

ANTIBACTERIAL EFFECT OF *Gmelina arborea* LEAF EXTRACTS ON *Pseudomonas aeruginosa* AND *Staphylococcus aureus* CLINICAL ISOLATES

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are bacterial species that have earned the reputation of “super bugs” due to their resistance to multiple classes of antimicrobial agents. The search for alternative therapies continues in the face of life-threatening infections caused by these organisms. *Gmelina arborea*, a deciduous tree native to the tropical regions of Asia, has been traditionally used in folk medicine for its potential therapeutic properties. **Objectives:** This study aimed to investigate the antibacterial activity of the ethanolic and aqueous extracts of *Gmelina arborea* leaves on *P. aeruginosa* and *S. aureus* isolates. The study also compared the antibacterial activity of the *Gmelina arborea* leaf extracts with those of commonly used antibiotics, which served as control. **Methods:** Clinical isolates were obtained from clinical samples. Fresh *Gmelina arborea* leaves were obtained and authenticated by a Botanist. Aqueous and ethanolic extracts were obtained using the Soxhlet extractor. Isolates were subjected to *in vitro* extract antibacterial testing in replicates using agar well diffusion method. One-way ANOVA was used to analyze for zones of inhibition. **Results:** Ethanolic leaf extract of *Gmelina arborea* at concentrations of 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL exhibited significant antibacterial activity with zone of inhibition diameters ranging from 30.2±0.2-17.1±0.14mm for *P. aeruginosa* and 28.5±0.15-16.0±0.17mm for *S. aureus* isolates. The MICs of ethanolic extracts varied between 3.125-6.25mg/mL and 6.25-12.5mg/mL while the MBCs were 6.25mg/mL and 12.5mg/mL for *P. aeruginosa* and *S. aureus* respectively. These results compared favourably with those of the standard antibiotic discs tested. In contrast, isolates were resistant to the aqueous extract. **Conclusion:** Ethanolic leaf extract of *Gmelina arborea* had antibacterial activity on *P. aeruginosa* and *S. aureus*. It could be a potential source of natural antimicrobial agents for the development of novel therapeutic strategies against *P. aeruginosa* and *S. aureus* infections.

Keywords: *Gmelina arborea*, leaf extracts, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, antibacterial activity

Introduction

Antibiotic resistant bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* pose a significant threat to public health worldwide. Infections caused by these resistant

pathogens have become difficult to treat with conventional antimicrobials (Harrison *et al.*, 2014; Ettu *et al.*, 2021; Eyo *et al.*, 2024). Microorganisms have developed numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the increase. This

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resistance could be conferred by Multidrug Resistance Pumps (MDRPs) and membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials (Eyo *et al.*, 2015; Pang *et al.*, 2019).

Furthermore, multiple drug resistance in human microbial pathogens has developed due to indiscriminate use of commercial antimicrobial agents commonly used in the treatment of infectious diseases (Leuthner *et al.*, 2016; Alabi *et al.*, 2023). The antimicrobial resistance situation in Nigeria is particularly worrisome due to the extensive use and misuse of antibiotics across various sectors and poor antimicrobial stewardship programmes. Several studies conducted recently in Nigeria have reported high prevalence of Gram-negative bacteria from clinical samples, with extended-spectrum beta-lactamase (ESBL) producers accounting for 60-80% of cases while methicillin-resistant *S. aureus* (MRSA) exceeded 80% (Chukwu *et al.*, 2022; Alabi *et al.*, 2023; Eyo *et al.*, 2024). This has led to a growing need for alternative antimicrobial agents. Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases, especially in light of the emergence of drug resistant microorganisms such as *P. aeruginosa* and *S. aureus* and the need to produce more effective antimicrobial agents.

Medicinal plants have a crucial role in health care needs of populations around the world especially in developing countries (Abdallah *et al.*, 2023). People living in remote areas depend more extensively on traditional medicine as modern systems are out of reach (Pang *et al.*, 2019). Medicinal plants are pivotal in the development and advancement of modern studies by serving as a starting point for the development of novelties in drugs (Khameneh *et al.*, 2021). Approximately 25% of drugs in modern Pharmacopoeias originate from plants and many others are synthetic analogues built on prototype compounds isolated from plants. It was estimated that the total number of medicinal plants in international trade is about 2500 species

(Abdallah *et al.*, 2023). The medicinal value of plants have assumed important dimensions in the past few decades, owing mainly to the discovery that extracts from plants contain not only minerals and primary metabolites, but also a variety of secondary metabolites with therapeutic values such as anti-inflammatory, antioxidant and antimicrobial potentials (Chaacchouay *et al.*, 2024).

Gmelina arborea commonly known as “*Gmelina*” is a tropical tree species found in various regions of Africa. *Gmelina arborea* Roxb. (Verbenaceae) is native to Asia and known by different names, e.g. Yemane, Gamar and Gumhar (Satyanarayana *et al.*, 1985). This species has been introduced in several countries, particularly in West Africa and especially in Nigeria and Côte d'Ivoire among others. It is a rapidly growing tree reaching three meters in height within the first year and can grow up to 30 meters tall with a straight cylindrical stem and widespread canopy. The tree produces edible fruits with a bitter-sweet taste and flowers that are also edible. It also finds usefulness in Agroforestry, construction and furniture. The leaves of *Gmelina arborea* have been traditionally used in folk medicine for their potential antimicrobial properties (Abdallah *et al.*, 2023). Ethnobotanical studies report that the species is widely used to treat many diseases including diarrhea, hypertension and malaria, among others (Shirwaikar *et al.*, 2023).

The antimicrobial properties of plants have been investigated by a number of scientists worldwide. Secondary metabolites from plants resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters which thus have beneficial medicinal effects on humans due to their recognition in potential target sites (El-Mahmood *et al.*, 2010). The use of plant extracts and phytochemicals can be of great impact in therapeutic medicine and could help curb the problem of these multi-drug resistant organisms (Camara *et al.*, 2022). The main objective of study was to determine the *in vitro* antibacterial activity of crude leaf extracts of *Gmelina arborea* on *P. aeruginosa* and *S. aureus* isolates from clinical infections.



Plate 1: *Gmelina arborea* leaves

Materials and Methods

Fresh leaves of *Gmelina arborea* were collected from the University of Calabar Botanical Garden and transported to the laboratory in a clean sterile container, maintaining appropriate temperature and humidity conditions to preserve the sample integrity. The leaf was properly identified and authenticated in the herbarium of Plant and Ecological Studies Department. A specimen voucher number BOT/HERB/UCC/583 was deposited.

Gmelina arborea leaf extraction

The leaves of the *Gmelina arborea* were washed thoroughly with sterile distilled water to remove any dirt or debris. Five hundred grams of the shade-dried leaves was pulverized and packed.

Preparation of aqueous extract

The extraction was performed by soaking 100g of dried *Gmelina* leaf powder in 600ml of distilled water for 24 hours. The residue and the filtrate were obtained by filtering the soaked *Gmelina* leaves using Whatman No.1 filter paper. The residue was dried on a cardboard paper and the filtrate was obtained as an extract.

Preparation of ethanolic extract using Soxhlet method

Twenty five grams (25g) of dried leaf powder was thoroughly mixed with 250ml of organic solvent (ethanol) in a round bottom flask attached to the Soxhlet extractor which had been

appropriately pre-assembled. Crushed material was loaded into the timble placed inside the Soxhlet extractor. The side arm which is lagged (padded) with glass wool was heated using the hexating mantle. The mixture was allowed to evaporate moving through the apparatus. The condensate was allowed to drip into the reservoir containing the timble. The process continued until all pigments were washed into the flask. The filtrate thus obtained was concentrated by complete evaporation of ethanol using a rotary evaporator to yield the pure extract.

Preparation of stock concentration of extract

A stock solution of crude extracts was prepared by properly mixing the appropriate amount of evaporated extracts with ethanol. The method of Akujobi *et al.* (2004) was used to determine the concentrations of *Gmelina arborea* leaf extracts. The reconstituted solution was stored at 15°C in sterilized bottles until required for use.

Phytochemical screening of extracts

Qualitative Phytochemical screening was conducted on the leaf extracts using standard methods (Udoh *et al.*, 2023) with slight modifications. The screening tests were used to determine the presence of alkaloids, saponins, tannins, flavonoids, phenols, and glycosides.

Preparation of bacterial inoculum

Test bacterial organisms comprised *P. aeruginosa* isolated from wound infections and *S. aureus* isolated from respiratory tract infections. Identity of organisms was confirmed using the Vitek 2® compact system. Inocula of the test organisms were prepared from young subcultures on Nutrient agar (Oxoid, UK) slants. The isolates were inoculated into Brain Heart Infusion broth (Oxoid, UK) and incubated for one hour at 37°C. The broth culture of each isolate was diluted with the same broth to approximately 1×10^8 cfu/mL equivalent to 0.5 McFarland standards.

Extract susceptibility testing

Agar well diffusion susceptibility testing of isolates was performed using 100mg/mL, 50mg/mL, 25mg/mL and 12.5mg/mL extract concentrations. The standardized inoculum suspensions were evenly inoculated on Mueller Hinton agar (Oxoid, UK) plates using sterile

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swab sticks. Wells were bored in the agar plates with sterile pipette tip. A drop or 0.1ml of plant extract was carefully added into wells. Controls with standard antibiotic discs; ciprofloxacin (5µg), ceftriaxone (30µg), cefuroxime (30µg), gentamicin (10µg) and augmentin (30µg) were set up using the Kirby Bauer disc diffusion method. The agar plates were incubated at 37°C for 24 hours. The zones of inhibition around wells were measured using a ruler calibrated in millimeters and compared with CLSI standards (CLSI, 2020).

Determination of MIC and MBC

Minimum inhibitory concentration of the ethanolic extract was determined for both bacterial isolates using the broth dilution method. Two-fold serial dilutions of the extracts were made using dimethyl sulfoxide (DMSO) in test tubes and the following concentrations were obtained and tested 12.5mg/mL, 6.25mg/mL, 3.125mg/mL and 1.56mg/mL. Three drops of overnight broth culture of test organisms were inoculated into the dilutions and incubated at 37°C for 24 hours with positive and negative controls. The lowest concentration of the extracts that inhibited the growth of the test organisms were recorded as the MIC. The minimum bactericidal concentration was determined by sub-culturing dilutions of the MIC tube and other tubes that showed no bacterial growth on Nutrient agar plates and incubated at 37°C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms were recorded as the MBC.

Statistical analysis

Data obtained were expressed as Mean ± Standard deviation (SD). One-way ANOVA was done using SPSS software package for windows (version 23) for differences between means.

Results

The results of antibacterial activity of *Gmelina arborea* leaf extract on *P. aeruginosa* clinical isolates is presented on Table 1 and that of *S. aureus* clinical isolates is presented on Table 2. The ethanolic extract possessed antibacterial activity on isolates tested whereas the aqueous extract showed very low activity on *P. aeruginosa* at 100mg/mL concentration and no

activity at other concentrations of extract on both organisms. The MICs of ethanolic extracts varied between 3.125-6.25mg/mL and 6.25-12.5mg/mL, while the MBCs were 6.25mg/mL and 12.5mg/mL for *P. aeruginosa* and *S. aureus* respectively. The results of the control experiments with the following antibiotics; Augmentin, Ciprofloxacin, Gentamicin, Cefuroxime and Ceftriaxone on the test organisms are presented on Table 3. The result of qualitative phytochemical screening of *Gmelina arborea* leaf extracts is shown on Table 4.

Table 1: Antibacterial activity of *Gmelina arborea* leaf extracts on *P. aeruginosa*

Extract	Concentration of extracts (mg/mL)/zones of inhibition (mm)			
	100	50	25	12.5
Ethanolic	30.2±0.2	27.4±1.03	21.3±0.01	17.1±0.14
Aqueous	10.0±0.01	0	0	0

MIC = 3.125-6.25mg/mL; MBC = 6.25mg/mL

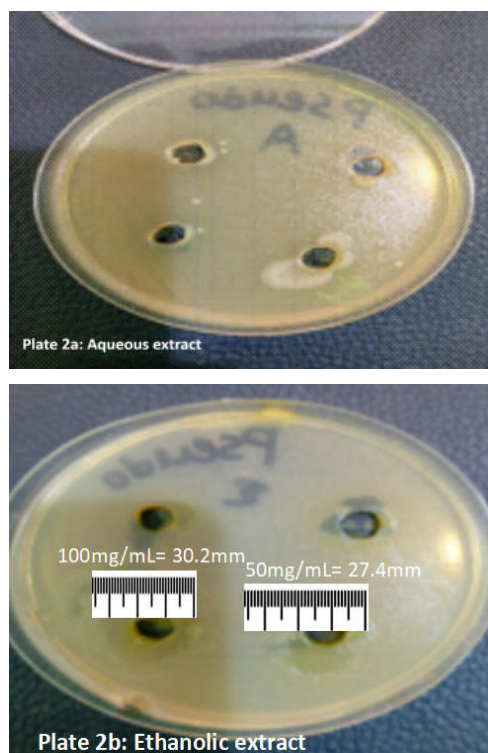


Plate 2: Antibacterial activity of a. Aqueous extract, b. Ethanolic extract of *Gmelina arborea* leaf on *Pseudomonas aeruginosa* isolate.

Table 2: Antibacterial activity of *Gmelina arborea* leaf extracts on *S. aureus*

Extract	Concentration of extracts (mg/mL)/zones of inhibition (mm)			
	100	50	25	12.5
Ethanolic	28.5±0.15	26.2±0.23	21.9±0.11	16.0±0.17
Aqueous	0	0	0	0

MIC = 6.25-12.5mg/mL; MBC = 12.5mg/mL

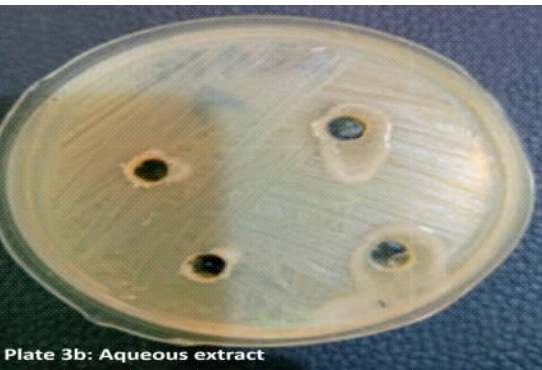
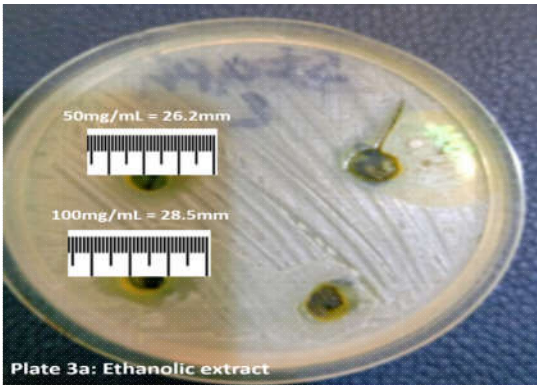


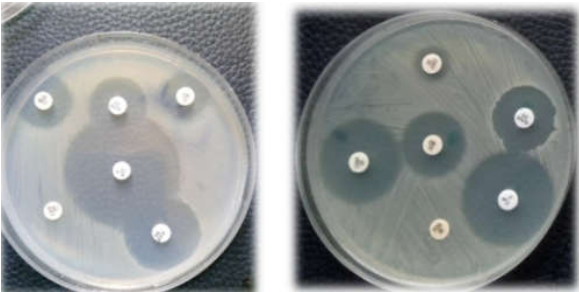
Plate 3: Antibacterial activity of a. Ethanolic extract, b. Aqueous extract of *Gmelina arborea* leaf on *Staphylococcus aureus* isolate

Discussion

The findings of this study revealed that *Gmelina arborea* crude leaf extracts have some antibacterial activity on *P. aeruginosa* and *S. aureus* (Table 1 & Table 2). The antibacterial activity of ethanolic extract on test organisms was comparable to those of standard antibiotic discs tested. However, the activity was concentration dependent. Higher concentrations of extract (100mg/mL and 50mg/mL) gave larger zones of inhibition than lower concentrations. Ethanolic extract showed higher activity, ranging

Table 3: Antibacterial activity of control antibiotics on test isolates

Bacterial isolate	Antibiotics (µg/mL)/zones of inhibition (mm)				
	CIP(5)	GEN(10)	CEF(30)	CXM(30)	AUG(30)
<i>P. aeruginosa</i>	38.3±0.04	19.0±0.01	12.0±0.01	13.9±0.10	15.0±0.03
<i>S. aureus</i>	27.2±0.15	18.4±0.02	0	26.8±0.13	17.6±0.05



a) Antibiotic control for *P. aeruginosa* b) Antibiotic control for *S. aureus*

Plate 4: Antibacterial activity of standard antibiotics on *P. aeruginosa* and *S. aureus* test isolates

Table 4: Phytochemical constituents of crude leaf extracts of *Gmelina arborea*

Solvent used	Phytochemical constituents screened						
	Alkaloids	Tannins	Flavonoids	Saponins	Lignans (Phenols)	Iridoid glycosides	Flavone glycosides
Ethanol	+++	++	++	+++	++	+++	+++
Aqueous	++	-	+	++	+	++	++

Key: + present in small concentration, ++ present in moderately high concentration, +++ present in very high concentration

between 30.2±0.2mm and 26.2±0.23 mm (zone diameter of inhibition) comparable to Ciprofloxacin (38.3±0.04mm) and Cefuroxime (26.8±0.13mm) for both test organisms (Table 3).

The aqueous extract showed very insignificant activity on *P. aeruginosa* (10.0±0.01mm), but none on *S. aureus*. This finding relates to the study that reported chloroform extracts of *Gmelina arborea* stem bark to demonstrate very high activity while the hexane extract had no activity on *S. dysenteriae* and *S. typhi* (Moges *et al.*, 2014; Vichakshana *et al.*, 2022). Thus,

indicating that extractability of phytoconstituents differs from solvent to solvent depending on their polarities. This accounts for differences observed in activity of solvent extracts.

Gmelina arborea crude leaf and stem bark extracts were reported to show significant antimicrobial activities on some Gram-positive and Gram-negative organisms. *In vitro* studies of both leaf and stem bark extracts showed significant activity on *E. coli*, *K. pneumoniae*, *S. dysenteriae* and *S. typhi* (Araboki *et al.*, 2012; Shepherd *et al.*, 2023). Through advanced analytical techniques such as High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), the phytoconstituents and properties of medicinal plants can be derived. It is suggested that the presence of saponins, tannins, flavonoids, phenolics, alkaloids and glycosides in *G. arborea* leaf extract is responsible for antimicrobial action of metabolites (Table 4) (Tiwari 2008; Akhtar and Mirza, 2018), while stem bark possesses saponins, carbohydrates, alkaloids, tannins and anthraquinone but no phenolics (Edeoga *et al.*, 2005; Akinmoadun *et al.*, 2007). Crude extracts had been reported to facilitate wound-healing (Kulkarni *et al.*, 2009). This could be due to presence of tannins in extract because, tannins and tannic acids owe their astringent action to their ability to precipitate proteins and this may form a protective coating over the wound, thus preventing external irritation which promotes healing (Bishnu *et al.*, 2009; Azzopardi *et al.*,

2011). Hence, the observed antibacterial activity of *Gmelina arborea* ethanolic extract on test organisms may be due to tannins saponins, flavonoids, steroids and anthraquinone or other phenolics, that may be present in extracts. However, cytotoxicity profiling of the leaf extracts is recommended to ensure safe and standardized *in vivo* use of this extract.

Conclusion

Gmelina arborea leaf extracts has been shown to possess antibacterial activities on *P. aeruginosa* and *S. aureus* clinical isolates. These findings confirm the traditional therapeutic claims for this plant in the treatment of wound infections and other disease conditions. The study also affirms that *Gmelina arborea* leaves contain bioactive compounds. Further studies into the isolation and formulation of these compounds are recommended, which could be explored in the development of new antimicrobial agents to combat antibiotic resistant bacterial infections.

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Conflict of Interest

The authors declare no conflict of interest. Funding for the study was privately sourced by authors on mutually agreed terms.

References

- Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., and Martins, C. H. G. (2023). Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era. *Plants (Basel, Switzerland)*, 12(17), 3077. <https://doi.org/10.3390/plants12173077>
- Akhtar, N., and Mirza, B. (2018). Analysis and comprehensive evaluation of Antimicrobial and Antioxidant properties of 61 Medicinal plant species. *Arab Journal of Chemistry*, 11, 1223-1235. <https://doi.org/10.1016/j.arabjc.2015.01.013>
- Akinmoladun, A. C., Ibukun, E. O., Afor, E. Akinrinlola, B. L., and Onibon, T. R, Akinboboye, A. O., Obuotor, E. M., and Farombi E. O. (2007). Chemical constituents and antioxidant activity of *Alstonia boonei*. *African Journal of Biotechnology*, 6(10), 1197-1201. <http://www.academicjournals.org/AJB>
- Akujobi, C., Anyanwu, B., Onyeze, N., and Ibekwe, V. (2004). Antibacterial activities of and preliminary phytochemical screening of four medicinal plants. *Journal of Applied Sciences*. 7: 4328-4338. <https://www.researchgate.net/publication/202902416>

- Alabi, E. D., Bindawa, B. L., and Mzungu, I. (2023). Characterization of selected multidrug-resistant bacteria from clinical and hospital environmental sources using Vitek 2 compact system. *Applied Microbiology: Open Access*. 9: 258. <https://doi.org/10.21203/rs.3.rs-2790096/v1>
- Araboki, M., Cluk, A. W., Czerwonka, G, Lankiff, A., and Kaca, W. (2012): Effect of Saponin against Clinical *E. coli* Strains Eukaryotic Cell Lines. *Journal of Biomedicine and Biotechnology*, 12, 6. <http://dx.doi.org/10.1155/2012/286216>
- Azzopardi, E. A, Azzopardi. S. M, Boyce, D. E., and Dickson, W. A. (2011). Emerging Gram-negative infections in burn wounds. *Journal of Burn Care Research*, 32(5), 570-576. <https://doi.org/10.1097/BCR.0b013e31822ac7e6>
- Bishnu, J. U., Sunil, L., and Anula, S. (2009): Antibacterial Properties of Different Medicinal plants; *Ocimum sanctum*, *Cinnamum zeylanicum*, *Xanthoxylum arimaticum* and *Origanum masorana*. *Kathmandu University Journal of Sciences, Engineering and Technology*, 25(5), 143-150. <https://doi.org/10.3126/kuset.v5i1.2854>
- Camara, J. S., Perestrelo, R, Berenguer, C. V., Andrade, C. F., Gomes, T. M., Olayanju, B., Kabir, A, Rocha, C., Teixeira, J. A., and Pereira, J. A. (2022). Green Extraction Techniques as Advanced sample preparation approaches in Biological food and Environmental matrices: A Review. *Molecules*. 27, 2953. <https://doi.org/10.3390/molecules27092953>
- Chaachouay, N., and Zidane, L. (2024). Plant derived natural products: A source for drug discovery and development. *Drugs Drug Candidate*, 3, 184-207. <https://doi.org/10.3390/ddc3010011>
- Chukwu, E. C., Awoderu, O. B., and Ewuru, C. A. (2022). High prevalence of resistance to third-generation cephalosporins detected among clinical isolates from sentinel healthcare facilities in Lagos, Nigeria. *Antimicrobial Resistance and Infection Control*, 11(1): 134. <https://doi.org/10.1186/s13756-022-01171-2>
- Clinical and Laboratory Standards Institute (2020). Performance standards for antimicrobial susceptibility testing: 30th informational supplement. CLSI document M100-S30. <https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf>
- Edeoga, H. O., and Eriata, D. O. (2005): Alkaloids, Tannins and Saponin Contents of Some Nigerian Medicinal and Aromatic Plants. *Sciences*, 23, 244-249. <http://www.academicjournals.org/AJB>
- El-Mahmood, A. M., Doughari, J. H., and Kiman, H. S. (2010). *In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceae. *African Journal of Pharmacy and Pharmacology*, 4(6), 355-361. <http://www.academicjournals.org/ajpp>
- Ettu, A. O., Oladapo, B. A., and Oduyebo, O. O. (2021). Prevalence of carbapenemase production in *Pseudomonas aeruginosa* isolates causing clinical infections in Lagos University Teaching Hospital, Nigeria. *African Journal Clinical Experimental Microbiology*. 22(4): 498-503. <https://doi.org/10.4314/ajcem.v22i4.10>
- Eyo, A. O., Udoh, D. I., Umoh, N. O., Ogba, O. M., Ibeneme, E. O., and Asuquo, A. E. (2024). Carbapenemase-producing *Pseudomonas aeruginosa* isolates: Prevalence and their Phenotypic Characteristics in Four Health Facilities in Calabar, Nigeria. *Acta Microbiologica Bulgarica*, 40(4), 1-7. <https://actamicrobio.bg/archive/issue-4-2024/amb-4-2024-article-5.pdf>
- Eyo, A. O., Ibeneme, E. O., Thumamo, B. P., and Asuquo, A. E. (2015). Antibiotic Resistance Profiles of Clinical and Environmental Isolates of *Pseudomonas aeruginosa* in Calabar, Nigeria. *Journal of Pharmaceutical and Biological Sciences*, 10(4), 9-15. <https://doi.org/10.9790/3008-10410915>
- Harrison, E. M., Weinert, L. A., Holden, M. T., Welch, J. J., Wilson, K., Morgan, F. J., Harris, S. R., Loeffler, A., Boag, A. K.,

- Peacock, S. J., Paterson, G. K., Waller, A. S., Parkhill, J., and Holmes, M. A. (2014). A shared population of epidemic methicillin-resistant *Staphylococcus aureus* 15 circulates in humans and companion animals. *MBio*, 5(3), e00985-13. <https://doi.org/10.1128/mBio.00985-13>
- Khameneh, B., Eskin, N. A. M., Iranshahy, M., and Fazly Bazzaz, B. S. (2021). Phytochemicals: A Promising Weapon in the Arsenal against Antibiotic-Resistant Bacteria. *Antibiotics (Basel, Switzerland)*, 10(9), 1044. <https://doi.org/10.3390/antibiotics10091044>
- Kulkarni, Y. A., and Addepalli, V. (2009). Antidiabetic activity of aqueous extract of *Gmelina arborea* bark in rats. *Alternative and Traditional Health Medicines* 15, 183-1834. <https://link.gale.com/apps/doc/A345108485/HRCA?u=anon~14fcd3e1&sid=googleScholar&xid=0e90568a>
- Leuthner, K.D., Buechler, K.A., Kogan, D., Saguros, A., and Lee, H.S. (2016). Clinical efficacy of dalbavancin for the treatment of acute bacterial skin and skin structure infections (ABSSSI). *Therapeutics and Clinical Risk Management*, 931-940. <https://doi.org/10.2147/TCRM.S86330>
- Moges, F., Endris, M., Mulu, A., Tessema, B., Belyhun, Y., Shiferaw, Y., Huruy, K., Unakal, C., and Kassu, A. (2014). The growing challenges of antibacterial drug resistance in Ethiopia. *Journal of Global Antimicrobial Resistance*, 2(3), 148-154. <https://doi.org/10.1016/j.jgar.2014.02.004>
- Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., and Cheng, Z. (2019). Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnological Advances*, 37(1), 177-192. <https://doi.org/10.1016/j.biotechadv.2018.11.013>
- Shepherd, M. A., Fleming, V. M., Connor, T. R., Corander-Fayisa, W. O., and Tuli, N. F. (2023). Review on *Staphylococcus aureus*. *International Journal of Nursing Care and Research*, 1(2), 1-8. <https://www.wecmelive.com/open-access/review-on-staphylococcus-aureus.pdf>
- Shirwaikar, A., Ghosh, S., and Rao, P. G. (2003). Effects of *Gmelina arborea* Roxb leaves on wound healing in rats. *Journal of Natural Remedies*, 3(1), 45-48. <https://www.scopus.com/inward/record.uri?eid=2-s2.0-0037218923&partnerID=40&md5=86c2d28011dc72ac7aac53322d2ca75c>
- Tiwari, S. (2008). Plants: A rich source of Herbal Medicine. *Journal of Natural Products*, 1, 27-35. https://www.researchgate.net/publication/28352248_Plants_A_rich_source_of_herbal_medicine
- Udoh, D. I., Ogbu, O. M., Eyo, A. O., and John, E. (2022). Resistance Patterns and Plasmid Profiles of Isolates from Diarrheic Stool Samples subjected to Susceptibility Testing on Crude Extracts of *Uvariachamae* (P. Beauv). *Acta Microbiologica Bulgarica*, 38(4): 1-7. <https://actamicrobio.bg/archive/issue-4-2022/amb-4-2022-article-10.pdf>
- Vichakshana, G. A. D., Young, D. J., and Choo, W. S. (2022). Extraction Purification, Food Applications and recent advances for enhancing the Bioavailability of 6-Gingerol from Ginger: A review. Quality Assurance and Safety of Crops. *Foods*, 14,67-83 <https://orcid.org/0000-0003-0495-3303>