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Phytochemical Screening and Comparative Antibacterial Activity of *Aframomum melegueta* Aqueous and Ethanolic Seed Extracts against Selected bacterial isolate in Idah, Kogi State

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Abstract

The growing occurrence of antibiotic-resistant bacterial pathogens demands the study of different antimicrobial agents from medicinal plants. This study assessed the phytochemical constituents and in vitro antibacterial activity of aqueous and ethanolic seed extracts of *Aframomum melegueta* (grains of paradise) against selected bacterial isolate. Seeds were purchased from the Egah market Idah in Kogi State, Nigeria. Samples were extracted using cold maceration with distilled water and 70% ethanol. Standard phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols in both extracts, with higher intensity observed in the ethanolic extract. The antibacterial assay using the agar well diffusion method demonstrated that both extracts exhibited dose-dependent inhibitory activity. The ethanolic extract showed significantly ($p < 0.05$) higher efficacy with mean inhibition zones ranging from 16.3 ± 0.6 mm to 22.7 ± 0.6 mm at 100 mg/mL concentration, compared to the aqueous extract (10.7 ± 0.6 mm to 14.3 ± 1.2 mm). Staphylococcus aureus was the most susceptible organism, while Escherichia coli showed the highest resistance. The Minimum Inhibitory Concentration (MIC) values for the ethanolic extract ranged from 12.5 mg/mL to 25 mg/mL, and the Minimum Bactericidal Concentration (MBC) values ranged from 25 mg/mL to 50 mg/mL. The results confirmed the traditional use of *A. melegueta* in ethno-medicine and recommend that the ethanolic extract possesses potent antibacterial activity, primarily attributed to its rich phytochemical constituents, suggest further analysis for therapeutic development.

Keywords: Aframomum melegueta, Antibacterial activity, Phytochemicals, Aqueous extract, Ethanolic extract

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1. Introduction

The global health burden enforced by bacterial infections is intensified by the quick emergence of multidrug-resistant (MDR) strains, rendering conventional antibiotics increasingly ineffective (World Health Organization, 2021). This crisis has restored scientific interest in medicinal plants as promising sources of different antimicrobial compounds with potentially different mechanisms of action (Diass *et al*., 2021; Guevara *et al*., 2023; Ouahabi *et al*., 2023; Tiwari *et al*, 2023). In Nigeria, especially among the Igala people of Kogi State, its seeds are extensively used in traditional medicine for treating ailments such as infections, inflammation, and gastrointestinal disorders (Osuntokun, 2020; Tiwari *et al*., 2023).

Existing studies have documented the antimicrobial potential of *A. melegueta* organic solvent extracts (Akinsuyi *et al*., 2020). However, there is a lack of comparative data on the efficacy of more traditional and readily accessible solvents like water and ethanol, particularly for plants sourced from specific geographical locations like Idah, Kogi State(Aina, 2023; Awoyemi *et al*., 2025).

The phytochemical composition and biological activity of plants can be significantly influenced by geographical and climatic factors (Tiwari *et al*., 2023; Kadda *et al*., 2026). This study, therefore, aims to investigate and compare the phytochemical profile and antibacterial activity of aqueous and ethanolic seed extracts of *A. melegueta* from Idah against selected three clinically significant bacterial pathogens: *Bacillus subtilis* (a Gram-positive model), *Staphylococcus aureus* (a major Gram-positive pathogen), and *Escherichia coli* (a common Gram-negative pathogen). The findings will provide a scientific basis for the local ethno-medicinal use and indicate the most effective extraction method for harnessing its antibacterial properties.

2. Materials and methods

2.1. Plant material

The dried seeds of *A. melegueta* were purchased in the month of November 2025 from Egah market in Idah local government, Kogi State Nigeria. The seed samples were manually separated, washed, and grounded into a fine powder using laboratory pestle and mortar in the Department of Science laboratory Technology, Biology laboratory Federal Polytechnic Idah Kogi state (Danjumma, *et al*., 2025; Kabir, 2024).

2.2. Preparation of the extracts

200 g of each powdered sample was cold-macerated in 1000 mL of distilled water and 70% ethanol for 48 hours with intermittent shaking. The mixture was filtered first with muslin cloth and then with Whatman No. 1 filters paper, filtrate was concentrated using a using a rotary evaporator. The

dried extracts were stored in airtight containers at 4°C until use (Islam *et al.* 2026; Kabir, 2024; Olajuyigbe *et al.*, 2020).

2.3. Phytochemical screening of the extracts

Qualitative phytochemical analysis of both extracts was performed using standard procedures as described by Tiwari *et al.*, 2023; Kabir, 2024; Danjuma *et al* 2025; El Amri *et al* 2025; Mustapha *et al*., 2025 to test for the presence of alkaloids (Dragendorff's test), flavonoids (Aluminum chloride test), tannins (Ferric chloride test), saponins (Foam test), terpenoids (Salkowski test), phenols (Lead acetate test), and cardiac glycosides (Keller-Killani test).

2.4. Test Microorganisms and Inoculum Preparation

Clinical bacterial isolates of *S. aureus*, and *E. coli*, and a standard strain of *B. subtilis* were obtained from the Microbiology Department, Science Laboratory Technology Federal Polytechnic Idah Kogi State. The isolates were revived on Nutrient Agar slants. Bacterial suspensions were prepared in sterile normal saline and standardized to 0.5 McFarland turbidity (≈ 1.5 x 10^8 CFU/mL) (Ogodo *et al.*, 2017; Yuni *et al.*, 2026).

2.5. Antibacterial Susceptibility Testing

The antibacterial activity was evaluated using the agar well diffusion method describes by Danjumma 2025; Kabir and Lawan 2025; Yuni *et al*., 2026 200 mL of sterile Mueller-Hinton Agar was poured into plates and allowed to solidify. The standardized inoculum was swabbed evenly on the agar surface. Wells (6 mm diameter) were bored, and 100 µL of each extract at concentrations of 25, 50, and 100 mg/mL (dissolved in 10% DMSO for EE and distilled water for AE) were introduced into respective wells. Ciprofloxacin (10 µg) and 10% DMSO served as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours. The antibacterial activity was measured as the diameter of the zone of inhibition (IZD) in millimetres (mm). All tests were performed in triplicate.

2.6 Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method. Two-fold serial dilutions of the extracts (6.25 to 100 mg/mL) were prepared in Mueller-Hinton Broth. Each tube was inoculated with the standardized bacterial suspension and incubated at 37°C for 24 hours. The MIC was recorded as the lowest concentration that showed no visible turbidity. From the clear tubes, a loopful was sub-cultured onto fresh Nutrient Agar plates to determine the

Minimum Bactericidal Concentration (MBC), defined as the lowest concentration that killed 99.9% of the inoculum (Kebede *et al*., 2021; Yusuf *et al*., 2022).

2.7 Statistical Analysis

Data were presented as mean ± standard deviation (SD) of three replicates. Analysis was performed using GraphPad Prism 9.0. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Phytochemical screening of the extracts

Qualitative phytochemical screening showed that both extracts confirmed the presence alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. However, the intensity of the reactions, particularly for flavonoids, terpenoids, and phenols, was markedly stronger in the ethanolic extract as shown in **Table 1.**

Table 1. Comparative results of phytochemical screening of extracts

Phytochemical Constituent	Aqueous Extract (AE)	Ethanolic Extract (EE)
Alkaloids	+	++
Flavonoids	+	+++
Tannins	++	++
Saponins	++	+
Terpenoids	+	+++
Phenols	+	+++
Cardiac Glycosides	-	-

Key: (+) = Present in low concentration, (++) = Present in moderate concentration, (+++) = Present in high concentration, (-) = absent.

The comparative results of phytochemical screening of extracts from **Table 1** highlights that Ethanol extract (EE) is generally a more potent solvent for a wider range of bioactive compounds (Altemimi *et al*., 2017). The high concentration of phenols and flavonoids in the EE suggests it may possess stronger antioxidant and antimicrobial activities, as these compounds are primary drivers of such bioactivities (Nadeem *et al*., 2022). In contrast, the Aqueous Extract (AE) is more effective specifically for saponins, which are often used for their foaming and detergent-like properties in pharmaceutical applications (Sadasivam, *et al*., 2025; Soetan *et al*., 2014).

The higher yield and more intense phytochemical reactions in the ethanolic extract align with the principle that ethanol, being a polar organic solvent, is more efficient at extracting a wider range of medium to high polarity bioactive compounds like phenols, flavonoids, and terpenoids from plant backgrounds (Akinsuyi *et al.*, 2020; Bae *et al* 2022).

3.2 Antibacterial Activity

This study confirms that *A. melegueta* seeds extracts from Idah local government, Kogi State, possess significant antibacterial properties, with the efficacy heavily dependent on the extraction solvent. Both extracts exhibited concentration-dependent antibacterial activity. The ethanolic extract consistently produced significantly larger ($p < 0.05$) zones of inhibition than the aqueous extract against all tested bacteria at all concentrations as shown in **Table 2.** At the highest concentration (100 mg/mL), the EE was most active against S. aureus (22.7 ± 0.6 mm), followed by B. subtilis (19.3 ± 0.6 mm), and least active against E. coli (16.3 ± 0.6 mm). The positive control (Ciprofloxacin) showed IZDs > 25 mm.

Table 2. Zones of inhibition than the aqueous extract against all tested bacteria at all concentrations

Bacterial Isolate	Extract	25 mg/mL	50 mg/mL	100 mg/mL
S. aureus	AE	7.0±0.0	10.7±0.6	14.3±1.2
	EE	14.0±1.0	18.3±0.6	22.7±0.6
B. subtilis	AE	6.3±0.6	9.7±0. 6	12.0±1.0
	EE	12.7±0.6	16.0±1.0	19.3±0.6
E. coli	AE	ND	7.7±0.6	10.7±0.6
	EE	10.3±0.6	13.3±0.6	16.3±0.6

Values are Mean ± SD (n=3); ND = Not Detected; AE = Aqueous Extract; EE = Ethanolic Extract.

The observed antibacterial activity of the extracts is directly correlated with this enriched preliminary phytochemical. Flavonoids and phenols are well-documented for their ability to disrupt microbial cell membranes, inhibit enzymes, and interfere with nucleic acid synthesis (Chandrasekaran *et al.*, 2020; Olajuyigbe *et al.*, 2020). The higher activity of both extracts against Gram-positive (S. aureus and B. subtilis) bacteria compared to the Gram-negative (E. coli) is a common finding in phytomedicine research (Imran *et al.*, 2021; Elisha *et al.*, 2017). This is primarily attributed to the structural difference in cell walls; the outer lipopolysaccharide membrane of Gram-negative bacteria acts as a formidable barrier to the penetration of many antimicrobial compounds (Benedetto et *al.*, 2018; Lehman and Grabowicz, 2019). Extracts from *A. melegueta* therefore have potent antiseptic or bactericidal properties (Yu *et al.*, 2019). This finding supports the use of extracts from *A. melegueta* in treating wounds that not only heals fast but also prevents the formation of infections (Khan *et al*., 2024).

3.3 Minimum Inhibitory and Bactericidal Concentrations

The MIC and MBC results confirmed the higher potency of the ethanolic extract as showed from **Table 3**. The EE had lower MIC values (12.5-25 mg/mL) compared to the AE (25-50 mg/mL). The MBC values followed a similar pattern, with the ethanolic extract indicating bactericidal action (MBC/MIC ratio ≤ 4) against all tested organisms. The significantly higher potency of the ethanolic extract of *A. melegueta* over the aqueous extract, as evidenced by greater IZDs and lower MIC/MBC values, has serious effects (Yazdanian *et al*., 2022).

Table 3: MIC and MBC Values (mg/mL) of A. melegueta Extracts

Bacterial Isolate	Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio
S. aureus	AE	25	100	4
	EE	12.5	25	2
B. subtilis	AE	50	>100	>2
	EE	25	50	2
E. coli	AE	50	>100	>2
	EE	25	50	2

It suggests that traditional preparations using water (infusions or decoctions) may not fully harness the plant's antibacterial potential. For optimal therapeutic effect in ethnomedicine, an ethanolic tincture might be more effective (Abdallah *et al*., 2023; Hlatshwayo *et al*., 2025). The bactericidal nature (MBC/MIC ≤ 4) of the ethanolic extract against all test organisms, particularly *S. aureus*, is promising for developing treatments for resistant infections (Mogana *et al*., 2020)

Conclusion

The findings from this study determined that *A. melegueta* seed extracts from Idah possess appreciable in vitro antibacterial activity, authenticating its use in Igala traditional medicine. The 70% ethanolic extract showed significantly greater potency than the aqueous extract, attributable to its richer concentration of flavonoids, phenols, and terpenoids. The extract was particularly effective against the Gram-positive pathogens, with Staphylococcus aureus being the most susceptible.

Conflict of Interest. The author declares that the was no conflict of interest

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