P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2026.035

Antibacterial Potential of Geraniol against *Escherichia coli* Strains Isolated from Urinary Clinical Samples of Dogs and Cats

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Article History: 25-347 Received: 12-Dec-25 Revised: 15-Feb-26 Accepted: 20-Feb-26 Online First: 10-Mar-26

ABSTRACT

Urinary tract infections (UTIs) are an important cause of morbidity in dogs and cats and are often associated with clinically relevant complications. *Escherichia coli* is the primary etiological agent of these infections. The increasing advancement of bacterial resistance has compromised the effectiveness of conventional antimicrobials, highlighting the need for new therapeutic alternatives. Geraniol, a natural monoterpene with well-recognized antimicrobial activity, has emerged as a promising therapeutic candidate. This study evaluated the antibacterial activity of geraniol against *E. coli* strains isolated from urinary samples of dogs and cats. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration of Adherence (MICA), and the effect of its association with synthetic antimicrobials were determined. Geraniol exhibited an MIC of 500µg/mL for 87.5% of the strains and bactericidal activity in five of the eight strains tested, with MBC values ranging from 500 to 1000µg/mL. Regarding MICA, the compound demonstrated anti-adherence activity for both strains under study, reaching ratios of 1:128 for strain Ecc 42 and 1:16 for strain Ecg 14. The associations showed synergistic effects, especially with gentamicin and amikacin. These findings reinforce the innovative potential of geraniol as a natural agent for controlling UTIs in companion animals and highlight its relevance to veterinary practice in the face of the growing challenge of antimicrobial resistance, although further studies are still required to enable its clinical application.

Keywords: Phytotherapy, Urinary tract infection, Veterinary medicine, Microbiology, Antimicrobial resistance.

INTRODUCTION

Urinary tract infections (UTIs) rank among the leading causes of morbidity in companion animals, being associated with clinical signs of varying severity, reduced quality of life, and, when left untreated, the development of potentially fatal complications (Scarpellini et al. 2025). These infections may affect different segments of the urinary system, including kidneys, ureters, bladder, and urethra, with the lower urinary tract being most frequently involved, which explains their high relevance in routine veterinary practice (Sakauchi et al. 2025).

Among the etiological agents, *Escherichia coli* stands out as the main bacterial species involved in UTIs in dogs and cats. It is a Gram-negative commensal bacterium of the intestinal tract of humans and homeothermic animals, capable of expressing a wide repertoire of virulence factors whose distribution varies according to the site of infection, including urinary, septicemic, meningeal, and enteric conditions and strains carrying multiple virulence factors tend to exhibit greater pathogenic potential and are frequently associated with more severe infections (Abdel-Kader et al. 2025).

In veterinary medicine, antimicrobials represent the primary therapeutic approach for the treatment of UTIs in companion animals (Oh and Park 2025). The inappropriate and widespread use of these agents in veterinary settings and public health systems accelerates antimicrobial resistance, which, although a natural phenomenon, has come to represent a significant and growing risk (Patra et al. 2025).

In this context, the One Health approach stands out, integrating human and veterinary medicine, environmental sciences, and related fields in the fight against the spread of resistant microorganisms (Vernaccini et al. 2024). Thus, natural products have emerged as promising sources of

Cite This Article as: Simões MM, Farias JHAd, Pereira CT, Santos B, Marques FMC, Alves MdS, Medeiros MAAd and Filho AAdO, 2026. Antibacterial potential of geraniol against *Escherichia coli* strains isolated from urinary clinical samples of dogs and cats. International Journal of Veterinary Science 15(3): 845-850. https://doi.org/10.47278/journal.ijvs/2026.035

compounds with antibacterial activity, both through direct action on microorganisms and by reversing bacterial resistance (Araújo et al. 2025). Among these compounds, geraniol, an acyclic monoterpene alcohol of natural occurrence, extracted mainly from essential oils such as those of *Cymbopogon martinii* (palmarosa), *Citrus aurantium* (neroli), and *Rosa* spp., stands out for its remarkable antibacterial and antifungal properties (Zou et al. 2025). In this context, the present study aimed to evaluate the antibacterial potential of geraniol against *Escherichia coli* strains isolated from urinary clinical samples of dogs and cats, with the hypothesis of finding an antibacterial agent to broaden therapeutic alternatives in veterinary medicine.

MATERIALS AND METHODS

Research location and test substance

The laboratory experiments were conducted at the Biochemistry Laboratory of the Federal University of Campina Grande (UFCG), affiliated with the Center for Health and Rural Technology (CSTR). The geraniol (purity grade 97.99%, batch GER8HB43) was obtained from Quinarí Industry. For the pharmacological assays, the compound was initially solubilized in dimethyl sulfoxide (DMSO) under stable conditions at room temperature and subsequently diluted in distilled water, maintaining the final DMSO concentration below 0.1% v/v.

Culture media and bacterial strains

The culture media used in the assays for the evaluation of antimicrobial activity were Mueller-Hinton broth and Mueller-Hinton agar, both purchased from Difco® and prepared according to the manufacturer's instructions. *Escherichia coli* strains isolated from urinary clinical samples of dogs and cats (Ecc 30, Ecc 42, Ecc 46, Ecc 49, Ecg 13, Ecg 14, Ecg 40, and Ecg 48) were used. The project was evaluated and approved by the Ethics Committee on the Use of Animals of the Federal University of Campina Grande (CEUA/CSTR/UFCG) under protocol number 022026. All strains were maintained on Mueller-Hinton Agar (MHA) at 4 °C. Inocula were prepared from cultures incubated overnight on MHA at 37 °C and subsequently diluted in sterile saline solution to reach a final concentration of approximately 1.5×10^8 colony-forming units per mL (CFU/mL). Adjustment of bacterial density was performed by comparing turbidity with tube 0.5 of the McFarland scale (Bona et al. 2014).

Antimicrobials

Ampicillin (10μg), gentamicin (10μg), ceftriaxone (30μg), norfloxacin (10μg), and amikacin (30μg) were used according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2018).

Determination of the Minimum Inhibitory Concentration (MIC)

Microdilution assays were performed in 96-well U-bottom plates containing 100μL of double-strength Mueller-Hinton broth and 100μL of geraniol at concentrations ranging from 1000 to 31.2μg/mL. The MIC was determined by inoculating 10μL of the bacterial suspension (approximately 1.5×10^8CFU/mL) into each well. The penultimate well served as the sterility control, and the last well as the growth control. Assays were conducted in duplicate, with incubation at 35 ± 2°C for 24h. After the initial reading, 20μL of resazurin were added, followed by further incubation and reading to confirm the MIC (Palomino et al. 2002; Ostrosky et al. 2008; CLSI 2012; Bona et al. 2014).

Determination of the Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of geraniol was determined after the MIC reading. Inocula of 10μL (approximately 1.5×10^8CFU/mL) from the corresponding dilutions were transferred to wells containing 100μL of Mueller-Hinton broth and incubated at 35 ± 2 °C for 24 h. Subsequently, 20μL of resazurin were added, followed by further incubation to confirm growth inhibition. The MBC was defined as the absence of color change in the indicator (Ncube et al. 2008; Queiroga et al. 2012).

Determination of the Minimum Inhibitory Concentration of Adherence (MICA)

The Minimum Inhibitory Concentration of Adherence (MICA) of geraniol was determined in the presence of 5% sucrose, according to Albuquerque et al. (2010), with modifications. The compound was tested in dilutions up to 1:128. After bacterial growth in Mueller-Hinton broth at 35±2°C, 0.9mL of the subculture were mixed with 0.1mL of the compound solutions in test tubes. Incubation was carried out at 35±2°C for 24 h, with tubes inclined at 30°. Readings were performed by visual inspection of adherence to the tube walls after agitation. Assays were conducted in duplicate, with chlorhexidine digluconate at 0.12% used as the positive control. The MICA was defined as the lowest concentration capable of preventing bacterial adhesion to the glass tube and was analyzed as the mean±S.E.M. (Standard Error of the Mean).

Study of the association of the product (geraniol) with synthetic antibacterials

The association between geraniol and synthetic antimicrobials was evaluated using the disk diffusion technique on Mueller-Hinton agar, employing filter paper disks impregnated with the antimicrobials and supplemented with 20μL of the MIC of the compound. As a negative control, plates containing only agar with the bacterial suspension and the antimicrobial disks were used. Plates were incubated at 35 ± 2°C for 24–48h, and inhibition zones were measured. The effect was considered synergistic when there was an increase of ≥ 2mm in the inhibition halo diameter (HI), antagonistic when smaller than that observed for the antimicrobial alone, and indifferent when equivalent (Cleeland and Squires 1991; Oliveira et al. 2006). All assays were performed in duplicate was analyzed as the mean±S.E.M. (Standard Error of the Mean).

RESULTS

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of geraniol against *Escherichia coli* Strains

The antibacterial activity of geraniol against

Escherichia coli strains was evaluated by MIC and MBC (Table 1). The compound exhibited a strong inhibitory effect, with an MIC of 500µg/mL in 87.5% of the strains, and bactericidal activity in 5 out of 8 isolates, with MBC values ranging from 500 to 1000µg/mL.

Minimum Inhibitory Concentration of Adherence (MICA) of geraniol against *Escherichia coli* Strains

Table 2 Minimum Inhibitory Concentration of Adherence (MICA) of geraniol and 0.12% Chlorhexidine against *Escherichia coli* Strains (Ecc 42 and Ecg 14) Isolated from Urinary Samples of Dogs and Cats. Geraniol demonstrated anti-adherence activity against both strains, reaching a ratio of 1:128 for Ecc 42 and 1:16 for Ecg 14. Chlorhexidine, in turn, exhibited activity at 1:128 against both strains.

Association of the compound geraniol with synthetic antimicrobials of geraniol against *Escherichia coli* Strains

The association between geraniol and synthetic antimicrobials against *Escherichia coli* strains isolated from urinary samples of dogs and cats revealed distinct interaction patterns (Table 3), with synergistic effects observed in combinations with gentamicin and amikacin.

DISCUSSION

A classification of the antimicrobial potential of phytotherapeutic products was adopted based on Minimum Inhibitory Concentration (MIC) values, considering strong activity between 50–500µg/mL, moderate activity between 600–1500µg/mL, and weak activity above 1600µg/mL (Sartoratto et al. 2004; Simões et al. 2025). According to this criterion, the results of the present study demonstrate that geraniol exhibited strong antibacterial activity, since 87.5% of *Escherichia coli* strains presented MIC values of 500µg/mL. This performance confirms the high antimicrobial potential of this monoterpene, widely described in the literature.

Regarding the Minimum Bactericidal Concentration (MBC), it was established that an MBC/MIC ratio between 1:1 and 2:1 characterizes bactericidal compounds, while values greater than 2:1 indicate a bacteriostatic effect (Hafidh et al. 2011; Medeiros et al. 2023). Applying this criterion, geraniol showed a variable action profile,

Table 1: MIC and MBC (µg/mL) of geraniol against *Escherichia coli* from Urinary Samples of Dogs and Cats

Geraniol								
	Bacterial Strains							
	Ecc 30	Ecc 42	Ecc 46	Ecc 49	Ecg 13	Ecg 14	Ecg 40	Ecg 48
MIC (µg/mL)	500	250	500	500	500	500	500	500
MBC (µg/mL)	1000	>1000	500	>1000	1000	>1000	1000	500

Note: MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration.

Table 2: Minimum Inhibitory Concentration of Adherence (MICA) of geraniol and 0.12% Chlorhexidine Digluconate against *Escherichia coli* Strains (Ecc 42 and Ecg 14)

Ecc 42									
geraniol	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	-	-	-	-	-	-	-	-	+
0.12% Chlorhexidine Digluconate	-	-	-	-	-	-	-	-	+
Ecg 14									
geraniol	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
	-	-	-	-	-	+	+	+	+
0.12% Chlorhexidine Digluconate	-	-	-	-	-	-	-	-	+

Note: (+) Adherence to the tube wall; (–) No adherence to the tube wall.

Table 3: Inhibition zones (mm) of geraniol/synthetic antimicrobial associations against *Escherichia coli* strains isolated from urinary samples of dogs and cats

geraniol						
Strains	Association	AMP	GEN	CRO	NOR	AMI
Ecc 30	AIH	0mm	20mm	10mm	0mm	22mm
	AIH with GE	0mm (*)	20mm (*)	0mm (↓)	0mm (*)	24mm (↑)
Ecc 42	AIH	0mm	20mm	10mm	30mm	20mm
	AIH with GE	0mm (*)	24mm (↑)	12mm (↑)	28mm (↓)	26mm (↑)
Ecc 46	AIH	0mm	20mm	10mm	34mm	20mm
	AIH with GE	0mm (*)	26mm (↑)	0mm (↓)	32mm (↓)	30mm (↑)
Ecc 49	AIH	0mm	22mm	0mm	30mm	28mm
	AIH with GE	0mm (*)	28mm (↑)	0mm (*)	30mm (*)	30mm (↑)
Ecg 13	AIH	0mm	8mm	0mm	0mm	20mm
	AIH with GE	0mm (*)	8mm (*)	0mm (*)	0mm (*)	28mm (↑)
Ecg 14	AIH	0mm	8mm	0mm	0mm	20mm
	AIH with GE	0mm (*)	10mm (↑)	0mm (*)	0mm (*)	28mm (↑)
Ecg 40	AIH	0mm	28mm	0mm	0mm	0mm
	AIH with GE	0mm (*)	0mm (↓)	0mm (*)	0mm (*)	28mm (↑)
Ecg 48	AIH	0mm	28mm	8mm	30mm	28mm
	AIH with GE	0mm (*)	24mm (↓)	14mm (↑)	30mm (*)	30mm (↑)

Note: AIH: Antimicrobial Inhibition Halo. GE: geraniol. Synergistic effect (↑); antagonistic effect (↓); indifferent effect (*); AMP: ampicillin; GEN: gentamicin; CRO: ceftriaxone; NOR: norfloxacin, AMI: amikacin.

with bactericidal behavior against certain strains and bacteriostatic activity against others. This pattern suggests that its efficacy may be directly related to the phenotypic and genetic characteristics of the evaluated isolates. Such variability is consistent with studies showing that uncatalogued genetic variation in resistance-associated gene families influences the phenotypic susceptibility of isolates (Lipworth et al. 2024).

Recent MIC and MBC values of geraniol have also been reported against methicillin-resistant *Staphylococcus aureus* (MRSA), with MIC of 625µg/mL and MBC ranging from 1,250 to 2,500µg/mL, confirming its antibacterial activity against clinically relevant pathogens (Castro et al. 2025). Similarly, the essential oil of *Eucalyptus radiata* presented MIC values between 500 and 1,000µg/mL and MBC ranging from 500 to 1,024µg/mL against *E. coli* (Santos et al. 2024), reinforcing the robustness of geraniol's antimicrobial activity and the potential of monoterpenes as alternatives in the context of bacterial resistance.

Furthermore, other studies have evaluated the antibacterial activity of geraniol, such as that of Simões et al. (2025), against *Enterobacter cloacae* strains, finding that the compound showed a significant inhibitory effect on the growth of different strains, with a MIC_{50} value equal to 500µg/mL, also demonstrating bactericidal action in three of the five strains analyzed, with MBC values of 500 and 1000µg/mL, which reinforces the potential of geraniol as a promising alternative in the control of foodborne pathogenic microorganisms and suggests its applicability both in isolation and in combination with synthetic antibacterials.

Similarly, the antimicrobial effect of *Cymbopogon martini* essential oil against clinical human bacterial isolates from the skin and respiratory tract was explored by Cebollada et al. (2026), revealing significant activity against Gram-positive bacteria of the genera *Streptococcus* and *Staphylococcus*, with MIC values ranging from 125 to 250µg/mL for *Streptococcus agalactiae*, *Streptococcus anginosus*, *Streptococcus dysgalactiae*, and *Streptococcus pyogenes*. It also demonstrated activity against some tested Gram-negative bacteria, namely *Escherichia coli* (MIC 350µg/mL), *Moraxella catarrhalis* (MIC 250µg/mL), and *Morganella morganii* (MIC 350µg/mL). These findings reinforce the relevance of the essential oil as a potential broad-spectrum antimicrobial agent.

Additionally, the findings of this study corroborate those of Madeira et al. (2025), who demonstrated antibacterial and anti-adherence effects of geraniol against MRSA. Consistently, in the present study, geraniol inhibited biofilm formation in *E. coli* strains, maintaining activity up to a 1:16 dilution. Complementarily, Aslan and Alim (2025) reported that combinations of antimicrobials with bioactive plant compounds, including geraniol, promoted significant suppression of biofilm formation in multidrug-resistant *Acinetobacter baumannii* isolates, highlighting the clinical impact of this property.

Other research reinforces the effect of terpenes in preventing the formation of *Escherichia coli* biofilms, such as that of Wang et al. (2025), in which they analyzed the implications of linalool in the development of the biofilm of this bacterial species and in the production of its extracellular polysaccharides, evaluating its effects in both planktonic and biofilmed states, observing that the minimum biofilm inhibitory concentrations (MBICs) were twice as high as the conventional minimum inhibitory concentrations.

The associations of geraniol with synthetic antimicrobials showed predominantly synergistic effects, especially with gentamicin and amikacin, indicating that its modulatory action may be dependent on the pharmacological class. Similar results were observed with (R)-(+)-limonene, which demonstrated synergy mainly with gentamicin and ciprofloxacin against most tested strains (Alves et al. 2024). Convergently, Huang et al. (2024) also reported synergy between citral and geraniol combined with amikacin, clarithromycin, and linezolid.

The investigation conducted by Diniz et al. (2024) reinforces the versatility of *Origanum vulgare* essential oil as a modulating agent of bacterial resistance in foodborne isolates. When tested in association with antimicrobials such as ampicillin, gentamicin, ceftazidime, and ciprofloxacin, the authors observed that, although indifference was predominant (50%), a significant rate of synergism (37.5%) was detected. This phenomenon suggests that oregano components may increase the susceptibility of pathogens such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus*, demonstrating both direct inhibition capacity and potentiation of the action of other drugs.

Taken together, these data reinforce the role of natural products as enhancers of antimicrobial activity, representing a promising strategy in tackling bacterial resistance. Above all, the antimicrobial effect of geraniol is associated with its ability to destabilize the lipoprotein structures of the microbial outer membrane, which increases permeability and leads to the loss of essential cellular constituents (Fajdek-Bieda et al. 2025).

Conclusion

Geraniol exhibited both antibacterial and anti-adherence activity against *Escherichia coli* strains isolated from urinary infections in dogs and cats, in addition to showing synergistic effects with conventional antimicrobials. Although promising, the results should be interpreted considering the experimental conditions of the *in vitro* model. The transition to clinical application requires further investigations of toxicity, pharmacokinetics, and *in vivo* studies to confirm the safety, efficacy, and therapeutic viability of the compound in the management of veterinary urinary tract infections.

DECLARATIONS

Funding: The study was funded by the National Council for Scientific and Technological Development (CNPq).

Acknowledgement: The authors thank the National Council for Scientific and Technological Development (CNPq) for the financial support provided, as well as the Federal University of Campina Grande (UFCG) for the institutional support necessary for the accomplishment of this work.

Conflict of Interest: The authors declare that they have no competing interests.

Data Availability: Data will be available on request from the corresponding author.

Ethics Statement: This research was evaluated and approved by the Ethics Committee on the Use of Animals of the Federal University of Campina Grande (CEUA/CSTR/UFCG) under protocol number 022026.

Author's Contribution: MMS conducted the antibacterial assays, performed data analysis, and drafted the manuscript. JHAF assisted with sample processing and contributed to data interpretation. CTP and BS supported laboratory procedures and data acquisition. FMCM and MSA contributed to the execution of microbiological tests and to the methodological review of the study. MAAM assisted in the discussion of the results and in the manuscript revision. AAOF supervised the study, contributed to the research design, and critically reviewed the final manuscript. All authors read and approved the final version.

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