

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(1): 1005-1007
www.biochemjournal.com
Received: 08-10-2025
Accepted: 12-11-2025

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In-vitro analysis of the antibacterial activity of curcumin against *Escherichia coli*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1m.7167>

Abstract

Colibacillosis of birds due to *Escherichia coli* infection is an important poultry disease and production risk factor, particularly in Third World countries. Rising antibiotic resistance necessitates a change of approach. Curcumin, a bioactive molecule isolated from *Curcuma longa*, has been found to exhibit antimicrobial activity. The present communication evaluated the in-vitro antibacterial action of curcumin against a disease-causing poultry isolate of *E. coli* (O78, MTCC No. 723). In disc diffusion and broth dilution tests, curcumin inhibited in dose-dependent pattern with zones of 8.33 ± 0.58 mm to 15.33 ± 1.00 mm and a minimum inhibitory concentration (MIC) of 1.5 mg/mL. The findings validate the moderate antibacterial efficacy of curcumin and suggest its utilization as a natural remedy in poultry disease management. *in vivo* experiments are recommended to establish its therapeutic potency.

Keywords: Curcumin, *Escherichia coli*, antibacterial, *in-vitro*, MIC, disc diffusion, poultry

Introduction

Poultry production is the cornerstone of the world's food economy, especially in developing countries like India. The poultry production industry, however, is gravely threatened by bacterial infection, and among the most common and economically important conditions is avian colibacillosis, whose causative organism is *Escherichia coli*. Avian colibacillosis typically causes respiratory distress, septicemia, and mortality among poultry flocks. The unchecked and uncontrolled application of antibiotics in poultry for the prevention and treatment of disease has been a factor in the evolution of drug-resistant *E. coli* strains. This not only hinders successful therapy but also creates risks to resistance development and antibiotic residue in human pathogens (Hasan *et al.*, 2011) [3].

Curcumin, the principal curcuminoid present in turmeric, is a drug that has been traditionally used in Indian medicine due to its diverse therapeutic potentials. Current scientific research has confirmed many of these observations, demonstrating curcumin's antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities (Moghadamtousi *et al.*, 2014) [9]. The growing demand for phytotherapeutics, coupled with the need to discover alternative antibiotics in veterinary medicine, makes curcumin a compound worth exploring. The goal of this research is to examine the in-vitro antibacterial activity of curcumin against a pathogenic *E. coli* strain to investigate its potential use for poultry disease control.

Avian colibacillosis is a global prevalent disease in poultry that normally causes severe economic losses. It is brought on by avian pathogenic *Escherichia coli* (APEC), most often serotype O78. The bacteria have been found to cause a chain of systemic infections including perihepatitis, pericarditis, and air sacculitis. Previous studies by, for instance, McPeake *et al.* (2005) [7] have touched on the role played by *E. coli* in exacerbating respiratory infections, especially in those birds co-infected with respiratory viruses.

The widespread application of antibiotics like tetracycline, sulfonamides, and ampicillin has led to resistance among *E. coli* bacteria. Hasan *et al.* (2011) [3] exhibited significant resistance among poultry isolates, which limits the effectiveness of traditional therapeutic practices and necessitates the use of alternative approaches. Curcumin is a potential bioactive compound exhibiting a wide spectrum of biological activities. Its antibacterial activity is based on disruption of bacterial cell membrane, inhibition of nucleic acid production, and quorum sensing interference. Experiments by Kaur *et al.* (2010) [5] revealed curcumin prevents polymerization of FtsZ, a crucial bacterial cytokinesis protein,

thereby preventing bacterial growth. Mishra *et al.* (2005) [8] have also synthesized curcumin bioconjugates and confirmed them to be more active than some conventional antibiotics. Several studies have revealed the potent antimicrobial activity of *Curcuma longa* (turmeric) and its bioactive compounds against an array of bacterial and fungal pathogens. Turmeric ethyl acetate extracts have been shown to display stronger antibacterial effect compared to methanol or water extracts, and notably enhance the activity of β -lactam antibiotics like ampicillin and oxacillin against MRSA, and successfully hinder its invasion into human mucosal fibroblasts (Kim *et al.*, 2005) [6].

Curcumin conjugates, such as acetyl and piperoyl-linked derivatives, were found to be more active antimicrobially than native antibiotics Cefepime, suggesting the medicinal potential of bioengineered curcuminoids (Mishra *et al.*, 2005) [8].

The antibacterial efficacy of curcumin is mediated by interference with cell division in bacteria by disrupting the FtsZ protein complex crucial for cytokinesis in bacteria like *E. coli* and *Bacillus subtilis* (Kaur *et al.*, 2010) [5]. Both crude curcuminoids and essential oils of various turmeric types have displayed broad-spectrum activity with the Kasur type of highest activity, especially against *B. subtilis* (Shagufta *et al.*, 2010) [12]. Ethanolic extracts of *C. longa* and *C. aromatica* have been found to possess antibacterial and antifungal activity against pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus flavus* (Jha *et al.*, 2013) [4]. Antibacterial screening of the aqueous and methanolic extracts of turmeric showed activity against general pathogens like *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Escherichia coli*, and ethanol was found to be the most active (Moghadamtousi *et al.*, 2014) [9]. Turmeric fractions were found to be more sensitive to clinical isolates of *S. aureus* compared to standard lab strains, with electron microscopy establishing membrane damage and cell disruption, confirming the broad-spectrum antimicrobial potential of turmeric (Gupta *et al.*, 2015) [2]. Besides, aqueous extracts and paste of turmeric have been effective in the inhibition of bacterial growth in culture media, although with a higher MIC than traditional antibiotics like amikacin (Afrose *et al.*, 2015) [1].

Curcumin I, a major constituent of turmeric, displayed significant activity against Gram-positive and Gram-negative bacteria by causing membrane disruption, as confirmed through membrane permeability bioassays and microscopy, highlighting its value as a phytochemical

natural antimicrobial agent to combat drug-resistant infections (Tyagi *et al.*, 2015) [15].

Materials and Methods

The present study was carried out at the Department of Veterinary Pharmacology and Toxicology and Poultry Research Station, under the Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, an associate of Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur.

One avian-pathogenic strain of *Escherichia coli* (MTCC No. 723, serotype O78) was procured from the Institute of Microbial Technology, Chandigarh, India. Bacterial culture was cultivated on nutrient broth, and an experimental colibacillosis induction was made with a suspension of approximately 1×10^8 CFU/mL.

Bacterial density was estimated based on spectrophotometric measurement via the optical density (OD) at 600 nm (OD₆₀₀), widely utilized for estimating cell density of bacteria. Optical density at 600 nm is performed by transmitting light with a known wavelength through the bacterial suspension in a cuvette and measuring absorbance, OD₆₀₀ = 1 to be approximately equal to 1×10^8 *E. coli* cells/mL of culture (Pelczar *et al.*, 1986) [10]. For the current study, analytical grade curcumin was procured from Hi-Media Laboratories and used for the determination of its in-vitro antibacterial activity against *E. coli*.

Disc diffusion assay was conducted to assess the zone of inhibition, following the protocol given by Savitha and Rathnavijaya (2011) [11]. Besides, the Minimum Inhibitory Concentration (MIC) of curcumin was assessed according to the method of dilution given by Pelczar *et al.* (1986) [10], to determine the minimum concentration required to inhibit perceptible bacterial growth. Experimental data were statistically analyzed using a Completely Randomized Design (CRD) by Snedecor and Cochran (1989) [14] to provide proper evaluation of treatment effects.

Results

Antibacterial activity of curcumin on *Escherichia coli* is presented in Table 1 and Fig 1.

The zone of inhibition for curcumin at 2.5, 5, 10, and 20 mg per disc was 8.33 ± 0.58 mm, 9.67 ± 0.58 mm, 12.33 ± 0.58 mm, and 15.33 ± 1.00 mm, respectively. The results clearly indicate a dose-dependent antibacterial activity, as higher concentrations of curcumin resulted in larger zones of inhibition than lower concentrations.

Table 1: Effect of curcumin (zone of inhibition) against *E. coli* Replication

Replication	Zone of inhibition (mm)			
	Concentration of curcumin			
	2.5 mg	5 mg	10 mg	20 mg
1	8	9	12	13
2	8	10	12	12
3	9	10	13	14
Mean $\pm$ SE	8.33 ± 0.58	9.67 ± 0.58	12.33 ± 0.58	15.33 ± 1.00

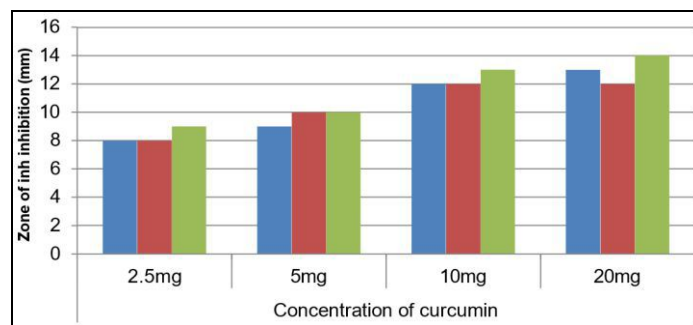


Fig 1. Graphical representation of zone inhibition of curcumin against *E. coli*

Among the concentrations attempted, 20 mg/disc showed greatest antibacterial activity with a mean inhibition zone value of 15.33 ± 1.00 mm. Minimum inhibitory concentration (MIC) of curcumin was assessed in terms of optical density (OD) readings and was 1.5 mg/mL. These results show that curcumin has moderate antibacterial activity against *E. coli*, in partial agreement with Singh and Jain (2011) [13] findings where there was a 18 mm inhibition zone at the same concentration of curcuminoids (20 mg/disc). In general, the in-vitro study confirms that curcumin has a moderate but noticeable antibacterial action against *E. coli*.

Discussion

Curcumin exhibited antibacterial action against *E. coli* as seen through zones of inhibition of 8.33 ± 0.58 mm, 9.67 ± 0.58 mm, 12.33 ± 0.58 mm, and 15.33 ± 1.00 mm at 2.5, 5, 10, and 20 mg/disc, respectively. Minimum inhibitory concentration (MIC), as measured by optical density readings, was 1.5 mg/mL. These results confirm that curcumin is actually antibacterial, particularly at the higher concentrations. Results are consistent with previous evidence that curcumin disrupts bacterial membranes and suppresses essential cellular functions (Afrose *et al.*, 2015) [1]. The dose-dependent increase in antibacterial activity observed verifies curcumin's activity *in vitro*. Its well-established mechanisms of action, including inhibition of the prokaryotic cell division protein FtsZ and interference with membrane integrity, indicate its potential for use in Gram-negative bacterial infection treatment (Kaur *et al.*, 2010) [5]. However, the comparably elevated MIC relative to traditional antibiotics highlights the necessity of additional improvement of its antimicrobial activity, possibly through new formulation strategies like nanoparticle-based delivery or synergistic combination therapy (Mishra *et al.*, 2005) [8].

Conclusion

Curcumin had profound antibacterial activity against avian pathogenic *Escherichia coli* *in vitro*. Its dose-response nature and moderate MIC values are in its favor for potential use as a natural antimicrobial drug in poultry. Curcumin may reduce the consumption of synthetic antibiotics and promote sustainable poultry production. Additional research is required to enhance its bioavailability and establish its efficacy *in vivo*.

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