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In vitro evaluation and mechanistic insights into the antimicrobial activity of *Aframomum melegueta* and *Curcuma longa* blends against wound isolates

Abstract

This study investigates the antibacterial efficacy of plant extract extracted from *Aframomum melegueta* (Grains of Paradise) and *Curcuma longa* against clinical wound isolates collected from the Adekunle Ajasin University Akungba-Akoko (AAUA) Health Centre in Ondo State, Nigeria. The increasing prevalence of antibiotic-resistant bacteria has led to a renewed interest in traditional herbal remedies for alternative treatment options. The plant extract were extracted using ethanol and n-hexane using a rotary evaporator and tested at varying concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml). Antimicrobial assays, including zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), were conducted using the agar well diffusion method to evaluate their effectiveness against bacterial strains such as *Escherichia coli, Klebsiella ornithinolytica, Streptococcus sp., Staphylococcus aureus,* and *Acinetobacter haemolyticus*. Results showed that *Aframomum melegueta* demonstrated stronger antibacterial activity, with an inhibition zone between 4-17mm at 100mg/ml compared to *Curcuma longa*, with an inhibition zone of between 6 to no inhibition at 100 mg/ml, particularly against *Acinetobacter haemolyticus*, with MIC of 12.5mg/ml and MBC of 25 mg/ml. Killing time assays done using a spectrophotometer at 600 wavelengths further indicated that higher concentrations of these oils were more effective in reducing bacterial growth. The study suggests that these plant-based extract could be potential alternatives to synthetic antibiotics for treating wound infections, especially in light of rising antibiotic resistance. Further research is recommended to explore the clinical applications, optimal extraction methods, and possible synergistic effects with conventional antibiotics.

Keywords: synergistic efficacy, *aframomum melegueta, curcuma longa,* plant extract

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Introduction

Aframomum melegueta, commonly known as Grains of Paradise, is an herbaceous perennial plant native to West Africa, widely valued for its medicinal properties. The plant is particularly rich in secondary metabolites such as flavonoids, tannins, and phenolic compounds, which are largely responsible for its pharmacological activities. These compounds have been shown to exhibit strong antioxidant activities, which play a significant role in neutralizing harmful free radicals that contribute to oxidative stress and related health issues, such as cardiovascular diseases and cancers.[1]

The antibacterial activity of *Aframomum melegueta* has been well-documented in several studies, demonstrating its effectiveness against both gram-positive and gram-negative bacteria.[2] The active compounds disrupt bacterial cell walls, leading to cell lysis and death, making the plant a promising candidate for further research into natural antibacterial agents. In addition to its antibacterial properties, *Aframomum melegueta* has also been shown to have anti-inflammatory and analgesic effects, making it an all-round therapeutic plant (Figure 1).[3]

In the pharmaceutical industry, *Aframomum melegueta's* antimicrobial properties have made it a key ingredient in the formulation of herbal remedies. The plant's bioactive compounds, such as gingerol and shogaol, have been shown to have antibacterial and anti-inflammatory effects, making it valuable for treating infections and digestive disorders.[4-6] *Aframomum melegueta* is also used in the production of nutraceuticals, where its health benefits are harnessed for dietary supplements aimed at improving digestion, reducing inflammation, and boosting immunity (Figure 2).

Figure 1 Dried *Aframomum melegueta* (Grains of Paradise) Seeds and Pod.[4]

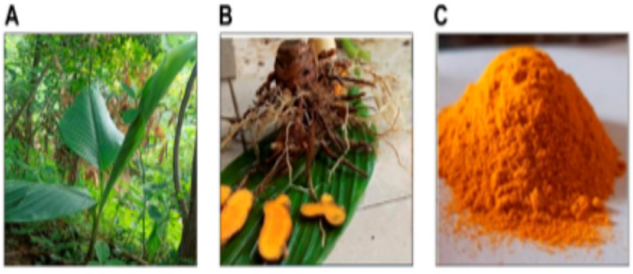

Figure 2 Important parts of C. longa.(A) C. longa in natural habitat, (B) medicinally important part of C. longa (rhizome), and (C) powder of dried rhizome of C. longa (used as a coloring agent in food).

Curcuma longa, commonly known as Turmeric, is another member of the Zingiberaceae family, widely cultivated for its rhizomes in tropical and subtropical regions. The rhizomes of *Curcuma longa* contain several bioactive compounds, particularly curcumin, a polyphenolic compound known for its potent antibacterial, antioxidant, and anti-inflammatory properties.[7-9] Turmeric has a long history of use in traditional medicine for treating various ailments, including bacterial infections, digestive disorders, and skin conditions.[10]

Research has highlighted *Curcuma longa's* antibacterial efficacy, particularly in inhibiting the growth of multidrug-resistant bacteria.[11] The antimicrobial mechanism of curcumin involves the disruption of bacterial cell membrane integrity and the inhibition of key bacterial enzymes. The versatility of *Curcuma longa* in traditional medicine has made it a focus of modern pharmacological research, particularly in the development of natural antimicrobial agents.[12]

Both *Aframomum melegueta* and *Curcuma longa* offer promising potential for the development of new antibacterial treatments, especially in light of rising antibiotic resistance. Their phytochemical constituents provide a natural, low-toxicity alternative to conventional antibiotics, making them valuable in the fight against bacterial infections.

The dried rhizomes of *Curcuma longa* are cylindrical in shape, typically 2-5 cm in length, and are known for their strong, earthy aroma and bright yellow color. The drying process intensifies the rhizome's color and enhances its medicinal properties. Dried turmeric has been used for centuries in Ayurveda and other traditional systems of medicine to treat various ailments, including inflammation and infections. It is also widely used as a natural dye and in cooking for its distinctive color and flavor. Wound infections represent a significant clinical challenge, especially following surgical procedures. These infections often lead to prolonged hospital stays, increased morbidity, and higher healthcare costs due to the reliance on antibiotics and antiseptics. However, the overuse of antibiotics has led to the emergence of antibiotic-resistant bacteria, rendering many traditional treatments ineffective. As a result, there is an urgent need for alternative treatments that are both effective and less likely to contribute to resistance.[11,12]

Aframomum melegueta and *Curcuma longa* have emerged as promising candidates in the search for alternative antibacterial agents. Both plants possess strong antibacterial properties, which have been demonstrated in various studies. The bioactive compounds in *Aframomum melegueta*, such as 6-gingerol and paradol, and curcumin from *Curcuma longa*, have shown the ability to inhibit bacterial growth by disrupting bacterial cell membranes. These properties make them suitable candidates for the treatment of wound infections, particularly those caused by antibiotic-resistant strains.[13]

Despite their potential, there is a gap in the literature regarding the specific antibacterial effects of *Aframomum melegueta* and *Curcuma longa* on wound isolates. Most existing studies have focused on their general antimicrobial properties, without delving into their specific applications in wound care. This study seeks to fill this gap by investigating the antibacterial efficacy of these plant extracts against bacterial isolates from wound infections. Given the rise in natural and integrative medicine, this research also holds significant value in promoting the use of natural remedies in clinical settings. By exploring the potential of *Aframomum melegueta* and *Curcuma longa* as natural alternatives to conventional antibiotics, this study aims to contribute to the broader discourse on sustainable healthcare solutions. The outcomes of this research could offer new insights into the development of plant-based antibacterial therapies for wound infections, potentially reducing reliance on synthetic antibiotics and addressing the growing issue of antibiotic resistance.

Material and methods

Collection of clinical wound isolates

In this study, 15 different surgical wound isolates were collected aseptically in sterile universal bottles from the Microbiology section of the University's Health Centre at Adekunle Ajasin University, Akungba-Akoko, in Ondo State. The samples were then transported to the laboratory within 15 minutes for microbial analysis. The isolates were maintained on nutrient agar slants and stored at 4°C in a refrigerator to preserve their viability.

Plant collection and identification

The dried seeds and buds of *Aframomum melegueta* and fresh rhizomes of *Curcuma longa* were purchased in June 2024 from the local market in Ikare, Akoko, Ondo State, Nigeria. Ondo State is located in the southwestern region of Nigeria and shares boundaries with neighboring states such as Ekiti, Kogi, and Edo. The *Aframomum melegueta* seeds and buds were cleaned to remove any dirt before being dry-blended into a powder for use in the study. The fresh *Curcuma longa* rhizomes were washed thoroughly under running tap water to remove adhered soil and foreign matter. They were then diced into small pieces to prepare them for further processing in the laboratory.

The plant specimens were identified by a botanist and voucher specimens were deposited in the herbarium of the Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Extraction methods

Two different solvents were used to extract the oils from the plant materials: ethanol and n-hexane. 95% ethanol and 70% n-hexane were used to extract the plant extract from *Aframomum melegueta* (grounded seeds and buds) as well as *Curcuma longa* rhizomes.

Aframomum melegueta and Curcuma longa extracted with N-hexane and ethanol

A 500g sample of grounded *Curcuma longa* and *Aframomum melegueta* seeds and buds was soaked in 1L of n-hexane and ethanol in a glass jar. The jar was sealed and kept in a dark place for 10 days. During this period, the jar was agitated (shaken) periodically to ensure proper contact between the plant material and the solvent. After 10 days, the soaked sample was filtered using a clean mesh cloth. The filtrate, containing the n-hexane and oil, was transferred to a round-bottom flask and connected to a rotary evaporator. The solvent was evaporated at 56°C under reduced pressure, leaving the extract in the flask. The extract was collected and stored in dark glass bottles at 4°C until further analysis.[13]

Preparation of extract concentration

Previously prepared and stored extracts were reconstituted using DMSO. Extract concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml were prepared by dissolving the concentrated extract in DMSO and adjusting the volume with sterile water to achieve the desired concentrations. Amoxicillin was used as a positive control at a concentration of 25mg/ml. The dilution medium used for the positive control was sterile distilled water.[13]

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Isolation, identification and characterization of clinical wound isolated

Preparation of wound samples for culture

Sterile physiological saline (9ml) was dispensed into a series of 10 sterile test tubes. The test tubes were sealed with cotton wool covered in aluminum foil, and each was labelled with dilution factors ranging from 10^-1 to 10^-6. Wound swabs were then immersed in 9ml of sterile saline.1 ml of the stock culture was sequentially transferred into the subsequent test tubes, each containing 9 ml of saline, to create serial dilutions up to the sixth dilution. The pour plate method was utilized to enumerate bacterial growth. A 0.5ml aliquot from the 10^-3 and 10^-5 dilutions was inoculated onto sterile Petri dishes for bacterial culture.[13,14]

Identification of isolates

Cultural and microscopic examinations were conducted to identify the isolates obtained from the wound samples. The cultural characteristics of the isolates were noted, including creamy pigmentation, round and slightly elevated shape, and some isolates exhibited an irregular or thread-like appearance. Additionally, certain isolates were swarmy, lacking distinct colony boundaries or cellular morphology.

Microscopic examination of isolates from wound sample

Microscopic observations were complemented by a series of biochemical tests to identify the isolates. These included gram staining, motility tests, and fermentation of sugars such as sucrose, lactose, and dextrose. Other tests performed were for indole production, urease activity, hydrogen sulfide production, gelatin liquefaction, and nitrate reduction.[15]

Microscopic and microscopic examination of isolates from clinical wound sample

Cultural and microscopic examinations were performed to determine the identity of the isolated wound microorganisms. The cultural characteristics of the isolates were examined; the creamy pigmentation, round and slightly elevated shape, irregular and thread-like some of which are swarmy, with no distinct colony and cellular morphology characteristics. Furthermore, conventional identification of the isolates was carried out using various biochemical tests such as, indole, motility, gram staining, fermentation of sugars (Sucrose, Lactose, Dextrose), Urease, Hydrogen sulphide, gelatin liquefaction and nitrate reduction test.[15]

Preparation of oil extract for antimicrobial susceptibility test

Preparation of extract concentration

Previously prepared and stored extracts were reconstituted using distilled water. A 100 mg/ml concentration of the extract was prepared by dissolving 0.5 g of the concentrated extract in 50 ml of distilled water. This was done for all extracts. Simultaneously, amoxicillin was used as the positive control at a concentration of 25 mg/ml. The dilution medium used for the positive control was sterile distilled water.

Antimicrobial assay of the plant extract

The antimicrobial activity of the plant extract was evaluated using the agar well diffusion method against seven bacterial strains: *Escherichia coli, Klebsiella ornithinolytica, Streptococcus sp., Budvicia aquatica, Staphylococcus aureus, Acinetobacter haemolyticus, and Citrobacter gillericus.* The bacterial strains were obtained from the Microbiology section of the University's Health Centre at Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The bacterial cultures were maintained on nutrient agar plates at 4°C and subcultured before each experiment.

Preparation and standardization of inoculum suspension

The direct colony suspension technique was used for the preparation and standardization of the inoculum. Pure cultures of the test organisms were transferred into sterile screw-capped McCartney bottles containing normal saline (0.90% w/v) using a sterilized inoculating loop. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, which was prepared and used as a reference for ensuring uniform turbidity. Both the inoculum suspensions and the McFarland standard were compared visually against a white background with black lines. After achieving the desired turbidity, the inoculum suspensions were stored under refrigeration until use.[14,15]

Determination of minimum inhibitory concentration

Mueller-Hinton agar plates were freshly prepared during the experiment. After inoculating the plates with the test organisms using sterile swabs, inoculum was transferred from the standardized inoculum suspension into sterile plates containing 100mL of Mueller-Hinton broth and 100mg/mL of the plant extract were introduced into the plates using sterile syringes and two fold serial dilution was performed to have different concentrations of the extracted plant extract (50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml). The positive control (Amoxicillin) was incorporated into one of the designated plates. The plates were incubated at 37°C for 24 hours. After incubation, the plate was observed for growth. The lowest concentration showing no growth is the minimum inhibitory concentration.

Determination of minimum bactericidal concentration

All MIC plates that showed no visible growth (clear wells) were selected, mixed gently well and sub cultured onto the Mueller-Hinton agar plate free of antimicrobial agents and label according to their concentration. The plates were then incubated at 37°C for 24 hours. After incubation, the plate was observed for the one that show no colony growth.

Measurement of death rate of the isolates using ultra-violet spectrophotometer

The growth dynamic test was used to determine the growth and killing rate of the isolates. A loopful of the colony from the stocked culture slant was inoculated into 9ml nutrient broth and incubated for 24 hours at 37°C. After incubation, a loopful of the organism was transferred into another 9ml nutrient broth in five sets, labeled A, B, C, D, and E. The growth rate was measured using ultraviolet (UV-Vis) spectrophotometer at 600 nm wavelength, which was calibrated with sterilized nutrient broth before measurement. The killing rate was determined using sterilized nutrient broth containing the oil extract. The first reading was taken at zero hours, followed by readings every 8 hours for six intervals to monitor the killing effect over time.[16,17]

Results

The following figures and table show the results as follows

Table 1 Describes the morphology and colonial characteristics of the clinical wound isolates. The most common colony color was creamy, and most isolates exhibited a flat or convex elevation. The majority of the isolates were Gram-negative rods, which are typically

associated with higher resistance to antimicrobial agents. However, two isolates, AG4 and AG10, were Gram-positive cocci, which are usually less resistant. The combination of these characteristics can provide an early indication of the type of bacteria involved in the infections and potential antimicrobial resistance.

Table 1 Morphology colonial characteristics of clinical wound isolate on agar

Isolate code	Colony colour	Elevation	Opacity	Margin	Colony Shape	Gram stain	Shape
AG1	Creamy	Flat	Opaque	Entire	Circular	-ve	rod
AG2	Creamy	Convex	Opaque	Entire	Circular	-ve	rod
AG4	Yellow	Convex	Translucent	Entire	Circular	+ve	cocci
AG5	White	Flat	Opaque	Irregular	Circular	-ve	rod
AG10	Golden Yellow	Convex	Opaque	Entire	Circular	+ve	cocci
AG11	White	Flat	Opaque	Irregular	Circular	-ve	rod
AG15	Creamy	Flat	Opaque	Irregular	Circular	-ve	rod

Keys: AG: University's Health Centre at Adekunle Ajasin University, Akungba-Akoko.

Table 2 outlines the results of the biochemical tests and sugar fermentation for wound isolates. All isolates tested positive for glucose, dextrose, fructose, and sucrose fermentation. The majority of the isolates, except AG2 and AG10, were positive for motility, catalase, and indole tests. However, methyl red and oxidase tests were negative for all isolates except AG2 and AG4. The Voges-Proskauer test was positive for AG2 and AG4, while other isolates were negative. Coagulase and urease tests showed varied results, and gas production (H_2S) was observed only in AG1 and AG4. These findings highlight the diversity in biochemical characteristics across the clinical wound isolates.

Table 3 Shows the identification of clinical wound isolates characterized using Bergey's Manual of Determinative Bacteriology. Based on biochemical tests and sugar fermentation results, AG1 was identified as *Escherichia coli*, AG2 as *Klebsiella ornithinolytica*, AG4 as *Streptococcus sp*., AG5 as *Budvicia aquatica*, AG10 as *Staphylococcus aureus*, AG11 as *Acinetobacter haemolyticus,* and AG15 as *Citrobacter gillericus*. Each isolate's biochemical characteristics and sugar fermentation patterns aided in confirming these identifications.

Table 3 List of clinical wound isolate characterized using Bergey's manual of determinative bacteriology

Isolate code	Probable Isolate
AG1	*Escherichia coli*
AG2	*Klebsiella ornithinolytica*
AG4	*Streptococcus sp*
AG5	*Budvicia aquatic*
AG10	*Staphylococcus aureus*
AG11	*Acinetobacter haemolyticus*
AG15	*Citrobacter gillericus*

Keys: AG: University's Health Centre at Adekunle Ajasin University, Akungba-Akoko

Figure 3 Presents the number of bacterial colonies found on nutrient agar from clinical wound isolates at different dilution factors. The isolate AG11 had the highest colony count of 203 at a dilution factor of 10^3, indicating a high bacterial load, which could suggest a potentially serious infection. On the other hand, AG1 had the lowest colony count of 53 at a dilution factor of 10^5, implying a lower bacterial load. Other isolates, like AG4 and AG10, show moderate colony counts, which may indicate varying infection severity levels.

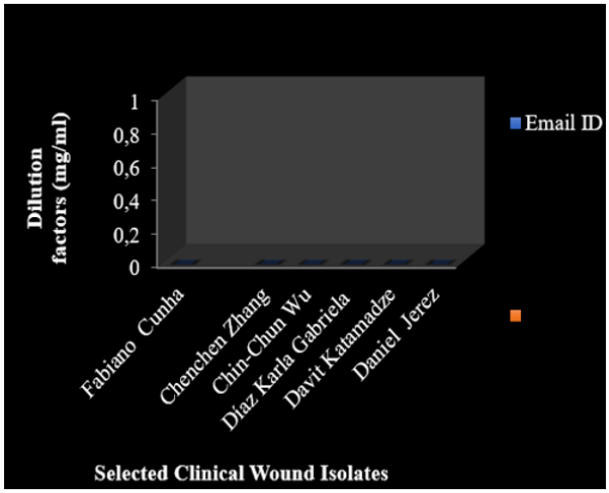

Figure 3 Dilution factor against number of colonies found on nutrient agar on selected clinical wound isolates

Figure 4 compares the zone of inhibition of bacterial isolates when treated with different solvent extracts of Alligator pepper and Turmeric at 100 mg/ml concentration. *Acinetobacter haemolyticus* showed the highest sensitivity with a 18mm zone of inhibition using Dry Alligator Pepper in N-Hexane (DAN), while *Budvicia aquatica* also showed significaant sensitivity (9 mm with DAN and 10 mm with DAE). Generally, the Fresh Turmeric extracts had minimal or no inhibition, and the antibiotic control (Amoxicillin) produced the largest inhibition zones across all isolates, demonstrating higher bacterial susceptibility to the control.

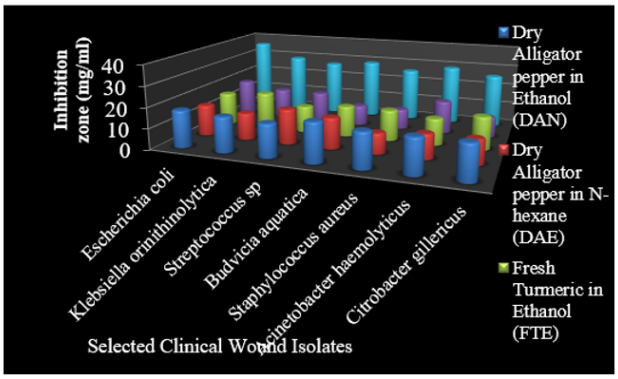

Figure 4 Zone of Inhibition of plant extract extracted at 100mg/ml against selected clinical wound isolates.

Figure 5.1 the antimicrobial activity of solvents and plant extract extracted at 50 mg/ml against various clinical wound isolates. The dry alligator pepper extract in both N-hexane and ethanol exhibited better antimicrobial effects than turmeric extracts. *Acinetobacter haemolyticus* was the most susceptible isolate, showing the largest inhibition zone of 14 mm with N-hexane. In contrast, *Streptococcus sp* had the lowest inhibition, with only 7 mm for ethanol and 3 mm for N-hexane. Overall, dry alligator pepper showed stronger antimicrobial properties compared to turmeric extracts.

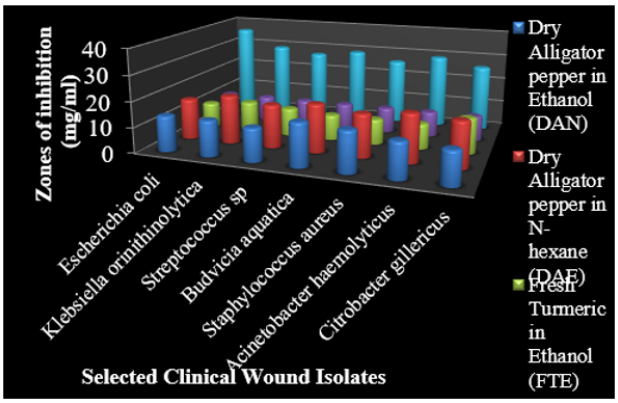

Figure 5.1 Zone of Inhibition of plant extract extracted at 50mg/ml against selected clinical wound isolates.

Figure 5.2 shows the antibacterial activity of the solvents and plant extract extracted at 25mg/ml on clinical wound isolates. Dry Alligator pepper in both ethanol (DAE) and N-hexane (DAN) exhibited the most notable inhibition zones, particularly against isolates like *Acinetobacter haemolyticus* and *Budvicia aquatica*, with inhibition zones of 6mm for each. Other isolates, such `as *Escherichia coli* and *Staphylococcus aureus*, had inhibition zones of 4mm and 3mm, respectively, when treated with DAE. On the other hand, Fresh Turmeric in ethanol (FTE) and N-hexane (FTN) showed minimal to no inhibition across the isolates, indicating weaker antibacterial activity at this concentration.

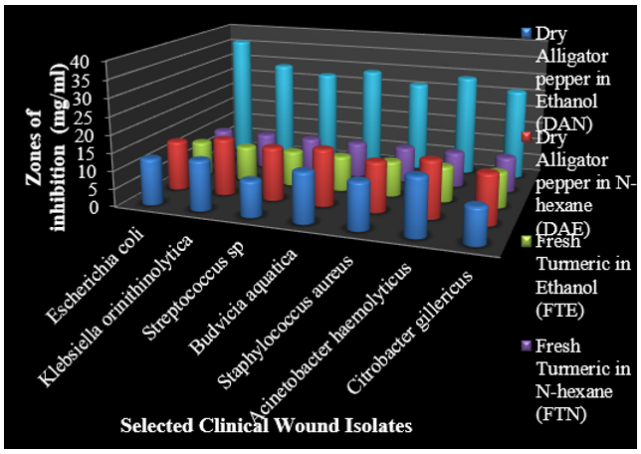

Figure 5.2 Zone of Inhibition of plant extract extracted at 25mg/ml against selected clinical wound isolates.

Figure 5.3 shows the inhibition zones at 12.5 mg/ml concentration. Dry Alligator pepper in N-hexane (DAN) had the largest inhibition zone (4 mm) against *Acinetobacter haemolyticus*, while no activity was observed for *Escherichia coli* and *Citrobacter gillericus*. Dry Alligator pepper in ethanol (DAE) displayed 4 mm against *Klebsiella ornithinolytica* and *Acinetobacter haemolyticus.* Neither Fresh Turmeric in ethanol (FTE) nor in N-hexane (FTN) showed inhibition for any isolates. The positive control, Amoxicillin, consistently produced large inhibition zones, with 36 mm against *Escherichia coli*.

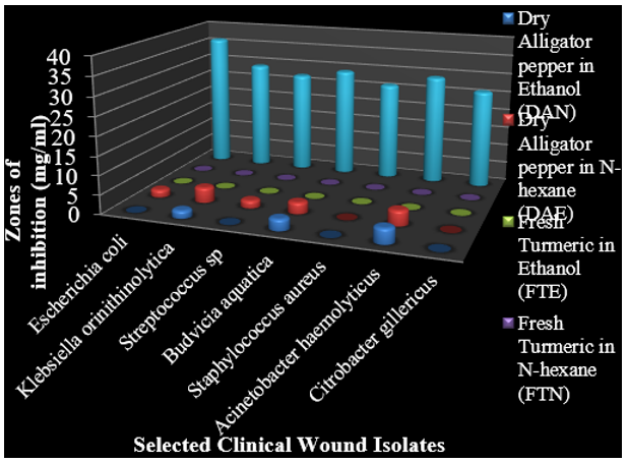

Figure 5.3 Zone of Inhibition of plant extract extracted at 12.5mg/ml against selected clinical wound isolates.

Figure 5.4 shows that, at 6.25 mg/ml, Dry Alligator pepper in Ethanol (DAE) showed slight activity against *Klebsiella ornithinolytica* (3 mm) and *Acinetobacter haemolyticus* (2 mm). No inhibition was observed for other isolates, and Fresh Turmeric in both solvents showed no effect across all isolates. Amoxicillin (CON) had consistent activity, with zones ranging from 26 mm to 36 mm across all isolates.

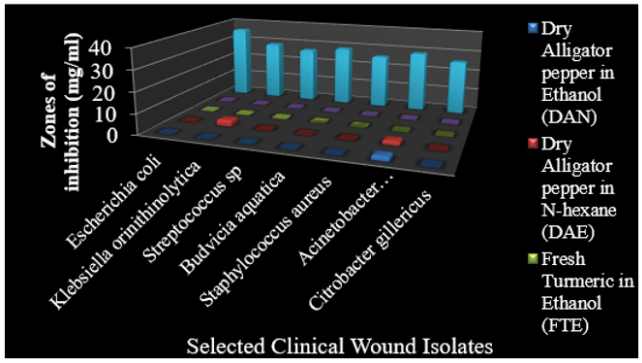

Figure 5.4 Zone of Inhibition of plant extract extracted at 6.25mg/ml against selected clinical wound isolates.

Figure 6 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Aframomum melegueta* against clinical wound isolates. The fig indicates that the MIC of *Aframomum melegueta* oil against the isolates ranges from 12.5 mg/ml to 25 mg/ml, while the MBC values range from 25 mg/ml to 50 mg/ml. For example, the oil exhibited MIC values of 12.5 mg/ml for *Escherichia coli, Klebsiella ornithinolytica*, *and Budvicia aquatica,* while the MBC was 25 mg/ml for these isolates. On the other hand, *Streptococcus* sp., *Staphylococcus aureus*, and *Citrobacter gillericus* exhibited higher MIC values of 25 mg/ml and MBC of 50 mg/ml. This suggests that *Aframomum melegueta* plant extract is effective against these isolates at these concentrations.

Figure 7 shows the killing time of clinical wound isolates treated with Aframomum melegueta oil at 100mg/ml. The results indicate that *Acinetobacter haemolyticus* exhibited the fastest killing time, with complete inhibition after 24 hours, starting from an initial

absorbance of 0.103 at 0 hours, and reducing steadily to 0.002 by the 24th hour. *Klebsiella ornithinolytica* followed, showing a reduction from 0.071 at 0 hours to complete inhibition at 32 hours. Meanwhile, *Escherichia coli* demonstrated the longest survival, with a reduction from 0.047 to 0.001 by 32 hours. Other isolates like Streptococcus sp., *Budvicia aquatica*, and *Citrobacter gillericus* showed similar patterns, achieving complete inhibition within the same timeframe.

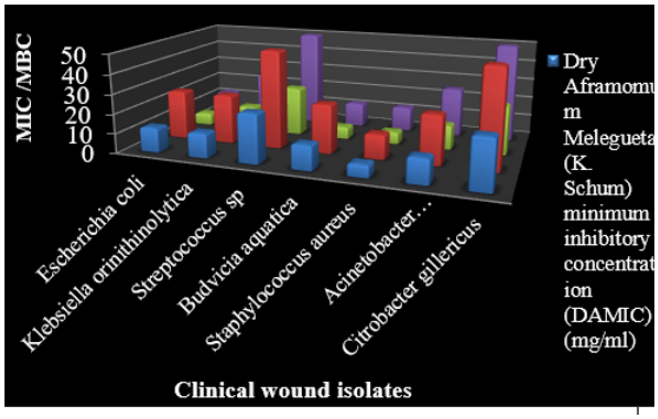

Figure 6 Minimum inhibitory concentration and minimum bactericidal concentration of *Aframomum melegueta* and *Curcuma longa* against clinical wound isolates.

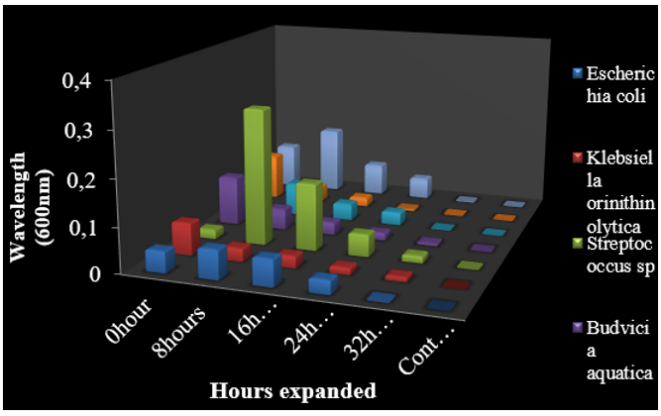

Figure 7 Effect of 100 mg *Aframomum melegueta* plant extract on the growth and killing time of various bacterial isolates.

Figure 7.1 shows killing time of clinical wound isolates treated with *Aframomum melegueta* extract at 50mg/ml is shown. *Acinetobacter haemolyticus* demonstrated the fastest reduction, starting with an absorbance of 0.146 at 0 hours and dropping steadily to 0.004 by 32 hours. *Klebsiella ornithinolytica* had a similar trend, showing a reduction from 0.123 to complete inhibition at 32 hours. *Escherichia coli* and *Citrobacter gillericus* displayed longer survival, requiring up to 32 hours for complete inhibition. Other isolates like *Budvicia aquatica* and *Staphylococcus aureus* also showed significant reduction, reaching complete inhibition within 32 hours.

Figure 7.2 Figure presents the killing time of bacterial isolates treated with *Aframomum melegueta* extract at 25mg/ml. *Acinetobacter haemolyticus* continued to show strong inhibition, with a reduction in absorbance from 0.102 at 0 hours to 0.002 at 32 hours. *Klebsiella ornithinolytica* followed a similar pattern, reducing from 0.068 to 0.002 by 32 hours. *Streptococcus sp.* and *Staphylococcus aureus* showed slower reduction, requiring the full 32 hours for complete inhibition. In contrast, *Escherichia coli* and *Citrobacter gillericus* took longer to achieve complete inhibition.

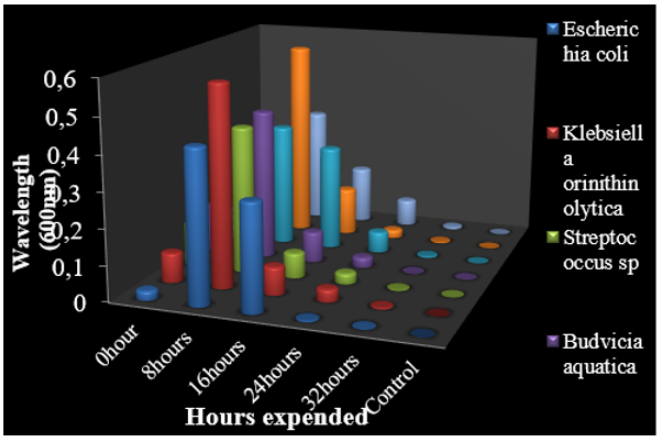

Figure 7.1 Effect of 50 mg *Aframomum melegueta* plant extract on the growth and killing time of various bacterial isolates.

Figure 7.3 shows the killing time of bacterial isolates treated with 12.5mg of Aframomum melegueta extract. All isolates showed a gradual reduction in growth over time, with complete inhibition by 32 hours. *Klebsiella ornithinolytica* and *Streptococcus sp.* had faster responses, while *Acinetobacter haemolyticus* and *Citrobacter gillericus* took longer to show growth reduction. Overall, the oil was effective in eliminating bacterial growth within the 32-hour period.

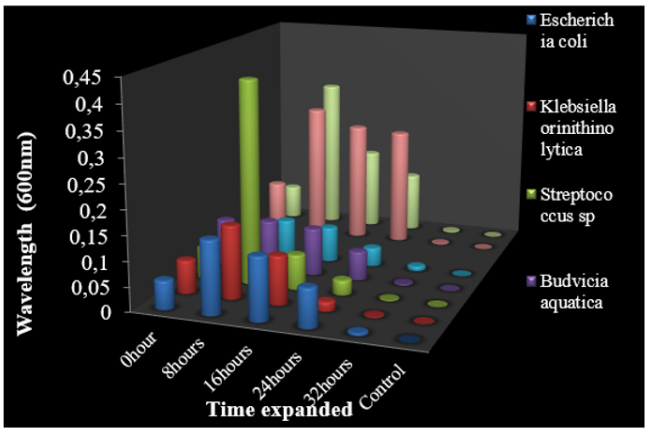

Figure 7.1 Effect of 12.5mg *Aframomum melegueta* plant extract on the growth and killing time of various bacterial isolates.

Figure 7.4 The killing kinetics of *Aframomum melegueta* extract at 6.25 mg/ml showed gradual reductions in absorbance across all bacterial isolates, indicating a decline in bacterial growth over time. By 32 hours, all isolates demonstrated near-complete inhibition, with the most significant reduction seen in *Streptococcus sp*. and *Klebsiella ornithinolytica*. This suggests that *Aframomum melegueta* extract is effective in killing or significantly inhibiting these bacterial isolates at this concentration.

Figures 4, 5.1, 5.2, 5.3 and 5.4 Measuring synergistic zone of inhibition of plant extract extracted at 100, 50, 25, 12, 25 and 6.25 mg/ml against selected clinical wound isolates

Figure 5.1 Measuring synergistic zone of inhibition of solvent and plant extract extracted at 50mg/ml against selected clinical wound isolates

Figure 6 Measuring minimum inhibitory concentration and minimum bactericidal concentration of *Aframomum melegueta* against clinical wound isolates

Citation: Osuntokun OT, Mitchell K. *In vitro* evaluation and mechanistic insights into the antimicrobial activity of *Aframomum melegueta* and *Curcuma longa* blends against wound isolates. *J Bacteriol Mycol Open Access*. 2026;14(2):63–72. DOI: 10.15406/jbmoa.2026.14.00432

Figures 7, 7.1, 7.2,.7,3 and 7.4 Killing time of bacteria isolates with 100, 50, 25, 12.5, and 6.25 mg of *Aframomum melegueta* plant extract using ultraviolet spectrophotometer with wavelength 600.

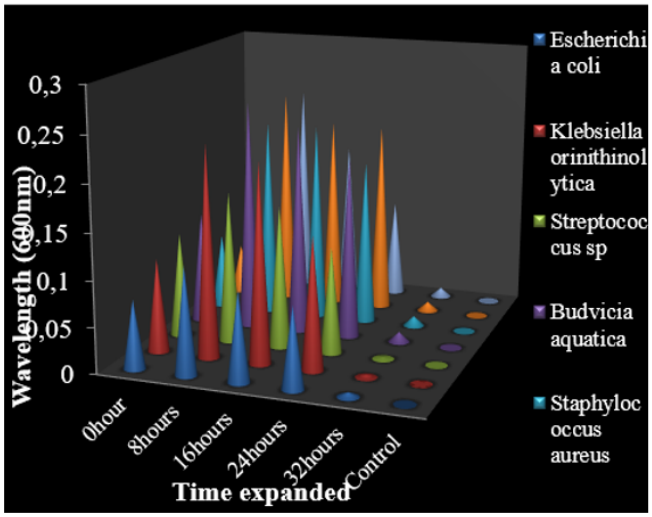

Figure 7.4 Effect of 6.25 mg *Aframomum melegueta* plant extract on the growth and killing time of various bacterial isolates.

Figures 8, 8.1, 8.2, 8.3 and 8.4 Killing time of bacteria isolates with 100, 50, 25, 12.5 and 6,25 mg of *Curcuma Longa (L.)* plant extract using Ultraviolet spectrophotometer with wavelength 600.

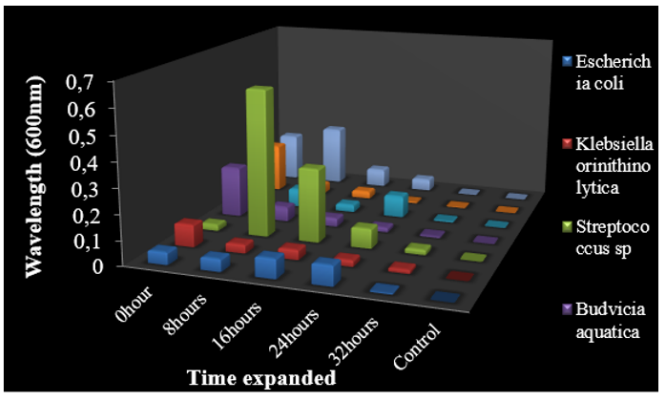

Figure 8 Effect of 100 mg *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

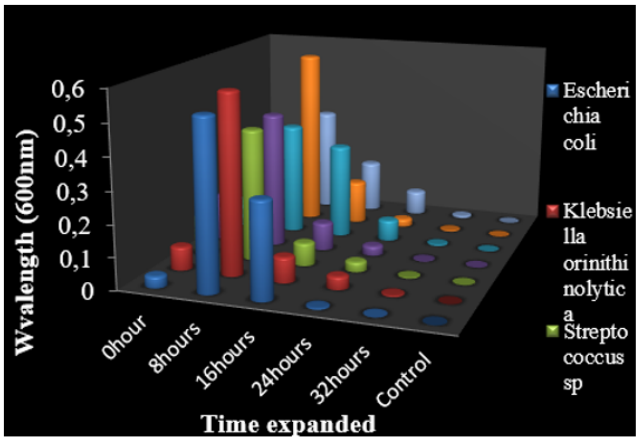

Figure 8.1 Effect of 50 mg *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

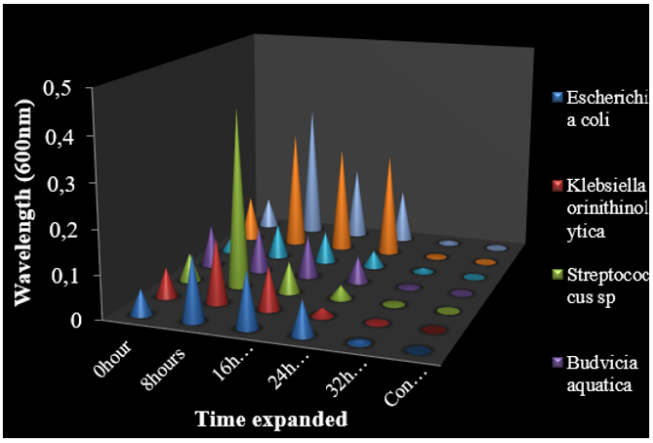

Figure 8.2 Effect of 25 mg *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

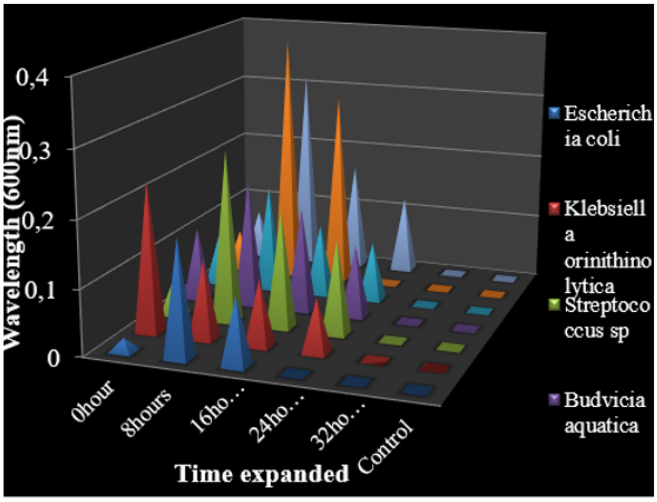

Figure 8.3 Effect of 12.5 mg *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

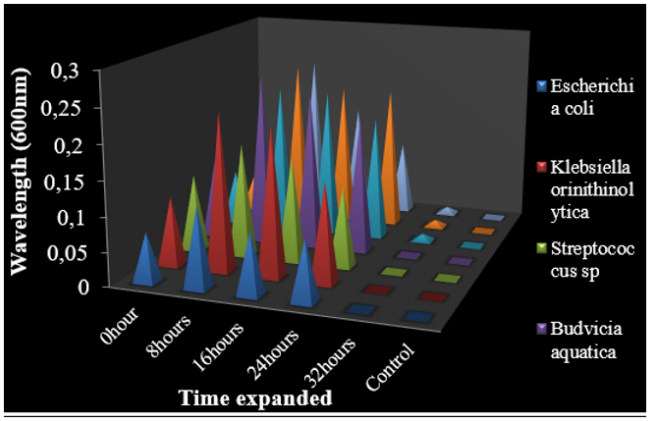

Figure 8.4 Effect of 6.25 mg *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

Figure 9 Killing time of bacteria isolates with 100mg of *Aframomum melegueta* and *Curcuma longa* (L.) Essential using Ultraviolet spectrophotometer with wavelength 600.

Citation: Osuntokun OT, Mitchell K. *In vitro* evaluation and mechanistic insights into the antimicrobial activity of *Aframomum melegueta* and *Curcuma longa* blends against wound isolates. *J Bacteriol Mycol Open Access*. 2026;14(2):63–72. DOI: 10.15406/jbmoa.2026.14.00432

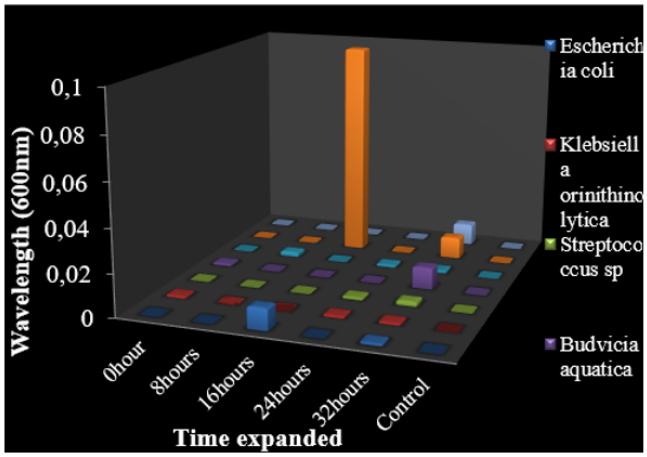

Figure 9 Effect of 100 mg *Aframomum melegueta* and *Curcuma longa* (L.) plant extract on the growth and killing time of various bacterial isolates.

Discussion

This study highlights the antibacterial efficacy of *Aframomum melegueta* (Grains of Paradise) and *Curcuma longa* (Turmeric), plant extract demonstrating their potential in treating wound infections. Both plants are traditionally known for their medicinal uses, but their specific antibacterial effects against clinical wound isolates have remained under searched. The findings of this research offer insights into the bioactivity of these plant-derived extract, reinforcing their relevance as alternative antimicrobial agents.

The antibacterial mechanisms of *Aframomum melegueta* and *Curcuma longa* plant extract, which were extracted using ethanol and n-hexane, were explored in this study. The analysis revealed significant zones of inhibition against the selected bacterial isolates, indicating that these plant extract have potent antibacterial activity. Notably, *Aframomum melegueta* extract exhibited strong inhibition against *Acinetobacter haemolyticus* and *Citrobacter gillericus*, while *Curcuma longa* demonstrated considerable activity against *Klebsiella ornithinolytica* and *Streptococcus sp*. These findings are in line with previous research that has documented the efficacy of plant-based extracts in combating bacterial infections, particularly multidrug-resistant pathogens.[9,18,19]

Aframomum melegueta and *Curcuma longa* plant extract showed a concentration-dependent inhibition of bacterial growth, as indicated by the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. For instance, the MIC and MBC values of *Aframomum melegueta* were lower for Gram-negative bacteria like *Escherichia coli* and *Klebsiella ornithinolytica,* suggesting that these oils may be more effective against these organisms. This is consistent with other studies that highlight the effectiveness of *Aframomum melegueta* in inhibiting both Gram-positive and Gram-negative bacteria due to the presence of bioactive compounds like 6-gingerol and paradol.[19-21] *Curcuma longa*, while effective, exhibited comparatively lower antibacterial activity, which may be due to the specific extraction methods used or the concentration of bioactive compounds in the samples tested.[22]

The killing kinetics experiment provided further evidence of the extract' antibacterial potential, with *Acinetobacter haemolyticus* showing the fastest reduction in bacterial growth. This aligns with existing research, which has noted the fast-acting antibacterial effects of *Aframomum melegueta* due to its disruption of bacterial cell membranes.[21,23] The time-kill assay also demonstrated that higher concentrations of the oils resulted in more rapid bacterial eradication, supporting the use of these oils in concentrated forms for clinical applications.[24]

Killing time of bacteria isolates with 100, 50, 25, and 12.5 mg of *Aframomum melegueta* plant extract using an ultraviolet spectrophotometer with wavelength 600. It was observed that between the 0th hour and 24th hours, some of the organisms were still active, i.e, the action of the *Aframomum melegueta* plant extract was bacteriostatic in action, while at the 32nd hour, they were bacteriocide in action. This depicts that, before any action of the medicinal plant on the wound isolate, it will take a whole day, thereby supporting the healing of the wound and totally eradicating the causative and opportunistic organisms.[24,25]

Killing time of bacteria isolates with 100, 25, 50, and 12.5mg of *Curcuma longa(L.)* plant extract using an ultraviolet spectrophotometer with wavelength 600, it was also observed that *Curcuma longa* were bacteriostatic in action between 0th and 16th hours and bacteriocide in action between 24th and 32nd hours In Synergy combining *Aframomum melegueta* and *Curcuma longa,* the result is magnificent; it was observed that at 100 mg/mL, all test organisms were totally inhibited (bactericidal). it shows that the two plants have a promising ability to reduce the microbial load in wound infection. It can also be deduced that both plants have a broad spectrum in action.[25,26]

The effectiveness of *Curcuma longa* was also validated through its performance in inhibiting bacteria like *Streptococcus sp.* and *Staphylococcus aureus*, which are commonly associated with wound infections. Curcumin, the primary bioactive compound in turmeric, is known for its ability to inhibit bacterial enzymes and disrupt membrane integrity, as evidenced by its inhibition of bacterial biofilm formation.[27,28] These results reinforce the potential application of turmeric in modern wound care.

In conclusion, this study demonstrates the antibacterial potential of *Aframomum melegueta* and *Curcuma longa* plant extract against bacterial isolates from wound infections. *Aframomum melegueta* exhibited stronger antibacterial activity than *Curcuma longa*, possibly due to extraction methods and bioactive compound concentrations. Both plants extract showed concentration-dependent inhibition, suggesting their effectiveness as natural alternatives to synthetic antibiotics.[29-34] While *Curcuma longa* was effective against specific strains, *Aframomum melegueta* showed faster and more potent bacterial inhibition. These findings are preliminary and limited to in vitro observations.

Based on the results of the study, some recommendations are as follows:

a) **Further research on extraction methods:** Since the antibacterial activity of *Curcuma longa* was lower compared to *Aframomum melegueta*, future studies should focus on optimizing extraction methods to maximize the yield of bioactive compounds, potentially enhancing its efficacy.

b) **Synergistic studies with conventional antibiotics:** Investigating the potential synergistic effects of these plant extract with conventional antibiotics could reveal whether they can be used in combination to enhance the overall treatment of wound infections.

c) **In vivo studies:** To validate the findings from this in vitro study, in vivo experiments are recommended to assess the safety, efficacy, and bioavailability of *Aframomum melegueta* and *Curcuma longa* oils in animal models or clinical trials involving human subjects.

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d) **Development of formulations**: Researchers should explore the formulation of these plant extract into topical creams, gels, or ointments for wound care, providing practical and easily applicable treatment options.

e) **Toxicological studies:** Comprehensive toxicological assessments are necessary to determine the safe dosage ranges and minimize any potential adverse effects, particularly for long-term use in clinical settings.

f) **Exploration of other plant extracts:** Expanding research to include other medicinal plants with antibacterial properties could further contribute to the development of natural alternatives for managing bacterial infections, enhancing the repertoire of plant-based treatments available for wound care.

Limitation of the study

This study was subject to several limitations that should be considered when interpreting the findings.

a) **Limited number of isolates**: The study utilized relatively small number of microbial isolates, which may not fully represent the diversity and variability of wound pathogens present in a wider population.

b) **Absence of molecular identification:** Identification of the test microorganisms was based primarily on conventional cultural, morphological, and biochemical methods. Molecular techniques such as 16S rRNA sequencing, polymerase chain reaction (PCR), MALDI-TOF MS, or automated identification systems were not employed, which limited the accuracy of microbial identification.

c) **Lack of phytochemical characterization**: Although the antimicrobial activity of the plant extract was evaluated, detailed phytochemical characterization and quantification of the bioactive compounds responsible for the observed effects were not conducted. Therefore, the specific constituents contributing to the antimicrobial activity was not be determined.

d) **Absence of toxicity studies:** The safety profile of the plant extract was not assessed through acute, sub-acute, or chronic toxicity studies. As a result, conclusions regarding the safety and potential adverse effects of the extract cannot be made based on the findings of this study.

e) **Lack of animal and clinical validation:** This study was limited to in vitro experiments, and the efficacy of the extract was not evaluated in animal models or human subjects. You may also Despite these limitations, the study provides valuable preliminary data on the antimicrobial potential of the investigated extract and serves as a basis for future studies involving molecular identification, phytochemical profiling, toxicity assessment, and *in vivo* validation.

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Conflicts of interest

The authors declare that there are no conflicts of interests.

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