# Comparative *in Vitro* Antimicrobial Activity of Solvent-Partitioned *Curcuma longa* L. Leaf Fractions against Selected Bacterial and Fungal Isolates

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## ABSTRACT

*Curcuma longa* L. leaves remain underused despite reports that they contain antimicrobial constituents. This preliminary study evaluated the concentration-dependent inhibition-zone activity of solvent-partitioned leaf fractions against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Trichophyton tonsurans*, and *Microsporum audouinii*. A 70% methanol leaf extract was successively partitioned into n-hexane, dichloromethane, ethyl acetate, n-butanol, and residual aqueous fractions. Agar-well diffusion assays were conducted as three replicate plate determinations per condition. Inhibition-zone diameters were expressed as mean ± standard error of the mean and analysed separately for each organism within each fraction using one-way analysis of variance followed by Tukey's honestly significant difference test at $p < 0.05$. At 100 mg $mL^{-1}$, the ethyl acetate fraction produced inhibition zones of 20.67 ± 0.67 mm against *S. aureus*, 20.00 ± 0.58 mm against *T. tonsurans*, and 19.67 ± 0.88 mm against *M. audouinii*. The dichloromethane fraction produced a 20.67 ± 0.67 mm zone against *S. aureus* at 100 mg $mL^{-1}$, whereas the residual aqueous fraction showed measurable activity against *C. albicans* and *T. tonsurans*. The n-hexane and n-butanol fractions had narrower inhibition profiles. These agar-diffusion findings identify fractions for further chemical and broth-microdilution studies but do not establish minimum inhibitory concentrations, clinical efficacy, or therapeutic potency.

**Keywords:** antimicrobial screening; *Curcuma longa*; dermatophytes; leaf fractions; solvent partitioning

## INTRODUCTION

Antimicrobial resistance is a major global health threat that compromises the treatment of common bacterial infections and increases morbidity and mortality. A comprehensive analysis estimated that bacterial antimicrobial resistance was associated with 4.95 million deaths worldwide in 2019 (Antimicrobial Resistance Collaborators, 2022). Fungal infections also impose a substantial and often overlooked burden, particularly among immunocompromised individuals and in settings with limited diagnostic capacity (Bongomin *et al*., 2017). These challenges support the continuing search for chemically diverse antimicrobial candidates from medicinal plants.

The test organisms represent clinically important bacteria, yeasts, and dermatophytes. *Staphylococcus aureus* causes infections of the skin, soft tissues, bloodstream, and medical devices, whereas *Escherichia coli* commonly causes urinary, gastrointestinal, and systemic infections. *Candida albicans* is an opportunistic fungus associated with mucosal and invasive candidiasis. *Trichophyton tonsurans* and *Microsporum audouinii* are important causes of tinea capitis, particularly among children in Africa (Nweze & Eke, 2018). Differences

in the cellular structures and physiological characteristics of these organisms provide a useful basis for preliminary evaluation of antimicrobial spectrum.

*Curcuma longa* L. (Zingiberaceae), commonly known as turmeric, is widely cultivated in tropical and subtropical regions. Its rhizome contains curcuminoids and volatile compounds with reported anti-inflammatory, antioxidant, and antimicrobial effects (Fuloria *et al*., 2022; Gupta *et al*., 2015). The leaves are sometimes treated as agricultural waste, although they contain phenolics, flavonoids, and volatile terpenoids of biological and nutritional significance (Braga *et al*., 2018). Essential oils obtained from *C. longa* leaves have demonstrated antibacterial and antifungal activity, with chemical profiles dominated by monoterpenes and oxygenated terpenoids in some accessions (Albaqami *et al*., 2022; Essien *et al*., 2015; Sharma *et al*., 2022). Aqueous and hydroalcoholic leaf extracts may additionally recover polar constituents that are absent from essential oils.

Solvent partitioning separates chemically complex extracts into fractions according to polarity. Non-polar solvents preferentially recover lipophilic constituents, whereas ethyl acetate, n-butanol, and water progressively retain more polar compounds. Fraction-specific antimicrobial profiles can therefore help localise activity and guide subsequent isolation (Oreopoulou *et al*., 2021). Comparative information across *C. longa* leaf fractions and bacterial, yeast, and dermatophyte targets remains limited. This study consequently evaluated the *in vitro* antimicrobial activity of n-hexane, dichloromethane, ethyl acetate, n-butanol, and residual aqueous fractions of Nigerian *C. longa* leaves against *S. aureus*, *E. coli*, *C. albicans*, *T. tonsurans*, and *M. audouinii*.

## MATERIALS AND METHODS

### Materials and equipment

Methanol, n-hexane, dichloromethane, ethyl acetate, and n-butanol were analytical-grade reagents. Dimethyl sulfoxide (DMSO), distilled water, nutrient agar, nutrient broth, Sabouraud dextrose agar, and clotrimazole were used for microbiological analysis. The principal equipment included an ESJ210-4A analytical balance, a laboratory hammer mill, and a rotary evaporator.

### Plant collection and authentication

Fresh *Curcuma longa* leaves (200 g) were collected in April 2024 in Uyo, Akwa Ibom State, Nigeria. Professor Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, authenticated the specimen. A voucher specimen was deposited in the University of Uyo Herbarium under accession number UUPH80(b).

### Preparation and hydroalcoholic extraction

The leaves were dried at ambient temperature and pulverised using a hammer mill. The powdered material (118.4 g) was macerated in 70% methanol for 72 h with intermittent stirring. The mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to obtain 10.30 g of crude extract, corresponding to an extraction yield of 8.70% w/w. The crude extract was refrigerated until fractionation.

### Solvent partitioning

A 3.05-g portion of the crude hydroalcoholic extract was dissolved in 20 mL of distilled water and successively partitioned with n-hexane, dichloromethane, ethyl acetate, and n-butanol. Solvents were removed from the organic phases, and the remaining aqueous phase was retained as the residual aqueous fraction. All fractions were stored under refrigeration until analysis. The recovered masses and apparent yields relative to the 3.05-g portion were 0.10 g (3.28% w/w) for n-hexane, 0.84 g (27.54% w/w) for dichloromethane, 1.49 g (48.85% w/w) for ethyl acetate, 0.06 g (1.97% w/w) for n-butanol, and 0.28 g (9.18% w/w) for the residual aqueous fraction. The combined recovered mass was 2.77 g, corresponding to a total fraction recovery of 90.82% w/w.

### Microorganisms

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Trichophyton tonsurans*, and *Microsporum audouinii* were obtained from the Pharmaceutical Microbiology Laboratory, University of Uyo. The bacteria were maintained on nutrient agar, whereas the yeast and dermatophytes were maintained on Sabouraud dextrose agar. All procedures were performed aseptically.

### Preparation of test concentrations

Stock dispersions were prepared in distilled water containing 1% DMSO. Six two-fold concentrations were evaluated for each fraction: 0.625-20 mg $mL^{-1}$ for the n-hexane fraction; 3.12-100 mg $mL^{-1}$ for the dichloromethane, ethyl acetate, and residual aqueous fractions; and 0.36-11.6 mg $mL^{-1}$ for the n-butanol fraction. Distilled water containing 1% DMSO served as the vehicle control.

### Antibacterial agar-well diffusion assay

A loopful of each bacterial stock culture was inoculated into 5 mL of nutrient broth and incubated at 37 °C for 24 h. A 0.1-mL aliquot was diluted with 9.9 mL of sterile distilled water (1:100), and 0.2 mL of the diluted inoculum was incorporated into nutrient agar cooled to approximately 45 °C. The inoculated medium was poured into sterile Petri dishes and allowed to solidify for 60 min. Wells measuring 8 mm in diameter were prepared aseptically and filled with the test fractions and vehicle control. Plates were allowed to pre-diffuse for 2 h and were then incubated at 37 °C for 24 h. Inhibition-zone diameters were measured in millimetres. Plates were prepared in triplicate for each condition.

### Antifungal agar-well diffusion assay

Sabouraud dextrose agar was prepared at 62 g $L^{-1}$ according to the manufacturer's instructions. A 0.2-mL aliquot of the diluted fungal inoculum was distributed evenly over the agar surface, and 8-mm wells were prepared aseptically. The test fractions and vehicle control were introduced into separate wells. Following a 120-min pre-diffusion period, the plates were incubated at 28 °C for 48 h. Inhibition-zone diameters were measured in millimetres. Each fraction was tested in triplicate.

### Statistical analysis

Results are presented as mean ± standard error of the mean (SEM), calculated as the sample standard deviation divided by the square root of three replicate plate measurements. Separate one-way analyses of variance were performed for each organism within each solvent fraction, with concentration as the sole factor (six levels; n = 3 replicate determinations per level). When an omnibus test was significant, Tukey's honestly significant difference procedure was used for all 15 pairwise comparisons among the six concentrations, controlling the family-wise error rate at 0.05 within that organism-fraction analysis. No global multiplicity adjustment was applied across the separate organism-fraction analyses. Analyses were performed in Python using SciPy version 1.17.0.

## RESULTS

### Overall activity profile

The five fractions differed in inhibition-zone magnitude and apparent antimicrobial spectrum. The ethyl acetate fraction showed the broadest activity, inhibiting *S. aureus*, *C. albicans*, *T. tonsurans*, and *M. audouinii*. The dichloromethane fraction produced its largest zones against *S. aureus* and showed measurable activity against *E. coli* and *M. audouinii* only at the highest concentration. The residual aqueous fraction showed its clearest responses against *C. albicans* and *T. tonsurans*. The n-hexane and n-butanol fractions had comparatively narrow inhibition profiles. No fraction inhibited all five organisms across the complete concentration range.

### n-Hexane fraction

The n-hexane fraction produced concentration-dependent inhibition of *S. aureus*, with mean zones of 9.67 ± 0.33, 12.67 ± 0.67, and 14.33 ± 0.33 mm at 5, 10, and 20 mg mL⁻¹, respectively. At 20 mg mL⁻¹, modest inhibition was also observed against *E. coli* (9.00 ± 0.58 mm), *T. tonsurans* (9.00 ± 0.58 mm), and *M. audouinii* (10.00 ± 0.58 mm). *C. albicans* was not inhibited at any tested concentration. Concentration significantly affected every organism that showed measurable inhibition ($p < 0.001$).

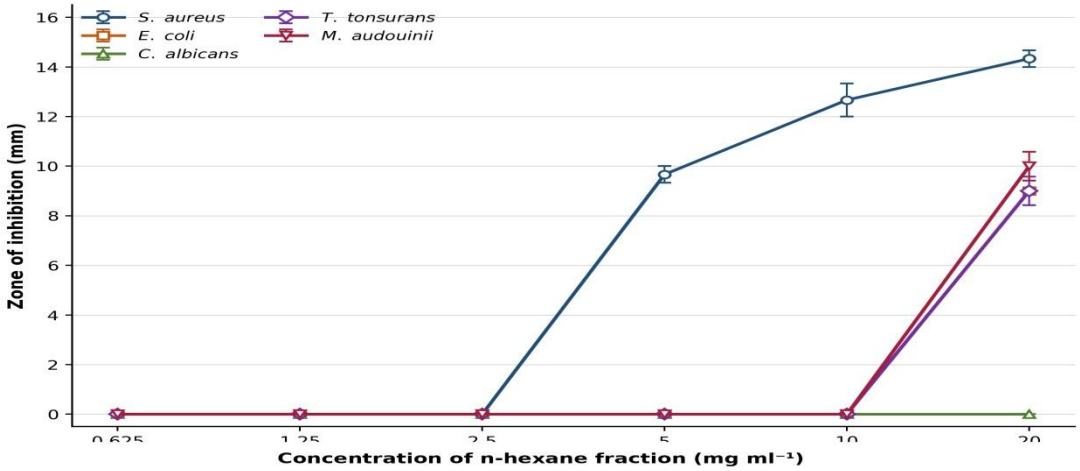


Figure 1. Antimicrobial activity of the n-hexane fraction of *Curcuma longa* leaves. Points represent mean inhibition-zone diameter ± standard error of the mean (n = 3 replicate plate determinations). Absence of a visible error bar indicates zero standard error or an error bar smaller than the plotting symbol.

### Dichloromethane fraction

The dichloromethane fraction produced concentration-dependent inhibition zones against *S. aureus*, increasing from 9.00 ± 0.58 mm at 6.25 mg mL⁻¹ to 20.67 ± 0.67 mm at 100 mg mL⁻¹, $F(5, 12) = 238.68$, $p < 0.001$. At 100 mg mL⁻¹, measurable zones were also observed against *E. coli* (9.33 ± 0.67 mm), $F(5, 12) = 196.00$, $p < 0.001$, and *M. audouinii* (10.00 ± 0.58 mm), $F(5, 12) = 300.00$, $p < 0.001$. No measurable activity was observed against *C. albicans* or *T. tonsurans*.

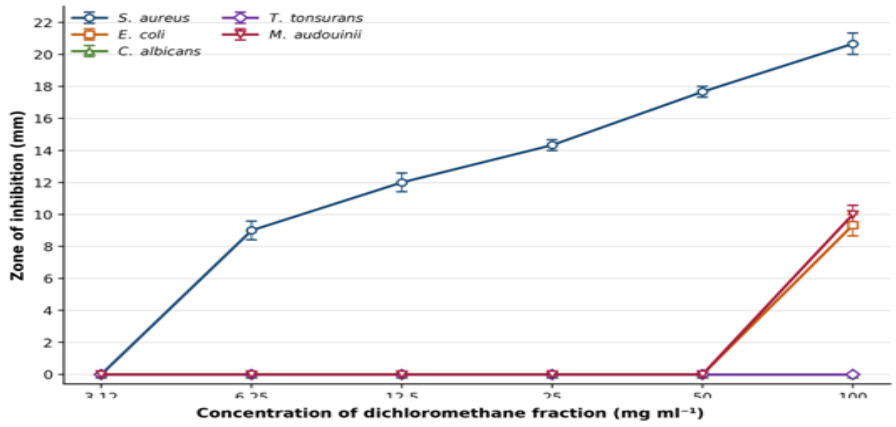


Figure 2. Antimicrobial activity of the dichloromethane fraction of *Curcuma longa* leaves. Points represent mean inhibition-zone diameter ± standard error of the mean (n = 3 replicate plate determinations). Concentrations are expressed in mg mL⁻¹.

### Ethyl acetate fraction

The ethyl acetate fraction showed the broadest activity. Inhibition of *S. aureus* increased from 6.00 ± 0.58 mm at 3.12 mg mL⁻¹ to 20.67 ± 0.67 mm at 100 mg mL⁻¹, $F(5, 12) = 100.19$, $p < 0.001$. Both dermatophytes were

inhibited throughout the tested range: *T. tonsurans* increased from 7.67 ± 0.33 to 20.00 ± 0.58 mm, $F(5, 12) = 80.91$, $p < 0.001$, and *M. audouinii* increased from 8.00 ± 1.15 to 19.67 ± 0.88 mm, $F(5, 12) = 36.80$, $p < 0.001$. *C. albicans* was inhibited from 25 mg mL$^{-1}$ and reached 11.00 ± 0.58 mm at 100 mg mL$^{-1}$, $F(5, 12) = 78.24$, $p < 0.001$. *E. coli* was not inhibited.

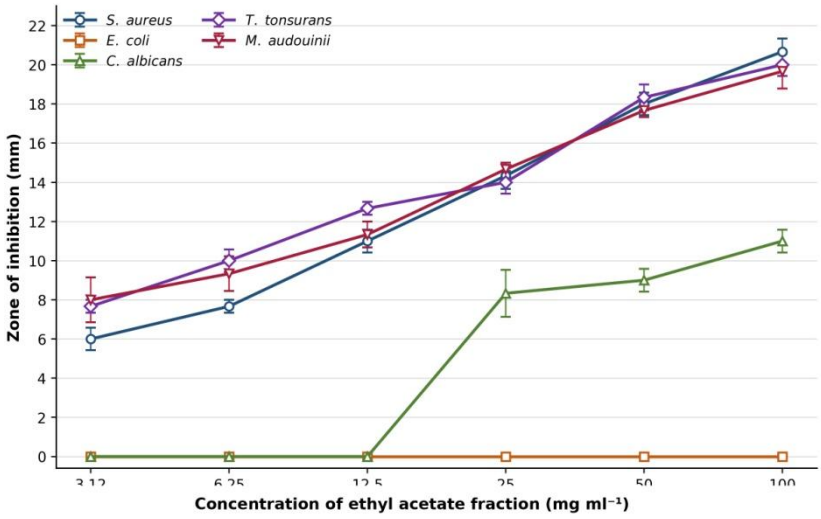


Figure 3. Antimicrobial activity of the ethyl acetate fraction of *Curcuma longa* leaves. Points represent mean inhibition-zone diameter ± standard error of the mean (n = 3 replicate plate determinations). Concentrations are expressed in mg mL$^{-1}$.

**n-Butanol fraction**

The n-butanol fraction produced limited activity at the upper end of its concentration range. *S. aureus* was inhibited at 5.8 mg mL$^{-1}$ (8.00 ± 1.15 mm) and 11.6 mg mL$^{-1}$ (9.67 ± 0.33 mm), $F(5, 12) = 87.58$, $p < 0.001$. At 11.6 mg mL$^{-1}$, inhibition zones of 9.67 ± 0.33 mm against *C. albicans*, $F(5, 12) = 841.00$, $p < 0.001$, and 9.00 ± 0.58 mm against *T. tonsurans*, $F(5, 12) = 243.00$, $p < 0.001$, were observed. *E. coli* and *M. audouinii* were not inhibited.

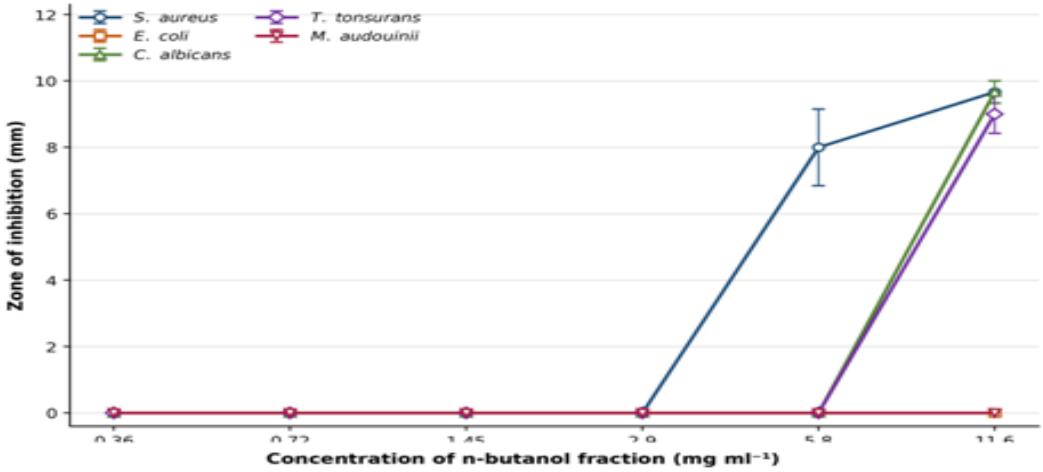


Figure 4. Antimicrobial activity of the n-butanol fraction of *Curcuma longa* leaves. Points represent mean inhibition-zone diameter ± standard error of the mean (n = 3 replicate plate determinations). Concentrations are expressed in mg mL$^{-1}$.

**Residual aqueous fraction**

The residual aqueous fraction showed its most consistent activity against the fungal isolates. Inhibition of *C. albicans* began at 12.5 mg mL$^{-1}$ (9.33 ± 0.67 mm) and increased to 14.33 ± 0.67 mm at 100 mg mL$^{-1}$, $F(5, 12)$

= 125.05, $p < 0.001$. *T. tonsurans* was inhibited from 6.25 mg $mL^{-1}$ (10.00 ± 0.00 mm), reaching 14.67 ± 0.33 mm at 100 mg $mL^{-1}$, $F(5, 12) = 156.44$, $p < 0.001$. At 100 mg $mL^{-1}$, the fraction also produced modest inhibition of *S. aureus* (9.67 ± 0.33 mm), $F(5, 12) = 841.00$, $p < 0.001$; *E. coli* (9.33 ± 0.67 mm), $F(5, 12) = 196.00$, $p < 0.001$; and *M. audouinii* (10.00 ± 0.58 mm), $F(5, 12) = 300.00$, $p < 0.001$.

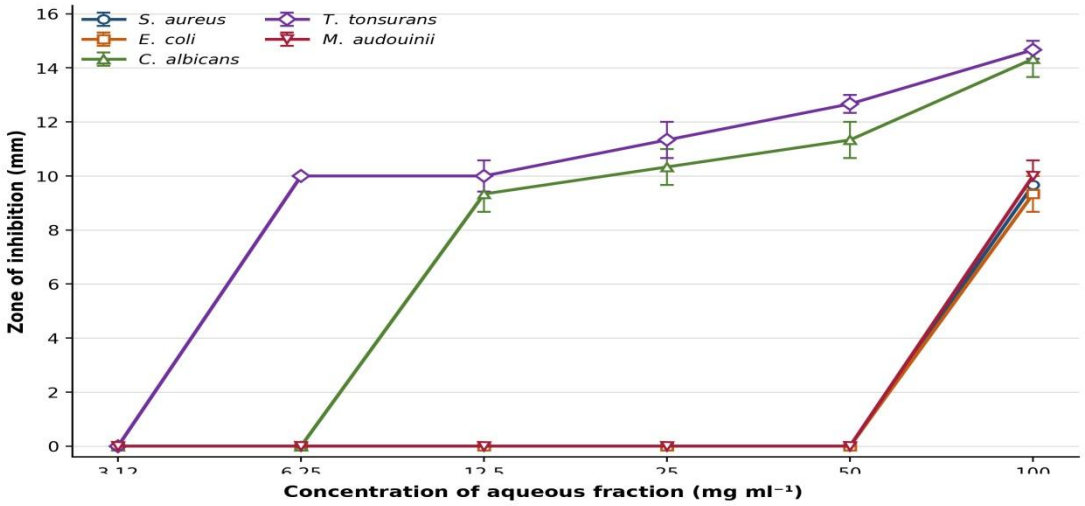


Figure 5. Antimicrobial activity of the residual aqueous fraction of *Curcuma longa* leaves. Points represent mean inhibition-zone diameter ± standard error of the mean (n = 3 replicate plate determinations). Concentrations are expressed in mg $mL^{-1}$.

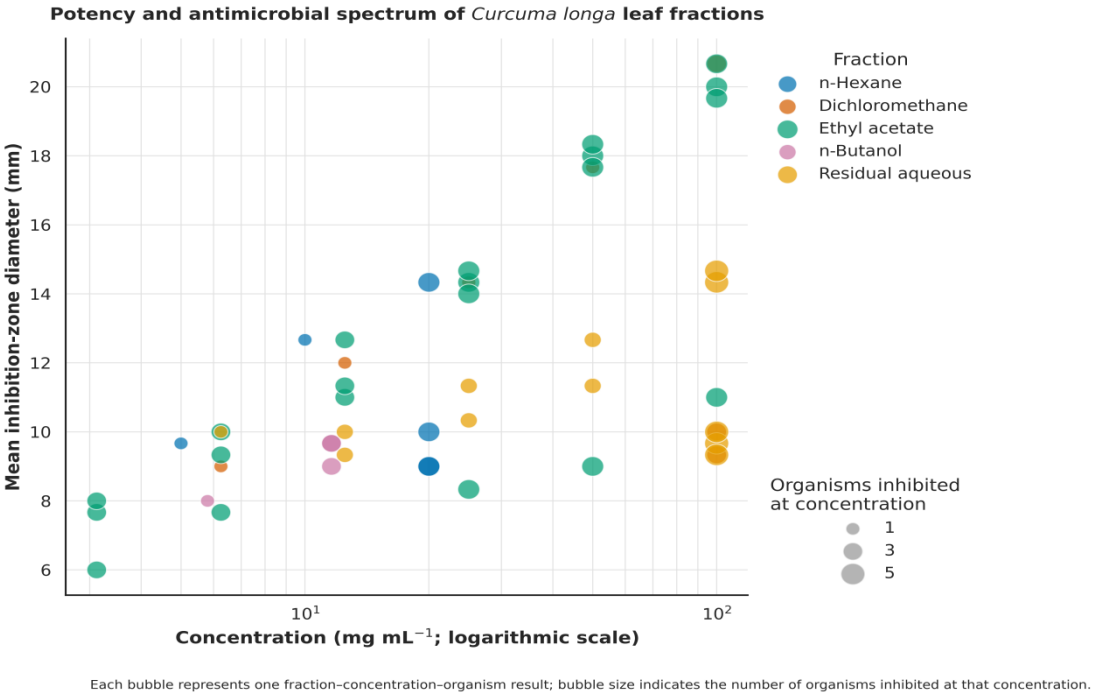


Figure 6. Comparative inhibition-zone profiles and antimicrobial spectrum of solvent-partitioned *Curcuma longa* leaf fractions. Each bubble represents one fraction-concentration-organism result. Bubble position indicates the tested concentration and mean inhibition-zone diameter, bubble colour identifies the solvent fraction, and bubble size represents the number of organisms inhibited at that concentration. Concentrations are shown on a logarithmic scale; values are means of three replicate plate determinations (n = 3).

## DISCUSSION

The inhibition-zone activity of *Curcuma longa* leaf fractions varied with solvent fraction, concentration, and test organism. The ethyl acetate fraction showed the broadest measurable spectrum, the dichloromethane

fraction produced its largest zones against *S. aureus*, and the residual aqueous fraction retained activity against *C. albicans* and *T. tonsurans*. These patterns are consistent with solvent partitioning producing chemically different fractions, but the present assay does not establish the identity, abundance, or intrinsic potency of the active constituents.

The comparative inhibition-zone plot (Figure 6) summarises the relationships among tested concentration, zone diameter, and the number of organisms showing measurable inhibition. The ethyl acetate fraction occupied the upper part of the plot and produced the largest bubbles at higher concentrations, consistent with its broader activity against *S. aureus* and the dermatophytes. Dichloromethane produced a larger but narrower response driven largely by *S. aureus*, whereas the residual aqueous fraction formed a lower-zone cluster that reflected measurable antifungal activity. The n-hexane and n-butanol fractions produced smaller and more restricted activity clusters. This integrated comparison does not estimate minimum inhibitory concentration, and the fractions cannot be ranked directly because their maximum tested concentrations were not identical and agar diffusion is influenced by compound mobility.

*S. aureus* susceptibility to several fractions agrees with previous findings on the activity of turmeric-derived constituents against this organism (Gupta *et al*., 2015). Greater susceptibility of this Gram-positive organism than *E. coli* is biologically plausible because the outer membrane of Gram-negative bacteria limits the penetration of several hydrophobic phytochemicals. In the present study, *E. coli* did not respond to the ethyl acetate or n-butanol fractions and responded to the n-hexane, dichloromethane, and residual aqueous fractions only at their highest concentrations. These findings are specific to the organisms tested and should not be interpreted as a general distinction between Gram-positive and Gram-negative bacteria because only one species from each group was examined.

The inhibition profile of the ethyl acetate fraction is consistent with enrichment of constituents of intermediate polarity. Turmeric leaves contain phenolics, flavonoids, and volatile terpenoids. Investigations of leaf essential oils have identified compounds such as α-phellandrene, 2-carene, and eucalyptol and have reported antibacterial activity (Albaqami *et al*., 2022). Essential oil from *C. longa* leaves cultivated in Nigeria has also shown antimicrobial activity, indicating that geographic origin and chemotype may influence activity (Essien *et al*., 2015). These studies provide a plausible chemical context, but the fractions in the present investigation were not chemically characterised. Activity therefore cannot be assigned to specific constituents.

The antifungal findings were also notable. The ethyl acetate fraction inhibited both dermatophytes at every tested concentration, whereas the residual aqueous fraction inhibited *T. tonsurans* from 6.25 mg $mL^{-1}$ and *C. albicans* from 12.5 mg $mL^{-1}$. These results agree with evidence that *C. longa* leaf essential oil possesses antifungal activity, although extraction procedures and chemical compositions differ among studies (Sharma *et al*., 2022). Retention of antifungal activity in the aqueous fraction suggests that comparatively polar leaf constituents may contribute to the response. The weaker and threshold-dependent activity of the n-butanol fraction may reflect a lower concentration of active constituents, antagonistic matrix effects, or limited diffusion through agar.

## STUDY LIMITATIONS

This study was an exploratory agar-well diffusion screening experiment. Inhibition-zone diameter depends on both antimicrobial activity and diffusion through agar; it cannot be used to infer minimum inhibitory concentration, minimum bactericidal or fungicidal concentration, clinical effectiveness, or therapeutic potency. The fractions were tested over different concentration ranges, which precludes direct potency ranking. The methods describe three replicate plate determinations but do not document independent biological repetitions. Formal inoculum-density standardisation, laboratory strain identifiers, the exact well-loading volume, positive-control results, and detailed drying conditions were not reported in the source manuscript and require author confirmation before they can be added. The small replicate sample also limited meaningful assessment of analysis-of-variance assumptions. These constraints require cautious interpretation and should be addressed in confirmatory studies using authenticated reference strains, standardised broth-microdilution methods, positive antimicrobial comparators, independent experiments, and chemical profiling.

## CONCLUSION

Solvent-partitioned *Curcuma longa* leaf fractions produced distinct concentration- and organism-dependent inhibition-zone profiles. Within the agar-well diffusion assay, the ethyl acetate fraction showed the broadest measurable activity, the dichloromethane fraction produced its largest zones against *Staphylococcus aureus*, and the residual aqueous fraction inhibited *Candida albicans* and *Trichophyton tonsurans*. These preliminary findings support further investigation of turmeric leaves. Standardised broth-microdilution assays with authenticated reference strains, complete positive controls, independently repeated biological experiments, fraction-yield reporting, chemical characterisation, and cytotoxicity testing are required before antimicrobial potency or pharmaceutical applicability can be assessed.

## ACKNOWLEDGEMENTS

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### Conflict of Interest

The authors declare no conflict of interest.

### Funding

This research received no external funding.

### Data Availability

The three replicate inhibition-zone observations underlying each reported condition are available from the corresponding author on reasonable request.

### Ethical Considerations

Ethical approval was not required because this *in vitro* study used existing microbial isolates and involved neither human participants nor animals.

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