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Research Article

Phytochemical screening and *in vitro* antibacterial activity of methanolic rhizome extract of *Curcuma longa*

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Background: Since ancient times, people have utilized plants as medicine. Rwandans frequently use *Curcuma longa* as a cosmetic, culinary spice, and to treat many different types of diseases, including arthritis, digestive disorders, respiratory infections, allergies, liver disease, and inflammation. Therefore, the purpose of this study was to detect phytochemicals and investigate the antibacterial activity of *Curcuma longa* rhizome extract using an in vitro method.

Methods: The Soxhlet extraction of 40 g of powdered rhizomes with methanol yielded 1.84 g of crude extract, corresponding to an extraction yield of 4.6 %. Thin-layer chromatography analysis, phytochemical screening, and the Agar diffusion method were performed.

Results: The phytochemical screening identified steroids, flavonoids, phenols, terpenoids, saponins, alkaloids, and tannins; coumarins were absent. The results of the antibacterial activity assay showed that the *Curcuma longa* rhizomes' methanolic extract exhibited significant antibacterial activity against *E. coli* and *S. aureus*. For a prepared suspension of bacteria with a concentration of 1.5×10^8 CFU/ml, undiluted extract (10°) produced an inhibition zone of 13 mm against *E. coli* and 15 mm against *S. aureus*. At dilution (0.1g/ml) of the extract, the inhibition zones were 12 mm for *E. coli* and 13 mm for *S. aureus*, while at dilution of 0.01g/ml, inhibition zones of 10 mm and 12 mm were recorded, respectively. No inhibition was observed for the negative control, which was sterile distilled water, whereas the positive control (undiluted ampicillin) yielded inhibition zones of 30 mm on both bacterial strains.

Conclusion: These results confirm the dose-dependent antibacterial effectiveness of *Curcuma longa* methanolic extract against tested bacterial isolates. However, more research, including the isolation of different phytochemicals from this plant, is needed to confirm its use by traditional healers.

Keywords: *Curcuma longa, phytochemical screening, antibacterial activity, E. coli, S. aureus*

Introduction

The Zingiberaceae family includes the rhizomatous herbaceous perennial plant known as turmeric (*Curcuma longa*). It is indigenous to Southeast Asia and is abundantly grown there, especially in India, where it is widely used in cooking and medicine. Because of their therapeutic qualities, the rhizomes, or underground stems, are used as a spice and in traditional Chinese and Ayurvedic medicine, two ancient Eastern medical systems (Tian et al., 2025). Turmeric has traditionally been used as a dietary supplement to treat several conditions, including liver illness, allergies, respiratory infections, digestive issues, and arthritis (Sharifi-Rad et al., 2020). Flavonoids, saponins, and polyphenolic compounds are only a few of the

many bioactive phytochemicals found in *Curcuma longa* that are thought to be important to its medicinal potential (Permatananda et al., 2021). Anti-inflammatory, antibacterial, antioxidant, cardioprotective, anticancer, antiviral, and antiparasitic qualities are just a few of the numerous biological activities these substances display (Fuloria et al., 2022). For example, flavonoids reduce inflammation by scavenging free radicals and preventing the synthesis of inflammatory mediators (Mahmud et al., 2023). *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtillis*, *Bacillus cereus*, *Escherichia coli*, and *Bacillus coagulans* are just a few of the bacterial diseases that curcumin, the main polyphenolic molecule in turmeric, can fight (Adamczak et al., 2020). *Curcuma longa* is known for its bright yellow-orange rhizomes, which are called Ikinzari locally in Rwanda. The plant thrives in rich, well-drained soils with a slightly acidic to neutral pH and needs warm, humid weather (Tudu et al., 2024). It is valued for its culinary and medicinal uses and is typically grown in home gardens and small farms (Kaliyadasa & Samarasinghe, 2019). People in rural areas frequently apply topical turmeric paste to their skin to treat injuries and sports-related ailments (Paul et al., 2015).

Materials and methods

Collection of plant materials

Fresh *Curcuma longa* rhizomes were collected in March 2025 from the Go Green Rwanda market in the Kimihurura Sector of Gasabo District, Kigali City. The market received the rhizomes from the Rukira sector in the Ngoma District of the Eastern Province, Rwanda. After collection, plant material was identified by a botanist in the Department of Biology. The samples were transported to the Chemistry laboratory after being labeled (sample ID: 20250505-KGL-CL) and put in polyethylene bags. After the rhizomes were peeled to remove the roots and outer layers, they were dried **(Figure 1)** and ground into a fine powder using a lab electric blender and kept in a glass beaker covered with aluminum foil at room temperature (25°C) in a dry, cool environment for later use **(Figure 2)**.



Figure 1. Dried sample



Figure 2. Powdered *Curcuma longa* rhizomes

Extraction procedure

Methanol extract

Forty grams of dried *Curcuma longa* rhizome powder were placed in a Soxhlet extraction thimble and subjected to extraction **(Figure 3),** with 250 mL of methanol using the Soxhlet method (Kumar et al., 2020). In order to optimize the recovery of bioactive chemicals, the extraction was carried out using the method described by Joshi et al, which involved

constantly evaporating, condensing, and siphoning the solvent at 65 °C for eight hours (Joshi et al., 2018). The methanolic extract was concentrated and dried in accordance with the procedure described by Chauhan to eliminate any remaining solvent before being kept at 4°C until further analysis (Chauhan et al., 2017).



Figure 3. Soxhlet extraction of phytochemicals from *Curcuma longa* rhizomes

Microbial culture maintenance and storage

Samples of toilet water and skin swabs were taken, serially diluted, and grown on selective media: Chro Med TBX agar for *Escherichia coli* to isolate the target bacterium (Jozić et al., 2019). For *Staphylococcus aureus*, we used mannitol salt agar to acquire separate colonies for analysis (Missiakas & Schneewind, 2013). The plates were incubated at 37 °C for 24 hours (Arduino et al., 1991).

Phytochemical screening of *Curcuma longa* methanolic crude extract (Joshi et al., 2018).

Test for alkaloid

Wagner's test: Six drops of Wagner's reagent, iodine in potassium iodide, were added to one milliliter of the plant extract. Precipitate development in the solution was noted. A reddish-brown precipitate formation in the test tube indicates the presence of alkaloids.

Test for flavonoids

Alkaline reagent test: Two to three drops of a 5% sodium hydroxide solution were mixed with the plant extract. The presence of flavonoids is suggested by the strong yellow color. After a little while, a few drops of 1% diluted hydrochloric acid are added, and the yellow color disappears, leaving a colorless solution.

Test for steroids

Ten milliliters of chloroform were used to dissolve one milligram of the crude extract. Concentrated sulfuric acid was then carefully measured and added to the test tube. The sulfuric acid layer appeared yellow with a fluorescent green hue. The presence of steroids is indicated by a greenish bottom layer and a red top layer.

Test for saponins

Foam test: To one milligram of *Curcuma longa* extract, add 5 milliliters of distilled water and shake well. The formation of foam indicates the presence of saponins.

Test for tannins

Ferric chloride test: Add 200 microliters of the extract and a few drops of a 10% ferric chloride solution to five milliliters of distilled water and apply heat. Tannins are present when a blue-black precipitate forms.

Test for coumarins

To 2 milliliters of *Curcuma longa* rhizome extract, add 3 milliliters of a 10% NaOH solution. The formation of yellow color indicates a positive test.

Test for phenols

Mix a small amount of plant extract with a few drops of 5% ferric chloride solution. Phenolic compounds are present when a deep blue or black color forms.

Test for terpenoids

Salkowski test: Mix 5 milliliters of extract with 2 milliliters of chloroform, then carefully add 3 milliliters of strong sulfuric acid to create a layer. Terpenoids are present when the interface becomes reddish-brown.

Thin layer chromatography (TLC) analysis of *Curcuma Longa* extract

Three 2 mm thick, glass-backed gel 60F 254 plates were used for thin layer chromatography (TLC) examination. Cyclohexane with Ethyl acetate (3:7) were used as mobile phase in various solvent system combinations. The samples were spotted 1 cm from the base of the TLC plates, along the spotting line. The plates were then placed in the TLC chamber and allowed to develop until solvent migration was complete. The solvent front was then marked, and the plates were air-dried. Separated compounds were visualized under UV light at 254 nm and 365 nm using a herolab UV-6S/L lamp, exploiting the characteristic UV absorbance at different types of phytochemicals. Compound identification and comparison were based on the retention factor (Rf), calculated as the ratio of the distance traveled by the compound to the distance traveled by the solvent front, where a higher Rf value reflects weaker affinity for the stationary phase (silica gel) and stronger affinity for the mobile phase (solvent system).

Antimicrobial activity

Escherichia coli and *Staphylococcus aureus* were cultured on ChroMed TBX and Mannitol salt agar, respectively. Both *E. coli* and *S. aureus* were characterized by Gram staining process based on their Gram reaction and cellular morphology. *E. coli* appeared as Gram-negative, pink/red rod-shaped cells, whereas *S. aureus* appeared as Gram-positive, violet, spherical cells arranged predominantly in clusters. Using sterilized swabs and water bottles, samples were taken and inoculated into the proper media. Freshly grown cultures were then used for antimicrobial activity testing. The antibacterial activity of the *Curcuma longa* crude extract was evaluated using the agar diffusion method on Mueller-Hinton agar, with ampicillin serving as the positive control. Wells were created in plates containing 100 µL of bacterial suspension (1.5×10^8 CFU/ml, McFarland standard) (Andrews, 2001) and filled with varying concentrations of *Curcuma longa* crude extract diluted with sterile distilled water. Plates were incubated at 37 °C for 24 hours. Inhibition zones were then measured.

Results and Discussion

Acute oral toxicity

Phytochemical screening

Table 1. Phytochemical screening of the methanol crude extract of *Curcuma longa*

Phytochemicals for *Curcuma longa* methanol extract	Reagent used	Observation	Conclusion
Alkaloids	Wagner's reagent	Reddish brown	+
Flavonoids	5% NaOH and 1%HCl	Acute yellow changes to colorless	+
Steroids	Chloroform and concentrated H_2SO_4	Reddish brown color	+
Saponins	Distilled water	Foams formation	+
Tannins	10 % $FeCl_3$	Blue-black precipitate	+
Coumarins	10 % NaOH	Orange (color of extract)	_
Phenols	Aqueous 5% $FeCl_3$	Blue-black color	+
Terpenoids	Chloroform and concentrated H_2SO_4	2 layers, including the reddish brown one.	+

Key: (+) Positive test, (-) Negative test (absent)

Phytochemical analyses were performed using the following methods: alkaloids by Wagner test, flavonoids by alkaline reagent test, saponins by foam test, tannins by ferric chloride test, terpenoids by Salkowski test, and phenols by ferric chloride test. Comparative phytochemical screening of the dried *Curcuma longa* methanolic crude extracts (Table 1) showed that they contained alkaloids, flavonoids, steroids, saponins, tannins, phenols, and terpenoids. The presence of alkaloids, flavonoids, tannins, phenols is consistent with previous reports on *Curcuma longa* rhizomes (Gupta et al., 2015).

Thin-Layer Chromatography (TLC)-based component separation of turmeric extract

Before TLC analysis, cyclohexane and ethyl acetate were used as solvent systems. During this experiment, different solvent ratios were tried. TLC analysis cyclohexane /ethyl acetate (3:7) was selected as an appropriate solvent system, as shown by **Figure 4**, while other ratios did not show clear spots. The TLC analysis shows that the sample contains different phytochemicals; some colors are visible to the naked eye, whereas the presence of other compounds becomes visible under a UV lamp.

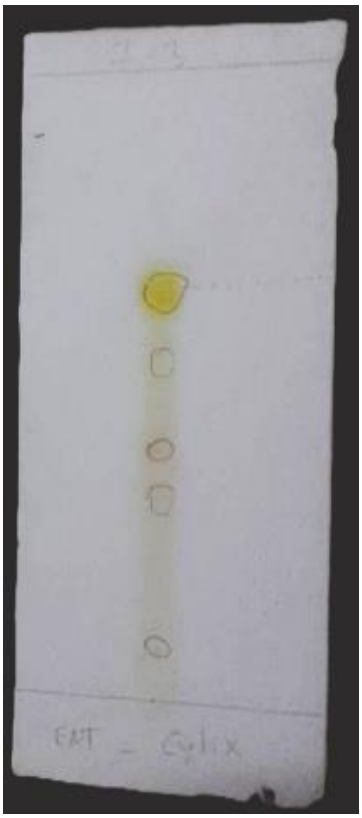


Figure 4. Thin-layer chromatogram (TLC) of *Curcuma longa* extract

The TLC plate displayed multiple distinct spots, indicating the presence of multiple substances. The retention factor (Rf) of one spot was found to be 0.707, whereas another spot had an Rf of 0.53. These spots, along with others, exhibit distinct phytochemicals (Ranganathan et al., 2020). The TLC profile indicated the presence of several separated components; however, individual compounds could not be definitely identified based solely on Rf values.

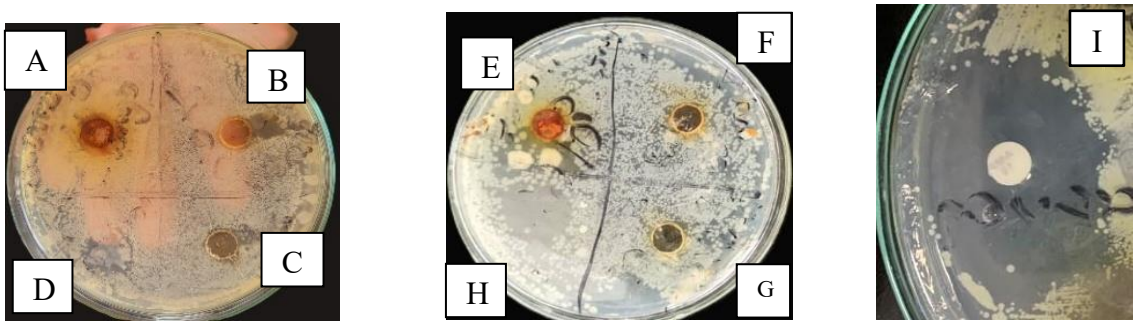
Antimicrobial activity testing of *Curcuma longa* methanolic crude extract

This study explores the antibacterial properties of freshly prepared methanolic *Curcuma Longa* crude extract on key pathogens. Notably, the dried rhizome extract showed activity against *Escherichia coli* and *Staphylococcus aureus*, which is consistent with the previous studies (Kasta, 2020). The antibacterial effect of different concentrations of *Curcuma longa* crude extract was evaluated against *S. aureus* and *E. coli*. As detailed in Table 2, the extent of bacterial inhibition varied with concentration, indicating a dose-dependent response. These results represent mean values from two replicates with the corresponding standard deviations.

Table 2. The antibacterial activity of variable concentrations of *Curcuma longa* crude extract against *S. aureus* and *E. coli*.

Bacteria tested	Bacterial concentration (CFU/ml)	Quantity of Innocum used (µL)	Quantity of Extract used (µL)	Culture media	Positive control (inhibition zone) in mm	Negative control (Inhibition zone)	Extract	Concentration of the extract	Inhibition zones by the extract in mm
E. coli	1.5×10^8	100	10	ChroMed TBX agar	30	No inhibition zone	Methanol	Full concentration	12.70 ± 1.29
								0.1g/ml	12.20 ± 1.29
								0.01g/ml	10.00 ± 1.29
S. aureus	1.5×10^8	100	10	Mannitol salt agar	30	No inhibition zone	Methanol	Full concentration	14.50 ± 1.28
								0.1g/ml	12.80 ±1.28
								0.01g/ml	12.00 ± 1.28

Clear inhibition zones were seen against *S. aureus* and *E. coli*, as shown in figure 5, whereas the positive control showed a noticeable inhibition zone.



(a) **(b)** **(c)**

Figure 5. Zones of inhibition of the methanolic crude extract of *Curcuma longa* against bacterial strains and control: (a) *S. aureus*, (b) *E. coli*, and (c) positive control on the concentration of 10^{-2} of inoculums.

Legend:

- **A**: Methanolic extract (undiluted and applied in solid form) with the zone of inhibition of 15 mm on *S. aureus* (1.5×10^8 CFU/ml).
- **B**: Methanolic extract (0.1g/ml) with the zone of inhibition of 13 mm on *S. aureus* (1.5×10^8 CFU/ml).
- **C**: Methanolic extract (0.01g/ml) with the zone of inhibition of 12 mm on *S. aureus* (1.5×10^8 CFU/ml).
- **D**: Negative control (sterile distilled water) with zero inhibition zone on *S. aureus* (1.5×10^8 CFU/ml).
- **E**: Methanolic extract (undiluted and applied in solid form) with the zone of inhibition of 13 mm on *E. coli* (1.5×10^8 CFU/ml).
- **F**: Methanolic extract (0.1g/ml) with the zone of inhibition of 12 mm on *E. coli* (1.5×10^8 CFU/ml).
- **G**: Methanolic extract (0.01g/ml) with the zone of inhibition of 10 mm on *E. coli* (1.5×10^8 CFU/ml).
- **H**: 95% (v/v) aqueous methanol.
- **I**: Positive control (undiluted ampicillin) with the inhibition zone of 30 mm on *E. coli* (1.5×10^8 CFU/ml).

For all tests, the concentration of bacteria is 1.5×10^8 CFU/ml. The methanolic extract of *Curcuma longa* exhibited antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with greater inhibitory activity against *S. aureus*. At the highest extract concentration, inhibition zone of 15 mm and 13 mm were observed against *S. aureus* and *E. coli*, respectively, indicating that the Gram-positive bacterium was more susceptible to the extract **(Figure 5)**. This difference can be explained by the structural differences between Gram-positive and Gram-negative bacteria. Gram-positive bacteria are often more susceptible to antimicrobial agents (including antibiotics and plant extracts) than Gram-negative bacteria because of differences in their cell envelope structure. Gram-positive bacteria possess a thick peptidoglycan cell wall but lack the outer lipopolysaccharide membrane characteristic of Gram-negative bacteria. In contrast, Gram-negative bacteria such as *E. coli* have an additional outer membrane composed of lipopolysaccharides, which acts as an effective permeability barrier and restricts the penetration of many antimicrobial compounds, particularly hydrophobic phytochemicals. This difference may be related to bioactive compounds present in *Curcuma longa* which can more readily penetrate the cell wall of Gram-positive-bacteria, resulting in greater antibacterial activity. The antibacterial activity found in this study is likely owing to the combined action of various phytochemical elements detected in the methanolic extract, including flavonoids, phenols, alkaloids, tannins, terpenoids, saponins, and steroids. Curcumin, the main polyphenolic component of *Curcuma longa*, is one of these substances whose antibacterial qualities have been well investigated. Furthermore, tannins can precipitate proteins and obstruct vital microbial enzymes, whereas phenolic

chemicals and flavonoids may increase membrane permeability, resulting in intracellular constituent leakage. The possible explanation is that the antibacterial activity seen in this study is probably the consequence of synergistic interactions among several bioactive elements. Also, methanol produced inhibition zones. Although methanol was used as the extraction solvent, it was not included as the negative control.

Conclusion

The results of this study, which yielded 4.6% using the Soxhlet extraction method, demonstrate the effectiveness of methanolic extraction in separating a variety of bioactive phytochemicals from the rhizomes of *Curcuma longa*. Pharmacologically significant substances such as flavonoids, phenols, alkaloids, tannins, saponins, and terpenoids were found during the phytochemical screening, which may have contributed to the plant's traditional use in herbal medicine. The extract showed inhibitory effects against *Escherichia coli* and *Staphylococcus aureus*, with higher activity against *S. aureus,* as determined by antimicrobial activity assays. The methanolic extract exhibited greater inhibitory activity against *S. aureus* than against *E. coli* under the conditions of this study. Overall, this research supports *Curcuma longa* as a valuable source and the results supports preliminary antibacterial potential of this plant. Further studies are recommended to isolate and identify the specific phytochemicals responsible for their antibacterial activity and to explore their mechanisms of action.

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Author contribution

JN and YI both contributed to designing, writing, and reviewing the manuscript. JH participated in the manuscript review process. JCS contributed in laboratory analyses.

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Conflict of interest

The authors declare that there are no conflicts of interest related to this work.

Ethical approval

Not applicable.

AI tool declaration

The authors have not used AI and its related tools to write this manuscript. The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data availability

The data generated during the present study are available from the corresponding author upon reasonable request.

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