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Evaluation of phytochemical constituents and antibacterial properties of crude ethanolic extract from turmeric (*Curcuma longa* L.) rhizomes against *Escherichia coli*

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Abstract

Antimicrobial resistance (AMR) is a critical global health concern that necessitates the discovery of novel therapeutic agents, including plant-derived compounds. Turmeric (*Curcuma longa* L.), widely recognized in traditional medicine, contains bioactive constituents with reported antimicrobial potential. This study evaluated the antibacterial activity of crude ethanolic extract from *C. longa* rhizomes against *Escherichia coli*, a Gram-negative pathogen prioritized by the World Health Organization due to its rising multidrug resistance. Fresh turmeric rhizomes were collected, shade-dried, pulverized, and subjected to Soxhlet extraction using 80% ethanol. The crude extract yield was 8.78%. Phytochemical screening revealed the presence of flavonoids, saponins, and tannins, compounds known for their diverse pharmacological activities. Antibacterial activity was assessed using the disk diffusion method at concentrations of 25, 50, and 100 mg/mL. Ciprofloxacin (5 mcg/disc) served as the positive control, while 1% dimethyl sulfoxide (DMSO) acted as the negative control. Results indicated that the crude ethanolic extract did not produce measurable zones of inhibition against *E. coli* at any tested concentration, while ciprofloxacin exhibited significant inhibition (25.67 ± 12.70 mm). Statistical analysis (ANOVA, $p = 0.073$) confirmed the absence of significant antibacterial activity. The findings suggest that crude ethanolic extracts of turmeric may have limited efficacy against Gram-negative bacteria under standard laboratory conditions. Nonetheless, the presence of bioactive phytochemicals highlights turmeric's potential as a natural antimicrobial source. Further research employing optimized extraction methods, advanced formulations, or synergistic approaches is recommended to unlock its therapeutic potential in addressing AMR

Keywords: Antimicrobial Resistance; Natural Products; Phytochemical; Turmeric; Ethanolic

1. Introduction

Antimicrobial resistance (AMR) is a mounting global health crisis that threatens the effectiveness of antibiotics and complicates infection management [1]. *Escherichia coli*, a leading cause of urinary tract infections, sepsis, and bloodstream infections, has been classified by the World Health Organization (WHO) as a high-priority pathogen due to its rising multidrug resistance [2]. Resistant *E. coli* strains are associated with higher mortality, prolonged illness, and increased healthcare costs [3]. In the Philippines, Corpuz [4] reported alarming resistance rates in both community and hospital isolates of *E. coli*, underscoring the urgent need for alternative therapeutic strategies. Plant-derived compounds offer a promising avenue for antimicrobial discovery. Turmeric (*Curcuma longa*), widely used in traditional medicine, contains curcuminoids and other phytochemicals with documented antibacterial, antioxidant, and anti-inflammatory activities [5]. Curcumin, its principal bioactive compound, exhibits antibacterial activity against both gram-positive and gram-negative bacteria, including *E. coli*, through mechanisms such as membrane disruption, biofilm inhibition, and interference with quorum sensing [6, 7]. These properties highlight turmeric's potential as a sustainable and locally available adjunct to conventional antibiotics, particularly in low-resource settings. Nevertheless, findings on turmeric's

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efficacy remain inconsistent, influenced by extraction methods, solvent type, and bacterial strain. Moreover, limitations such as poor solubility and low bioavailability continue to hinder clinical translation [8, 9]. Despite these challenges, the WHO and the United Nations (UN) Sustainable Development Goals emphasize the importance of investigating natural antimicrobials to address AMR in a sustainable manner [10]. This study evaluates the antibacterial activity of crude ethanolic extract from turmeric rhizomes against *E. coli*, focusing on phytochemical composition and concentration-dependent effects to inform its potential role in AMR mitigation.

2. Materials and Methods

2.1. Ethical Considerations

The present study did not involve human participants or animal subjects, as all experiments were conducted *in vitro* using bacterial strains. All experimental procedures adhered strictly to established laboratory protocols and recognized guidelines for in vitro research.

2.2. Reagents

Ethanol (MW: 46.07 g/mol; GPR grade) was purchased from Belman Laboratories (Quezon City, National Capital Region, Philippines). Dimethyl sulfoxide (MW: 78.13 g/mol; ≥99.5% purity) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Reagents used for phytochemical testing were supplied by the laboratory of Lourdes College, Inc. (Cagayan de Oro City, Misamis Oriental, Philippines). Unless otherwise stated, all materials and reagents were of analytical grade.

2.3. Instruments

The dried rhizomes were pulverized using a cutting mill (Wiley™ Model 4, Thomas Scientific, New Jersey, USA) and extracted with a Soxhlet apparatus (LSOA-A11, Labtron Equipment Ltd., Surrey, United Kingdom). The crude extract and subsequent fractions were concentrated using a rotary evaporator (RV 10™, IKA®, Selangor, Malaysia). Test organisms for the antimicrobial assay were cultured in an electro-thermostatic incubator (DRP-9082, Senxin®, Shanghai, China).

2.4. Preparation of Plant Material

Fresh rhizomes of *Curcuma longa* L. were collected in February 2025 from a designated site in Esperanza, Sultan Kudarat. A voucher specimen was submitted to the herbarium of Far Eastern University for taxonomic verification. The rhizomes were washed with distilled water, sliced into thin sections, shade-dried for five days, and subsequently ground using a cutting mill.

2.5. Preparation of Crude Ethanolic Extract

Approximately 83 g of pulverized rhizomes were subjected to Soxhlet extraction for 8 hours using 200 mL of 80% ethanol. The combined extracts were concentrated at 60 °C for 5 hours using a rotary evaporator, yielding a dark yellow, viscous residue. The crude ethanolic extract was stored in an amber, airtight container under refrigeration until further use. The extraction yield was calculated using the following formula, where CEE represents the weight of the crude ethanolic extract and DR denotes the weight of the dried rhizome.

$$percentage\ (\%)\ yield = \left(\frac{CEE}{DR}\right) x\ 100$$

2.6. Qualitative Phytochemical Analysis

To determine the presence of various phytochemicals, 1 mL of the crude ethanolic extract was placed in labeled test tubes and subjected to a series of standard qualitative tests, as outlined below:

2.6.1. Flavonoids

A few drops of 10% sodium hydroxide solution were added to the extract, producing an intense yellow coloration. The addition of dilute hydrochloric acid caused the color to fade, confirming the presence of flavonoids.

2.6.2. Saponins

The extract was vigorously shaken with 5 mL of distilled water. The formation of a stable and persistent froth indicated the presence of saponins.

2.6.3. Tannins

Five drops of a 10% ferric chloride solution were added to the extract. The development of a blue-black or greenish-black coloration was taken as evidence of tannins.

2.7. Evaluation of Antibacterial Activity

The crude ethanolic extract was evaluated for antibacterial activity against *Escherichia coli* (BIOTECH 1634) using the disk diffusion method.

2.7.1. Preparation of Inoculum

The test strain, initially maintained in sterile sodium chloride–peptone buffer, was subcultured on freshly prepared Mueller-Hinton agar (MHA) plates and incubated at 37 °C for 24 hours. Well-isolated colonies were then suspended in sterile saline, and the turbidity was adjusted to match 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using a spectrophotometer or visual comparison. The suspension was mixed thoroughly with a vortex mixer and used within 15 minutes of preparation.

2.7.2. Disk Diffusion Assay

MHA (20 mL per plate) was poured into sterile Petri dishes and allowed to solidify. A standardized inoculum of the test bacterium was uniformly spread over the agar surface using a sterile cotton swab. Sterile paper discs (6 mm diameter) were placed aseptically on the inoculated agar, and each disc was loaded with 20 mcL of the crude ethanolic extract at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL. The plates were incubated at 37 °C for 18–24 hours. Zones of inhibition (ZOI) were measured in millimeters using a digital Vernier caliper, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Ciprofloxacin (5 mcg/disc) was used as the positive control, while 1% dimethyl sulfoxide (DMSO) served as the negative control.

2.8. Statistical Analysis

The assay was conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (version 20; IBM Corp., Armonk, NY, USA). A one-way analysis of variance (ANOVA) was applied to determine significant differences in antibacterial activity among treatments, with a significance threshold set at $p < 0.05$.

3. Results and Discussion

3.1. Percentage Yield

Approximately 7.29 g of crude ethanolic extract were recovered from 83.00 g of dried, ground rhizomes, yielding 8.78%. This yield aligns with typical ranges reported for ethanol-based extractions; for instance, an accelerated solvent extraction study found an ~8.4% physical extract yield using ethanol [11]. Similarly, temperature- and time-optimized ethanol extraction protocols have produced curcumin yields of approximately 9.15% [12]. In contrast, advanced techniques such as microwave-assisted extraction (MAE) have achieved significantly higher yields—with one study reporting an extraction yield of 17.89% using 95% ethanol [13]. These discrepancies can be attributed to differences in extraction methodologies, solvent concentration, temperature control, and equipment efficiency [14]. The moderate yield observed in the present study likely reflects standard laboratory extraction parameters, with room for optimization—such as exploring MAE or refining solvent conditions—to enhance recovery of bioactive components.

3.2. Qualitative Phytochemical Analysis

Table 1 presents the results of phytochemical tests conducted to identify the presence of compounds such as flavonoids, saponins, and tannins. The phytochemical screening of the crude ethanolic extract from turmeric rhizomes revealed the presence of flavonoids, saponins, and tannins. The detection of flavonoids is notable, as these secondary metabolites are widely recognized for their antioxidant, anti-inflammatory, and chemopreventive properties, which may contribute to the therapeutic potential of turmeric [15]. Similarly, the presence of saponins suggests possible biological activities such as antimicrobial, hypocholesterolemic, and immunomodulatory effects, which could enhance the medicinal relevance

of the extract [16]. The identification of tannins further strengthens the pharmacological profile of the extract, as tannins are associated with astringent, antimicrobial, and free radical scavenging activities [17]. Recent investigations on turmeric extracts confirm these findings, reporting the presence of flavonoids, saponins, and tannins, along with significant antimicrobial and antioxidant activities [18]. Collectively, the presence of these bioactive constituents underscores the medicinal value of turmeric and supports its traditional use in ethnomedicine. Moreover, the findings provide a foundation for further investigations on the quantitative determination of these metabolites and their specific contributions to the biological activities of turmeric extracts.

Table 1 Phytochemical tests to detect the presence of flavonoids, saponins, and tannins in the crude ethanolic extract from turmeric rhizomes.

Phytochemical	Result
Flavonoids	Present
Saponins	Present
Tannins	Present

3.3. Results of Antibacterial Test

The results of the antibacterial assay **(Table 2)** revealed that the crude ethanolic extract of *Curcuma longa* (turmeric) did not produce any measurable zone of inhibition against *Escherichia coli* at concentrations of 25, 50, and 100 mg/mL. In contrast, the positive control, ciprofloxacin, exhibited substantial inhibition across all trials, thereby confirming both the susceptibility of *E. coli* and the validity of the assay protocol. Statistical analysis supported these findings, indicating no significant antibacterial effect ($p = 0.073$). This lack of activity may be attributed to several factors: the concentration of active compounds in the crude extract may have been insufficient; the phytochemicals may have exhibited limited diffusion within the agar medium; and *E. coli*, being a Gram-negative bacterium with a robust outer membrane, may have resisted penetration by plant-derived agents—a well-documented challenge in antimicrobial research [19]. Broader antimicrobial studies indicate that certain turmeric extracts can inhibit *E. coli*, but typically under different conditions, such as higher concentrations, alternative extraction solvents, or advanced formulations. For example, ethanolic extracts of *C. longa* demonstrated inhibitory zones against *E. coli* at 150 mg/mL, although the effect was more pronounced with petroleum ether extracts [20]. In another widely referenced study using turmeric extract equivalents (up to 10 mg/mL), complete inhibition of *E. coli* growth was observed only at the highest concentration tested (10 mg TE/mL) [21]. Moreover, Al-Najjar et al. [22] reported that ethanolic extracts exhibited no inhibitory activity against *E. coli* or other tested microbes, reaffirming the limited efficacy observed under certain extraction conditions. In contrast, Hashim and Bakar [23] documented effective antibacterial activity against *E. coli*, although only at concentrations ranging from 100 to 500 mg/mL—further suggesting that higher doses or specific extraction protocols may significantly influence efficacy. These conflicting findings highlight the complexity of antibacterial efficacy derived from turmeric and suggest that both the extraction method and solvent selection play critical roles in determining outcomes. Importantly, the present study is consistent with reports indicating that crude ethanolic extracts may lack sufficient antibacterial potency against Gram-negative organisms such as *E. coli*, unless higher concentrations or alternative solvents are employed.

Table 2 Results of the antibacterial assay between the crude ethanolic extract from turmeric rhizomes and the positive control against *E. coli*.

Sample	Concentration	ZOI (mm)
CEE	100 mg/mL	-
	50 mg/mL	-
	125 mg/mL	-
Ciprofloxacin	5 mcg	25.67 ± 12.70
DMSO	1%	-

4. Conclusions

Antimicrobial resistance continues to pose one of the most pressing threats to global health, challenging the efficacy of existing antibiotics and disproportionately affecting low- and middle-income countries where access to advanced therapeutics is limited. Within this context, turmeric (*Curcuma longa* L.) represents an important natural resource with long-standing ethnomedicinal relevance and a reservoir of bioactive compounds that may be harnessed to combat resistant pathogens. While current evidence, including the present investigation, indicates that crude ethanolic extracts alone may offer limited antibacterial activity against *E. coli*, this should not diminish the value of turmeric as a candidate for further exploration. Rather, it underscores the critical need for more rigorous and innovative approaches in natural product research. Future studies should prioritize optimizing extraction methods, testing a wider range of solvents, and employing advanced techniques such as nanoformulation, encapsulation, or synergistic combinations with standard antibiotics to enhance bioavailability and potency. Furthermore, integrating phytochemical quantification with mechanistic studies can help establish a clearer link between specific compounds and observed antimicrobial effects. Such approaches may bridge the gap between traditional knowledge and clinical application, ultimately contributing to the development of sustainable, cost-effective, and culturally appropriate antimicrobial therapies. By investing in the systematic investigation of turmeric and other medicinal plants, researchers can not only expand the arsenal of tools available to address antimicrobial resistance but also promote the utilization of indigenous resources that align with global health priorities and the United Nations Sustainable Development Goals. This direction of inquiry holds significant promise for both the scientific community and public health, offering pathways toward innovative and accessible solutions in the fight against drug-resistant infections.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper's content.

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