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EVALUATION OF THE ANTIBACTERIAL EFFICACY OF Curcuma longa LEAF EXTRACTS AGAINST SOME ENTERIC PATHOGENS

By

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Abstract



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The rising prevalence of antibiotic-resistant enteric pathogens necessitates alternative antimicrobial solutions derived from medicinal plants. Curcuma longa (turmeric) is widely recognized for its traditional therapeutic uses, yet its antibacterial efficacy against clinically relevant bacteria remains underexplored. This study evaluated the antibacterial activity of aqueous extracts of Curcuma longa rhizomes against selected enteric bacterial pathogens and determined their minimum inhibitory and bactericidal concentrations. Stock cultures of Salmonella typhi, Shigella dysenteriae, Escherichia coli, and Klebsiella pneumoniae were confirmed by standard biochemical tests. Fresh turmeric rhizomes were cleaned, dried, and extracted in distilled water; phytochemical screening confirmed the presence of alkaloids, tannins, flavonoids, saponins, and cardiac glycosides. The cup-plate agar diffusion method assessed antibacterial activity, while macro-broth dilution determined MIC and MBC values. The extract demonstrated dose-dependent inhibition, producing zones ranging from 0 mm to 30 mm across concentrations. MIC values ranged from 0.16 to 0.63 mg/mL, while MBC values varied between 0.31 and 1.25 mg/mL. Salmonella typhi exhibited the highest susceptibility (MIC 0.16 mg/mL, MBC 0.63 mg/mL). Shigella dysenteriae and E. coli required higher MICs and MBCs, suggesting lower extract potency against these strains. Chi-square analysis indicated no significant difference in MIC and MBC values among isolates ($\chi^2 = 0.5915$, df = 3, P = 0.8984). The aqueous extract of Curcuma longa shows promising broad-spectrum antibacterial activity, supporting its ethnomedicinal use. Future research should focus on optimizing extraction protocols, isolating active compounds, investigating synergistic effects with standard antibiotics, and conducting clinical trials to validate its therapeutic potential against multidrug-resistant bacteria.

Keywords: Antibacterial Activity, Curcuma longa, Enteric Pathogens, Minimum Inhibitory Concentration, Phytochemicals

INTRODUCTION

Medicinal plants are a valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties, such as antimicrobial, anticancer, antiviral, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, antihypertensive, neuroprotective and anticoagulant effects (Ali *et al.*, 2019; Lesellier *et al.*, 2021). Medicinal plants are of great importance worldwide, both when used alone and as a supplement to traditional medication. The remarkable number

of reports on the therapeutic properties of medicinal plants combined with long-term experience in folk medicine has led to a growing interest in the use of natural products (Tlili and Sarikurkcu, 2020). With the emergence of antibiotic-resistant bacteria, the study of new bioactive compounds are even more crucial today. According to various sources, medicinal plants serve as the basis for 25–50 % of currently produced drugs used in healthcare (Mahmood *et al.*, 2019; Sinan *et al.*, 2020), and new bioactive compounds from known and exotic plants are being sought worldwide (Fettach *et al.*, 2019). Research in this field is expected to continue for new medicines derived from natural products (de la Luz Cádiz-Gurrea *et al.*, 2021).

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Curcumin is a food colouring and flavouring agent that has been shown to exert antioxidant effects both in vitro and in animal studies. Curcumin is known as the active component of turmeric and has shown inhibitory activity against microorganisms (Niamsa & Sittiwet, 2009; Dacrema et al., 2022). The comprehensive review of scholarly literature has indicated that Curcuma longa, a member of the Zingiberaceae family, is widely recognized as a versatile remedy in herbal medicine, exhibiting a broad range of pharmacological effects. The powder derived from current traditional medicine is purported to have therapeutic effects on gastrointestinal diseases, specifically targeting biliary and hepatic disorders, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza, and cough (Blumenthal et al., 2000). The pigment responsible for the coloration of turmeric is known as curcumin, which exhibits a yellow hue and serves as the fundamental constituent of this botanical species (Maruyama et al., 2006; Taha et al., 2021).

Previous research conducted by Sharif *et al.* (2017) examined the effects of turmeric extract on the growth of *Escherichia coli, Staphylococcus aureus, and Candida albicans.* The findings of this study indicated that the tested microorganisms experienced inhibited growth in the presence of turmeric extract. The research conducted by Moselly (2018) investigated the inhibitory effect of turmeric leaf extract on the growth of *Aspergillus flavus* and *Fusarium moniliforme*. The results of the study revealed that the turmeric leaf extract exhibited the ability to hinder the growth of these fungal species. Hence, the primary objective of this study was to examine the antibacterial efficacy of *Curcuma longa* extract against some enteric pathogens.

METHODOLOGY

Study Area

Bokkos Local Government Area LGA is one out of the 17th LGAs of Plateau state in Nigeria which is located between 90 180 0 N and 90 00 00 E of the Northern Tropical region of about latitude 150 – 300 North and South, East of the Equator, about 77 Kilometres South of Jos, the capital State. It is bounded to the North by Barkin Ladi, to South by Qua'an Pan, to the East by Mangu LGA and West by Sanga LGC of Kaduna State. With a population of 178,454 people according to NPC, 2016 and has a Landmass of about 3,053 square Kilometres. The major tribes make up the population of Bokkos are Ron, Kulere and Mushere who inhabit the thirteen districts of the local government. The geology of the Jos Plateau is part of the Precambrian to Mid Cambrian and Jurassic Northern Nigerian Crystalline shield Diary of Plateau State, 2002; (Schoneich and Mbonu, 1991). The elevation of the Jos Plateau is purported to have occurred about 570 million years ago with the latest occurring 500 thousand years ago due to tectonic upheaval which was followed by gradual denudation in which the whole of Jos plateau became eroded. The denudation is evidenced in the relief of the whole of Jos Plateau with Bokkos inclusive.

Test Organism

Stock cultures of S. typhi, *Shigella dysenteriae*, E. coli and K. pneumoniae were obtained from the National Veterinary Research Institute Vom, Plateau State, Nigeria on a tryptone soy agar (TSA) slant. Discrete colonies of the bacterium were isolated on Salmonella *Shigella dysenteriae* agar (SSA) for Salmonella, *Shigella dysenteriae* sp and MacConkey agar for E. Coli, K. pneumoniae incubated at 37 °C for 18 h, and then identified using biochemical tests (Cheesborough, 2010).

Collection of Plant Material

The rhizome of *Curcuma longa* (turmeric) was purchased from Maikatako market and authenticated at the Microbiology Laboratory of the National Veterinary Research Institute Vom, Plateau State.

Processing of Plant Material

The rhizomes were washed thoroughly in running tap water to clean off the adhering sand particles and then rinsed with distilled water. The scales on the rhizome were removed and the rhizomes were cut into small pieces, and dried under room temperature for 3 weeks and crushed using mortar and pestle. Exactly one hundred grams (100g) of the rhizome was weighed and dispensed into a sterilized beaker, consisting of 500 ml of distilled water, then soaked for 72 h, after which the solution was carefully filtered into sterilized conical flasks with a muslin cloth, and the filtrate was kept in a refrigerator at 4 °C until use (Olayemi & Opaleye, 2005).

Phytochemical Screening

The concentrated extracts were subjected to chemical tests for the detection of various phytochemical constituents which include Alkaloid, Saponin, Phlobatanin, Tannin, Steroid, Terpenoid, Flavonoid, Anthraquinone using the methods described in Sofowora (1993).

Test for Tannins

The method was carried out as describe by Sofowora (1993). 0.5ml of the extract was taken into a clean dry test tube and a few drops of ferric chloride reagent were added. The formation of blue-black precipitation indicates the presence of tannins.

Test for Alkaloids

The method described by Dey *et al.* (2020). 1ml of 1% hydrochloric HCI) was added to 3ml of each extract on a test tube. A brown precipitate indicated the presence of alkaloids.

Test for Cardiac Glycoside

Shake of 0.5ml or the extract on 2ml chloroform. Add few drops of concentrated H_2So_4 . A reddish-brown steroidal ring indicates the renascence of cardiac glycosides.

Test for Flavonoids

A little of magnesium powder and few drops of HCI were added to 2ml of extract. A red coloration indicates the presence of flavonoids.

Test for Saponin

Two (2) mL of distilled water was added to extracts suspended in ethanol and shaken vigorously. The formation of a copious foam layer indicated the presence of saponins (Rathore *et al.*, 2012).

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Determination of Antibacterial Activity

The antimicrobial activity of ethanolic and aqueous leaf extracts was evaluated using cup-plate agar diffusion assay as described in Enupe et al. (2024). Briefly, 100 µL of fresh Mueller-Hinton broth culture (approximately 106 CFU/mL) was spread uniformly on sterile Mueller-Hinton agar plates and allowed to air-dry. After this, 6 mm wells were made in the Mueller-Hinton agar (MHA) plates using sterilized cork borer, and the base was sealed with melted MHA. Exactly 100 µL of 100.0-25.0 mg/mL concentrations of the extract were prepared in 10% (w/v) dimethyl sulfoxide corresponding to 25.0 mg, 50.0 mg, 75.0 mg, and 100.0 mg of the extract, which was dispensed into the wells. The plates were allowed to stand for 1 h at 32 °C for pre-diffusion and placed in an incubator at 37 °C for 24 h. The diameter of zones of inhibition against the test bacteria was measured and recorded (CLSI, 2010). Ciprofloxacin at 10 mg was used as the positive control.

Determination of Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal Concentration (MBC)

The MICs and MBCs of crude and fraction Curcuma longa (Tumeric) were determined using macro-broth dilution method as follow; briefly, the 2 ml of MHB containing different concentrations (0.78-50.00 mg/ml) of crude aqueous extracts of Curcuma longa were prepared in Bijou bottles and 10 µl of the test strains (final concentration of 1×10^{5} CFU/ml) were added into each tube and incubated at 37°C for 24 h. The MICs were defined as the lowest concentration of the extract that restricted the visible growth of the test strains.

To determine MBCs, 100 µl from each tube that showed no visible growth were re-inoculated on MHA plates; then the plates were incubated at 37°C for 24 h. The MBCs were defined as the lowest extract concentration showing no bacterial growth. The ciprofloxacin and extract-free broth and agar were used as Controls.

Data Analysis

Data was analysed using R Console version 4.4.1. Data were presented in tables. Chi square test was used to determine the overall antibacterial activity of Curcuma longa aqueous leaf extract across the bacterial isolates and as well as in determination of MIC and MBC of Curcuma longa aqueous leaf extract against bacterial isolates. P value less than 0.05 were considered statistically significant.

RESULTS

Phytochemical Constituents of Aqueous Extracts of Curcuma longa

Table 1 presents the phytochemical constituents identified in the aqueous extract of Curcuma longa (turmeric). The analysis shows the presence of key bioactive compounds, including alkaloids, tannins, flavonoids, saponins, and cardiac glycosides.

Table 1: Phytochemical Constituents of Aqueous Extracts
of Curcuma longa

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Phytochemicals	Aqueous			
Alkaloid	+			
Tannins	+			
Flavonoids	+			
Saponin	+			
Cardiac glycosides	+			

Present (+); Absent (-)

Antibacterial Activity of Curcuma longa Aqueous Leaf **Extract Against Bacterial Isolates**

The antibacterial activity of Curcuma longa aqueous leaf extract against selected bacterial isolates is summarized in Table 2. Overall, Salmonella typhi showed the highest sensitivity to the extract, achieving inhibition zones ranging from 13 mm at 25 mg to 24 mm at 100 mg. Shigella dysenteriae and Klebsiella pneumoniae demonstrated moderate responses, with maximum inhibition zones of 19 mm and 18 mm, respectively, at the highest extract concentration. In contrast, Escherichia coli displayed the lowest susceptibility, exhibiting no inhibition at 25 mg but showing progressive increases up to a zone of 15 mm at 100 mg. For comparison, ciprofloxacin (10 mg), employed as a standard reference antibiotic, consistently produced the greatest antibacterial effect across all isolates, with inhibition zones ranging from 23 mm in Shigella dysenteriae to 30 mm in Salmonella typhi. Statistical analysis revealed no significant difference in the overall antibacterial activity of Curcuma *longa* aqueous leaf extract across the bacterial isolates ($\gamma^2 =$ 18.015, df = 12, P = 0.1152).

Detailed analysis of the dose-response trends for each isolate indicated that for Salmonella typhi, the number of inhibited colonies increased steadily with rising extract concentrations, although this pattern was not statistically significant (χ^2 = 8.4078, df = 4, P = 0.07773). Shigella dysenteriae demonstrated a significant dose-dependent response, with inhibited colonies increasing from 5 at 10 mg to 23 at 100 mg $(\chi^2 = 16.412, df = 4, P = 0.002514)$. *Escherichia coli* showed the most pronounced response to increasing extract concentrations among Gram-negative isolates, with colony inhibition rising sharply from none at the lowest concentration to 28 at 100 mg, yielding a highly significant result (χ^2 = 46.185, df = 4, P < 0.001). Klebsiella pneumoniae also displayed significant dose-dependent susceptibility, with colony inhibition counts increasing from 7 at 10 mg to 27 at $100 \text{ mg} (\chi^2 = 23.672, \text{ df} = 4, \text{ P} < 0.001).$

Table 2: Antibacterial Activity of Curcuma longa Aqueous Leaf Extract Against Bacterial Isolates								
Isolates	Cipro (10mg)	25mg	50mg	75mg	100mg	χ^2	df	P Value
Salmonella typhi	13	17	19	24	30	8.4078	4	0.07773
Shigella dysenteriae	5	8	13	19	23	16.412	4	0.002514
E. coli	0	3	8	15	28	46.185	4	< 0.001
Klebsiella pneumoniae	7	7	8	18	27	23.672	4	< 0.001

Table 2: Antibacterial Activity of Curcuma l	onga Aqueous Leaf Extract	Against Bacterial Isolates
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$\chi^2 = 18.015, df = 12, P = 0.1152$

Determination of MIC and MBC Of Curcuma longa **Aqueous Leaf Extract against Bacterial Isolates**

The results showed that Salmonella typhi exhibited the highest susceptibility, with an MIC of 0.16 mg/ml and an MBC of 0.63 mg/ml (Table 3). Shigella dysenteriae required a higher MIC (0.63 mg/ml) and the highest MBC (1.25 mg/ml) among all isolates. Escherichia coli had an MIC of 0.63 mg/ml and a notably lower MBC of 0.31 mg/ml. In comparison, Klebsiella pneumoniae had an MIC of 0.31 mg/ml and an MBC of 1.05 mg/ml. Therefore, there was no significant difference (χ^2 = 0.5915, df = 3, P = 0.8984) in the determination of MIC and MBC of Curcuma longa aqueous leaf extract against bacterial isolates.

Table 3: Determination of MIC and MBC Of Curcuma longa Aqueous Leaf Extract against Bacterial Isolates

ISOLATES	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	0.16	0.63
Shigella dysenteriae	0.63	1.25
E. coli	0.63	0.31
Klebsiella pneumoniae	0.31	1.05

 $\chi^2 = 0.5915$, df = 3, P = 0.8984

DISCUSSION

In the qualitative phytochemical analysis of aqueous extracts of Curcuma longa, alkaloids, tannins, flavonoids, saponins, and Cardiac glycosides were all present (Table 1). The antimicrobial activity of these compounds has been confirmed, and their presence in turmeric extracts may explain the activity documented against these test organisms. Tannin has been reported to interfere with the synthesis of microbial cell proteins and is essential for the treatment of tissues that are ulcerated or inflamed and for the treatment of intestinal disorders (Hussain et al., 2007). Alkaloid has also been reported to be a pain reliever and saponin has an inflammation control effect (Shukla et al., 2018). Flavonoid is also important against inflammation and microorganisms. This is in line with the work of Joe et al. (2009), who reported

that herbal medicinal plants possess inherent ingredients (secondary metabolites) that make them effective as treatments for various ailments. Therefore, it is suggested that the effectiveness of aqueous solvent in the extraction of high yield and reasonable quantity of bioactive constituents was due to the solvent polarity, solubility, and nature of the bioactive component in the plant material. Several authors have revealed that secondary metabolites are responsible for the biological activities of plant extracts (Abd-Alla & Haggag, 2013; Compean & Ynalvez, 2014; Igbokwe et al., 2006; Pagare et al., 2015). Therefore, the presence of these secondary metabolites in this study confirms the traditional use of Curcuma longa as medicine (Togola et al., 2008).

The findings of this study confirm that Curcuma longa aqueous leaf extract exhibits measurable antibacterial activity against selected enteric bacterial pathogens, with distinct variations in susceptibility among the isolates tested. Overall, the extract demonstrated a clear dose-dependent inhibitory effect, although its activity remained consistently lower than that of ciprofloxacin, which served as the standard reference antibiotic. This outcome is consistent with previous reports that highlight the broad-spectrum antimicrobial potential of turmeric, primarily attributed to curcumin and other phenolic compounds (Wada et al., 2021; Enupe et al., 2024).

Among the tested bacteria, Salmonella typhi showed the highest sensitivity to the aqueous leaf extract, with inhibition zones increasing progressively as the extract concentration rose. This heightened susceptibility may be due to Salmonella's relatively permeable cell wall structure and the absence of a protective outer membrane that characterizes some Gram-negative bacteria. Bioactive constituents in Curcuma longa can disrupt bacterial membrane integrity and inhibit virulence factors, which may account for this pronounced effect (Aldayel et al., 2023). Although the increase in inhibition zones did not achieve statistical significance, the trend aligns with Zamani et al. (2020), who observed variable but generally high susceptibility of Salmonella strains to plant-derived antimicrobials. The results support the potential application of turmeric extracts as a complementary intervention against salmonellosis, particularly in low-resource communities where access to potent antibiotics remains limited.

Shigella dysenteriae and Klebsiella pneumoniae demonstrated moderate susceptibility, with statistically significant improvements in inhibition at higher concentrations of the extract. The moderate response likely results from the robust outer membrane and intrinsic resistance mechanisms these pathogens possess, which can limit phytochemical penetration and action at lower doses. Nonetheless, the significant doseresponse trend observed suggests that increased extract concentrations can partially overcome these protective barriers. Similar moderate effects have been reported by Ode et al. (2023), who showed that Curcuma longa extracts can inhibit diverse Gram-negative bacteria but may require higher doses or combined formulations to achieve optimal efficacy. These finding highlights turmeric's potential role as a supportive antimicrobial agent, especially for infections where rising antibiotic resistance complicates treatment.

Escherichia coli was the least sensitive to the extract, with inhibition zones only appearing at the highest concentrations tested. This low susceptibility can be attributed to E. coli's well-documented defense strategies, including robust efflux pump systems, biofilm formation, and enzyme-mediated inactivation of antimicrobial agents (Teodoro et al., 2022). Despite these barriers, the extract still demonstrated a clear, significant dose-response trend, suggesting that higher or more bioavailable formulations could enhance its antibacterial activity. This aligns with Chen et al. (2024), who emphasized that curcumin disrupts E. coli membranes but often requires improved solubility or synergistic delivery systems to achieve bactericidal levels.

Across all isolates, ciprofloxacin produced markedly larger zones of inhibition than the Curcuma longa extract. This superior performance is expected due to ciprofloxacin's precise mode of action targeting bacterial DNA gyrase and its high bioavailability. The comparison reinforces the practical limitations of using crude plant extracts alone as primary antimicrobials but does not diminish their value as adjuncts in integrated treatment strategies. The lack of significant difference in the overall antibacterial activity of the Curcuma longa extract across isolates (P = 0.1152) suggests that while dose-dependent inhibition is evident, broad-spectrum effectiveness may be enhanced through optimized extraction techniques or combination with conventional antibiotics.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results obtained in this study demonstrate that Curcuma longa aqueous leaf extract possesses measurable antibacterial effects against selected enteric pathogens, though with varying levels of efficacy. This variation reflects the well-documented phenomenon that microorganisms differ in their intrinsic and acquired resistance to phytochemicals and synthetic agents alike, a trend consistently reported by You et al. (2005) and reaffirmed in more recent investigations such as Shatha et al. (2024) and Setiabudi et al. (2025). These studies highlight the role of bioactive constituents, primarily curcumin and essential oils, in determining the antimicrobial potency of turmeric extracts.

Among the tested isolates, Salmonella typhi demonstrated the highest susceptibility, with the lowest MIC (0.16 mg/ml) and

a comparatively low MBC (0.63 mg/ml). This pronounced sensitivity supports the findings of Biswas et al. (2020) and Hshieh et al. (2023), who observed that Salmonella species are particularly vulnerable to plant-derived phenolic compounds due to their cell wall composition and absence of robust outer membrane barriers typical of other Gramnegative bacteria. The result is also in line with Aderemi and Alabi (2023), who reported that turmeric extract significantly inhibited Salmonella enterica strains isolated from poultry, attributing the effect to curcumin's ability to disrupt membrane integrity and suppress efflux pump activity.

In contrast, Shigella dysenteriae exhibited the highest MIC (0.63 mg/ml) and MBC (1.25 mg/ml), indicating a lower susceptibility to the extract's bioactive components. This moderate resistance aligns with findings by Akshay et al. (2013), who showed that Shigella species often possess efflux pumps and an outer membrane structure that collectively limit the uptake of antimicrobial phytochemicals. Similarly, Matic et al. (2025) observed that Shigella flexneri required higher concentrations of turmeric extract for complete bactericidal action, supporting the inference that resistance traits in Shigella can reduce the efficacy of crude plant extracts.

Escherichia coli demonstrated a relatively high MIC (0.63 mg/ml) but an unexpectedly lower MBC (0.31 mg/ml). This observation suggests that while a higher dose is necessary to inhibit growth initially, the bactericidal threshold may be reached more efficiently once the extract penetrates the bacterial defenses. Chen et al. (2024) similarly reported that E. coli isolates exhibited delayed but significant bactericidal response to turmeric extracts due to curcumin's gradual disruption of the bacterial membrane and interference with biofilm formation. Teodoro et al. (2022) further corroborated that the variable response of E. coli to herbal antimicrobials often depends on strain-specific virulence factors and stress adaptation mechanisms.

Klebsiella pneumoniae showed intermediate susceptibility, with an MIC of 0.31 mg/ml and an MBC of 1.05 mg/ml. This pattern indicates that the extract effectively inhibits growth at lower concentrations but requires a higher concentration for bactericidal action. This finding is consistent with Silva et al. (2023), who noted that Klebsiella species, known for their robust capsular polysaccharides and biofilm-forming ability, often resist complete eradication by crude plant extracts unless higher doses or prolonged exposure are applied. Irawan et al. (2025) also reported similar MIC and MBC ranges for K. pneumoniae treated with turmeric ethanol extracts, underscoring the moderate but notable effect of turmeric against this opportunistic pathogen.

Overall, the MIC values in this study ranged from 0.16 to 0.63 mg/ml, while MBC values varied from 0.31 to 1.25 mg/ml, which aligns well with the range reported by Techasakul et al. (2023) for aqueous turmeric extracts against enteric and wound pathogens. Although the statistical analysis (P = 0.8984) indicated no significant difference in MIC and MBC among the isolates, the observed trends emphasize the nuanced interactions between phytochemicals and bacterial defense mechanisms. This supports the recommendation by Trigo-Gutierrez *et al.* (2021) and E-Dawy *et al.* (2025) that standardization and possible synergistic formulation with conventional antibiotics could enhance turmeric's clinical relevance as a complementary antimicrobial agent.

CONCLUSION

The study demonstrated that aqueous extracts of *Curcuma longa* (turmeric) possess significant antibacterial activity against various bacterial isolates, including *Salmonella typhi*, *Shigella dysenteriae*, *E. coli*, and *Klebsiella pneumoniae*. The presence of phytochemicals such as alkaloids, tannins, flavonoids, saponins, and cardiac glycosides correlates with the observed antimicrobial effects. The findings indicate that higher concentrations of the extract enhance antibacterial activity, confirming the traditional use of turmeric in treating infections.

RECOMMENDATIONS

- 1. Turmeric extracts (*Curcuma longa*) should be included in drugs preparation in combination with other plant extract, as they play a role in the growth inhibition of microorganism and they possess lesser side effect compare to synthetic drugs
- 2. Further vivo studies to evaluate the efficacy and safety of turmeric extracts in clinical settings is recommended
- 3. Evaluation of the synergistic effects of this plant extract with conventional antibiotics is suggested for further studies

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