



In Vitro Assessment of Aqueous, Acetone and Ethanolic Extracts of Garlic and Turmeric as Plant-based Antimicrobial Agents

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Abstract	Article History
This study assessed the <i>in vitro</i>, antimicrobial activity of aqueous, acetone, and ethanol extracts of <i>Allium sativum</i> and <i>Curcuma longa</i> against selected bacterial pathogens in order to determine their potential as plant-based antimicrobial agents. The methodology involved preparing different concentrations of extracts using water and ethanol solvents. The antibacterial activity was tested against <i>Escherichia coli</i> and <i>Staphylococcus aureus</i> using the disc diffusion method, with zone diameters measured to determine susceptibility, while minimum inhibitory and bactericidal concentrations were determined using tube dilution and streak plating respectively. The results revealed that garlic extracts exhibited significant antimicrobial effects against all tested bacteria, including <i>Escherichia coli</i> and <i>Staphylococcus aureus</i>. Ethanolic extracts of garlic showed higher efficacy than aqueous extract, with maximum zone inhibition diameters of 18 mm for <i>E. coli</i> and 20 mm for <i>S. aureus</i> at 200 mg/mL for acetone garlic extract. Turmeric extracts demonstrated varying levels of antimicrobial activity, with ethanolic extracts generally more reactive than aqueous extracts. The aqueous turmeric shows no activity at all against <i>S. aureus</i>, and acetone/ethanol turmeric show 18/15 mm at 400 mg/mL. The ethanolic extracts of both garlic and turmeric showed better results compared to aqueous extracts, indicating that organic solvents may be more effective in extracting bioactive compounds responsible for antimicrobial activity. The study highlights the potential of garlic and turmeric as natural antimicrobial agents, with garlic exhibiting superior antibacterial properties. These findings suggest that these spices could be used to develop alternative and complementary medicines. Further research is needed to validate their clinical effectiveness and explore their therapeutic potential. Keywords: Antibacterial activity, Antimicrobial agent, Garlic, therapeutic potential, Tumeric	Received: 28 Apr 2026 Accepted: 24 May 2026 Published: 13 Jun 2026  Scan QR code to view* License: CC BY 4.0*  Open Access article.
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Introduction

Since ancient times, spices are commonly used for flavouring foods and as natural medicines against different forms of human diseases. Spices could be a seed, fruit, root, bark, berry, bud or vegetable substance that is primarily used as natural colourants, flavours, antioxidants (Chude *et al.*, 2023) and antimicrobials (Dokubo and Uba 2023; Ezenwata *et al.* 2022a; 2022b). The use of spices corresponds mainly with the food industry, but they are also used in medicine (Umeh *et al.*, 2020; 2021; Ele *et al.* 2025), cosmetics, perfumery and pharmaceuticals industry (Igbojika *et al.*, 2014; Ubani *et al.* 2024a; 2024b; 2025). In medicine, spices are used for different applications, such as stimulants, diuretics, carminatives, anti-inflammatory, stomachic, antibiotics, digestives, astringents, anthelmintics, expectorants and tonics etc (Agu *et al.*, 2024;

Dibua *et al.*, 2025a; Duru *et al.*, 2025; Umennadi *et al.*, 2026; Ndife *et al.*, 2026).

Garlic belongs to the genus *Allium* and is closely related to the onion, rakkoyo (an onion found in Asia), scallion, chire, leek and shallot. Garlic has been used all over the world for thousands of years. Records indicate that garlic was in use when the Giza pyramids were built, about 5,000 years ago. Richard S. Rivlin wrote in the 'Journal of nutrition' that the ancient Greek physician Hippocrates (circa. 460-370BC), known today as the 'Father of Western Medicine', prescribed garlic for a wide range of conditions and illnesses. Hippocrates promoted the use of garlic for treating respiratory problems, parasites, poor digestion, and fatigue. Currently garlic is widely used for several conditions linked to the blood system

and heart, including atherosclerosis (hardening of the arteries), high cholesterol, heart attack, coronary heart disease, and prevention of lung cancer, prostate cancer, breast cancer et cetra (Uba 2018; 2019a; 2019b; Egrefa *et al.*, 2020a; 2020b).

Tumeric (*Curcuma longa*), is a bright yellow spice used throughout Asia for centuries, it has in recent decades been embraced by the west, not just for its ability to satisfy our appetite for curry, but for its impressive list of health benefits (Alisa *et al.*, 2020; Chude *et al.*, 2023; Umennadi *et al.* 2026a; 2026b). Turmeric is quite literally, hot stuff. It is grown for its root, it has an ancient history of uses in cooking, fabric dyeing, cosmetics and traditional medicine in China and India. Its potent ingredient, curcumin, not only gives turmeric its golden colour, but also has a dazzling array of properties that are beneficial to health. Long term inflammation has been implicated in most chronic conditions such as heart disease and cancer so, must be controlled. The curcumin in turmeric has proven, strong anti-inflammatory that block bowel diseases, amongst others. Turmeric is also a powerful antioxidant as curcumin has been shown to be a robust scavenger of oxygen free radicals, which are chemically active molecules that cause damage to the body cells, it can play a part in preventing and managing heart disease (Anukam *et al.*, 2020a; Anukam *et al.*, 2020b; Uba and Umennadi, 2026).

The increasing prevalence of antibiotic-resistant bacteria has necessitated the exploration of alternative antimicrobial agents (Uba *et al.* 2020a; 2020b). Garlic and turmeric, known for their medicinal properties, have been studied for their potential antibacterial effects (Ekwenze *et al.*, 2025; Mere *et al.*, 2025; Ubajekwe *et al.*, 2025). However, several studies have reported that the effectiveness of aqueous, acetone, and ethanol extracts of these plants against bacterial growth remains unclear (Uba *et al.*, 2018a; 2018b; 2019a; 2019b; Uba and Anidu, 2023; Uba and Obiefuna 2023). This study was designed to investigate the antibacterial activity of aqueous, acetone, and ethanol extracts of *Allium sativum* and *Curcuma longa* against selected bacterial pathogens in order to evaluate their potential as plant-based antimicrobial agents.

Material and Methods

Collection of Sample

The fresh bulbs of garlic and tumeric were purchased from Ogwashi Ukwu modern market, Delta State, Nigeria by adopting the method of Mere *et al.* (2025).

Preparation of Crude Extracts

Fresh bud samples were brought to the lab where they were washed thoroughly under running tap water, and after being air dried for 1 h, they were ground separately in an electric grinder. To 100 g of each powdered bulb sample in a conical flask, 300 mL of 70:30 ratio mixture ethanol, acetone and water were added. A cotton plug and aluminium foil were then placed at the top of each flask to prevent evaporation, after which the flasks were kept on a shaker at room temperature to allow the extraction process to take place (Uba *et al.*, 2020c; 2020d). After seven days, the aqueous, acetone and ethanolic extracts were double-filtered using a muslin cloth and then through a filter paper (Whatman No. 1). The filtrates were concentrated to dryness in a water bath at 45 °C. The extracts were stored in a desiccator until needed (Oso *et al.*, 2018).

Phytochemical analysis

The qualitative phytochemical screening for alkaloids, tannins, anthraquinones, terpenoids, glycoside, saponins, flavonoids, steroids, protein and carbohydrate of the aqueous, acetone and ethanol extracts of garlic and tumeric were performed by the following standard methods as reported by Okaiyeto *et al.* (2019) and Karthigaiselvi and Rameshwari (2016) with slight modifications. The obtained results were qualitatively expressed as positive (+) or negative (-) as follow:

Test for alkaloid

The solution of the crude extract (50 mg/mL) was added to Mayer's reagent (0.68 g of HgCl₂ in 30 mL of distilled water, 2.5 g of KI in 5 mL of distilled water) solution. Formation of whitish yellow (or) cream coloured precipitate indicated the absence of alkaloids.

Test for tannin

The solution of the crude extract (50 mg/mL) was prepared, 1 mL was transferred in test tube, and 2 drops of 5 % FeCl₃ solution was added thereafter. Dirty green precipitate indicated the presence of tannin.

Test for flavonoid

The solution of the crude extract at a volume of 1 mL (50 mg/mL) was transferred into a test tube and 3 mL of Pb(C₂H₃O₂)₂ solution was added. A bulky white Lead precipitate indicated the test was positive.

Test for saponin

The solution of the crude extract (100 mg) was mixed with 5 mL of distilled water in a test tube, and it was shaken vigorously. The formation of stable foam indicated the presence of saponins.

Test for glycoside

A volume of 2 mL glacial CH₃COOH containing one drop of FeCl₃ solution was added to 5 mL of the solution of the crude extract (50 mg/mL). A 1 mL volume of concentrated H₂SO₄ was poured along the sides of the tube. Formation of a brown ring at the interface indicated the presence of glycosides.

Test for terpenoids

The solution of the crude extract (50 mg) was added with chloroform and H₂SO₄. Red violet colour indicated the presence of terpenoids.

Test for protein and amino acids

Ninhydrin solution (two drops of 10 mg of Ninhydrin in 200 mL of acetone) was added to 2 mL of the solution of the crude extract (50 mg/mL). A characteristic purple colour indicated the presence of amino acid.

Test for carbohydrates

The solution of the crude extract (50 mg) was mixed with 2 mL of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for anthraquinone

The solution of the crude extract (50 mg) was boiled with 10 mL of H₂SO₄ and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was

transferred into another test tube. The resulting solution was observed for colour changes to violet indicating the presence of anthraquinone.

Test for steroids

The solution of the crude extract (50 mg) was taken in a test tube and dissolved with chloroform (10 mL), then had added an equal volume of concentrated H₂SO₄ to the test tube by sides. The upper layer in the test tube turned into a red and sulphuric acid layer showing yellow with green fluorescence, showing the presence of steroids.

Preparation of extract concentrations

Stock solutions (100 mg/mL) of the ethanol/acetone/aqueous leave extracts of garlic and turmeric bulb was prepared by weighing 1 g of the extracts and dissolving in 10 mL of dimethyl sulpho-oxide (DMSO). Thereafter, different concentrations were obtained from the stock solutions as described by Oso *et al.* (2018).

Test organisms

The test human bacterial pathogens used in this study include *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli* isolates. These isolates were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria. They were re-subcultured in Nutrient Agar (NA) slant contained in Bjou bottles and preserved in refrigerator at 4 °C for further analysis.

Inocula Preparation

Each bacterial strain was sub-cultured overnight at 35 °C in Nutrient Agar (NA) slants. The bacterial growth was harvested using 5 mL of sterile saline water; its absorbance was adjusted at 600 nm and diluted to attain viable cell count of 1.5 X 10⁸ CFU/mL using spectrophotometer (Mostafa *et al.*, 2018; Okoye *et al.*, 2024a).

Antibacterial activity of the tumeric and garlic bulb extract

The disk diffusion method was used to evaluate antibacterial activity of the extract. The plant extract residues (400 mg) were diluted in two folds using DMSO to obtain 200 and 100 mg/mL concentrations respectively. Fifteen millilitres of Muller Hinton agar (MHA) medium were poured into sterile Petri dishes followed by spreading with swab sticks previously

inoculated with each bacterial suspension (1.5 x 10⁸ CFU) and appropriately labelled. Sterile filter paper discs loaded with tumeric and garlic bulb extract concentration of 400, 200 and 100 mg/mL was placed aseptically on the top of MHA plates. Filter paper discs loaded with 1 mg of ciprofloxacin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h to permit plant extracts diffusion and then incubated 28 °C for 48 – 72 h, respectively. The presence of inhibition zones was measured by millimeter calibrated metre rule, recorded and considered as indication for antibacterial activity (Mostafa *et al.*, 2018; Uba *et al.*, 2018c; Okoye *et al.*, 2024a; Okoye *et al.*, 2024b).

Determination of minimal inhibitory and bactericidal concentration of the tumeric and garlic bulb extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 48 h and 72 h of incubation. Using the tube dilution technique, 1 g was dissolved in 5 ml sterile nutrient broth; this gave 200 mg/mL. Thereafter, two-fold serial dilutions were made from the original stock of 5 mL, using Nutrient broth to achieve the following concentrations: 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/mL (Agu *et al.*, 2024; Dibua *et al.*, 2025a; Dibua *et al.*, 2025b). Having obtained different dilutions and concentrations, 0.1 mL of standardized test organisms was inoculated into the dilutions and incubated at 28 °C for 48 – 72 h for bacterial isolates. Using solvents and test organisms without extract, positive controls was equally set up. The lowest concentration of the extracts that inhibited the growth of the test organism was recorded as the MIC. Bacterial tubes showing no visible growth from the MIC test were sub-cultured into MHA and incubated at 28 °C for 48 – 72. The lowest concentration of the extracts yielding no growth on sub-culturing was recorded as the minimum bactericidal concentration (MBC) for fungi as described by Adegoke *et al.* (2010).

Data Analysis

The data were presented in tables and charts and the means statistically analyzed using GraphPad Prism version 9.0.0 by adopting Analysis of Variance (ANOVA). Values less 0.05 are considered significant (Uba *et al.*, 2020e; 2020f; 2020g; 2020h; Uba *et al.*, 2024; Uba *et al.*, 2025; Chude *et al.*, 2021).

Results

Tables 1 and 2 present the qualitative phytochemical profiles of aqueous, acetone, and ethanolic extracts of turmeric and garlic.

Table 1: Qualitative phytochemical profile of the aqueous, acetone and ethanolic extracts of tumeric

Component	Aqueous	Acetone	Ethanol
Anthroquinone	-	-	-
Tannins	-	-	-
Terpenoids	+	+	+
Flavonoids	-	-	-
Carbohydrate	-	+	-
Alkaloids	+	+	+
Protein and amino acid	-	-	+
Cardiac glycoside	+	+	+
Saponins	-	-	-
Glycoside	+	+	+

Key: + = Positive, - = Negative

Table 2: Qualitative phytochemical profiles of the aqueous, acetone and ethanolic extracts of garlic

Component	Aqueous	Acetone	Ethanol
Anthroquinone	-	-	-
Tannins	-	-	-
Terpenoids	+	+	+
Flavonoids	+	+	+
Carbohydrate	+	+	+
Alkaloids	+	+	+
Protein and amino acid	+	-	-
Cardiac glycoside	-	-	-
Saponins	-	-	-
Glycoside	-	-	-

Key: + = Positive, - = Negative

Table 3 presents the antibacterial activity of different plant extracts (aqueous, acetone, and ethanol) of turmeric and garlic against three bacterial strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*.

Table 3: Antibacterial activity (mm) of different plant extracts against the tested bacterial organisms

Test Organisms/ strain code	Extract type	CIP (mm)	DMSO (mm)	400 mg/mL (mm)	200 mg/mL (mm)	100 mg/mL (mm)
<i>Pseudomonas aeruginosa</i>	Aqueous tumeric	23.00±0.76	-	17.00 ±0.26	14.00 ±0.20	12.00 ±0.10
	Acetone tumeric	23.00±0.76	-	14.00±0.20	-	-
	Ethanol tumeric	23.00±0.76	-	18.00 ±0.30	15.00 ±0.10	13.00±0.15
	Aqueous garlic	23.00±0.76	-	16.00±0.20	14.00±0.10	-
	Acetone garlic	23.00±0.76	-	11.00±0.10	10.00 ±0.13	-
	Ethanol garlic	23.00±0.76	-	14.00±0.30	12.00 ±0.30	-
<i>Staphylococcus aureus</i>	Aqueous tumeric	-	-	-	-	-
	Acetone tumeric	-	-	18.00±0.15	13.00±0.20	-
	Ethanol tumeric	-	-	15.00±0.23	13.00±0.10	10.00±0.13
	Aqueous garlic	-	-	14.00±0.15	13.00±0.12	11.00 ±0.20
	Acetone garlic	-	-	23.00±0.35	20.00±0.25	19.00±0.20
	Ethanol garlic	-	-	16.00±0.15	13.00 ±0.10	-
<i>Escherichia coli</i>	Aqueous tumeric	23.00±0.35	-	14.00±0.12	-	-
	Acetone tumeric	23.00±0.35	-	13.00±0.15	12.00±0.20	-
	Ethanol tumeric	23.00±0.35	-	11.00±0.20	-	-
	Aqueous garlic	23.00±0.35	-	12.00±0.25	-	-
	Acetone garlic	23.00±0.35	-	14.00±0.10	11.00±0.35	-
	Ethanol garlic	23.00±0.35	-	18.00±0.25	16.00±0.10	14.00±0.15

Table 4: Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of different plant extracts against the tested bacterial organisms

Test organisms/strain code	Extract type	MIC (mg/mL)	MBC (mg/mL)
<i>Pseudomonas aeruginosa</i>	Aqueous turmeric	400	>400
	Acetone turmeric	400	>400
	Ethanol turmeric	400	>400
	Aqueous Garlic	100	400
	Acetone Garlic	400	>400
	Ethanol Garlic	>400	>400
	Aqueous turmeric	NT	NT
	Acetone turmeric	400	>400
	Ethanol turmeric	200	400
	Aqueous Garlic	400	>400
<i>Staphylococcus aureus</i>	Acetone Garlic	200	400
	Ethanol Garlic	100	200
	Aqueous turmeric	>400	>400
	Acetone turmeric	400	>400
	Ethanol turmeric	>400	>400
	Aqueous Garlic	400	>400
	Acetone Garlic	>400	>400
	Ethanol Garlic	400	>400
	Aqueous turmeric	>400	>400
	Acetone turmeric	400	>400
<i>Escherichia coli</i>	Ethanol turmeric	>400	>400
	Aqueous Garlic	400	>400
	Acetone Garlic	>400	>400
	Ethanol Garlic	400	>400
	Aqueous turmeric	>400	>400
	Acetone turmeric	400	>400
	Ethanol turmeric	>400	>400
	Aqueous Garlic	400	>400
	Acetone Garlic	>400	>400
	Ethanol Garlic	400	>400

Discussion

The results indicate the presence or absence of various bioactive compounds in each extract. Terpenoids was present in all three extracts (aqueous, acetone, and ethanol) for turmeric and garlic bulbs; cardiac glycosides are positive across the board for turmeric but negative across the board for garlic ;alkaloids was present in aqueous and acetone extracts, but not specified for ethanol extract in the table provided however under the ethanol column for alkaloids it is "+", glycosides: present in acetone and ethanol extracts, protein and amino acids: present only in ethanol extract, carbohydrates: present only in acetone extract, while anthroquinone, tannins, flavonoids, and saponins: Absent in all three extracts turmeric and garlic bulbs, respectively. The presence of various phytochemicals in turmeric extracts may contribute to their medicinal properties, including antimicrobial, anti-inflammatory, and antioxidant activities (Okoye *et al.*, 2016a; 2016b; Uba *et al.*, 2021; Anameze *et al.* 2023; Obiefoka *et al.*,2023).

For *Pseudomonas aeruginosa*, all extracts showed antibacterial activity, with ethanolic extract of turmeric

exhibiting the highest activity at 400 mg/mL (18.00 ± 0.30 mm) while aqueous turmeric extract showed activity at all concentrations (400, 200 and 100 mg/mL) (Table 3). For *Staphylococcus aureus*, acetone garlic extract exhibited the highest activity at all concentrations (400, 200 and 100 mg/mL), with the highest activity at 400 mg/mL (23.00 ± 0.35 mm) while aqueous garlic and ethanol turmeric extracts also showed activity. For *E. coli*, ethanol garlic extract exhibited the highest activity at all concentrations (400, 200, and 100 mg/mL), with the highest activity at 400 mg/mL (18.00 ± 0.25 mm) while aqueous turmeric and acetone garlic extracts also showed activity (Table 3). The results suggest that different plant extracts exhibit varying levels of antibacterial activity against different bacterial strains (Ezeamama *et al.* 2025a; 2025b; Uba *et al.*, 2020b; Afulukwe *et al.*2025, Afulukwe *et al.* 2026; Okoye *et al.* 2020a; 2020b; 2020c).

Table 4 presents the MICs and MBCs of different plant extracts (aqueous, acetone, and ethanol) of turmeric and garlic against three bacterial strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli*. For *Pseudomonas aeruginosa*, aqueous garlic extract showed the lowest MIC

(100 mg/mL), indicating its effectiveness in inhibiting bacterial growth. All extracts had MBCs greater than 400 mg/mL, suggesting that they may not be bactericidal at the tested concentrations. For *Staphylococcus aureus*, ethanol garlic extract showed the lowest MIC (100 mg/mL) and MBC (200 mg/mL), indicating its effectiveness in inhibiting and killing bacterial cells while acetone turmeric and ethanol turmeric extracts also showed some activity. For *E. coli*, most extracts had MICs and MBCs greater than 400 mg/mL, suggesting that they may not be effective against this bacterial strain. The results suggest that garlic extracts, particularly ethanol garlic extract, show promise as potential antimicrobial agents (Iheukwumere *et al.*, 2012a; 2012b; Mundi *et al.*, 2013; 2014; Okoye *et al.* 2013; Okoye *et al.* 2014, Anyanele *et al.*, 2023a; Anyanele *et al.*, 2023b).

Conclusion and Recommendations

The differences in phytochemical profiles across extraction methods highlight the importance of selecting the appropriate solvent for specific applications. The effectiveness of an extract depends on the type of plant, solvent used, and concentration. Garlic and turmeric extracts have potential applications in medicine and food preservation.

The study recommends further studies on quantifying the phytochemicals present in each extract, investigating the biological activities of individual phytochemicals, identifying the bioactive compounds responsible for the antibacterial activity, investigating the mechanisms of action of the extracts, and exploring the potential applications of turmeric and garlic extracts in medicine, food, and cosmetics.

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