

Comparative Antibacterial Efficacy of Aqueous and Ethanolic Turmeric Extracts versus Pure Curcumin against Uropathogenic *Escherichia Coli*.

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المقارنة بين الفعالية المضادة للبكتيريا لمستخلصات الكركم المائية والإيثانولية مقارنةً بالكرمين النقي ضد بكتيريا الاشريكية القولونية الممرضة للمسالك البولية

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Abstract

Urinary tract infections are among the most common bacterial infections, and *Escherichia coli* is a leading causative pathogen. Increasing antimicrobial resistance among uropathogenic *E. coli* has encouraged investigation of plant-derived compounds with potential antibacterial activity. Turmeric (*Curcuma longa*) and its principal bioactive constituent, curcumin, have been reported to exhibit antimicrobial effects in vitro. this study aimed to evaluate the in-vitro antibacterial activity of aqueous turmeric extract, ethanolic turmeric extract, and curcumin against *E. coli*



isolates recovered from urine samples of patients with urinary tract infections. Methods This in-vitro laboratory study evaluated *E. coli* isolates obtained from urine samples of patients with urinary tract infections. Three test preparations were assessed: aqueous turmeric extract, turmeric extract prepared by maceration with 75% ethanol, and curcumin dissolved in dimethyl sulfoxide. Antibacterial activity was evaluated using the disk-diffusion method at serial concentrations of 25%, 50%, 75%, and 100%. the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of curcumin were determined using the standard broth tube dilution assay. Inhibition-zone diameters, MIC, and MBC values were recorded and compared. The Results showed that ethanolic turmeric extract demonstrated a concentration-dependent inhibitory effect against *E. coli*. In contrast, the aqueous turmeric extract showed minimal antibacterial activity. Curcumin demonstrated the greatest in-vitro antibacterial activity among the tested preparations exhibiting an MIC of 128 µg/mL and an MBC of 256 µg/mL. The differences in antibacterial activity among the tested preparations were statistically significant ($P < 0.001$).

Conclusion, Ethanolic turmeric extract and curcumin demonstrated in-vitro antibacterial activity against urinary *E. coli* isolates, whereas the aqueous turmeric extract showed minimal activity. The limited activity of the aqueous extract may be related to the poor aqueous solubility and limited diffusion of curcuminoids. Further studies using standardized extract concentrations, appropriate solvent and antibiotic controls, and broth tube dilution assay methods to determine minimum inhibitory and minimum bactericidal concentrations are required before any clinical application can be considered.

Key words: UTIs, *E. coli*, ethanolic extract, ethanolic turmeric extract

المستخلص

تُعد التهابات المسالك البولية من أكثر أنواع العدوى البكتيرية شيوعاً، وتُعد بكتيريا الاشريكية القولونية (*Escherichia coli*) من أبرز مسبباتها. وقد أدى تزايد مقاومة الاشريكية القولونية المسببة لالتهابات المسالك البولية للمضادات الميكروبية إلى زيادة الاهتمام بدراسة المركبات المشتقة من النباتات ذات النشاط المحتمل المضاد للبكتيريا. وقد أظهرت الكركم (*Curcuma longa*) ومكونه الحيوي الرئيسي، الكركمين (Curcumin)، تأثيرات مضادة للميكروبات في الدراسات المخبرية هدفت هذه الدراسة إلى تقييم النشاط المضاد للبكتيريا في المختبر (in vitro) لكل من المستخلص المائي للكركم، والمستخلص الكحولي للكركم، والكركمين، ضد عزلات *E. coli* المعزولة من عينات بول المرضى المصابين بالتهابات المسالك البولية. أجريت هذه الدراسة المخبرية in vitro باستخدام عزلات *E. coli* تم الحصول عليها من عينات بول مرضى مصابين بالتهابات المسالك البولية. أجريت هذه الدراسة وتم تقييم ثلاثة مستحضرات اختبارية: المستخلص المائي للكركم، ومستخلص الكركم المحضر بالنقع باستخدام إيثانول بتركيز 75%، والكركمين المذاب في ثنائي ميثيل سلفوكسيد (DMSO). وتم تقييم النشاط المضاد للبكتيريا باستخدام طريقة الانتشار بالأقراص (Disk-diffusion method) عند تراكيز متسلسلة بلغت 25%، 50%، 75%، و100%. بالإضافة إلى ذلك تم تحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل (MBC) للكركمين باستخدام اختبار تخفيف المرق في الانابيب Broth tube dilution assay) وتم قياس أقطار مناطق تثبيط النمو ومقارنتها بين المستحضرات المختبرة، وظهرت النتائج أن مستخلص الكركم الكحولي تأثيراً مثبطاً لنمو *E. coli* يعتمد على التركيز. في المقابل، أظهر المستخلص المائي للكركم نشاطاً مضاداً للبكتيريا محدوداً. وأظهر الكركمين أعلى نشاط مضاد للبكتيريا في المختبر مقارنةً بالمستحضرات المختبرة الأخرى. حيث بلغ التركيز المثبط الأدنى (MIC) للكركمين 128 ميكروغرام/مل، والتركيز القاتل الأدنى (MBC) 256 ميكروغرام/مل. وكانت الفروق في النشاط المضاد للبكتيريا بين المستحضرات المختبرة ذات دلالة إحصائية ($P < 0.001$) وفي الخلاصة أظهر كل من مستخلص الكركم الكحولي والكركمين نشاطاً مضاداً للبكتيريا في المختبر ضد عزلات *E. coli* البولية، بينما أظهر المستخلص المائي للكركم نشاطاً محدوداً. وقد يرتبط ضعف نشاط المستخلص المائي بانخفاض ذوبانية الكركمين في الماء ومحدودية انتشارها في الوسط. وهناك حاجة إلى دراسات مستقبلية تستخدم تراكيز موحدة للمستخلصات وضوابط مناسبة للمذيبات والمضادات الحيوية، بالإضافة إلى طريقة التخفيف المرق Broth tube dilution assay) لتحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى للبكتيريا (MBC)، وذلك قبل إمكانية النظر في أي تطبيق سريري.

الكلمات المفتاحية: *E. coli*، التهابات المسالك البولية، المستخلص الكحولي للكركم، والكركمين، طريقة القرص المنتشر.

1. Introduction

1.1 *Escherichia coli* and Urinary Tract Infections

Urinary tract infections are among the most common bacterial infections of humans and constitute an important cause of outpatient visits, antimicrobial prescribing, and healthcare-associated morbidity. Uropathogenic *Escherichia coli* (UPEC) is the predominant etiological agent of urinary tract infections and accounts for approximately 80–90% of community-acquired urinary tract infections. Other microorganisms, including *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus species*, and *Staphylococcus saprophyticus*, may also be responsible for urinary tract infections, especially in complicated or healthcare-associated cases (Klein & Hultgren, 2020; Pal & Mahawal, 2021).

Escherichia coli is a Gram-negative, non-spore-forming bacillus that belongs to the family *Enterobacteriaceae*. Many strains are motile due to the presence of peritrichous flagella. It is a common commensal organism of the intestinal microbiota of humans and animals; however, certain strains can act as opportunistic pathogens when they colonize sites outside the gastrointestinal tract. UPEC strains can ascend from the intestinal and perineal reservoir to the urinary tract, where they adhere to urothelial cells, evade host defenses. Cause cystitis, pyelonephritis, and, in severe cases, bloodstream infection (Wagenlehner et al., 2020).

1.2 Antibiotic Resistance and the Need for New Antimicrobial Agents

The appearance of antimicrobial resistance among uropathogenic bacteria is one of the most concerning issues for public health and human health as this problem leads to the decreased efficacy of commonly prescribed drugs in the treatment of UTIs (Medina & Castillo-Pino, 2019). Moreover, the emergence and persistence of resistance are determined by antibiotic misuse and underuse, insufficient antimicrobial stewardship, and the transmission of resistant strains and resistance mechanisms, which significantly complicates the management of UTIs (The global burden of antimicrobial resistance – urinary tract infections, 2024). Thus, the rising prevalence of strains with acquired resistance to multiple antibiotics, especially uropathogenic *Escherichia coli* has prompted the scientific community to explore other sources of antimicrobial agents (Gajdács & Urbán, 2019).

Medicinal plants and their extracts have been considered as an alternative source of antimicrobial agents. However, the empirical evidence on the effectiveness of treatment is insufficient, and antibacterial efficiency should not be taken for granted when extrapolating these results to patients with UTIs. Thus, such plant preparations and extracts should be regarded as a possible subject of further research and not as an alternative to standard antibiotic therapy.

1.3 Turmeric and Curcumin

Turmeric, scientifically known as *Curcuma longa* L., is a medicinal plant widely used as a dietary spice and in traditional medicine. It contains several bioactive compounds, particularly curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the principal polyphenolic compound in turmeric and has been reported to possess antioxidant, anti-inflammatory, and antimicrobial properties in experimental studies (Hewling & Kalman, 2019).

Turmeric also contains volatile oils, including turmerones and zingiberene, which may contribute to its biological activity. The reported antibacterial activity of turmeric and curcumin varies according to the extraction solvent, concentration, formulation, bacterial species, and laboratory method used. In particular, curcumin has limited solubility in water, which may influence its

extraction efficiency and apparent antibacterial activity in diffusion-based assays (Odogbu Odo et al., 2023); (Górnicka et al., 2023).

Therefore, this study aimed to compare the in-vitro antibacterial activity of aqueous and ethanolic turmeric extracts and pure curcumin against *Escherichia coli* isolated from urine samples of patients with urinary tract infections.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Dried turmeric rhizomes of *Curcuma longa* L. were obtained from a local market in Misurata, Libya. The rhizomes were inspected to remove visible impurities and were further dried before processing. The dried material was ground using an electric grinder to obtain a fine, homogeneous powder. The turmeric powder was stored in a clean, dry, light-protected container until extraction.

2.2 Preparation of Turmeric Extracts

2.2.1 Aqueous Extract

A cold aqueous extract was prepared by adding 20 g of turmeric powder to 100 mL of sterile distilled water. The mixture was left to macerate for 24 hours at room temperature and was mixed intermittently. The mixture was then filtered through filter paper to remove plant debris. The filtrate was dried in an oven at 40°C for 12–16 hours until the solvent had evaporated and a dry concentrated extract was obtained. The dried aqueous extract was stored in a sterile glass container protected from light with aluminum foil and maintained at 4°C until use.

2.2.2 Ethanolic Extract

An ethanolic extract was prepared by adding 20 g of turmeric powder to 100 mL of 75% ethanol (v/v). The mixture was macerated for 24 hours at room temperature with intermittent mixing and was subsequently filtered through filter paper. The filtrate was dried at 40°C until complete evaporation of the solvent and formation of a concentrated dry extract. The dried ethanolic extract was stored in a sterile, light-protected container at 4°C until use.

2.3 Preparation of Test Concentrations

Aqueous and ethanolic turmeric extracts were prepared for antibacterial testing at four concentrations: 25%, 50%, 75%, and 100%. The 100% concentration represented the undiluted stock preparation, whereas the remaining concentrations were prepared by serial dilution of the stock solution using the appropriate sterile solvent.

Pure curcumin was used as a reference compound and was dissolved in dimethyl sulfoxide to prepare the stock solution. Curcumin was tested at four concentrations corresponding to 25%, 50%, 75%, and 100% of the prepared stock solution. All curcumin preparations were protected from light during preparation and storage because curcumin is sensitive to light exposure.

2.4 Bacterial Isolates and Identification

Pure clinical isolates of *Escherichia coli* were obtained from the microbiology laboratory. The isolates had been recovered from urine samples of patients with urinary tract infections. Presumptive identification of *E. coli* was based on colony morphology and culture characteristics. The isolates produced pink colonies on MacConkey agar, indicating lactose fermentation, and showed a characteristic green metallic sheen on eosin methylene blue agar.

2.5 Preparation of Bacterial Inoculum

A bacterial suspension was prepared from fresh *E. coli* colonies in sterile normal saline. The turbidity of the suspension was adjusted to the 0.5 McFarland standard, corresponding approximately to 1.5×10^8 colony-forming units per milliliter. The standardized bacterial suspension was used immediately for antimicrobial testing.

2.6 Antibacterial Activity Testing

The antibacterial activity of the aqueous turmeric extract, ethanolic turmeric extract, and curcumin was evaluated using the disk-diffusion method. Mueller–Hinton agar plates were inoculated uniformly with the standardized *E. coli* suspension. Sterile filter-paper disks were impregnated with the four concentrations of each test preparation and placed on the surface of the inoculated Mueller–Hinton agar plates.

The plates were incubated for 24 hours at 37°C. After incubation, the diameter of the growth-inhibition zone surrounding each disk was measured in millimeters. Each test condition was performed in triplicate, and the mean inhibition-zone diameter was calculated.

Appropriate controls were included in each experiment. A standard antibiotic disk was used as a positive control, whereas disks containing the respective solvent without turmeric extract or curcumin were used as negative controls. A dimethyl sulfoxide control disk was included for the curcumin preparation to confirm that any observed inhibition was attributable to curcumin rather than the solvent. Positive antibiotic controls and solvent controls are essential for interpreting antibacterial activity in plant-extract assays.

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of curcumin were evaluated using the standard broth tube dilution assay. To prepare the test concentrations, two-fold serial dilutions of curcumin were performed in sterile test tubes containing nutrient broth, yielding a concentration range from 1024 down to 32 µg/mL (1024, 512, 256, 128, 64, and 32 µg/mL).

Each tube was subsequently inoculated with a standardized suspension of the test bacteria. For quality control, a growth control tube containing only nutrient broth and the bacterial inoculum (without curcumin) was maintained throughout the test. All tubes were then incubated at 37 °C for 24 hours.

Following incubation, the tubes were inspected visually for turbidity as an indicator of bacterial growth. The MIC was established as the lowest concentration of curcumin that completely inhibited visible bacterial growth relative to the growth control.

To determine the MBC, a small aliquot was taken from each test tube that showed no visible turbidity and spread onto sterile nutrient agar plates. These plates were incubated at 37 °C for 24 hours. The MBC was recorded as the lowest concentration of curcumin that yielded no visible bacterial colonies on the agar plates.

3. Results

3.1 Effect of Aqueous Turmeric Extract on the Growth of *Escherichia coli*

The aqueous turmeric extract showed limited antibacterial activity against *Escherichia coli* at the tested concentrations. No inhibitory effect was observed at concentrations of 25% and 50%. At concentrations of 75% and 100%, low levels of growth inhibition were observed as presented in Table 1, with mean values of 1.67 ± 0.29 at both concentrations. No variability was observed at concentrations of 25% and 50%, whereas the standard deviation was 0.29 at concentrations of 75% and 100% (Table 1).

Table (1): Mean inhibition-zone diameter of aqueous turmeric extract on *Escherichia coli* (mm).

Extract concentration (%)	Mean value	Standard deviation
25	0.00	0.00
50	0.00	0.00

75	1.67	0.29
100	1.67	0.29

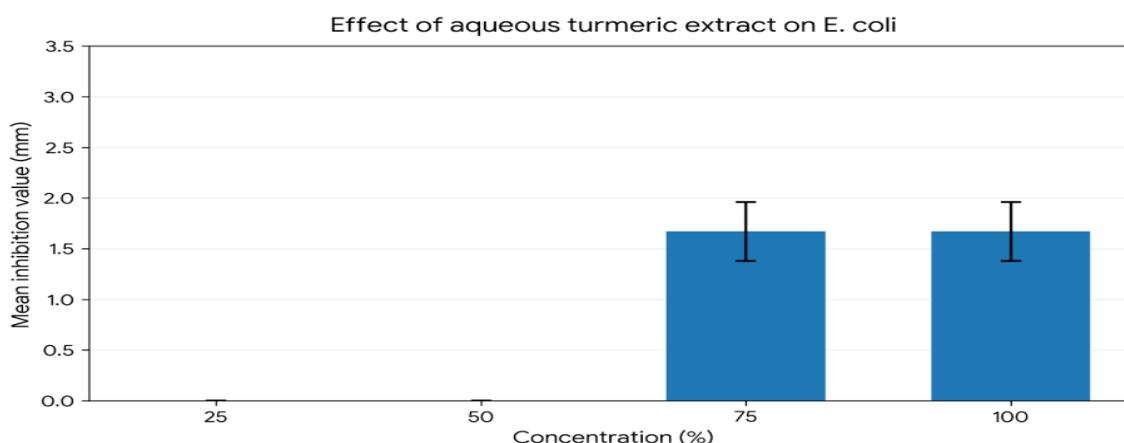


Figure (1): Mean inhibition-zone diameter of aqueous turmeric extract on *Escherichia coli* (mm).

3.2 Effect of Ethanolic Turmeric Extract on the Growth of *Escherichia coli*

The ethanolic turmeric extract demonstrated greater antibacterial activity against *Escherichia coli* than the aqueous extract. No inhibitory effect was observed at a concentration of 25%. At concentrations of 50%, 75%, and 100%, the mean values increased from 1.67 ± 0.29 to 3.33 ± 0.29 and 5.33 ± 0.06 , respectively. The highest observed value was recorded at the 100% concentration (Table 2).

Table (2): Mean inhibition-zone diameter of ethanolic turmeric extract against *Escherichia coli* (mm).

Extract concentration (%)	Mean value	Standard deviation
25	0.00	0.00
50	1.67	0.29
75	3.33	0.29
100	5.33	0.06

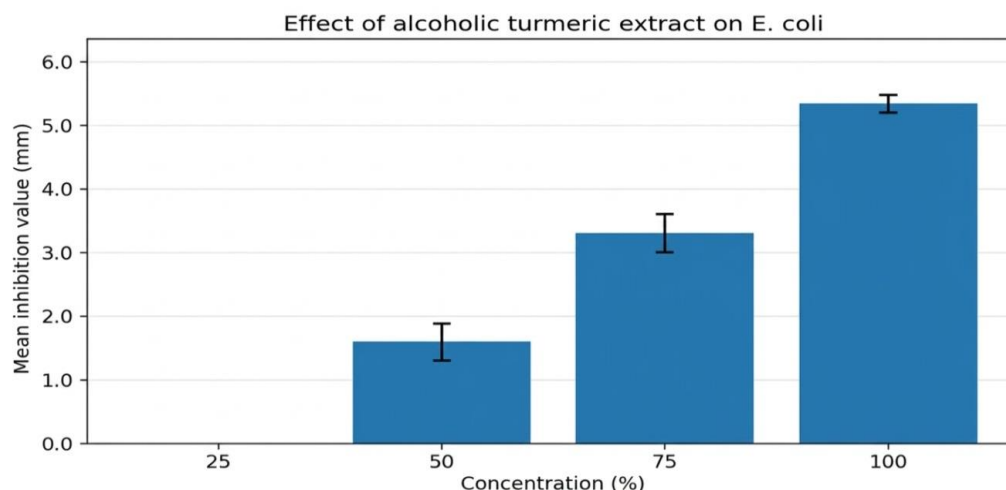


Figure (2): Mean inhibition-zone diameter of ethanolic turmeric extract against *Escherichia coli* (mm).

3.3 Statistical Analysis of the Effect of Curcumin Concentrations Against *Escherichia coli*

One-way analysis of variance demonstrated a statistically significant difference in mean inhibition-zone diameter among the tested curcumin concentrations against *Escherichia coli* ($F=11.29$, $P=0.0093P$). No measurable inhibition zone was observed at the 25% curcumin concentration. The mean inhibition-zone diameter presented in Table3 was 16.67 ± 0.58 mm at 50%, 19.00 ± 1.00 mm at 75%, and 20.00 ± 1.00 mm at 100%. The largest mean inhibition zone was observed at the 100% curcumin concentration.

Table (3): Mean inhibition-zone diameters produced by curcumin against *Escherichia coli*(mm).

Curcumin concentration (%)	Mean inhibition-zone diameter	Standard deviation
25	0.00	0.00
50	16.67	0.58
75	19.00	1.00
100	20.00	1.00

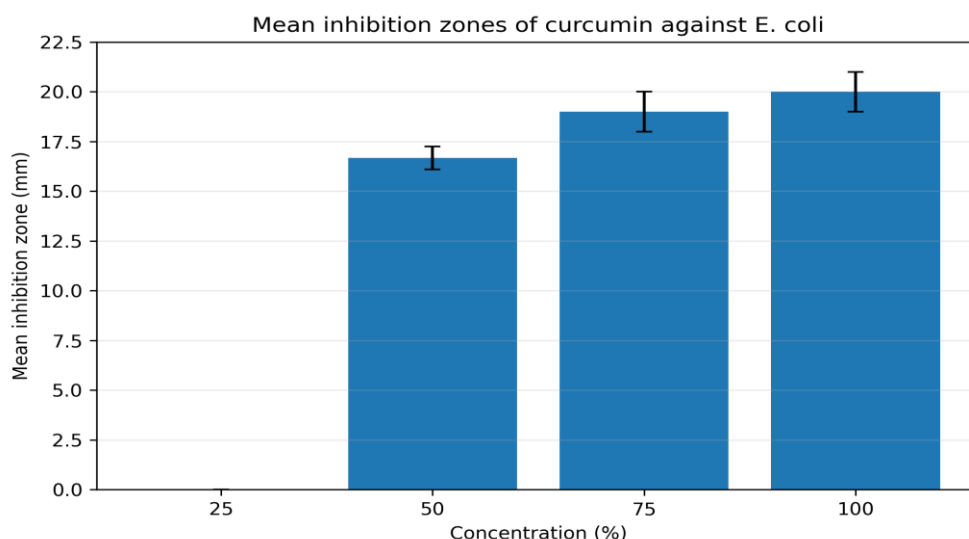


Figure (3): Mean inhibition-zone diameters produced by curcumin at different concentrations against *Escherichia coli*.

3.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Curcumin

The minimum inhibitory concentration of curcumin against *Escherichia coli* was 128 $\mu\text{g/mL}$, whereas the minimum bactericidal concentration was 256 $\mu\text{g/mL}$ (Table 4). The MBC/MIC ratio was 2.0, indicating bactericidal activity of curcumin against the tested *E. coli* isolate under the in-vitro conditions of this study.

Table (4): Minimum inhibitory concentration and minimum bactericidal concentration of curcumin against *Escherichia coli*.

Organism	MIC, $\mu\text{g/mL}$	MBC, $\mu\text{g/mL}$	MBC/MIC ratio
<i>E. coli</i>	128	256	2.0

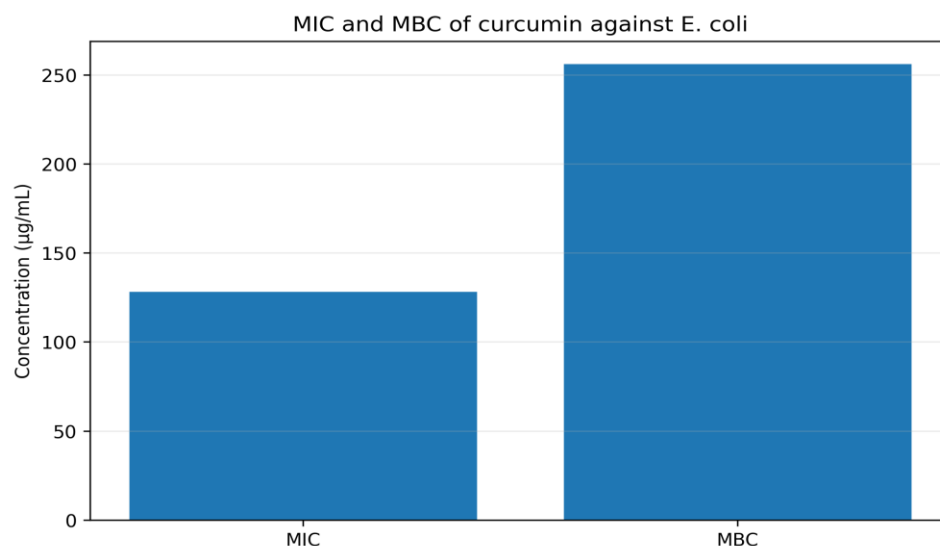


Figure (4): Minimum inhibitory concentration and minimum bactericidal concentration of curcumin against *Escherichia coli*

3.5 Comparison of Aqueous Turmeric Extract, Ethanolic Turmeric Extract, and Curcumin

A comparative analysis was performed to evaluate the antibacterial activity of aqueous turmeric extract, ethanolic turmeric extract, and curcumin against *Escherichia coli* at the tested concentrations. Curcumin demonstrated the greatest antibacterial activity, followed by the ethanolic turmeric extract, whereas the aqueous turmeric extract showed the lowest activity. The greatest inhibitory effect was observed with curcumin at the 100% concentration.

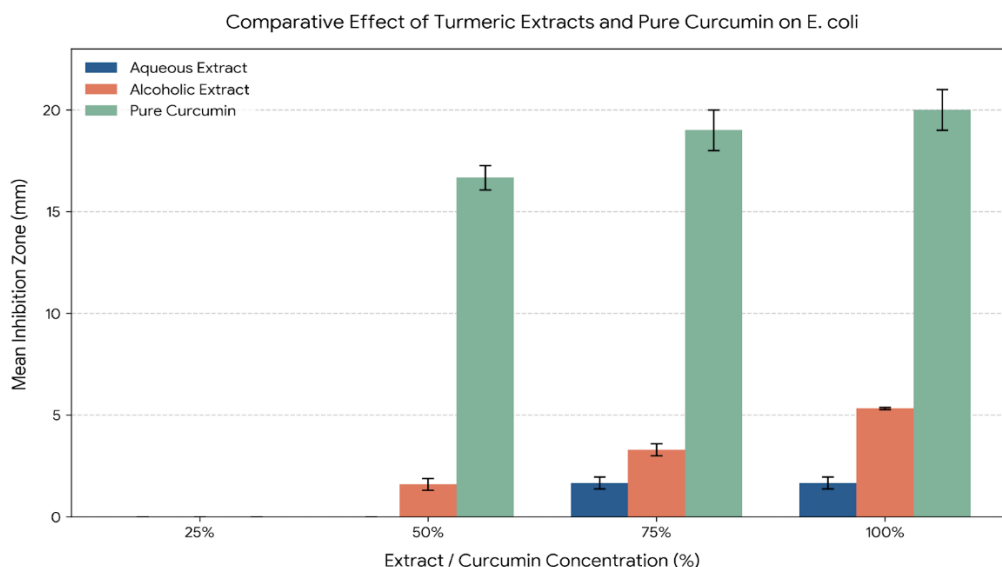


Figure (5): Comparison of the antibacterial activity of aqueous turmeric extract, ethanolic turmeric extract, and curcumin against *Escherichia coli* at different concentrate.

4. Discussion

4.1 Antibacterial Activity of the Aqueous Turmeric Extract

The aqueous turmeric extract showed little antibacterial activity towards *E. coli*. The inhibition was not observed at 25% and 50%, while only marginal inhibition could be observed at 75% and 100%. It could be postulated that the poor water solubility of curcuminoids, especially curcumin, and the low bioavailability of turmeric's lipophilic components might be the reason for the finding. Indeed, curcumin is a hydrophobic chemical with a very low solubility in water that might affect its ability to be extracted and diffuse through the antimicrobial agar assay medium (Odogbu Odo et al., 2023; Górnicka et al., 2023).

Based on the findings, it could be concluded that the aqueous extraction method might lead to the production of a preparation that showed limited antibacterial activity in the in vitro assay. However, it should be mentioned that the curcuminoid content, total phenolic content, and extraction yield of the aqueous extract were not determined, and therefore the findings cannot completely explain the observed phenomenon (Balahay et al., 2025).

4.2 Antibacterial Activity of the Ethanolic Turmeric Extract

The ethanolic turmeric extract showed greater antibacterial activity towards *E. coli* compared to aqueous extract. It was also observed that higher concentrations of extract showed higher percent inhibition. This may be because of higher number of bioactive compounds in the 75% ethanol extract such as curcuminoids and other moderately polar or lipid soluble metabolites that have antibacterial properties (Gow-Chyin et al., 2023). However, in the present study, the chemical composition of neither extract has been analyzed, hence the specific compounds responsible for the antibacterial effects could not be identified.

The observed higher antibacterial activity of the ethanolic extract must be treated with caution. The inhibition zone diameter, as determined by the modified Kirby-Bauer method is affected by a number of factors including antimicrobial activity, agar diffusion characteristics, solubility of the extract, the total quantity used and the concentration applied to the disk. Also, appropriate solvent controls are a must and interpretation of activity of ethanolic extract must take into account the possible contribution of residual ethanol. Hence, it must also be noted that activity of the ethanolic extract is preliminary evidence of in-vitro antibacterial activity only (Al-Bayati, 2008).

4.3 Antibacterial Activity of Curcumin

Curcumin showed the highest antibacterial activity of all the tested preparations. A visible inhibition zone was not observed at a 25% concentration, while mean inhibition-zone diameters increased at 50%, 75%, and 100% concentrations. Indeed, the mean inhibition-zone diameter increased from 16.67 mm to 19.00 mm (2.33 mm) at a 75% concentration and reached 20.00 mm (3.33 mm) at a 100% concentration.

The insignificant increase of the inhibition-zone diameter between the 75% and 100% concentrations could suggest the plateaus of the response function in disk-diffusion assays; however, it does not necessarily mean that the bacterial cell receptors were saturated at the 100% curcumin concentration. The observed response could be determined by the solubility of curcumin, its ability to diffuse into agar, the concentration loaded on each disk, and several other variables (Grover et al., 2021). Moreover, such variables as the minimum inhibitory concentration, inoculum size, and test methods contribute to the determination of whether an antibacterial agent is bactericidal or bacteriostatic. The minimum inhibitory and bactericidal concentrations of curcumin for the tested *E. coli* isolate were 128 µg/mL and 256 µg/mL, respectively. Thus, the MBC/MIC ratio was 2, indicating that curcumin might be classified as a bactericidal antibacterial agent under the in-vitro conditions of this study. However, this conclusion might not be completely correct because the determination of bactericidal activity for an antibacterial substance could depend on the test system, strains under the investigation, inoculum sizes, and certain other factors (Moghadamtousi et al., 2014; Górski et al., 2022).

5. Conclusion

In this in-vitro study, curcumin revealed the strongest antibacterial effect against the *Escherichia coli* isolate tested, followed by the ethanolic turmeric extract. The aqueous turmeric extract displayed only minimal antibacterial activity. The stronger activity seen with curcumin and the ethanolic extract could stem from better extraction or availability of turmeric's bioactive constituents relative to the aqueous extract; however, this mechanism was not directly assessed. Against the *E. coli* isolate tested, curcumin exhibited a minimum inhibitory concentration of 128 µg/mL and a minimum bactericidal concentration of 256 µg/mL, giving an MBC/MIC ratio of 2. One-way ANOVA revealed a statistically significant difference in mean inhibition-zone diameter across the curcumin concentrations tested ($P=0.0093P$).

These results offer preliminary evidence that curcumin and ethanolic turmeric extract possess in-vitro antibacterial activity against *E. coli*. Before their use in preventing or treating urinary tract infections can be considered, further standardized laboratory investigations as well as in-vivo and clinical studies are needed. Recommendations Assessment of Antibiotic Interactions: Exploring potential synergistic or antagonistic interactions between curcumin and conventional antibiotics currently used to treat urinary tract infections

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