and antibacterial activity of *Newbouldia laevis* (boundary tree) on *Escherichia Coli* and *Staphylococcus Aureus*. Asian Journal of Microbiology and Biotechnology, 2 (1):30-36
2. World Health Organization, 2023. Global antimicrobial resistance and use surveillance system (GLASS) report. Geneva: WHO Press.
3. Kaper JB, Nataro JP, Mobley HLT, 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology, 2(2):123–140.
4. Whelan S, Lucey B, Finn K, 2023. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. Microorganisms, 11(9):2169.
5. Osuala OJ, Maduakor JG, Ezemba CC, Etikudike VO, Ike VE, Nnadi CO, Oli AN, 2025. Isolation and characterisation of carbapenemase-producing *Escherichia coli* isolates from fomites in Madonna University, Elele Campus, Rivers State, Nigeria. Advanced Pharmaceutical and Biomedical Letters, 2(1):27–32.
6. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG, 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3):603–661.
7. Paczosa MK, Mecsas J, 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense.

- Microbiology and Molecular Biology Reviews, 80(3):629–661.
8. Wasfi R, Hamed SM, Amer MA, Fahmy LI, 2020. *Proteus mirabilis* biofilm: development and therapeutic strategies. *Frontiers in Cellular and Infection Microbiology*, 10:414.
  9. Moradali MF, Ghods S, Rehm BHA, 2017. *Pseudomonas aeruginosa* lifestyle: a paradigm for adaptation, survival, and persistence. *Frontiers in Cellular and Infection Microbiology*, 7:39.
  10. Odewade JO, Odewade LO, 2023. Evaluation of antibacterial and antioxidant properties of leaf extract of *Chrysophyllum albidum* against selected enteric bacterial pathogens. *FUDMA Journal of Sciences*, 7(2):131–140.
  11. Adonu C, Ali I, Omeh R, 2023. Phytochemical screening and antibacterial activity of *Chrysophyllum albidum* collected in Nsukka, South East Nigeria. *European Journal of Medicinal Plants*, 34(6):13-22.
  12. Azih I, Ijezie M, Ugariogu SN, Akalezi CO, 2020. Antibacterial activity and identification of metabolites from the semi-purified fraction of *Chrysophyllum albidum* leaf (African star apple). *Tropical Journal of Natural Product Research*, 4(7):262–269.
  13. Nartey D, Gyesi JN, Borquaye LS, 2021. Chemical composition and biological activities of the essential oils of *Chrysophyllum albidum* G. Don (African star apple). *Biochemistry Research International*, (1):9911713.
  14. Anaduaka EG, Ogugua VN, Egba SI, Apeh VO, 2013. Investigation of some important phytochemical, nutritional properties and toxicological potentials of ethanol extracts of *Newbouldia laevis* leaf and stem. *African Journal of Biotechnology*, 12(40):5941-5949
  15. Harborne AJ, 1998. *Phytochemical methods a guide to modern techniques of plant analysis*, 3rd ed., Chapman and Hall Ltd: London.
  16. Trease GE, Evans WC, 2002. *Pharmacognosy*. 15th ed. Saunders Publishers: London.
  17. Cheesbrough M, 2006. *District laboratory practice in tropical countries*, Part 2. 2nd ed. Cambridge University Press: Cambridge.
  18. Dike MC, Nnamdi EA, 2012. Comparative study of proximate, phytochemical and mineral compositions of edible plant fruits/seeds from Nigerian rainforest. *International Journal of Biological and Chemical Sciences*, 6(4): 1905-1909.
  19. Jubair N, Rajagopal M, Chinnappan S, Abdullah NR, Fatima A, 2021. Review on the antibacterial mechanism of plant-derived compounds against multidrug-resistant bacteria. *Evidence-Based Complementary and Alternative Medicine*, 2021:3663315.
  20. Ujam NT, Nwobodo VC, Adonu CC, Ali I, Okorie NH, Ejikeugwu CE, Ajaegbu EE, 2024. The phytochemical screening, antibacterial and antipyretic properties of extracts of *Chrysophyllum albidum* leaves. *International Journal of Research and Innovation in Applied Science*, 9(3):461-472.
  21. Akinpelu DA, Odewade JO, Aiyegboro OA, Ashafa AOT, Akinpelu OF, Agunbiade MO, 2016. Biocidal effects of stem bark extract of *Chrysophyllum albidum* G. Don on vancomycin-resistant *Staphylococcus aureus*. *BMC Complementary and Alternative Medicine*, 16:105
  22. Andrew UE, Mbanefo F, Osigwe CC, Okokon JE, Uwaeme UF, Johnny II, 2025. Evaluation of genotoxic and cytotoxic activities of stem extract of *Telfairia occidentalis*. *Advanced Pharmaceutical and Biomedical Letters*, 2(1):20–26.