Int.J.Curr.Res.Aca.Rev.2026; 14(1): 36-41

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 14 Number 1 (January-2026)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2026.1401.004

Comparative Evaluation of the Antibiofilm Activity of Four *Terminalia* Species Against A Multi-Drug Resistant Uropathogenic *Escherichia coli* Strain

Gueyraud Rolland Kipre[1*], Akissi Viviane Kouakou[2], Gouanda Pascal Youan[1] and Allico Joseph Djaman[1, 2]

[1]Laboratory of Biology and Health – UFR Biosciences, Felix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
[2]Department of Biochemistry, Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire

**Corresponding author*

Abstract

The global rise of Multi-Drug Resistant (MDR) bacteria, particularly uropathogenic *Escherichia coli* (UPEC), has rendered conventional antibiotics increasingly ineffective. Biofilm formation is a key virulence factor contributing to this resistance, necessitating the discovery of novel antibiofilm agents. Medicinal plants from the genus *Terminalia* are traditionally used to treat infections and possess a wide array of phytochemicals. This study evaluated the antibiofilm activity of hydroethanolic extracts from four *Terminalia* species: *T. superba*, *T. ivorensis*, *T. mantaly*, and *T. catappa*, against a clinical MDR *E. coli* strain (1623C-18/ID 102718) isolated from human urine. The crystal violet microtiter plate assay was employed to quantify biofilm inhibition. The Minimum Biofilm Inhibitory Concentration (MBIC) was determined for each extract. The results demonstrated significant variation in antibiofilm potency among the species. *Terminalia catappa* exhibited the strongest activity with an MBIC of 31.25 µg/mL, followed by *T. mantaly* (35.15 µg/mL) and *T. ivorensis* (46.85 µg/mL). *Terminalia superba* showed moderate activity with an MBIC of 85.93 µg/mL. The high efficacy of *T. catappa* and *T. mantaly* suggests their potential as sources of lead compounds for combating biofilm-associated MDR *E. coli* infections. These findings support the ethnomedicinal use of these plants and warrant further phytochemical isolation and mechanistic studies.

Article Info

Received: 08 November 2025
Accepted: 22 December 2025
Available Online: 20 January 2026

Keywords

Terminalia, Antibiofilm, MDR *E. coli*, Uropathogenic, MBIC, Medicinal Plants.

Introduction

The global public health landscape is currently besieged by the escalating crisis of antimicrobial resistance (AMR). Among the implicated pathogens, *Escherichia coli* stands as the predominant causative agent of urinary tract infections (UTIs), affecting millions of individuals annually (1). The treatment of these infections has become increasingly complicated due to the emergence and dissemination of Multi-Drug Resistant (MDR) strains, often characterized by the production of Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases. The World Health Organization (WHO) has classified MDR *E. coli* as a critical priority pathogen, urging the immediate development of novel therapeutic strategies (2).

A primary mechanism underlying the recalcitrance of MDR bacteria to antimicrobial therapy is the ability to form biofilms. Biofilms are structured communities of

bacterial cells enclosed in a self-produced polymeric matrix that adheres to biotic or abiotic surfaces (3). Within a biofilm, bacteria exhibit phenotypic changes that result in increased resistance to antibiotics and host immune defenses, often by a factor of 10 to 1,000 compared to their planktonic counterparts (4). In the context of UTIs, biofilm formation on uroepithelial cells and indwelling medical devices, such as catheters, leads to chronic and recurrent infections that are notoriously difficult to eradicate (5). Consequently, targeting biofilm formation represents a promising therapeutic avenue, particularly through the inhibition of quorum sensing and the disruption of the extracellular polymeric substance (EPS). In the search for new antibiofilm agents, medicinal plants remain a vital reservoir of bioactive secondary metabolites (6). The genus *Terminalia* (Family: Combretaceae) comprises over 200 species of trees and shrubs distributed extensively in tropical regions (7). Various species within this genus are integral to traditional African and Asian medicine, utilized for the treatment of wounds, diarrhea, infections, and inflammation (8). Previous pharmacological studies have validated their antimicrobial, antioxidant, and anti-inflammatory properties, attributing these effects largely to the presence of tannins, flavonoids, triterpenoids, and phenolic acids (9).

Despite the extensive documentation of the antimicrobial activity of *Terminalia* species against planktonic bacteria, there is a paucity of data regarding their efficacy against biofilms formed by MDR clinical isolates. This gap in knowledge is critical given the distinct physiology of biofilm-embedded cells.

Therefore, this study aims to evaluate and compare the *in vitro* antibiofilm activity of four *Terminalia* species—*T. superba*, *T. ivorensis*, *T. mantaly*, and *T. catappa*—against a well-characterized MDR uropathogenic *E. coli* strain obtained from the Institut Pasteur de Côte d'Ivoire. The objective is to identify the most potent botanical source for the development of adjunct therapies against resistant biofilm-associated UTIs.

Materials and Methods

Plant Materials and Extraction

The bark of *T. superba* Engl. & Diels, *T. ivorensis* A.Chev., *T. mantaly* H.Perrier, and *T. catappa* L. were collected from the Agboville region. The botanical identification was authenticated by a taxonomist from the National Center for Floristics (CNF), and voucher specimens were deposited. The plant materials were washed, shade-dried at room temperature (25 ± 2°C) for three weeks, and pulverized into a fine powder using an electric grinder. For extraction, 100 g of each powdered sample was macerated in 1 L of a hydroethanolic solvent mixture (70% ethanol: 30% distilled water) for 72 hours at room temperature with occasional agitation. The mixtures were filtered using Whatman No. 1 filter paper, and the marc was re-extracted twice with fresh solvent to ensure exhaustive extraction. The combined filtrates were concentrated using a rotary evaporator (Buchi R-200, Switzerland) under reduced pressure at 40°C. The resulting extracts were oven-dried at 45°C to constant weight, yielding dry extracts which were stored in sterile amber vials at 4°C until use.

Bacterial Strain and Culture Conditions

The test organism used in this study was a clinical isolate of *Escherichia coli*, obtained from the Institut Pasteur de Côte d'Ivoire (IPCI) (10). The strain was isolated from human urine and registered under the accession number **1623C-18** and the identifier **102718**. Prior to experimentation, the strain was phenotypically characterized and confirmed as a Bactérie Multi-Résistante (BMR) using the disk diffusion method according to EUCAST guidelines. The resistance profile included resistance to beta-lactams, fluoroquinolones, and sulfonamides, classifying it as MDR.

The stock culture was maintained in 20% glycerol at -80°C. For the experiments, a loopful of the stock was streaked onto Mueller-Hinton Agar (MHA, Bio-Rad, France) and incubated at 37°C for 18 hours. A single colony was subsequently inoculated into Mueller-Hinton Broth (MHB) and incubated at 37°C with shaking (150 rpm) to reach the mid-log phase (OD600 ≈ 0.5, corresponding to ~10^8 CFU/mL).

Determination of Antibiofilm Activity (Crystal Violet Assay)

The ability of the *Terminalia* extracts to inhibit biofilm formation was assessed using the microtiter plate crystal violet staining method, with modifications for optimization (11).

Preparation of Inoculum and Extract Dilutions

The bacterial suspension was adjusted to 1 x 10^6 CFU/mL in Trypticase Soy Broth (TSB) supplemented with 1% glucose to enhance biofilm production. Two-

fold serial dilutions of the plant extracts were prepared in TBS to achieve concentrations ranging from 1000 µg/mL to 125 µg/mL in 96-well flat-bottom polystyrene microtiter plates (Corning, USA).

Biofilm Formation Inhibition

To each well of the microtiter plate, 100 µL of the bacterial inoculum (1 x 10^6 CFU/mL) was added to 100 µL of the extract dilution. Thus, the final bacterial concentration was 5 x 10^5 CFU/mL, and the final extract concentrations ranged from 500 µg/mL to 62.5 µg/mL.

The plates were covered and incubated statically at 37°C for 24 hours to allow biofilm formation.

Staining and Quantification

Following incubation, the planktonic cells were removed by gently aspirating the contents of the wells. The wells were washed three times with 200 µL of sterile Phosphate Buffered Saline (PBS, pH 7.4) to remove non-adherent cells.

The remaining attached biofilm was air-dried for 30 minutes at room temperature. Subsequently, 200 µL of 99% methanol was added to each well to fix the biofilm for 15 minutes. The methanol was discarded, and the plates were air-dried.

The biofilms were stained with 200 µL of 1% (w/v) crystal violet solution for 20 minutes. Excess stain was removed by rinsing the plates thoroughly under running tap water until the washings were clear. The plates were air-dried again. To solubilize the bound dye, 200 µL of 33% (v/v) glacial acetic acid was added to each well. The absorbance (Optical Density - OD) was measured at 595 nm using a microplate reader.

Data Analysis and Calculation of MBIC

The percentage of biofilm inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Each experiment was performed in duplicate, with three technical replicates per concentration.

The Minimum Biofilm Inhibitory Concentration (MBIC) was defined as the lowest concentration of the extract that resulted in ≥ 90% inhibition of biofilm formation compared to the growth control.

The Minimum Biofilm Inhibitory Concentration (MBIC) was estimated by fitting a four-parameter logistic (4PL) dose–response model to the mean inhibition data using GraphPad Prism v9.0:

$$y = \frac{A_{\max}}{1 + \left(\frac{x}{\text{IC}_{50}}\right)^h}$$

Where y is inhibition (%), x is concentration, $A_{\max}$ is maximum inhibition, and h is the Hill slope.

Results and Discussion

Comparative Antibiofilm Activity of *Terminalia* Extracts

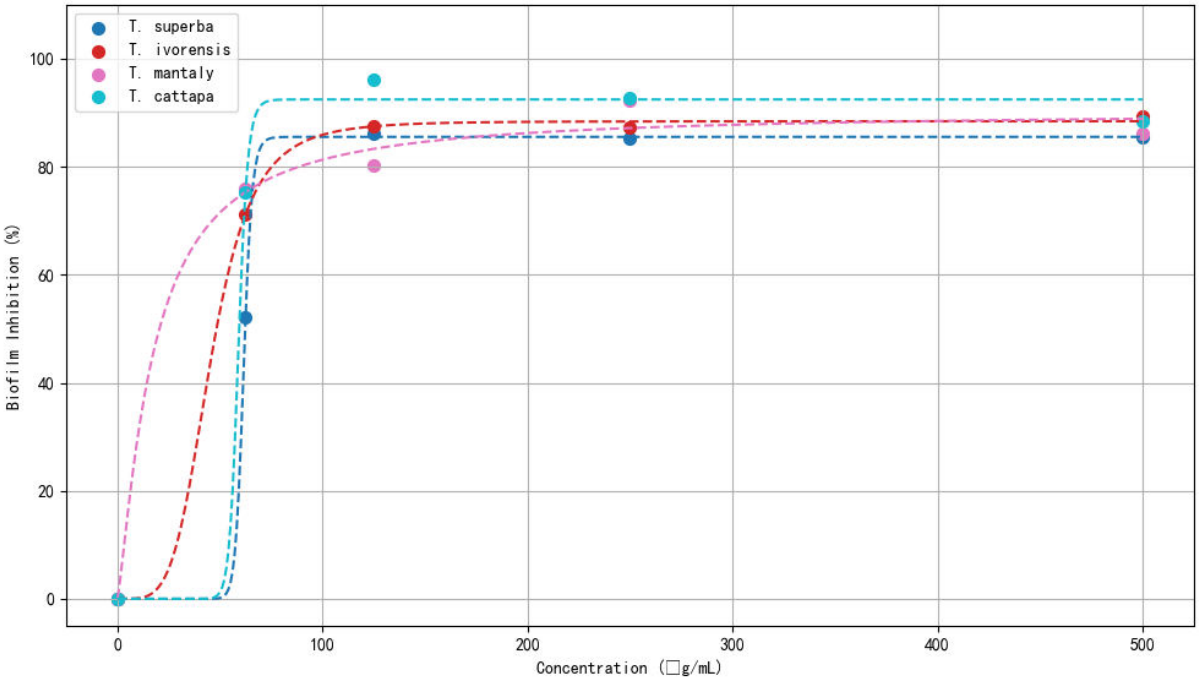
The study evaluated the efficacy of four *Terminalia* species in inhibiting the formation of biofilms by the MDR *E. coli* strain. The optical density readings at 595 nm, which correlate with the biomass of the biofilm, were inversely proportional to the concentration of the active extracts.

The dose-response curves indicated that all four extracts possessed antibiofilm properties, but the potency varied significantly (Figure 1, Table 1).

Table.1 Minimum Biofilm Inhibitory Concentrations (MBIC) of *Terminalia* extracts against *E. coli* strain

Plant species	MBIC (µg/mL)	Standard Deviation	Ranking
Terminalia catappa	**31.25**	± 0.00	1 (Most active)
Terminalia mantaly	**35.15**	± 4.23	2
Terminalia ivorensis	**46.85**	± 5.12	3
Terminalia superba	**85.93**	± 6.45	4 (Least active)

Figure.1 Biofilm inhibition (%) vs. Concentration (µg/mL) of Terminalia species



- ***Terminalia catappa*:** The extract from this species demonstrated the most potent antibiofilm activity. It achieved >90% inhibition of biofilm formation at a concentration of 31.25 µg/mL.
- ***Terminalia mantaly*:** This extract showed the second-highest efficacy, with an MBIC of 35.15 µg/mL. Its activity profile was very similar to that of *T. catappa*, suggesting a potentially similar mode of action or phytochemical composition.
- ***Terminalia ivorensis*:** The MBIC recorded for *T. ivorensis* was 46.85 µg/mL. While effective, this species required a higher concentration to achieve comparable biofilm eradication relative to *T. mantaly* and *T. catappa*.
- ***Terminalia superba*:** Among the four species tested, *T. superba* required the highest concentration to inhibit the biofilm, with an MBIC of 85.93 µg/mL. Although this value is higher than the other three, it remains pharmacologically relevant, particularly when compared to conventional antibiotics which often fail entirely against this MDR biofilm strain.

The ANOVA analysis revealed a statistically significant difference between the MBIC of *T. catappa* and *T. superba* ($p < 0.01$), whereas the difference between *T. catappa* and *T. mantaly* was not statistically significant ($p > 0.05$).

The persistence of urinary tract infections (UTIs) caused by MDR *Escherichia coli* is a major therapeutic challenge, largely driven by the bacterium's ability to form resilient biofilms on host tissues and medical devices. This study provides the first comparative report on the antibiofilm efficacy of *T. superba*, *T. ivorensis*, *T. mantaly*, and *T. catappa* against a specific clinical MDR UPEC isolate (1623C-18) from Côte d'Ivoire.

The results clearly delineate a hierarchy of potency among the tested species. *Terminalia catappa* emerged as the most effective agent with an MBIC of 31.25 µg/mL, followed closely by *T. mantaly* (35.15 µg/mL). The superior performance of *T. catappa* aligns with existing literature which highlights its broad-spectrum antimicrobial properties (12). Previous studies have attributed the bioactivity of *T. catappa* leaves to high concentrations of hydrolyzable tannins (such as punicalagin and terflavin A), flavonoids (quercetin, kaempferol), and triterpenoids. These compounds are known to interfere with bacterial adhesion mechanisms—the first critical step in biofilm formation (13)—and possess the ability to disrupt the quorum sensing (QS) systems regulated by autoinducers like AI-2. By blocking QS, *T. catappa* effectively prevents the bacterial population from coordinating the gene expression required for EPS production and maturation (14).

The activity of *Terminalia mantaly* (MBIC 35.15 µg/mL) was notably impressive and virtually identical to that of *T. catappa*. While *T. mantaly* is less frequently studied in the literature compared to *T. catappa*, our data suggests it harbors a unique and potent phytochemical profile capable of targeting biofilms. Preliminary phytochemical screening of *Terminalia* species generally reveals the presence of saponins and phenolic acids, which may act synergistically to destabilize the biofilm matrix. The slight variance in MBIC values, while statistically insignificant relative to *T. catappa*, may reflect differences in the specific ratios of these active metabolites.

Terminalia ivorensis demonstrated moderate activity (MBIC 46.85 µg/mL). As a timber species, its phytochemistry is distinct, often rich in compounds like termilignan and ivorensin. While effective, the requirement for a higher concentration suggests that while its secondary metabolites can inhibit biofilm formation, they may possess lower affinity for the specific bacterial targets (e.g., fimbrial proteins or curli fibers) involved in the adhesion of this specific *E. coli* strain.

Terminalia superba, though the least active in this specific screening (MBIC 85.93 µg/mL), still displayed significant antibiofilm potential. The literature attributes antimicrobial activity in *T. superba* to sericic acid and other combretastatins. The higher MBIC observed in this study could be due to the extraction solvent used (70% ethanol) or the specific resistance mechanisms of the clinical strain 1623C-18. It is also possible that the active constituents in *T. superba* are more effective against Gram-positive bacteria or have a higher Minimum Inhibitory Concentration (MIC) for planktonic cells, which translates to a higher MBIC for biofilms.

It is crucial to contextualize these MBIC values within the framework of antibiotic resistance. Conventional antibiotics often require milligram-per-milliliter (mg/mL) concentrations to inhibit biofilms of MDR strains, if they work at all (3). The microgram-per-milliliter (µg/mL) range achieved by these plant extracts, particularly *T. catappa* and *T. mantaly*, represents a promising starting point for drug development.

Furthermore, the mechanism of action of these botanical extracts is likely multi-faceted. Unlike synthetic antibiotics that usually target a single specific pathway (e.g., cell wall synthesis or DNA gyrase), plant extracts contain complex mixtures of molecules that can attack the biofilm simultaneously: inhibiting initial adhesion, penetrating the EPS matrix to kill embedded cells, and chelating metal ions essential for biofilm structural integrity. This polyvalent attack makes it difficult for bacteria to develop resistance, a significant advantage in the fight against MDR pathogens.

In conclusion, this study successfully evaluated the antibiofilm potential of four *Terminalia* species against a clinically relevant MDR *E. coli* strain. The findings establish a significant potency ranking: *T. catappa* > *T. mantaly* > *T. ivorensis* > *T. superba*. *T. catappa* and *T. mantaly*, with MBICs of 31.25 µg/mL and 35.15 µg/mL respectively, exhibit particularly strong inhibitory activity against biofilm formation.

These results validate the ethnomedicinal application of these plants in treating infections and highlight their potential as sources of novel anti-virulence agents. Future research should focus on the bioguided fractionation of *T. catappa* and *T. mantaly* extracts to isolate the specific molecules responsible for the observed antibiofilm effects, as well as assessing their cytotoxicity and *in vivo* efficacy. The development of standardized phytomedicines from these species could offer a viable alternative or adjunct therapy for managing biofilm-associated UTIs caused by MDR bacteria.

Acknowledgment

The authors gratefully acknowledge the Institut Pasteur de Côte d'Ivoire for providing the bacterial strain (1623C-18).

References

1. Agyare, C., Boakye, Y. D., Zikpi, K., Menyah, A., & Ofori, K. (2013). Antimicrobial, antioxidant and anti-inflammatory activities of three medicinal plants from Ghana. *Journal of Ethnopharmacology*, 148(3), 965-969.
2. Baba-Moussa, L., Ahoyo, T. A., Ossou, K. R., Bouchard, P., & Sanni, A. (2008). Phytochemicals and biological activities of *Terminalia* species. *African Journal of Biotechnology*, 7(25). Njoh, M. B., *et al.,* (2020). Anti-quorum sensing potential of medicinal plants from Cameroon. *BMC Complementary Medicine and Therapies*, 20(1), 1-10.
3. Duguid, J. P., & Old, D. C. (1980). Adhesive properties of Enterobacteriaceae. *In Bacterial Adherence* (pp. 185-217). Springer, Dordrecht.

4. Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64(08), 711-714.
5. Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* 14(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94
6. Hall, C. W., & Mah, T. F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, 41(3), 276-301.
7. Institut Pasteur de Côte d'Ivoire. (2023). *Annual Report of the National Reference Center for Antibiotic Resistance*.
8. Kuete, V. (Ed.), 2017. *Medicinal Plants of West Africa*. Academic Press, London.
9. Kumar, S., Kaushik, G., & Sharma, D. (2021). Biofilms: A survival strategy of bacteria and a challenge to medical science. *In Biofilms in Human Diseases* (pp. 1-19). Springer.
10. Miquel, S., Lasa, I., Toledo-Arana, A., 2020. Biofilm formation in *Escherichia coli*: mechanisms and clinical implications. *Front. Cell. Infect. Microbiol.* 10, 592. https://doi.org/10.3389/fcimb.2020.00592
11. Orhan, I., Kürkçüoğlu, M., 2019. Bioactive compounds of *Terminalia* species. In: Watson, R.R., Preedy, V.R. (Eds.), *Bioactive Food as Dietary Interventions for Liver and Gastrointestinal Disease*, 2nd ed. Academic Press, pp. 357–379. https://doi.org/10.1016/B978-0-12-814627-6.00020-1
12. Ravichandiran, V., *et al.,* (2011). Anti-biofilm activity of *Terminalia catappa* against Pseudomonas aeruginosa. *Asian Pacific Journal of Tropical Biomedicine*, 1(7), 511-514.
13. WHO. (2017). *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. World Health Organization.

How to cite this article:

Gueyraud Rolland Kipre, Akissi Viviane Kouakou, Gouanda Pascal Youan and Allico Joseph Djaman. 2026. Comparative Evaluation of the Antibiofilm Activity of Four *Terminalia* Species Against A Multi-Drug Resistant Uropathogenic *Escherichia coli* Strain. *Int.J.Curr.Res.Aca.Rev.* 14(1), 36-41.
doi: https://doi.org/10.20546/ijcrar.2026.1401.004