

## International Journal of Multidisciplinary Research in Biotechnology, Pharmacy, Dental and Medical Sciences (IJMRBPDMS)

### Antibacterial Activity of *Curcuma longa* (Turmeric) Ethanolic Extract Against *Staphylococcus aureus* and *E. coli*: An In Vitro Study

Oyegue, Anthonia O.<sup>1</sup>; Anslem S. Maichiki <sup>1</sup>; Olowosoye, Ebunoluwa Grace<sup>1</sup>

<sup>1</sup>Department of Microbiology, Veritas University, Abuja, Nigeria

**Corresponding Author:** Oyegue, Anthonia, Department of Microbiology, Veritas University, Abuja, Nigeria  
Email: osathoniaoyegue@gmail.com

#### ABSTRACT

##### Background:

Antimicrobial resistance (AMR) is a growing global public health crisis that undermines the effectiveness of conventional antibiotics. Natural plant-derived compounds have received renewed attention as alternative or adjunct antimicrobial agents. *Curcuma longa* (turmeric), a member of the Zingiberaceae family, contains bioactive constituents, notably curcumin, flavonoids, tannins, and alkaloids, with well-documented antibacterial properties. The aim of this study is to evaluate the in vitro antibacterial activity of the ethanolic extract of *Curcuma longa* rhizomes against *Staphylococcus aureus* and *E. coli*.

##### Methods:

Fresh rhizomes of *C. longa* were sourced from Dei Dei Market, Abuja, Nigeria, and extracted by cold maceration in 90% ethanol for 72 hours. *Staphylococcus aureus* was isolated from a throat swab and confirmed by Mannitol Salt Agar (MSA) morphology, Gram staining, and catalase and coagulase tests. *E. coli* was isolated from a campus tap water sample and confirmed on Eosin Methylene Blue (EMB) Agar with positive indole and methyl red tests. Antibacterial activity was assessed by agar well diffusion on Mueller-Hinton Agar (MHA) at six extract concentrations (50, 100, 200, 400, 800, and 1000 mg/mL). Ciprofloxacin (5 µg) and Ampicillin (10 µg) served as positive controls; DMSO served as the negative control.

##### Results:

No inhibitory activity was observed at 50–200 mg/mL for either organism. At 400, 800, and 1000 mg/mL, mean zones of inhibition (ZOI) against *S. aureus* were 15.0, 22.0, and 27.5 mm respectively; against *E. coli*, ZOI were 11.5, 17.0, and 21.5 mm. *Staphylococcus aureus* was consistently more susceptible than *E. coli* at all active concentrations. At 1000 mg/mL, the extract marginally exceeded both the Ciprofloxacin (26.0 mm) and Ampicillin (21.0 mm) positive controls.

##### Conclusion:

Locally sourced Nigerian turmeric demonstrates significant, concentration-dependent antibacterial activity against both test organisms, with *S. aureus* being more susceptible than *E. coli*, consistent with the structural differences between Gram-positive and Gram-negative cell walls. These findings support *C. longa* as a promising natural antimicrobial candidate in the context of antimicrobial resistance.

**Keywords:** *Curcuma longa*; turmeric; antibacterial activity; *Staphylococcus aureus*; *E. coli*; agar well diffusion; antimicrobial resistance.

##### Article Info

Received: 05 January 2026, received in revised form: 15 January 2026, accepted: 20 January 2026, available online: 30 January 2026; Volume: 2; Issue: 1; Pages: 35-42.

**Citation:** Anthonia O., 2026. Antibacterial Activity of *Curcuma longa* (Turmeric) Ethanolic Extract Against *Staphylococcus aureus* and *E. coli*: An In Vitro Study. *International Journal of Multidisciplinary Research in Biotechnology, Pharmacy, Dental and Medical Sciences (IJMRBPDMS)* 2(1): 35-42.

##### DOI:

## Introduction

The escalating global prevalence of antibiotic resistance in pathogenic microorganisms represents one of the most serious threats to modern medicine. The World Health Organization (WHO) has classified antimicrobial resistance (AMR) as one of the top ten threats to global public health (WHO, 2023). Resistance to antibacterial agents is increasingly identified as a primary cause of therapeutic failure, raising morbidity, mortality, and healthcare costs worldwide (WHO, 2023). This situation has galvanised the search for effective, safe, and economically accessible natural products and phytochemicals as alternatives or adjuncts to conventional antibiotics (Vaou *et al.*, 2021) and (Ekpe *et al.*, 2018).

*Curcuma longa* commonly referred to as Turmeric, belongs to the Zingiberaceae family and is prevalent in tropical regions, particularly across the Indian subcontinent and South Asia (Abdullahi *et al.*, 2024). The rhizomes of

turmeric contain curcuminoids, including curcumin, bisdemethoxycurcumin, and dimethoxycurcumin, alongside an array of flavonoids, tannins, alkaloids, and saponins, all of which contribute to its broad pharmacological profile (Fuloria *et al.*, 2022). Curcumin has been recognised as an anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory agent (Hamzah *et al.*, 2020) and (Obum-Nnadi *et al.*, 2022).

*Staphylococcus aureus* is a Gram-positive, facultatively anaerobic coccus and one of the most clinically relevant human pathogens, asymptotically colonising approximately 30% of the human population (Mun *et al.*, 2013). It is a leading cause of skin and soft tissue infections, bacteraemia, pneumonia, endocarditis, and toxic shock syndrome (Algammal *et al.*, 2021). Methicillin-resistant *S. aureus* (MRSA) has emerged as a global priority pathogen (Craft & Nguyen, 2019). *E. coli*, a Gram-negative facultatively anaerobic rod in the family Enterobacteriaceae, is a major cause of urinary tract infections, diarrhoeal disease, and neonatal sepsis; multidrug-resistant strains are increasingly isolated in clinical settings across Nigeria and globally (Nasrollahian *et al.*, 2024). Given the mounting evidence of AMR and the centuries-long ethnomedicinal use of turmeric in treating infectious diseases, evaluating the antibacterial efficacy of *C. longa* against *S. aureus* and *E. coli* constitutes a scientifically valid and globally relevant research pursuit (Vaou *et al.*, 2021; Zheng *et al.*, 2020). Understanding the antibacterial dose-response relationship of locally sourced Nigerian turmeric against these pathogens may provide empirical evidence to support the development of plant-based adjunct therapies in resource-limited settings where access to advanced antibiotics remains constrained.

The aim of this study was to evaluate the *in vitro* antibacterial activity of the ethanolic extract of *Curcuma longa* rhizomes against *Staphylococcus aureus* and *E. coli* using the agar well diffusion method. The specific objectives were: (i) to extract bioactive compounds from *C. longa* using ethanol maceration; (ii) to isolate and identify *S. aureus* and *E. coli* from clinical and environmental samples; and (iii) to assess the antibacterial activity of the extract at six concentration levels and compare results against standard antibiotic controls.

## Materials and Methods

### Study Design and Area of Study

This research employed an experimental, *in vitro* laboratory-based study design. All microbiological analyses were performed in the Microbiology Laboratory, Veritas University, Abuja, Nigeria. The experimental layout comprised six extract concentrations (50, 100, 200, 400, 800, and 1000 mg/mL), a positive control, and a negative control (DMSO). Outcomes were determined by measuring zones of inhibition (ZOI) under standardised laboratory conditions. All experiments were performed in duplicate.

### Plant Material Collection and Preparation

Fresh rhizomes of *Curcuma longa* were purchased from Dei Dei Market, Abuja, Federal Capital Territory, Nigeria, and selected based on freshness and absence of mould. Rhizomes were washed, peeled, thinly sliced (approximately 2–3 mm), and dried at room temperature (25–28°C) away from direct sunlight for 20 days (Salem *et al.*, 2022). The dried material was blended and sieved (0.5 mm pore size) to a uniform fine powder, then stored in a dry, airtight container.

### Ethanol Extraction by Maceration

A quantity of 344.06 g of turmeric powder was transferred into a sterile 1000 mL conical flask, and 1000 mL of 90% ethanol was added (solid-to-solvent ratio approximately 1:3 w/v). The mixture was sealed with aluminium foil and allowed to macerate at room temperature for 72 hours with intermittent stirring (Odo *et al.*, 2023). The macerate was filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated on a water bath at 50°C to yield a semi-solid paste, which was stored at 4°C in a sterile, dark-glass bottle until use. Sterility of the extract was confirmed by inoculation onto Nutrient Agar (incubated 37°C for 24 hours) prior to antibacterial testing.

#### Preparation of Extract Concentrations

Working concentrations of 50, 100, 200, 400, 800, and 1000 mg/mL were prepared by dissolving appropriate quantities of the paste extract in DMSO in sterile Eppendorf tubes. DMSO was selected as the solubilising agent because it dissolves hydrophobic plant extracts without exhibiting inherent antibacterial activity at the concentrations employed (Beshiru *et al.*, 2024).

### Isolation and Identification of Test Organisms

#### • *Staphylococcus aureus*

A throat swab was obtained from a consenting volunteer using a sterile cotton swab. The sample was enriched in sterile peptone water at 37°C for 24 hours, then streaked onto Mannitol Salt Agar (MSA) plates incubated at 37°C for 24–48 hours. Yellow colonies indicating mannitol fermentation were selected and subcultured onto Nutrient Agar. Identification was confirmed by: Gram staining (Gram-positive cocci in clusters), positive catalase test (3% H<sub>2</sub>O<sub>2</sub>), positive tube coagulase test (rabbit plasma, 37°C, 4–24 hours), and beta-haemolysis on Blood Agar (Tille,

2017; Beshiru et al., 2024).

- **Escherichia coli**

A 100 mL tap water sample was collected within the Veritas University campus. Following pre-enrichment in sterile peptone water at 37°C for 24 hours, the culture was streaked onto EMB Agar (37°C, 24 hours). Colonies exhibiting a characteristic metallic green sheen were selected. Confirmation was by Gram staining (Gram-negative rods), positive indole test (Kovac's reagent), and positive methyl red (MR) test (Odo *et al.*, 2023; Cheesbrough, 2017).

### Antibacterial Susceptibility Testing

Confirmed isolates were separately suspended in sterile peptone water and turbidity adjusted to 0.5 McFarland standard ( $\approx 1.5 \times 10^8$  CFU/mL). Mueller-Hinton Agar plates were inoculated by lawn swabbing in three directions (0°, 60°, 120°) and allowed to dry. Six wells (6 mm diameter, bored with a sterile cork borer) were loaded with 100 µL of each concentration. Positive controls were Ciprofloxacin (5 µg) for *S. aureus* and Ampicillin (10 µg) for *E. coli*, which were applied as discs on the agar surface. DMSO only served as the negative control. Plates were pre-diffused for 30–60 minutes at room temperature, then incubated inverted at 37°C for 24 hours. ZOI diameters (including well diameter) were measured in mm using a transparent ruler; results are reported as mean values of duplicate readings (Balouiri *et al.*, 2016; Beshiru *et al.*, 2024).

## Results

### Cultural and Biochemical Characterisation of Isolates

Table 1 presents the cultural characteristics of the isolates obtained on selective media. MSA isolates (MSA 001 and MSA 002) produced yellow, smooth, opaque colonies with positive mannitol fermentation. EMB isolates (EMB 001 and EMB 002) produced dark blue-black colonies with a positive metallic green sheen. Table 2 summarises the confirmatory biochemical and pathogenicity tests. Both MSA isolates were Gram-positive cocci in clusters, catalase-positive, coagulase-positive, and produced haemolysis on Blood Agar, confirming their identity as *Staphylococcus aureus*. Both EMB isolates were Gram-negative rods, indole-positive, and methyl red-positive, consistent with *E. coli* (Tille, 2017; Cheesbrough, 2017).

**Table 1: Cultural Characterisation of Isolates**

Isolate	Colony Colour	Shape	Surface	Elevation	Edge	Differential Reaction	Opacity
MSA 001	Yellow	Circular	Smooth/Shiny	Flat	Entire	Mannitol Ferm.: (+)	Opaque
MSA 002	Yellow	Oval	Smooth/Shiny	Slightly raised	Entire	Mannitol Ferm.: (+)	Opaque
EMB 001	Dark/Blue-black	Circular	Smooth/Shiny	Flat	Entire	Metallic Sheen: (+)	Opaque
EMB 002	Dark/Blue-black	Circular	Smooth/Shiny	Flat	Entire	Metallic Sheen: (+)	Opaque

Keys: MSA – Mannitol Salt Agar; EMB – Eosin Methylene Blue Agar

**Table 2: Biochemical and Pathogenicity Tests for Isolates**

Isolate	Gram Reaction	Catalase	Coagulase	Indole	Methyl Red	Haemolysis	Organism
MSA 001	G+ve cocci (clusters)	+	+	NT	NT	Beta (β)	<i>S. aureus</i>
MSA 002	G+ve cocci (clusters)	+	+	NT	NT	Alpha (α)	<i>S. aureus</i>
EMB 001	G-ve rods	NT	NT	+	+	Negative (-)	<i>E. coli</i>
EMB 002	G-ve rods	NT	NT	+	+	Negative (-)	<i>E. coli</i>

Keys: NT – Not Tested; G+ve – Gram-positive; G-ve – Gram-negative

### Antibacterial Activity Against *Staphylococcus aureus*

No zone of inhibition was produced at 50, 100, or 200 mg/mL. At 400 mg/mL the mean ZOI was 15.0 mm; at 800 mg/mL it was 22.0 mm; and at 1000 mg/mL it was 27.5 mm. The Ciprofloxacin positive control produced a ZOI of 26.0 mm. At 1000 mg/mL the extract marginally exceeded the antibiotic control. The DMSO negative control produced no inhibition (Table 3).

**Table 3: Antibacterial Activity of *C. longa* Extract Against *S. aureus***

Concentration (mg/mL)	Trial 1 – ZOI (mm)	Trial 2 – ZOI (mm)	Mean ZOI (mm)
50	0	0	0
100	0	0	0
200	0	0	0
400	16	14	15.0
800	22	22	22.0
1000	28	27	27.5
Positive Control (Ciprofloxacin 5 µg)	26	26	26.0
Negative Control (DMSO)	0	0	0

**Keys: ZOI – Zone of Inhibition; DMSO – Dimethyl Sulphoxide**

#### Antibacterial Activity Against *E. coli*

No zone of inhibition was detected at 50, 100, or 200 mg/mL. At 400 mg/mL the mean ZOI was 11.5 mm; at 800 mg/mL it was 17.0 mm; and at 1000 mg/mL it was 21.5 mm. The Ampicillin positive control produced 21.0 mm. The extract at 1000 mg/mL marginally exceeded the antibiotic control. DMSO produced no inhibition (Table 4).

**Table 4: Antibacterial Activity of *C. longa* Extract Against *E. coli***

Concentration (mg/mL)	Trial 1 – ZOI (mm)	Trial 2 – ZOI (mm)	Mean ZOI (mm)
50	0	0	0
100	0	0	0
200	0	0	0
400	11	12	11.5
800	18	16	17.0
1,000	22	21	21.5
Positive Control (Ampicillin 10 µg)	21	21	21.0
Negative Control (DMSO)	0	0	0

**Keys: ZOI – Zone of Inhibition; DMSO – Dimethyl Sulphoxide**

#### Comparative Antibacterial Activity

At all active concentrations, *S. aureus* consistently produced larger ZOI than *E. coli*. The differences in mean ZOI at 400, 800, and 1000 mg/mL were 3.5, 5.0, and 6.0 mm respectively, indicating that the differential susceptibility between the two organisms widened with increasing extract concentration (Table 5).

**Table 5: Comparative ZOI of *C. longa* Extract Against *S. aureus* and *E. coli***

Concentration (mg/mL)	Mean ZOI – <i>S. aureus</i> (mm)	Mean ZOI – <i>E. coli</i> (mm)	Difference (mm)
50	0	0	0
100	0	0	0
200	0	0	0
400	15.0	11.5	3.5

Concentration (mg/mL)	Mean ZOI – <i>S. aureus</i> (mm)	Mean ZOI – <i>E. coli</i> (mm)	Difference (mm)
800	22.0	17.0	5.0
1,000	27.5	21.5	6.0
Negative Control (DMSO)	0	0	0

Keys: ZOI – Zone of Inhibition; DMSO – Dimethyl Sulphoxide

## Discussion

### Antibacterial Activity Against *Staphylococcus aureus*

The ethanolic extract of *Curcuma longa* produced no measurable inhibitory activity against *S. aureus* at concentrations of 50, 100, and 200 mg/mL, but demonstrated clear, concentration-dependent antibacterial activity at 400, 800, and 1000 mg/mL, yielding mean zones of inhibition of 15.0, 22.0, and 27.5 mm respectively. This pattern is consistent with the findings of Momoh et al. (2022), who tested aqueous and methanolic extracts of *C. longa* against *S. aureus* at LASUSTECH, Lagos, and reported zones of inhibition ranging from 22.31 to 30.13 mm at concentrations of 250–500 mg/mL, values directly comparable to those obtained in the present study at 800–1000 mg/mL. Similarly, Abdullahi et al. (2024) working with turmeric rhizome extract from Kaduna State, reported inhibition zones of 10–17 mm against *S. aureus* clinical isolates using methanol extract, which falls below the 15.0–27.5 mm range recorded here. This discrepancy is most plausibly attributable to the higher concentration range employed in the present study (up to 1000 mg/mL) and to possible variation in curcuminoid content between geographically distinct plant material sources, since Salem et al. (2022) demonstrated that phytochemical composition varies significantly with geographical origin and post-harvest handling.

The positive control, Ciprofloxacin (5 µg), produced a ZOI of 26.0 mm against *S. aureus*. The extract at 1000 mg/mL (27.5 mm) marginally exceeded this value, consistent with the report by Mahmuda et al. (2015), who similarly found that ethanolic turmeric extract surpassed ciprofloxacin (18.2 ± 0.66 mm) under comparable conditions. This finding suggests that at sufficiently high concentrations, *C. longa* ethanolic extract can produce antibacterial effects against *S. aureus* at least comparable to a clinically relevant antibiotic. This comparison must, however, be interpreted with caution: the agar well diffusion method delivers a substantially larger volume of extract per well (100 µL) compared to the standardised dose on an antibiotic disc, and the two methods differ inherently in their diffusion kinetics (Balouiri et al., 2016). Nonetheless, the finding retains significance in the AMR context because, as Mun et al. (2014) demonstrated through transmission electron microscopy, curcumin causes direct cell membrane disruption, cytoplasmic damage, and cell lysis in *S. aureus* through a multi-target mechanism that is inherently more difficult for bacteria to evade through single resistance mutations than the single-target mechanism of fluoroquinolones such as ciprofloxacin.

The absence of inhibitory activity at 50–200 mg/mL against *S. aureus* in this study contrasts with Mohammed et al. (2021), who reported measurable zones at concentrations as low as 25 mg/mL in their Bauchi Teaching Hospital study. This discrepancy likely reflects differences in the bacterial strains used: Mohammed et al. (2021) tested clinical isolates obtained directly from hospitalised patients, who may exhibit different strain-specific susceptibility profiles from the throat swab isolate used here. Additionally, Odo et al. (2023) reported zones of 4–9 mm against *S. aureus* at the much lower range of 0.25–1.0 mg/mL, attributable to their specific extraction conditions and strain characteristics. These differences collectively highlight a well-known limitation of the agar well diffusion method when applied to hydrophobic compounds: the rate of diffusion through the aqueous agar matrix is constrained by curcumin's low water solubility, such that the detectable inhibition threshold in agar-based assays does not necessarily correspond to the true minimum inhibitory concentration of the compound (Balouiri et al., 2016; Abdullahi et al., 2024).

### Antibacterial Activity Against *E. coli*

Against *E. coli*, the extract produced mean ZOI of 11.5, 17.0, and 21.5 mm at 400, 800, and 1000 mg/mL respectively, with no activity below 400 mg/mL. These results are consistent with Momoh et al. (2022), who reported ZOI of 22.29 ± 2.35 to 29.56 ± 2.23 mm against *E. coli* at 250–500 mg/mL. The somewhat larger zones in that study may be explained by the higher concentrations tested and the specific methanolic solvent system employed, which may yield a phytochemical profile different from the 90% ethanol used here. Abdullahi et al. (2024) confirmed dose-dependent ZOI of 10–25 mm against *E. coli*, directly mirroring the pattern observed in this study.

In contrast, Mohammed et al. (2021) reported complete resistance of *E. coli* to turmeric extract across 25–100 mg/mL, attributing this to the protective function of the outer membrane lipopolysaccharide (LPS) layer. The present study also recorded no activity against *E. coli* within the overlapping 50–200 mg/mL range, which is consistent with Mohammed et al. (2021). However, substantial activity was observed at 400–1000 mg/mL, a range not tested in the Bauchi study. This indicates that the outer membrane barrier of *E. coli* is concentration-dependent and can be overcome at sufficiently high extract concentrations, rather than representing absolute resistance, in agreement with the structural analysis by Silhavy et al. (2010) and the broader experimental literature reviewed by Beshiru et al. (2024).

The Ampicillin positive control produced a ZOI of 21.0 mm against *E. coli*, marginally below the 21.5 mm produced by the extract at 1000 mg/mL. This is particularly noteworthy because ampicillin resistance is among the most widely reported resistance phenotypes in *E. coli*, driven by beta-lactamase production and efflux pump overexpression (Nasrollahian et al., 2024). The modest ampicillin zone therefore suggests that the *E. coli* isolate used in this study may exhibit reduced

susceptibility to the antibiotic, a scenario under which the extract's ability to match or exceed the antibiotic ZOI carries additional clinical relevance. Abdullahi *et al.* (2024) similarly proposed that plant-derived curcuminoid extracts may retain activity against organisms where conventional antibiotics are losing effectiveness, because curcumin's membrane-disruption mechanism does not share the beta-lactam antibiotic's molecular target.

### Differential Susceptibility: Gram-Positive vs. Gram-Negative

At all active concentrations, *S. aureus* produced consistently larger ZOI than *E. coli*, with differences of 3.5 mm, 5.0 mm, and 6.0 mm at 400, 800, and 1000 mg/mL respectively. This pattern of greater susceptibility of the Gram-positive organism, is the most consistently reported finding across the turmeric antibacterial literature. Momoh *et al.* (2022) and Abdullahi *et al.* (2024) all documented larger ZOI against *S. aureus* than against *E. coli* under the same experimental conditions. The structural basis for this differential is well established: *S. aureus* lacks the outer lipopolysaccharide membrane present in *E. coli*, allowing hydrophobic curcuminoids to reach the cytoplasmic membrane of *S. aureus* more readily than they can penetrate the tripartite envelope of *E. coli* (Silhavy *et al.*, 2010). At the multi-species level, Adamczak *et al.* (2020) documented consistently greater Gram-positive sensitivity to curcumin across over 100 strains belonging to 19 species, confirming that the pattern observed here is not isolate-specific but reflects a consistent pharmacological reality rooted in bacterial cell architecture. In this present study, rhizome extract was used, which is the pharmacologically richest botanical source, and the more typical pattern of greater *S. aureus* susceptibility was observed, consistent with the bulk of the evidence.

The widening of the inter-organism ZOI difference with increasing concentration (from 3.5 mm at 400 mg/mL to 6.0 mm at 1000 mg/mL) suggests that as concentration increases, the additional bioactive compounds penetrating *E. coli*'s outer membrane still reach *S. aureus*'s cytoplasmic membrane at a greater effective concentration, amplifying the differential. This has implications for concentration selection in future studies, particularly the threshold at which *E. coli* inhibition transitions from borderline to clinically meaningful.

### Phytochemical Basis of Observed Activity

The antibacterial activity observed in this study is attributable to the spectrum of bioactive secondary metabolites present in the ethanolic extract of *C. longa*. Ethanol is a polar protic solvent that efficiently extracts the hydrophobic curcuminoid fraction being curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which are the primary pharmacologically active constituents responsible for membrane disruption, FtsZ inhibition, and ROS generation (Teow *et al.*, 2016; Kali *et al.*, 2020). Flavonoids present in the extract contribute antibacterial activity through disruption of cell membrane function and inhibition of nucleic acid synthesis; tannins precipitate bacterial proteins and inhibit microbial enzymes; and alkaloids intercalate with bacterial DNA and inhibit topoisomerase (Adekunle *et al.*, 2022). This multi-constituent, multi-target pharmacological action is fundamentally different from the single-target mechanism of conventional antibiotics, and is a recognised reason why plant-derived extracts are inherently less likely to drive resistance development through single-step mutation (Adekunle *et al.*, 2022; Teow *et al.*, 2016). The combination of membrane-active curcuminoids and intracellular-targeting flavonoids and alkaloids in the same extract is likely responsible for the robust dose-dependent activity observed across both test organisms.

### Implications of *E. coli* Isolation from Campus Tap Water

The isolation of *E. coli* from a tap water sample collected within the Veritas University campus represents a public health observation of independent significance. *E. coli* is a widely adopted indicator organism for faecal contamination of water supplies, and its presence in treated tap water signals a potential breakdown in water treatment, storage, or distribution infrastructure (Cheesbrough, 2017 and Amaechi *et al.*, 2021). In the Nigerian context, faecal contamination of institutional water supplies has been repeatedly documented across university campuses and public facilities, and is a recognised driver of gastrointestinal disease in students and staff populations (Nasrollahian *et al.*, 2024). This finding, while incidental to the primary objective of the present study, warrants formal reporting to the relevant institutional and regulatory authorities and points to the need for systematic microbiological monitoring of campus water supplies.

### Study Strengths and Comparison with Published Literature

Several methodological decisions in the present study enhance the rigour and interpretability of the findings. The use of 90% ethanol as the extraction solvent is supported by multiple comparative studies demonstrating superior curcuminoid recovery relative to aqueous extraction (Amalraj *et al.*, 2021; Mohammed *et al.*, 2021). The use of Mueller-Hinton Agar as the assay medium, standardisation of inoculum to 0.5 McFarland standard, and inclusion of both positive and negative controls align with CLSI-endorsed protocols and maximise the comparability of results with the international literature (Balouiri *et al.*, 2016). The testing of a wide concentration range (50–1000 mg/mL) and the use of duplicate trials enable characterisation of the complete dose-response relationship. The simultaneous evaluation of both *S. aureus* and *E. coli* under identical experimental conditions provides direct and scientifically valid comparative data on Gram-positive versus Gram-negative susceptibility that many published studies have not generated.

### Conclusion

This study demonstrated that the ethanolic extract of locally sourced *Curcuma longa* rhizomes possesses significant, concentration-dependent antibacterial activity against both *Staphylococcus aureus* and *E. coli* under in vitro conditions. *Staphylococcus aureus* was successfully isolated from a throat swab and confirmed by MSA morphology, Gram staining, catalase and coagulase reactions, and beta-haemolysis. *E. coli* was isolated from campus tap water and confirmed by EMB metallic sheen, indole positivity, and methyl red positivity. The 90% ethanolic paste extract, prepared by cold maceration and confirmed sterile prior to use, produced no inhibitory activity at 50, 100, and 200 mg/mL but demonstrated clear, reproducible zones of inhibition at 400, 800, and 1000 mg/mL. Mean ZOI against *S. aureus* were 15.0, 22.0, and 27.5 mm respectively; against *E. coli* they were 11.5, 17.0, and 21.5 mm. *Staphylococcus aureus* was consistently more susceptible than *E. coli* at all

active concentrations, as predicted by structural differences between Gram-positive and Gram-negative cell envelopes. At 1000 mg/mL, the extract marginally exceeded the Ciprofloxacin positive control (26.0 mm) against *S. aureus* and the Ampicillin positive control (21.0 mm) against *E. coli*.

These findings contribute locally generated, empirically grounded evidence to the growing global body of research supporting *Curcuma longa* as a promising natural antimicrobial agent in the context of antimicrobial resistance. The results affirm the pharmacological potential of Nigerian-sourced turmeric and justify further investigation including MIC/MBC determination, phytochemical characterisation, multi-isolate testing, and in vivo evaluation toward the ultimate goal of contributing to AMR management in resource-limited healthcare settings.

### Recommendations

Future studies should formally determine MIC and MBC values for the *C. longa* ethanolic extract against *S. aureus* and *E. coli* using broth microdilution, to provide quantitative pharmacological endpoints that complement the ZOI data and enable comparison with clinical antibiotic breakpoints (Odo et al., 2023). The concentration range between 200 and 400 mg/mL should be investigated at finer increments to precisely define the inhibitory threshold for both organisms, given that the current data indicates an activity onset between 200 and 400 mg/mL for both. Additionally, a comparative extraction study should be conducted testing ethanol, methanol, and aqueous extracts from the same *C. longa* rhizome sample under identical conditions to establish which solvent system yields the most potent extract, providing evidence-based guidance for future extraction method selection (Momoh et al., 2022; Odo et al., 2023). Furthermore, phytochemical screening and HPLC quantification of the extract's bioactive constituents, particularly curcumin, flavonoids, tannins, and alkaloids should be performed so that observed antibacterial activity can be correlated with specific compounds and compared to reference standards (Salem et al., 2022). Likewise, cytotoxicity assays and in vivo safety evaluations should be conducted at the active concentrations (400–1000 mg/mL) identified here, prior to any consideration of translational or therapeutic application (Beshiru et al., 2024). Finally, the study should be extended to include multiple clinical isolates of *S. aureus* and *E. coli*, encompassing confirmed MRSA and ESBL-producing strains, to assess the practical utility of the extract against clinically relevant resistant phenotypes.

### References

1. Abdullahi, A. A., Bello, A., & Hassan, A. (2024). Phytochemical and antibacterial properties of *Curcuma longa* extracts from Kaduna State, Nigeria, against selected clinical bacterial isolates. *Nigerian Journal of Microbiology*, 38(1), 12–21.
2. Adamczak, A., Ōzarowski, M., & Karpiński, T. M. (2020). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine*, 9(1), 109. <https://doi.org/10.3390/jcm9010109>
3. Adekunle, A. I., Oduola, A. M. J., & Yusuf, B. O. (2022). Review on plant-based management in combating antimicrobial resistance: Mechanistic perspective. *Pharmaceutics*, 14(10), 2175. <https://doi.org/10.3390/pharmaceutics14102175>
4. Algammal, A. M., Hetta, H. F., Elkelish, A., Alkhalifah, D. H. M., Hozzein, W. N., Batiha, G. E., El Nahhas, N., & Mabrok, M. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA): One health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and Drug Resistance*, 13, 3255–3265. <https://doi.org/10.2147/IDR.S272733>
5. Amalraj, A., Varma, K., Jacob, J., Divya, C., Kunnumakkara, A. B., Stohs, S. J., & Gopi, S. (2021). Curcumin extraction, isolation, quantification and its application in functional foods: A review with a focus on immune enhancement activities and COVID-19. *Frontiers in Nutrition*, 8, 747956. <https://doi.org/10.3389/fnut.2021.747956>
6. Balouri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
7. Beshiru, A., Igbinosa, E. O., Igbinosa, I. H., Okonkwo, E. J., Obi, L. C., & Okoh, A. I. (2024). *Curcuma longa* rhizome extract: A potential antibiofilm agent against antibiotic-resistant foodborne pathogens. *Biofouling*, 40(10), 932–947. <https://doi.org/10.1080/08927014.2024.2432963>
8. Cheesbrough, M. (2017). *District laboratory practice in tropical countries* (2nd ed., updated). Cambridge University Press.
9. C.N. Obum-Nnadi, C.M Ezenwa, Dennis Amaechi, N.B. Ohabughiro, P.A. Nnagbo, K. S. Nwokorie, C.S. Okoli. Evaluation of the Antimicrobial and Phytochemical Properties of *Annona Muricata* L. (Soursop). *Journal of Biomedicine and Biosensors*. 2( 2): 43 – 59, 2022
10. Craft, K. M., & Nguyen, J. M. (2019). Methicillin-resistant *Staphylococcus aureus* (MRSA): Antibiotic-resistance and the biofilm phenotype. *MedChemComm*, 10(8), 1231–1241. <https://doi.org/10.1039/c9md00044e>
11. Dennis Amaechi , I. P. Ekpe , E. D. Edet and M. C. Madu . Hepatoprotective and Hematological Effects of *Solanum melongena* (Garden Egg), *Solanum lycopersicum* (Tomato) and *Daucus carota* Subsp. *Sativus* (Carrot) Extracts against Lead Induced Toxicity in Wistar Rats. *Asian Journal of Biochemistry, Genetics and Molecular Biology* 7(2): 10-18, 2021. <https://doi.org/10.9734/ajbgmb/2021/v7i230169>
12. Ekpe I.P., Udosen E. O., and Dennis Amaechi (2018). Evaluation of Some Vitamins and Macro-Nutrients Composition of Ethanolic Extract of *Tecomastans* and *Costusafer* Leaves. *International Journal of Biochemistry Research & Review* 23(4): 1-5
13. Fuloria, S., Mehta, J., Chandel, A., Sekar, M., Rani, N. N. I. M., Begum, M. Y., Subramaniyan, V., Chidambaram, K., Thangavelu, L., Nordin, R., Wu, Y. S., Sathasivam, K. V., Lum, P. T., Meenakshi, D. U., Kumarasamy, V., Azad, A. K., & Fuloria, N. K. (2022). A comprehensive review on the therapeutic potential of *Curcuma longa* Linn. in relation to its major active constituent curcumin. *Frontiers in Pharmacology*, 13, 820806. <https://doi.org/10.3389/fphar.2022.820806>
14. Grigor'eva, A., Bardasheva, A., Tupitsyna, A., Tikunova, N., & Pyshnyi, D. (2023). Antibiofilm activity of mitocurcumin

- against *Staphylococcus aureus*. *Antibiotics*, 12(4), 681. <https://doi.org/10.3390/antibiotics12040681>
15. Hamzah, H., Radu, S., Chabridon, S. M., & Saidi, N. (2020). The role of curcumin in inhibiting biofilm formation of *Staphylococcus aureus*. *Journal of Pure and Applied Microbiology*, 14(1), 303–312. <https://doi.org/10.22207/JPAM.14.1.31>
  16. Hussain, Z., Thu, H. E., Rawas-Qalaji, M., & Khan, S. (2022). Recent advancements in curcumin delivery and bioavailability: A review. *Drug Delivery*, 29(1), 1498–1507.
  17. Jyotirmayee, B., & Mahalik, G. (2022). A review on selected pharmacological activities of *Curcuma longa* L. *International Journal of Applied and Pure Science and Agriculture*, 8(2), 1–17.
  18. Kali, A., Bhuvaneshwar, D., Charles, P. M. V., & Seetha, K. S. (2020). Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates. *Journal of Basic and Clinical Pharmacy*, 7(3), 93–96. <https://doi.org/10.4103/0976-0105.183264>
  19. Mahmuda, A., Islam, M. S., & Rana, M. (2015). Antibacterial effect of *Curcuma longa* (turmeric) against *Staphylococcus aureus* and *E. coli*. *Asian Journal of Medical and Biological Research*, 1(2), 271–278. <https://doi.org/10.3329/ajmbr.v1i2.26193>
  20. Mbah-Omeje, K. N. (2019). In vitro study on the antimicrobial activity of *Curcuma longa* rhizome on some microorganism. *American Journal of Biomedical and Life Sciences*, 7(1), 1–5. <https://doi.org/10.11648/j.ajbls.20190701.11>
  21. Mohammed, Y., Danlami, H. U., & Mukhtar, M. D. (2021). Antimicrobial activity of extracts of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) against selected bacterial clinical isolates. *Mediterranean Journal of Infection, Microbes and Antimicrobials*, 10, 2020.6. <https://doi.org/10.4274/mjima.galenos.2021.2020.6>
  22. Momoh, J. O., Manuwa, A. A., & Bankole, Y. O. (2022). Phytochemical screening, atomic absorption spectroscopy, GC-MS and antibacterial activities of turmeric (*Curcuma longa* L.) rhizome extracts. *Journal of Advances in Microbiology*, 22(9), 116–131. <https://doi.org/10.9734/jamb/2022/v22i930498>
  23. Mun, S.-H., Joung, D.-K., Kim, Y.-S., Kang, O.-H., Kim, S.-B., Seo, Y.-S., Kim, Y.-C., Lee, D.-S., Shin, D.-W., Kweon, K.-T., & Kwon, D.-Y. (2013). Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. *Phytomedicine*, 20(8–9), 714–718. <https://doi.org/10.1016/j.phymed.2013.02.006>
  24. Mun, S.-H., Kim, S.-B., Kong, R., Choi, J.-G., Kim, Y.-C., Shin, D.-W., Kang, O.-H., & Kwon, D.-Y. (2014). Curcumin reverses methicillin resistance in *Staphylococcus aureus*. *Molecules*, 19(11), 18283–18295. <https://doi.org/10.3390/molecules191118283>
  25. Nasrollahian, S., Graham, J. P., & Halaji, M. (2024). A review of the mechanisms that confer antibiotic resistance in pathotypes of *E. coli*. *Frontiers in Cellular and Infection Microbiology*, 14, 1387497. <https://doi.org/10.3389/fcimb.2024.1387497>
  26. Odo, E. O., Okeke, I. C., Nnaji, J. C., Nweze, I. C., & Agwu, O. E. (2023). Analysis of the antibacterial effects of turmeric on particular bacteria. *Medicine*, 102(48), e36492. <https://doi.org/10.1097/MD.00000000000036492>
  27. Oghenejobo, M., Opajobi, O. A., Oghenejobo, B. U. S., & Uzoegbu, U. (2017). Antibacterial evaluation, phytochemical screening and ascorbic acid assay of turmeric (*Curcuma longa*). *MOJ Bioequivalence and Bioavailability*, 4(2), 232–239. <https://doi.org/10.15406/mojbb.2017.04.00063>
  28. Royal Botanic Gardens, Kew. (2023). *Curcuma longa* L. In *Plants of the World Online*. <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:796451-1/general-information>
  29. Salem, M. A., El-Shiekh, R. A., Fernie, A. R., Alseekh, S., & Zayed, A. (2022). Metabolomics-based profiling for quality assessment and revealing the impact of drying of turmeric (*Curcuma longa* L.). *Scientific Reports*, 12(1), 10288. <https://doi.org/10.1038/s41598-022-13882-y>
  30. Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 2(5), a000414. <https://doi.org/10.1101/cshperspect.a000414>
  31. Teow, S.-Y., Liew, K., Ali, S. A., Khoo, A. S.-B., & Peh, S.-C. (2016). Antibacterial action of curcumin against *Staphylococcus aureus*: A brief review. *Journal of Tropical Medicine*, 2016, 2853045. <https://doi.org/10.1155/2016/2853045>
  32. Tille, P. M. (2017). *Bailey and Scott's diagnostic microbiology* (14th ed.). Elsevier Mosby.
  33. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041. <https://doi.org/10.3390/microorganisms9102041>
  34. Wang, W., Liu, D., Zhang, X., Chen, D., Cheng, Y., & Shen, F. (2025). *Curcuma longa* (turmeric): From traditional applications to modern plant medicine research hotspots. *Chinese Medicine*, 20, 1–30. <https://doi.org/10.1186/s13020-025-01115-z>
  35. World Health Organization. (2023). Antimicrobial resistance [Fact sheet]. <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>