Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

The Antibacterial Effect of Cinnamon Ethanolic Extract against *Proteus* spp. Isolated from Dogs

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Abstract | This study aimed to investigate the antibacterial effect of *Cinnamomum verum* ethanolic extract against *Proteus mirabilis* isolated from the oral cavities of humans and dogs. Fifty samples, collected from both humans and dogs, corresponding to isolates obtained using the primary and VITEK 2 systems, yielded eight isolates that were positive for *P. mirabilis*. Different doses of the *Cinnamomum verum* extract were evaluated against the isolated *P. mirabilis* cultures, ranging from 125 to 2000 micrograms. Significant results were observed starting from 500 micrograms, yielding a 19 mm zone of inhibition. These results corroborate the findings of the GC-MS test on the extract, confirming the antibacterial properties of the active compounds resulting from the extraction process. Collectively, the results demonstrate the anti-microbial effect of *Cinnamomum verum* and may offer an alternative therapy against microbial infections.

Keywords | Antimicrobial effect, *Cinnamomum verum,* Ethanolic extract, *Proteus mirabilis*, Multidrug resistant

Received | July 02, 2025; **Accepted** | August 23, 2025; **Published** | September 03, 2025
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Citation | Lateef LI, Mohammed HF, Al-Nasiry BS, Kamal IM (2025). The antibacterial effect of cinnamon ethanolic extract against *Proteus* spp. isolated from dogs. J. Anim. Health Prod. 13(s1): 278-284.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.278.284
ISSN (Online) | 2308-2801

INTRODUCTION

Many bacterial species, including those of the Enterobacteriaceae family, have developed drug resistance as a result of the misuse and overuse of antibiotics in recent decades. Developed antibacterial medication resistance, resulting in decreased treatment effectiveness and increased morbidity (Mancuso *et al.*, 2021; Al-Nasiry, 2022; Al-Juburi and Al-Sammarraae, 2022). Zoonotic antibiotic-resistant bacteria constitute a health danger in Iraq, with research revealing that the primary public health risk comes from *Enterobacteriaceae* spp. The principal pathogen is *Klebsiella* sp. *E. coli*, followed by *Salmonella* spp., *Proteus*, and *Shigella* spp. (Al-Anbagi and Al-Khafaji, 2020; Al-Shattrawi, 2024).

Cinnamomum verum (C. verum), also known by its old name, *Cinnamomum zeylanicum*, is a species of spice plant that has been utilized in medicine since ancient times due to its pharmacological properties. It is endemic to the southern regions of India and Sri Lanka. The spice, which belongs to the Lauraceae family, is extracted from the plant's striped dry bark after it has been removed from the outer cork and the parenchyma below (Pathak and Sharma, 2021).

The plant contains major active components, including cinnamaldehyde, eugenol, caryophyllene, cinnamyl acetate, cinnamic acid, and coumarin, as well as minor other components that contribute to its pharmacological activities, such as antimicrobial, anti-inflammatory, antioxidant, anticancer, wound-healing, as well as other effects. These components have led to the use of this spice as an alternative medication to treat inflammation, asthma, bronchitis, diarrhoea, headaches, and heart issues (Singh *et al*., 2020; Al-Nasiry, 2022; Mohammed and Kamal, 2025).

Cinnamaldehyde is a natural, organic, botanical pale to yellow flavonoid substance with viscous consistency, part of the many species of the genus Cinnamomum, which gives cinnamon its intense flavour and odour. This material is often utilized as a natural alternative to medicine in India (Silva *et al*., 2020; Mohammed and Al-Gburi, 2023; Sharma *et al*., 2024).

The coumarin group is a collection of naturally occurring heterocyclic compounds belonging to the benzopyrone family, which are found in numerous plants, including cinnamon, tonka beans, and sweet clover. These compounds possess various bioactive characteristics used in medicine, including antibacterial, antifungal, anti-viral, anti-tubercular, anti-inflammatory, antioxidant, and other biological activities, due to their ability to interact non-covalently with various enzymes and receptors of living cells (Garg *et al*., 2020b; Kamal and Al-Hadad, 2023; Samanth and Bhat, 2024).

In the recent decade, research into different routes of antibacterial agents produced from plant compounds to replace commercial and widely used agents has yielded a safe and natural alternative answer. Such natural extracts may address the issue of antibacterial agents misuse, as many bacterial strains have developed resistance to these agents (Savoia, 2012; Al-Juburi and Al-Sammarraae, 2022; Barry, 2025). *C. verum* has been used as a natural antibacterial in alternative and traditional medicine in many countries (Pathak and Sharma, 2021; Singh *et al*., 2020; Kamal *et al*., 2025).

Yap *et al*. (2015) have found that cinnamon bark oil extract has the potential to eliminate germs and is likely to function synergistically with other chemicals to improve outcomes. The substance demonstrated the ability to break bacterial cell membranes, increasing permeability and causing permanent membrane damage, as well as reducing bacterial surface charge.

Proteus mirabilis belongs to the *Enterobacteriaceae* family. *P. mirabilis* is known to cause various illnesses in humans and animals, including urinary tract infections, particularly catheter-associated urinary tract infections (CAUTI), gastrointestinal tract infections, wounds, and eye infections (Armbruster *et al*., 2018). In Baghdad city *P. mirabilis* is considered the primary isolate, with a 66% of the total *Proteus spp*. isolates (Ahmed, 2015; Kamal and Al-haddad, 2022).

P. mirabilis expresses potent virulence factors, including adhesion proteins, lipopolysaccharides, quorum-sensing molecules, efflux pumps, and urease enzymes. These factors contribute to the bacteria's ability to form biofilms, which is the primary cause of its infectious abilities, as well as rendering the bacteria embedded in the biofilm effectively resistant to both antibacterial agents and the immune system as a whole (Wasfi *et al*., 2020).

MATERIALS AND METHODS

Preparation of *Cinnamomum* extract

The plant extract was produced in accordance with the method described by Mnge *et al*. (2025), with adjustments. Two hundred grams of cinnamon bark were powdered and placed in a 2-litre flask. 1400 mL of 99.9% ethanol was mixed with 600 mL of distilled water. The flask was immersed in the shaker for 24 hours to steep and shake. The substance was filtered using a Buchner funnel and a vacuum. The resultant liquid was evaporated in large trays at room temperature.

Gas chromatography-mass spectrometry (GC-MS) Analysis

GC-MS is an effective and widely used separation technique for analysis in various fields of science and technology through principles of adsorption and partition. The method involves the separation and identification of different compounds at the molecular level (Maji *et al*., 2023).

Antibacterial susceptibility testing and minimum inhibitory concentration (MIC) determination

MIC assay was performed using the agar dilution method with serial dilution (50%) of *C. verum* ethanolic extract. The dilutions were (2000 μg, 1000 μg, 500 μg, 250 μg, and 125 μg). The results were compared to a negative control standard (distilled water). Inhibition zone of 20 mm and above was considered sensitive (susceptible), above 15 mm to be intermediate susceptibility, and below it to be resistant.

RESULTS AND DISCUSSION

The GC-MS results show five major components in the *C. Verum* extract, with Cinnamaldehyde being the main component, followed by Coumarin, Phthalic acid, and O-Methoxycinnamaldehyde (E)-, another form of Cinnamaldehyde, accounting for a total percentage of 65.55%. These compounds exhibit antibacterial activity (Yu *et al*., 2020) and other bioactive properties (Table 1).

Table 1: Identified phytochemical profile of *C. verum* ethanolic extract (GC-MS Analysis).

Compound name	Retention time (RT)	Peak area (%)	CAS No.	Potential bioactive properties
Cinnamaldehyde	8.748	59.48	104-55-2	Antimicrobial, Antioxidant, Anti-inflammatory, Anticancer, Neuroprotection, Diabetes management, (Wang *et al*., 2025)
Coumarin	11.298	7.92	91-64-5	Antimicrobial, Antioxidant, Anti-inflammatory, Anticancer, Anti-coagulant activities, (Tsivileva *et al*., 2022)
Cinnamaldehyde (2-Propenal, 3-(2-methoxyphenyl)-) (O-Methoxycinnamaldehyde, (E)-)	12.511	6.07	1504-74-1	Antibacterial, Anti-inflammatory, Antitumor, Neuroprotection, (Wu *et al*., 2025)
Phthalic acid	25.562	6.5	131-20-4	Antimicrobial, Antioxidant, Anti-inflammatory, (Enikeev, 2025)
Benzene, (2-nitropropen-1-yl)- (Phenyl-2-nitropropene)	11.102	5.45	705-60-2	Antioxidant, Anti-inflammatory, Anticancer, (Kapısuz *et al*., 2024)
Other Minor Compounds (25%)	N/A	14.58	N/A	Antioxidant, Anti-inflammatory, Reduce cholesterol, (Petkova *et al*., 2025)

Isolation of Proteus mirabilis

The samples were collected from the oral cavities of dogs using sterile swabs, then grown on TSB to enhance bacterial growth. The samples were subsequently placed on TSA for 24 hours at 37°C, after which swarming phenomena was observed in the culture. A colony was placed on MacConkey agar, a differential medium, and colonies appeared within 24 hours (Figure 1). The cultures were then sent for testing using the VITEK2 system to confirm the species.

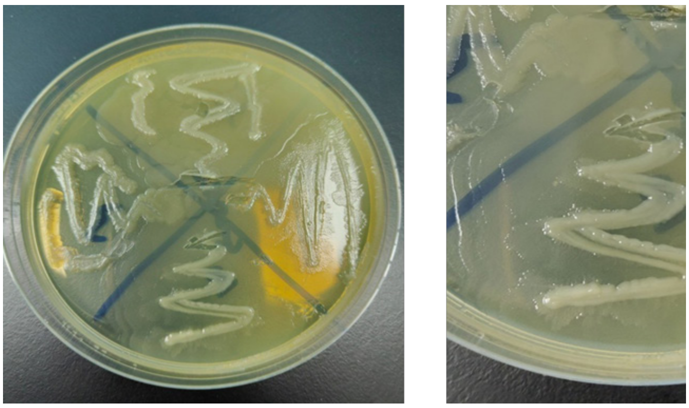

Figure 1: Growth of *Proteus* on TSA displaying the swarming phenomenon.

The number of isolates taken from humans and dogs was 25 samples each. The primary suspected isolates were 16 for humans and 9 for dogs, with 5 confirmed to be *Proteus mirabilis*, resulting in the highest percentage of isolates for humans at 20%, compared to 3 isolates, which amounted for 12% of the isolates from dogs (Table 2).

Four confirmed isolates were obtained from females, with a rate of 36%, and one confirmed isolate was obtained from a male, with a rate of 7% (Table 3).

Table 2: Carriage of *P. mirabilis* isolates according to sample sites.

Isolation site	No. of samples	Primary isolate	VITEK2
Human	25	16	(5) 20%
Dogs	25	9	(3) 12%

Table 3: Positive samples isolated from humans according to gender.

Human isolates	Sample No.	+ve %
Male	14	(1) 7%
Female	11	(4) 36%

Three positive isolates were confirmed in 16% of female dogs, while none were confirmed in male dogs (Table 4).

Table 4: Positive samples isolated from dogs according to gender.

Dog isolates	Sample No.	+ve %
Male	7	0%
Female	18	(3) 16%

The ethanolic extract of *C. verum* was tested for its minimum inhibitory antibacterial activities against *Proteus mirabilis*. Results demonstrated that the extract has an inhibitory effect against *P. mirabilis*. The minimum sensitivity concentration was 500 µg, which yielded a 19 mm zone of inhibition. The 250 µg concentration resulted in an intermediate zone of inhibition (11 mm), while the 125 µg concentration showed resistance (4 mm) (Table 5, Figure 2).

Results confirmed that *C. verum* alcoholic extract can be an effective antibacterial agent against *P. mirabilis*, agreeing with Al-Yasiri (2017), who tested the ability of Cinnamon Oil to inhibit both *P. mirabilis* and *Staphylococcus aureus*, with results comparable to those of the Ciprofloxacin antibiotic. Aljeboury (2022) reported similar results on the antibacterial activity of cinnamon oil against various bacterial strains, including *P. mirabilis*, which yielded moderate to extremely sensitive results depending on the isolate. Vasconcelos *et al.* (2018) proposed that cinnamon with its derivatives of extracts, essential oils, or even their compounds can inhibit bacterial activity by altering the lipid profile of the cell membrane, damaging it, inhibiting ATPases and biofilm formation, as well as other possible activities, in both gram positive and negative bacteria.

Table 5: Inhibition zone of *C. verum* extract against *P. mirabilis.*

No.	Concentration	Zone of Inhibition (mm)
1	2000 µg	33 mm
2	1000 µg	24 mm
3	500 µg	19 mm
4	250 µg	11 mm
5	125 µg	4 mm
6	Control	0 mm

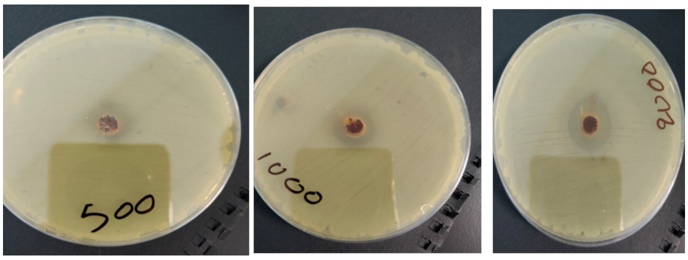

Figure 2: Minimum inhibitory concentration (MIC).

Plant extracts inhibit bacterial growth due to their multiple active ingredients, which can disrupt bacterial cell structures, interfere with their metabolism, and potentially suppress resistance mechanisms. These ingredients often work synergistically, enhancing the overall antibacterial effect (Orimaye *et al.*, 2024). Armbruster *et al.* (2018) investigated the phytochemical constituents and antibacterial potential of *Cinnamomum verum* extract against *Proteus mirabilis*, a substantially multi-resistant uropathogen isolated from cases of urinary tract infections. This study contributes to the existing body of literature that supports the importance of plant-derived antimicrobial agents as effective alternatives to synthetic antibiotics.

Exploration of the plant-based antibacterial *Cinnamomum verum* against resistant isolates of *P. mirabilis*. The increasing incidence of AMR, mainly because of the overuse and abuse of synthetic antibiotics (WHO, 2023), has attracted research efforts towards natural alternatives such as cinnamon, which are effective but relatively safe (Zhang *et al.*, 2021).

GC-MS analysis of *C. verum* ethanolic extract revealed the presence of various bioactive compounds, particularly cinnamaldehyde and coumarin, which significantly contributed to the composition of the extract. Cinnamaldehyde (59.48%) is known for its antimicrobial and anti-inflammatory properties (Sharma *et al.*, 2024), whereas coumarin (7.92%) has been reported to possess a wide range of pharmacological properties, including antibacterial and antioxidant effects (Samanth and Bhat, 2024). Results are based on the previous phytochemical evaluations of *C. verum*, which show strong medicinal potential (Singh *et al.*, 2020).

Current GC-MS analysis of *C. verum* has revealed that it contains a significant amount of cinnamaldehyde, coumarin, and numerous other bioactive components. These phytochemicals have broad-spectrum antimicrobial properties. The leading compound, cinnamaldehyde, exhibited great potency, with the potential to break bacterial cell walls and repress quorum sensing, as well as handle the quorum sensing system.

Cinnamon oil has an antibacterial mechanism of action by altering the cell membrane of bacteria, thereby enhancing its permeability and causing irreversible damage (Yap *et al.*, 2015). This effect is potentiated by the lipophilic character of cinnamaldehyde, which can integrate into and destabilise lipid membranes (Vasconcelos *et al.*, 2018). The concept of the synergistic ability of the extract, whether alone or in combination with other antibiotics, may provide an answer to antibiotic resistance, a growing problem worldwide (Barry, 2025).

The antibacterial effects of *C. verum* are multifactorial. Besides membrane depolarisation (Yap *et al.*, 2015), it has been demonstrated that cinnamon extracts lead to oxidative stress in bacterial cells by inducing ROS and consequent cell apoptosis (Marchese *et al.*, 2017). Additionally, the phenolic content in the extract may chelate bacterial enzyme-required metal ions, resulting in a decrease in pathogenic activity (Negi, 2012).

Wasfi *et al.* (2020) highlighted the difficulty encountered by *P. mirabilis*, which exhibits high biofilm formation and drug resistance, due to its large number of virulence factors. Because *Proteus* species are the most frequent isolate infecting many parts of Iraq (Ahmed, 2015), particularly in females, as patients' positive swab isolate rate exceeds that of men in this study, plant-derived antibacterial agents such as cinnamon that have been described to have antibacterial activity among others might have a promising value on treating infections.

P. mirabilis, which is distinguished by its ability to form strong biofilms and become resistant to the most potent broad-spectrum antibiotics, is a significant pathogen in hospitals (Schaffer and Pearson, 2015). The increasing rates of *P. mirabilis*-associated UTIs and their resistance patterns, particularly in developing countries such as Iraq, have strongly emphasised the necessity of new antimicrobial agents (Mancuso *et al*., 2021; Al-Shattrawi, 2024). The low MIC values exhibited by *C. verum* extract, with an inhibition zone of 19 mm at 500 µg, render it promising for further pharmaceutical developments.

The extract demonstrated appreciable antibacterial activity, with a minimum inhibitory concentration (MIC) of 500 µg, resulting in a 19 mm zone of inhibition. At 1000 µg and 2000 µg, the extract showed potent inhibitory effects, similar to those of ciprofloxacin, at concentrations of 24 mm and 33 mm (Al-Yasiri, 2017; Aljeboury, 2022). These outcomes are indicative of a dose-response relationship and further demonstrate the therapeutic potential of *C. verum* against resistant *P. mirabilis*.

Many reports have demonstrated the ability of cinnamon essential oil or extract to equal or exceed the antibacterial properties of conventional antibiotics, such as ciprofloxacin, particularly when used in combination with them (Gupta *et al*., 2008). A recent study published by Ribeiro-Santos *et al*. (2017) reported that cinnamon oil exhibits strong synergistic effects with other essential oils and antibiotics against both Gram-positive and Gram-negative bacteria. Such synergistic action is essential for the eradication of multidrug-resistant (MDR) organisms, such as biofilm producers, i.e., *P. mirabilis*.

In veterinary clinics, natural antimicrobials, such as cinnamon, may potentially function as prophylactic agents for use in animal feed or oral hygiene applications, thereby limiting the spread of zoonotic diseases (Nazzaro *et al*., 2013). This is not only for human health but also for One Health approaches, which are based on the interaction of human, animal, and environmental health.

CONCLUSION

P. mirabilis, which can coexist in human and canine oral cavities, as reported in the current study, suggests a potential zoonotic risk. The antibacterial activity of *C. verum*, particularly against *P. mirabilis*, is reaffirmed in this study, supported by both strong experimental evidence in animals and phytochemical validation. The presence of metabolites such as cinnamaldehyde and coumarin indicates cinnamon as a promising plant-based antimicrobial agent. Subsequent research may be directed toward its clinical application, synergistic effects with traditional antibiotics, and the formulation into therapeutic products.

ACKNOWLEDGEMENT

This work would not have been completed without the support of the University of Baghdad college of Veterinary Medicine, and the support of Zoonosis Diseases Research Unit.

NOVELTY STATEMENT

This manuscript presents *Cinnamomum verum* (*C. verum*) as a novel natural alternative to antibacterial agents, aiming to combat the rising threat of multi-drug resistant bacteria like *Proteus mirabilis* prevalent in the current global health crisis.

AUTHOR'S CONTRIBUTION

Linah Ibrahim Lateef proposed the idea, provided the extraction work, helped with the lab work, and part of the literature collection and reviewing. Hassan F. Mohammed contributed with the isolation and primary identification of the bacteria and the lab work as well as the writing of the results in relation to isolation and lab results. Ban S. Al-Nasiry provided literature writing and auditing. Ibrahim M. Kamal provided further auditing and supplying materials for the work.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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