

Antimicrobial Efficacy of Methanolic Extracts of *Curcuma longa* (Turmeric) and *Zingiber officinale* (Ginger): A Combined Approach

*¹Alaabo P. O., ²Onuoha U. N., ¹Muoneke B. S., ¹Ogbonna A. O., ¹Nwede C. A., ¹Nwankwo R. C.,
⁴Anyadike N. N., ¹Okezue J. D., ¹Chukwu, H. C., ⁵Udeh P. E., ⁶Njoku B., ¹Obasi V. N. and ⁴Odo V. C.

¹Department of Biochemistry, College of Natural Sciences, Michael Okpara University of
Agriculture, Umudike, Abia State, Nigeria

²Department of Microbiology, College of Natural Sciences, Michael Okpara University of
Agriculture, Umudike, Abia State, Nigeria

³Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria Nsukka, Enugu
State, Nigeria

⁴Department of Medical Laboratory Science, Faculty of Health Science and Technology, Tansian
University, Umunya, Anambra State, Nigeria

⁵Department of Biochemistry, Faculty of Biological Sciences, Federal University of Owerri, Imo
State, Nigeria

⁶Department of Pure and Industrial Chemistry, Faculty of Sciences, University of Port Harcourt,
Rivers State, Nigeria

*Corresponding Author: alaabo.prince@mouau.edu.ng

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Abstract

This study investigated the antimicrobial efficacy of methanolic extracts of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and their combination against selected clinical bacterial isolates: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species. Using the agar well diffusion method, extracts at concentrations of 50, 100, and 200 mg/ml were tested, with Gentamicin serving as a positive control. Ginger extract showed significant inhibitory activity, particularly against *Staphylococcus aureus* and *Salmonella* species, with zones of inhibition reaching up to 15.0 ± 0.14 mm. Turmeric also exhibited moderate antibacterial activity, with notable effects on *E. coli* and *Salmonella* species. However, neither extract showed any activity against *Pseudomonas aeruginosa* at any concentration. The combination of ginger and turmeric produced enhanced inhibitory effects compared to individual extracts, especially against *E. coli* and *Salmonella*, suggesting possible synergistic interactions. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays further confirmed the antimicrobial potential. The lowest MICs (12.5 mg/ml) were observed for ginger against *S. aureus* and the combination extract against *E. coli* and *Salmonella*. MBC values ranged from 25 to 100 mg/ml across the extracts. Notably, the combination extract achieved bactericidal effects at comparable or lower concentrations than the individual components, indicating enhanced efficacy. These findings support the potential application of ginger and turmeric extracts particularly in combination as natural antimicrobial agents. This study contributes

novel insights into their synergistic antibacterial properties and provides a foundation for further pharmacological and formulation studies targeting resistant pathogens.

Keywords: Ginger, turmeric, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration, bacterial isolates, natural products

Introduction

In response to the growing global crisis of antimicrobial resistance (AMR), there has been increasing scientific interest in alternative and complementary therapeutic agents derived from natural products [1]. For centuries, traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and African ethnomedicine have relied on plant-based remedies for the treatment of infectious diseases. Among the numerous medicinal plants investigated, *Curcuma longa* and *Zingiber officinale* have attracted considerable attention due to their rich phytochemical composition and diverse biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects [2]. Previous studies have demonstrated that turmeric extracts possess significant inhibitory activity against a wide range of clinical bacterial isolates, while related investigations have shown that plant-derived extracts can effectively suppress the growth of pathogenic microorganisms [3].

The antimicrobial potential of *Curcuma longa* has been extensively documented in recent literature. Crude and essential oil extracts of the plant have shown activity against multidrug-resistant organisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Acinetobacter baumannii* [4]. In addition, specific bioactive compounds such as ar-turmerone have been reported to inhibit bacterial growth and disrupt biofilm formation, providing insight into the mechanisms underlying its antimicrobial effects [5]. Similarly, *Zingiber officinale* has been reported to exhibit broad-spectrum antibacterial activity, attributed to its bioactive constituents such as gingerols and shogaols, which contribute to its therapeutic efficacy.

In spite of these promising findings, several gaps remain in the existing body of knowledge. Most previous studies have focused on the antimicrobial effects of individual plant extracts, with limited attention given to the combined or synergistic interactions between *Curcuma longa* and *Zingiber officinale*. Furthermore, many investigations have utilized standard laboratory strains of microorganisms, which may not accurately reflect the resistance patterns observed in clinical isolates. There is also a lack of comprehensive data on quantitative antimicrobial parameters such as minimum inhibitory concentration and minimum bactericidal concentration, as well as limited comparative evaluation of plant extracts relative to conventional antimicrobial agents. In addition, the effectiveness of these plant extracts against multiple clinically relevant and resistant pathogens has not been systematically explored.

In response to these limitations, the present study was designed to evaluate the antimicrobial efficacy of methanolic extracts of *Curcuma longa* and *Zingiber officinale*, both individually and in

combination, against selected clinical bacterial isolates. The study specifically investigated their inhibitory effects on clinically relevant pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* species. Emphasis was placed on assessing whether the combined application of both plant extracts produces enhanced or synergistic antimicrobial activity compared to their individual effects. Furthermore, the study determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts to establish their potency and therapeutic relevance. By exploring the combined antimicrobial potential of turmeric and ginger, this research contributes to the growing search for effective plant-based alternatives for managing infectious diseases and combating antimicrobial resistance.

Materials and Methods

Study Design

This study employed an experimental laboratory design to evaluate the antimicrobial activity of methanolic extracts of *Curcuma longa*, *Zingiber officinale*, and their combination against selected clinical bacterial isolates. The antimicrobial activity was assessed using the agar well diffusion method, while MIC and MBC assays were conducted to determine the lowest concentration required to inhibit and kill the test organisms respectively.

Plant Collection

Fresh rhizomes of *Curcuma longa* and *Zingiber officinale* were harvested from the National Root Crops Research Institute, Umudike, Abia State, Nigeria. The plant materials were authenticated at the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, and a voucher specimen was deposited in the departmental herbarium with voucher number MOUAU/PSB/2025/CL-ZO/014 for reference. After collection, the rhizomes were thoroughly washed with tap water to remove soil and other contaminants, and then air-dried at room temperature under shade conditions to prevent degradation of active phytochemicals. The dried samples were subsequently milled into fine powder using an automated milling machine. The powdered samples were weighed using an analytical weighing balance, yielding a total weight of 200 g [6].

Preparation of Plant Samples

The cleaned rhizomes were sliced into smaller pieces and air-dried at room temperature for approximately two weeks until a constant weight was obtained. The dried samples were then ground into a fine powder using an electric grinder. The powdered samples were stored in clean airtight containers until extraction [7-8].

Preparation of Methanolic Extracts

Extraction was carried out using the cold maceration method. Approximately 200 g of powdered turmeric and ginger were separately soaked in 1000 ml of 80% methanol in sterile conical flasks. The

mixtures were agitated intermittently and allowed to stand for 72 hours at room temperature to ensure complete extraction of phytochemicals. After maceration, the mixtures were filtered using Whatman No. 1 filter paper (Cytiva, United Kingdom). The filtrates were concentrated using a rotary evaporator (RE300 Rotary Evaporator, Yamato Scientific Co., Ltd., Japan) at 40–45°C to remove the solvent and obtain the crude methanolic extracts. The extracts were further dried in a water bath (HH-4 Digital Water Bath, Jintan Medical Equipment Co., Ltd., China) to obtain semi-solid residues and stored in sterile containers at 4°C until use [9].

Preparation of Extract Concentrations

Stock solutions of the extracts were prepared by dissolving measured quantities of the crude extract in dimethyl sulfoxide (DMSO), from the stock solution, different concentrations were prepared: 200 mg/ml, 100 mg/ml, 50 mg/ml. For the combined extract, equal proportions of turmeric and ginger extracts were mixed together to obtain the same concentration levels [10].

Test Microorganisms

The clinical bacterial isolates used in this study included:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Salmonella* species

These organisms were obtained from the Microbiology Laboratory of a recognized medical laboratory or teaching hospital. The isolates were confirmed using standard microbiological identification procedures and maintained on nutrient agar slants at 4°C prior to use. [11].

Preparation of Bacterial Inoculum

Pure colonies of each bacterial isolate were inoculated into sterile nutrient broth and incubated at 37°C for 18–24 hours. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/ml [10].

Antimicrobial Susceptibility Test (Agar Well Diffusion Method)

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method. Sterile Mueller–Hinton agar plates were prepared according to the manufacturer’s instructions and inoculated with standardized bacterial suspensions using a sterile cotton swab to ensure uniform distribution of the test organisms. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer, and 0.1 ml of the prepared extract concentrations (200, 100, and 50 mg/ml) were carefully introduced into each well. Gentamicin was used as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The plates were allowed to stand for 30 minutes to enable proper diffusion of the extracts into the agar medium and were subsequently incubated at 37°C for 24 hours. After

incubation, the zones of inhibition around the wells were measured in millimeters using a ruler or digital calliper, and the results were expressed as mean \pm standard deviation from duplicate experiments [12].

Determination of Minimum Inhibitory Concentration

The broth dilution method was used to determine the minimum inhibitory concentration of the extracts. Serial two-fold dilutions of each extract were prepared in sterile nutrient broth to obtain concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 mg/ml. Each tube was inoculated with 0.1 ml of standardized bacterial suspension and incubated at 37°C for 24 hours. After incubation, the tubes were examined for turbidity, which indicated bacterial growth. The MIC was defined as the lowest concentration of the extract that showed no visible growth, indicated by a clear solution [10].

Determination of Minimum Bactericidal Concentration

To determine the MBC, samples from tubes that showed no visible growth during the MIC test were streaked onto fresh nutrient agar plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the extract that completely prevented bacterial growth on the agar plate, indicating bacterial death [11].

Statistical Analysis

All experiments were carried out in duplicate, and results were expressed as mean \pm standard deviation (SD). Data obtained were analyzed using descriptive statistical methods with IBM SPSS Statistics. Comparisons between extract treatments were interpreted based on differences in inhibition zones and MIC/MBC values [13].

Results and Discussion

The values in Table 1 represent the mean diameter (in millimeters) of inhibition zones produced by different concentrations (200, 100, and 50 mg/ml) of methanolic ginger extract against selected clinical isolates, measured using the agar well diffusion method.

Table 1: Mean Diameter Zones of Inhibition (mm) produced by methanolic extracts of Ginger against selected clinical isolates.

Test Organisms	Concentration (mg/ml)			Control
	200	100	50	Gentamicin
<i>Escherichia coli</i>	10.5 \pm 0.70	0.0 \pm 0.00	0.0 \pm 0.00	25.0 \pm 1.41
<i>Staphylococcus aureus</i>	15.0 \pm 0.14	11.0 \pm 0.00	9.0 \pm 0.00	21.0 \pm 0.70
<i>Pseudomonas aeruginosa</i>	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	19.0 \pm 0.00
<i>Salmonella</i> species	12.0 \pm 0.00	10.0 \pm 0.00	0.0 \pm 0.00	22.0 \pm 0.70

Alaabo et al.: Antimicrobial Efficacy of Methanolic Extracts of Curcuma longa (Turmeric) and Zingiber officinale (Ginger): A Combined Approach

Values in the Table are the mean \pm standard deviation from the results of two replication of each experiment.

Gentamicin (standard antibiotic) was used as the positive control. A larger zone of inhibition indicates greater antimicrobial activity. "0.0 \pm 0.00" indicates no observable inhibitory effect. Data are expressed as mean \pm standard deviation from two replicates of each experiment.

The values in Table 2 represent the mean diameter (in millimeters) of inhibition zones produced by various concentrations (200, 100, and 50 mg/ml) of methanolic turmeric extract against selected clinical isolates, assessed using the agar well diffusion method

Table 2: Mean Diameter Zones of Inhibition (mm) produced by methanolic extracts of Turmeric against selected clinical isolates.

Test Organisms	Concentration (mg/ml)			Control
	200	100	50	Gentamicin
<i>Escherichia coli</i>	13.0 \pm 0.00	11.5 \pm 0.70	0.0 \pm 0.00	25.0 \pm 1.41
<i>Staphylococcus aureus</i>	11.0 \pm 0.02	10.0 \pm 0.70	0.0 \pm 0.00	21.0 \pm 0.70
<i>Pseudomonas aeruginosa</i>	0.0 \pm 0.00	0.0 \pm 0.00	0.0 \pm 0.00	19.0 \pm 0.00
<i>Salmonella</i> species	12.0 \pm 0.00	10.0 \pm 0.00	0.0 \pm 0.00	22.0 \pm 0.70

Values in the Table are the mean \pm standard deviation from the results of two replication of each experiment.

Gentamicin served as the positive control. A higher inhibition zone indicates stronger antimicrobial activity. A value of "0.0 \pm 0.00" denotes no detectable inhibition. Results are presented as mean \pm standard deviation from two independent replicates of each test.

Table 3 presents the mean diameter (in millimeters) of inhibition zones produced by combined methanolic extracts of ginger and turmeric at concentrations of 200, 100, and 50 mg/ml against selected clinical isolates, using the agar well diffusion method.

Table 3: Mean Diameter Zones of Inhibition (mm) produced by methanolic extracts of combined Ginger and Turmeric against selected clinical isolates.

Test Organisms	Concentration (mg/ml)			Control
	200	100	50	Gentamicin
<i>Escherichia coli</i>	14.0 ± 0.00	12.0 ± 0.00	9.0 ± 0.00	25.0 ± 1.41
<i>Staphylococcus aureus</i>	12.5 ± 0.14	10.0 ± 0.00	0.0 ± 0.00	21.0 ± 0.70
<i>Pseudomonas aeruginosa</i>	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	19.0 ± 0.00
<i>Salmonella species</i>	13.0 ± 0.00	11.5 ± 0.70	0.0 ± 0.00	22.0 ± 0.70

Values in the Table are the mean ± standard deviation from the results of two replication of each experiment.

Gentamicin was used as the positive control. Larger inhibition zones reflect greater antimicrobial potency. A reading of "0.0 ± 0.00" indicates no observable antimicrobial activity. Data are expressed as mean ± standard deviation from two replicates of each experimental condition.

Table 4 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values (in mg/ml) of methanolic extracts of turmeric, ginger, and their combination against selected clinical isolates.

Table 4: MIC and MBC values (mg/ml) of extracts of Ginger and Turmeric against the Isolates.

Extract	Organisms	100	50	25	12.5	6.25	3.12	1.56	0.78	MIC	MBC
Turmeric	<i>Escherichia coli</i>	-	-	-	+	+	+	+	+	12.5	25
	<i>Staphylococcus aureus</i>	-	-	+	+	+	+	+	+	25	50
	<i>Pseudomonas aeruginosa</i>	-	+	+	+	+	+	+	+	50	100
	<i>Salmonella species</i>	-	+	+	+	+	+	+	+	50	100
Ginger	<i>Escherichia coli</i>	-	-	+	+	+	+	+	+	25	50
	<i>Staphylococcus aureus</i>	-	-	-	+	+	+	+	+	12.5	25

	<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	+	+	25	50
	<i>Salmonella</i> species	-	+	+	+	+	+	+	+	50	100
Ginger + Turmeric	<i>Escherichia coli</i>	-	-	-	+	+	+	+	+	12.5	25
	<i>Staphylococcus aureus</i>	-	-	+	+	+	+	+	+	25	50
	<i>Pseudomonas aeruginosa</i>	-	+	+	+	+	+	+	+	50	100
	<i>Salmonella</i> species	-	-	-	+	+	+	+	+	12.5	100

+: growth of the organism indicated by turbidity in the broth medium; -= Absence of growth of the test organism shown by no form of turbidity in the medium.

MIC is the lowest concentration of extract that inhibited visible microbial growth (no turbidity), while MBC is the lowest concentration that killed the organism completely. The symbols “+” indicate visible growth (turbidity), while “-” indicates no growth. Results reflect the increasing antimicrobial potency at lower concentrations for certain extracts and highlight the relative effectiveness of each extract and their combination.

The antimicrobial activity of ginger, turmeric, and their combination displayed varying degrees of effectiveness against different clinical pathogens. Ginger showed the highest inhibitory effect against *Staphylococcus aureus* (15.0 mm at 200 mg/ml), while turmeric was most effective against *Escherichia coli* (13.0 mm at 200 mg/ml). However, both extracts showed no activity against *Pseudomonas aeruginosa*, indicating the pathogen's strong resistance. When combined, ginger and turmeric exhibited enhanced zones of inhibition, particularly against *E. coli* and *Salmonella* spp., suggesting a synergistic effect. This pattern highlights the potential advantage of combining plant extracts to improve antimicrobial efficacy and broaden the spectrum of activity.

The results in Table 1 indicate that ginger exhibited potent activity against *Staphylococcus aureus* (15.0 ± 0.14 mm at 200 mg/ml), consistent with previous studies reporting gingerol's effectiveness against Gram-positive bacteria due to its ability to disrupt microbial cell membranes [14-15]. However, ginger was ineffective against *Pseudomonas aeruginosa*, a bacterium known for its intrinsic resistance mechanisms, such as efflux pumps and low membrane permeability [16].

Table 2 shows that the turmeric extract demonstrated a zone of inhibition of 13.0 ± 0.00 mm against *E. coli* and moderate activity against *Staphylococcus aureus* and *Salmonella* spp. These results align with findings from Mahady et al. [17] and Gupta et al. [18], who reported the bacteriostatic and bactericidal properties of curcumin via inhibition of bacterial cell wall synthesis and modulation of

microbial enzyme systems. As with ginger, turmeric showed no activity against *P. aeruginosa*, confirming the need for enhanced formulations or combinations to overcome resistance in such organisms.

In Table 3, the combination of ginger and turmeric showed improved antimicrobial effects against *E. coli* (14.0 ± 0.00 mm) and *Salmonella* spp. (13.0 ± 0.00 mm), exceeding the activity of either extract alone at the same concentration. This enhancement suggests synergistic interactions between curcumin and gingerol, potentially via additive mechanisms of cell membrane disruption and inhibition of biofilm formation [19-20]. However, the combination remained ineffective against *P. aeruginosa*, underscoring the pathogen's formidable resistance profile and the limitation of phytochemicals in treating such infections.

The MIC and MBC data further supported the findings from the inhibition zone analysis. The lowest MIC value (12.5 mg/ml) was recorded for ginger against *S. aureus* and the combination extract against *E. coli* and *Salmonella* spp. Ginger and turmeric individually showed higher MICs and MBCs, confirming reduced potency when not combined. In all cases, MIC values were lower than MBC values, indicating a bacteriostatic effect at lower concentrations and a bactericidal effect at higher doses, consistent with the findings of Tyagi *et al.* [21]. This supports the conclusion that combining extracts reduces the concentration required to inhibit microbial growth, thus minimizing potential toxicity and side effects, which is aligned with prior research emphasizing the benefit of synergy in herbal medicine [22].

These findings align with multiple studies that have documented the antimicrobial activity of ginger and turmeric. Mathew and Hsu [22] found that curcumin could inhibit *E. coli* and *S. aureus* by blocking bacterial quorum sensing and inflammatory pathways. Ali *et al.* [14] and Mashhadi *et al.* [23] highlighted gingerol's ability to suppress Gram-positive pathogens and modulate oxidative stress. Teow *et al.* [20] specifically reported enhanced antimicrobial performance when curcumin was used alongside other phytochemicals, reinforcing the current study's findings on combination therapy.

However, no significant improvement was observed against *Pseudomonas aeruginosa*, as also noted by Gupta *et al.* [18], who stated that *P. aeruginosa*'s outer membrane restricts the entry of many antimicrobial agents, including polyphenols.

Conclusion

The findings of this study indicate that most of the stated objectives were successfully accomplished, as the methanolic extracts of *Curcuma longa* and *Zingiber officinale* exhibited notable antimicrobial activity when tested individually and demonstrated improved inhibitory effects when used in combination, especially against *Escherichia coli*, *Salmonella* species, and *Staphylococcus aureus*. In addition, the determination of minimum inhibitory concentration (MIC) and minimum bactericidal

concentration (MBC) values provided quantitative evidence supporting the antimicrobial effectiveness of the extracts. Nevertheless, the aim of validating these extracts as broad-spectrum alternatives to conventional antibiotics was only partially realized because no inhibitory activity was observed against *Pseudomonas aeruginosa*. This limitation may be explained by the inherent resistance characteristics of *P. aeruginosa*, including reduced outer membrane permeability, multidrug efflux systems, enzymatic degradation mechanisms, and strong biofilm-forming capacity. Additional contributing factors may include the crude nature of the extracts, relatively low concentrations of active phytochemical constituents, poor solubility and bioavailability of compounds such as curcumin, and the lack of advanced delivery or formulation techniques capable of improving extract penetration and antimicrobial performance.

Contribution to knowledge

This study contributes to knowledge by demonstrating that methanolic extracts of *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) possess measurable antimicrobial activity against selected clinical pathogens, and that their combined application produces enhanced inhibitory effects particularly against *Escherichia coli* and *Salmonella* species, indicating a synergistic interaction between *gingerol* and *curcumin*. The study further provides quantitative evidence through MIC and MBC analyses showing that the combined extract reduces the concentration required to inhibit microbial growth compared to individual extracts, thereby highlighting the potential of phytochemical combinations as complementary antimicrobial agents. Additionally, the study identifies the resistance of *Pseudomonas aeruginosa* to both individual and combined extracts, thereby contributing useful insight into the limitations of these phytochemicals and emphasizing the need for advanced formulations or adjunct therapies in combating highly resistant pathogens.

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