



## Original Article



## Antibacterial Efficacy of Cinnamon Essential Oil Compared to Standard Antibiotics on Diabetic Foot Ulcer Isolates at Tertiary Care Units Karachi, Pakistan

Ubaid Ullah<sup>1</sup>, Sameen Adib Rahman<sup>2</sup>, Kahkashan Perveen<sup>3\*</sup>, Benish Zafar<sup>3</sup>, Ayaz Khan<sup>1</sup> and Ashfaq Ahmad<sup>4</sup><sup>1</sup>Department of Medical Technology, Baqai Institute of Medical Technology, Baqai Medical University, Karachi, Pakistan<sup>2</sup>Jinnah Medical and Dental College, Karachi, Pakistan<sup>3</sup>Department of Biochemistry, Baqai Medical University, Karachi, Pakistan<sup>4</sup>Department of Hematology, Baqai Medical University, Karachi, Pakistan

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## \*Corresponding Author:

Kahkashan Perveen  
Department of Biochemistry, Baqai Medical University, Karachi, Pakistan  
[dr.kahkashan@baqai.edu.pk](mailto:dr.kahkashan@baqai.edu.pk)Received Date: 29<sup>th</sup> August, 2025Revised Date: 10<sup>th</sup> November, 2025Acceptance Date: 21<sup>st</sup> November, 2025Published Date: 30<sup>th</sup> November, 2025

## ABSTRACT

Lower limb complications associated with Type II Diabetes have become a serious public health issue in today's world. Diabetic patients with ulcers commonly experience infection due to Gram-positive, Gram-negative bacteria, as well as some anaerobes. **Objectives:** To investigate the antibacterial activity of Cinnamon essential oil on microbial isolates from diabetic foot ulcer. **Methods:** An experimental investigation was carried out on diabetic patients suffering from foot ulcers. A total of 115 pus swab samples from diabetic foot ulcer patients were acquired, who were recruited and processed at Baqai Institute of Diabetology and Endocrinology. Antibiotic sensitivity testing was performed by the Kirby agar Disc diffusion method. Antibacterial activity of cinnamon essential oil was checked by the Kirby agar well diffusion method. **Results:** Out of 115 samples, 138 bacteria were isolated. 80% of samples were mono-microbial and 20% were poly-microbial. *Pseudomonas Aeruginosa* was the most frequently encountered bacterium, followed by *E. Coli*, *S. Aureus*, *Klebsiella Pneumoniae*, *Proteus Mirabilis*, *Enterococcus* species, Coagulase-negative *Staphylococcus* Species, and *Proteus Vulgaris*. All the isolated bacteria showed different resistance patterns against commercially available antibiotics. Cinnamon essential oil showed antibacterial activity against all isolated bacteria. **Conclusions:** The study demonstrated a high bacterial isolation rate from diabetic foot ulcer. Antibiotic resistance varied among bacterial isolates, highlighting the challenge of treating bacterial infections. Cinnamon essential oil exhibited strong antibacterial activity against all isolated bacteria, suggesting its potency of being used as an alternative antimicrobial agent.

## INTRODUCTION

Diabetes is a chronic illness that develops when insulin is not produced in an adequate amount in the body, or it becomes resistant to its effects, or a combination of both, and has serious health issues, affecting millions of individuals worldwide [1, 2]. The dysfunction of insulin action, whether due to insufficient production or resistance, results in the onset of Type I and Type II diabetes, respectively. In type II diabetes, Lower limb

complications are rising worldwide wide which include diabetic foot ulcers, infections, and even amputations, particularly in underdeveloped countries [3, 4]. Limited access to quality healthcare, poor diabetes management, and lack of awareness contribute to the increasing burden of these conditions. Without timely intervention, lower limb complications can significantly affect a patient's lifestyle and put the patient under financial strain for



affording higher expenses for management of lower limb complications [5, 6]. Peripheral neuropathy, along with macrovascular and microvascular complications, plays an important role in increasing the risk of diabetic foot ulcers. Additionally, the duration of diabetes and traumatic injuries further contributes to the development of these ulcers [7]. Most Gram-positive bacteria, *S. Aureus*, *Enterococcus* species, Gram-negative bacteria, including *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella* species, *Proteus* species, as well as certain anaerobes are responsible for developing diabetic foot ulcers [8]. The delayed wound healing in Diabetic patients presents various complex pathological processes that interfere with the normal healing process, which tends to be a challenge for clinicians [9, 10]. In these patients, chronic hyperglycaemia causes microvascular damage, neuropathy, and immune dysfunction, all of which result in hindrance to the normal healing process [11, 12]. Specific features of diabetic wounds are associated with delayed healing, poor blood vessel formation, reduced collagen synthesis, and a high risk of infection, resulting in prolonged, non-healing ulcers. Current treatments for diabetic wounds mainly aim to control blood sugar, prevent infection, and relieve pressure on the wound area [13]. Despite the use of currently available antibiotics, diabetic foot ulcers often show poor response and repeated occurrence. Due to these facts, there is an urgent need for novel therapeutic approaches. Use of natural products with anti-inflammatory, antioxidant and healing and healing properties [14]. The quality of Cinnamon to cure wounds has already been studied in many recent studies, but none of them specifically demonstrate cinnamon's ability to heal wounds in diabetic conditions. It is observed that Cinnamon essential oil has strong antimicrobial and antioxidant effects, due to higher content of compounds like cinnamaldehyde. Cinnamon verum (commonly known as cinnamon) is a highly effective medicinal plant belonging to the Lauraceae family. Cinnamon essential oils can be extracted from leaves, fruits, and bark of the plants, which are known for their therapeutic and pharmacological properties [15]. Due to its multifaceted medicinal benefits, cinnamon continues to be explored as a natural remedy for various metabolic, infectious, and inflammatory conditions in both traditional and modern medicine [16]. Cinnamon extracts, essential oils, and their compounds have been reported to inhibit bacteria by damaging cell membranes; inhibiting cell division, membrane porins, and biofilm formation; and via anti-quorum-sensing effects. In view of the above-mentioned properties of cinnamon essential oil, the current study will be conducted to find out the antibacterial activity of cinnamon essential oil against isolated bacteria of DFU. Standard antibiotics refer to those antibiotics that are commonly used by physicians to

treat infection and are also used in microbiology laboratories for antibacterial susceptibility testing.

This study aimed to check the antibacterial activity of cinnamon essential oil against isolated bacteria from diabetic foot ulcer patients and compare it with standard antibiotics.

## METHODS

This in vitro comparative experimental study was conducted at Baqai Institute of Diabetology and Endocrinology. The research work was conducted over a period of nine months (April 2023 to January 2024) on 115 active diabetic foot ulcer patients. The study was approved by the Ethics Committee (Ref: BMU-EC/01-2023) of Baqai Medical University, Karachi, and the Board of Advanced Studies and Research (BASR), Baqai Medical University, Karachi, in accordance with the international guidelines outlined in the 2008 WMA Declaration of Helsinki for Medical Research involving Human Participants. The samples were collected and processed by simple techniques used in microbiology studies. These individuals, having been diagnosed as diabetic foot ulcer patients with type II diabetes and who did not receive systemic and topical antibiotics or other medications, including steroids, from 24 to 72 hours, were included in this study. Pus samples were collected from these patients, bacteria were isolated and grown, and both different standard antibiotics and cinnamon essential oils were used to check their antibacterial activity against these isolated bacteria. Patients who had received systemic and topical antibiotics and other medicines, including steroids, for 24 to 72 hours were excluded from this study. Patients having diabetes other than type II were also excluded from this study. The calculated Sample size was 115 by using Open Epi Version 3.0.1, named as Open-Source Epidemiologic Statistics for Public Health [17]. Samples were collected from diabetic patients after obtaining their consent. A pus sample was collected from subjects by a sterile amine transport swab. The sample was inoculated on Sheep Blood Agar, McConkey Agar, and Chocolate Agar medium and incubated at 37°C for 24-48 hours according to Clinical Laboratory Standardization Institution (CLSI). After 48 hours, bacterial growth was observed. Identification was carried out by evaluating the appearance of colonies by Gram staining. Biochemical tests were conducted for the identification of isolated bacterial pathogens according to the protocol described by CLSI guidelines. All isolated bacteria isolated from Diabetic Foot Ulcers were divided into two broad groups: mono-microbial isolates and poly-microbial isolates. They were further divided into Gram-positive and Gram-negative bacteria. Antibacterial activity of all the standard antibiotics and cinnamon essential oil was determined by

measuring the zone of inhibition. By the disc diffusion method, Antimicrobial Susceptibility Testing (AST) was performed. 0.5 MCF suspension of bacterial colony was prepared and was made uniform by spreading on Muller-Hinton Agar (MHA) medium and incubated at 37°C for 24 hours. After 24 hours, the zone of inhibition was measured and compared with the standard zone of inhibition as described by CLSI guidelines. Commercially available Cinnamon essential oil was purchased and sent to the PCSIR Laboratory Karachi to check purity with the designated Sr No PCSIR KLC 5022069. Cinnamon essential oil was used in various dilution forms, from high to low dilution. Dilution forms of cinnamon essential oil used in the study were 2.5 mg/ml, 5.0mg/ml, 10.0mg/ml, 20.0mg/ml, and 40.0mg/ml. Cinnamon essential oil was diluted in Propylene glycol as done in a previous study [18]. Antibacterial sensitivity of Cinnamon essential oil was performed by the Kirby-Bauer agar well diffusion method. A diluted sample of Cinnamon essential oil was poured by an adjustable micro pipette into the wells designed in the MHA media plate. After adding cinnamon essential oil, the plates were incubated at 37°C for 24 hours. After 24 hours, the zone of inhibition was measured. The sensitivity of cinnamon essential oil was calculated by measuring the increase in zone of inhibition by serial dilution from a lower concentration to a higher concentration. No statistical analysis software was used to interpret the results; it was a simple comparative study in which the antibacterial activity was observed by measuring and comparing the zone of inhibition in which the zone of inhibition of the standard antibiotic with that of cinnamon essential oil against the bacteria isolated from diabetic foot ulcer patients.

## RESULTS

All specimens showed growth of bacteria. Samples collected from 92 patients showed mono-microbial growth, and samples collected from 23 patients showed poly-microbial growth. Out of 115 collected specimens, a total of 138 bacterial species were isolated, of which 36 (25.94%) were gram-positive and 102 (73.73%) were gram-negative organisms. Gram-negative bacteria, *Pseudomonas Aeruginosa*, showed maximum prevalence in the isolates of DFU (Table 1).

**Table 1:** Distribution of Organisms Isolated from Diabetic Foot Ulcer Patients

Isolated Organism	Frequency (%)
Total Sample	115
Mono-microbial isolates	92 (80%)
Polymicrobial isolates	23 (20%)
Total bacterial Isolates	138

Gram Positive Bacteria	<i>Staphylococcus Aureus</i>	20 (14.4%)
	Coagulase-negative <i>Staphylococcus</i> Species (CONS)	02 (1.44%)
	<i>Enterococcus</i> Species	14 (10.1%)
Gram-negative Bacteria	<i>Pseudomonas Aeruginosa</i>	43 (31.1%)
	<i>E. coli</i>	29 (21.01%)
	<i>Klebsiella Pneumoniae</i>	19 (13.7%)
	<i>Proteus Mirabilis</i>	10 (7.2%)
	<i>Proteus Vulgaris</i>	01 (0.72%)
Methicillin-sensitive <i>Staphylococcus Aureus</i> (MSSA)	—	12
Methicillin-resistant <i>Staphylococcus Aureus</i> (MRSA)	—	08

Commercially available antibiotics show different sensitivity results. It is observed that Meropenem, Imipenem, and linezolid were the most sensitive antibiotics against isolated bacteria (Table 2).

**Table 2:** Antibiotic Profile of Gram-Negative Bacteria Isolated from Wounds of Diabetic Foot Ulcer

Antibiotics	P. Aeruginosa		P. Mirabilis		P. Vulgaris		E. Coli		K. Pneumoniae	
	S	R	S	R	S	R	S	R	S	R
Ampicillin	—	—	0	10	0	1	—	—	—	—
Amoxil/Clavulanic Acid	11	32	0	10	0	1	19	10	4	15
Piperacillin/Tazobactam	23	20	2	8	0	1	19	10	6	13
Ceftriaxone	39	4	0	10	0	1	19	10	4	15
Cefuroxime	39	4	0	10	0	1	19	10	4	15
Cefixime	25	19	0	10	0	1	19	10	4	15
Cefepime	25	19	0	10	0	1	—	—	—	—
Meropenem	25	19	9	1	1	0	1	28	19	0
Imipenem	10	33	9	1	1	0	1	28	19	0
Gentamicin	10	33	6	4	1	0	10	19	13	6
Amikacin	23	20	6	4	1	0	10	19	13	6
Tobramycin	11	32	6	4	1	0	10	19	13	6
Levofloxacin	23	20	4	6	0	1	13	16	10	9
Ciprofloxacin	39	4	4	6	0	1	13	16	10	9
Co-troximazole	39	4	4	6	0	1	13	16	10	9
Ceftazidime	25	19	4	6	1	0	—	—	—	—
Doxycycline	—	—	4	6	1	0	13	16	15	4
Polymyxin B	—	—	—	—	—	—	—	—	—	—
Colistin	4	0	1	0	—	—	1	0	—	—

Commercially available antibiotics show different sensitivity results (Table 3).

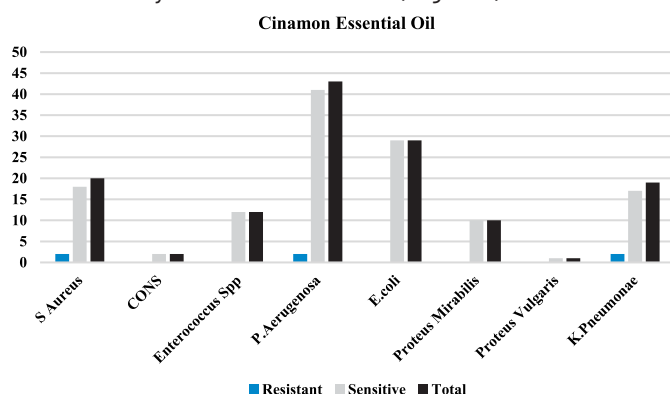
**Table 3:** Antibiotic Profile of Gram-Positive Bacteria Isolated from Wounds of Diabetic Foot Ulcers

Antibiotics	S. Aureus		CONS		Enterococcus species	
	S	R	S	R	S	R
Ampicillin	—	—	—	—	11	3
Cefoxitin	12	8	2	0	—	—
Ciprofloxacin	12	8	2	0	—	—

Co-troximazole	12	8	2	0	—	—
Erythromycin	12	8	2	0	4	10
Clindamycin	12	8	2	0	4	10
Linezolid	20	0	2	0	—	—
Fusidic Acid	11	9	2	0	—	—
Doxycycline	12	8	2	0	—	—
Vancomycin	8	0	—	—	14	0

S=Sensitive, R=Resistant

Cinnamon essential oil shows a wide range of antibacterial activity against different isolated bacteria compared to commercially available antibiotics (Figure 1).



**Figure 1:** Antibacterial Potential of Cinnamon Essential Oil Against Bacteria Isolated from Diabetic Foot Ulcer Patients

## DISCUSSIONS

Among the complications related to diabetes, Diabetic foot ulcer (DFU) is one of the most frequently occurring. Patients with peripheral neuropathy (PN) and peripheral vascular diseases (PVD) are more vulnerable to developing foot problems such as ulcers, infection, and if the condition is not managed properly, then it would lead to amputation. PN and PVD are one of the prevalent causes of non-traumatic lower limb amputation. DFU is influenced by several risk factors like neuropathy, vasculopathy, immunopathy, mechanical stress, and neuroarthropathy. These factors cause low blood flow and tissue hypoxia in various body parts, leading to a weak immune response toward pathogenic organisms, which then delays healing of tissues [19]. Various studies have conducted evaluations of the pathogenic profile of diabetic foot ulcer (DFU) patients, among which some have identified the presence of both mono-microbial and poly-microbial infections in the subjects included in those studies. The bacterial organisms isolated in previous studies include both Gram-positive and Gram-negative organisms. The Gram-negative bacteria include species of *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus species*, *Klebsiella pneumoniae*, *Acinetobacter*, and *Enterobacter species* are among the gram-negative bacteria which have been frequently observed. Meanwhile, the Gram-positive bacteria identified include *Staphylococcus aureus* and

various *Streptococcus* species. These findings highlight the diverse microbial landscape associated with DFU infections [20, 21]. In the present study, researchers collected a total of 115 samples from diabetic foot ulcers. Out of 115 samples, 92 samples (88%) were found to be mono-microbial, and 23 were found to be poly-microbial. A total of 138 bacteria were obtained from 115 patients. Different bacterial species were isolated from diabetic foot ulcer patients. Gram staining, colony morphology, and various biochemical tests were used to identify the bacterial isolates. The predominant bacteria isolated were *P. aeruginosa* found in 31.1% followed by *E. coli* 21.01%, *Staphylococcus Aureus* 14.4%, *Klebsiella Pneumoniae* 13.7%, *Enterococcus species* 10.1%, *Proteus Mirabilis* 7.2%, Coagulase-negative *staphylococcus species* 1.44% and *Proteus Vulgaris* found in 0.72%. Our findings are in alignment with the results reported by Singh et al. [15]. The authors reported a predominant prevalence of *P. aeruginosa* (27.3%) followed by *S. Aureus*, *E. coli*, *Proteus species*, and *Klebsiella species* in Diabetic Foot Ulcers (DFU). Our results are also supported by the findings of Naeem et al. and Miyan et al. which reported the presence of *S. aureus*, *S. epidermitis*, *Proteus species*, *Enterococcus species*, and *Klebsiella species* in the DFU samples [22, 23]. The antibiotic susceptibility profile of organisms isolated from diabetic foot ulcers exhibits varying resistance patterns. Previous studies have also reported the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant (MDR) bacteria. Among the antibiotics tested, linezolid (LZD) and vancomycin (VA) were found to be the most effective against Gram-positive bacteria, while meropenem (MEM) demonstrated the highest sensitivity against Gram-negative bacteria. Conversely, the most resistant antibiotics were penicillin G for Gram-positive isolates and amoxicillin-clavulanate (AMC) and trimethoprim-sulfamethoxazole (SXT) for Gram-negative isolates. These findings emphasize the need for continuous surveillance of antibiotic resistance patterns to guide effective treatment strategies for diabetic foot infections [24]. In the current study, antibiotic susceptibility testing showed different resistance patterns against these isolated bacterial species. Methicillin-resistant *Staphylococcus Aureus* (MRSA) and multidrug-resistant (MDR) organisms were found in 08 and 06 isolated organisms, respectively. All the bacterial isolates showed a significant resistance pattern towards most of the antibiotics. *Pseudomonas Aeruginosa* showed high resistance rates toward Ciprofloxacin and Levofloxacin and was sensitive toward Meropenem and Imipenem. *E. coli* resistance rates were at the highest percent toward Amoxil/Clavulanic Acid, Piperacillin/Tazobactam, Ceftriaxone, Cefuroxime, and Cefixime, and were sensitive

toward Meropenem and Imipenem. *Staphylococcus Aureus* shows a higher sensitivity rate toward Linezolid. Many traditional remedies have already been tested against different bacteria isolated from various infections to overcome the resistance of antibiotics against many bacteria. Considering these promising findings, the present research was conducted to assess the antibacterial activity of cinnamon essential oil. This investigation explored their potential as effective antimicrobial agents, which may contribute to the development of alternative therapeutic approaches for combating bacterial infections. In the present research project, Cinnamon essential oil was tested against bacteria isolated from diabetic foot ulcer patients in different dilutions. Cinnamon essential oil gives a maximum zone of 25mm against sensitive bacteria. Cinnamon essential oil inhibited the growth of *E. Coli*, *Proteus Mirabilis*, *Proteus Vulgaris*, *Enterococcus* species by 100%, *S. Aureus* was inhibited by 90%, *Pseudomonas Aeruginosa* by 95% and *Klebsiella Pneumoniae* was inhibited by 89%. Considering these promising findings, the present study was conducted to assess the antibacterial activity of cinnamon essential oil. This investigation explored their potential as effective antimicrobial agents, which may contribute to the development of alternative therapeutic approaches for combating bacterial infections. In the present study, Cinnamon essential oil was tested against bacteria isolated from diabetic foot ulcer patients in different dilutions. Cinnamon essential oil gives a maximum zone of 25mm against sensitive bacteria. Cinnamon essential oil inhibited the growth of *E. Coli*, *Proteus Mirabilis*, *Proteus Vulgaris*, *Enterococcus* species by 100%, *S. Aureus* was inhibited by 90%, *Pseudomonas Aeruginosa* by 95% and *Klebsiella Pneumoniae* was inhibited by 89%. This is also supported by a study conducted by Shu et al. and Radwan et al. [25, 26]. These results show the increasing challenge of antibiotic resistance in diabetic foot ulcers (DFUs), which can complicate treatment and lead to poor clinical outcomes if not effectively managed. The presence of multidrug-resistant (MDR) microorganisms, such as *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA), indicates that commercially available antibiotics may no longer be sufficient to treat these infections. In the present study, cinnamon essential oil (CEO) demonstrated even stronger antibacterial effects, effectively inhibiting a broader range of pathogens, making it a more potent candidate for DFU treatment.

## CONCLUSIONS

The study demonstrated a high bacterial isolation rate from diabetic foot ulcer. Antibiotic resistance varied among bacterial isolates, highlighting the challenge of treating bacterial infections. Cinnamon essential oil exhibited

strong antibacterial activity against all isolated bacteria, suggesting its potential as an alternative antimicrobial agent.

## Authors Contribution

Conceptualization: UU, KP

Methodology: UU, AK

Formal analysis: UU

Writing review and editing: UU, SAR, KP, BZ, AK, AA

All authors have read and agreed to the published version of the manuscript

## Conflicts of Interest

All the authors declare no conflict of interest.

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