Journal of Applied Pharmaceutical Science Vol. 15(09), pp 041-051, September, 2025
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2025.250483
ISSN 2231-3354

Antibiogram and antibacterial activity of *Crassocephalum crepidioides* (Thickhead) leaf extract against the wound isolates

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ARTICLE HISTORY

Received on: 02/04/2025
Accepted on: 06/07/2025
Available Online: 05/08/2025

Key words:
Wound infection, bacterial isolates, antibiotic sensitivity, *Crassocephalum crepidioides* leaf extract, antibacterial activity

ABSTRACT

This study aims to evaluate the antibiogram and antibacterial activity of *Crassocephalum crepidioides* leaf extract against the bacterial strains isolated from infected wounds. A total of 69 swab samples were obtained from various cases of infected wounds and 20 pure bacterial strains were isolated. The most prevalent organisms isolated from wound infections were *Staphylococcus* species and *Escherichia coli* (25%), followed by *Klebsiella* species (15%), *Proteus* species (10%), *Providencia* species (10%), *Pseudomonas aeruginosa* (5%), *Acinetobacter baumannii* (5%), and *Enterobacter hormaechei* (5%). The susceptibility pattern of all bacterial isolates was assessed against antibiotic discs using the Kirby Bauer Disc diffusion method. The results revealed that Gram-positive cocci exhibited 100 % susceptibility to Amikacin, Bacitracin, Oxytetracycline, and Vancomycin, however, showed 80% resistance to Novobiocin, Amoxicillin, Cephalothin, Erythromycin. Conversely, Gram-negative bacilli exhibited high resistance levels, including 86.7% to Ciprofloxacin, 80% to Carbericillin and Nitrofurantoin, 66.7% to Streptomycin and Tetracycline, 60% resistance to Co- Trimazine; however, they showed 73.3% sensitivity to Amikacin and 53.3% sensitivity to Kanamycin. Among the 20 bacterial strains, 13 (65%) were identified as multidrug-resistant (MDR) and 4 (20%) were extensively drug-resistant (XDR). *In vitro* antibacterial activity assay revealed that *C. crepidioides* leaf extract was found to be effective against all the *Staphylococcus* spp., *E. hormaechei*, *A. baumannii*, *Providencia* spp., two *E. coli* isolates and one *Klebsiella* spp. with the zone size ranging from 10.83 ± 0.28 to 25.83 ± 1.04, with minimum inhibitory concentration between 2.5 and 40 mg/ml, however, resistant to *P. aeruginosa*, *Proteus* spp., three *E. coli* isolates and two *Klebsiella* spp. *Staphylococcus* spp. was found to be the most inhibited wound isolates by *C. crepidioides* leaf extract. These findings suggest that *C. crepidioides* leaf extract has the potential to develop antibacterial agents against the MDR and XDR organisms causing wound infection, emphasizing the significant role of plant extracts in treating bacterial wound infections, thereby preventing the delay of the wound healing process.

INTRODUCTION

The skin serves as a protective barrier against bacterial infection. A wound occurs when its normal anatomical structure is disrupted due to surgical procedures or chemical, physical, mechanical, or thermal factors, resulting in impaired skin functions [1]. Polymicrobial organisms, including bacteria, viruses, and fungi, can easily penetrate wounds through subcutaneous tissues, where they can thrive and multiply in a

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supportive environment [2]. When microorganisms proliferate to the extent that they cause local and or systemic infection, the condition is referred to as a wound infection. Microorganisms in the wound lead to tissue damage locally and prevent the wound from healing [3]. The warm, moist, and nutrient-rich environment of wounds promotes microbial colonization and proliferation, increasing their contagiousness [4]. Wound infections resulting from microbial invasion are among the most common public health concerns. The most frequent pathogenic bacteria that cause wound infection includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Proteus* species [5,6]. These pathogens can cause significant tissue damage and non-healing wounds by impairing the immune system with its virulence factors [7]. Wounds are classified into two types: acute and chronic. Acute wounds, such as cuts, burns, abrasions, and surgical wounds, result from external factors and typically heal through the natural stages of wound repair [6]. An infected wound can delay the healing rate and negatively impact the quality of life [8]. Conversely, chronic wounds, such as leg or arterial ulcers, take a longer time to heal and are primarily caused by internal factors that can be associated with diseases such as diabetes, over weight, immune deficiencies, and microbial infections, which can further exacerbate the wound [7]. Wound infections constitute one third of nosocomial infections among surgical patients and contribute to 70%–80% of mortality [5,9,10]. The rise of antibiotic-resistant strains, coupled with the scarcity of new-generation antibiotics, and their high-cost increased wound-related morbidity and mortality [11].

Discovering a novel treatment approach to combat wound infections is crucial since these infections negatively impact the patient's mental health and result in exorbitant medical expenses. More than 80% of people worldwide still rely on traditional medicines to treat a variety of illnesses, according to a survey conducted by World Health Organization [12]. Many plants and natural products possess antibacterial, antifungal, and antiprotozoal properties, making them suitable for both systemic and local applications. The medicinal use of plants is widely preferred due to their strong pharmacological effects, minimal toxicity, and cost-effectiveness compared to synthetic drugs. Medicinal plants are rich in diverse bioactive secondary metabolites, including tannins, terpenoids, alkaloids, saponins, flavonoids, and phenolic compounds, and can have distinct physiological effects on the human body [13].

Medicinal plants are effective in treating infectious diseases and various types of external wounds, including chronic, deep suppurative, open, lacerated, incised, and ulcerated wounds, and have been utilized for these purposes in both humans and various animal species. Their use offers the advantage of minimizing many side effects commonly associated with synthetic antimicrobials [14,15].

Crassocephalum crepidioides, a wild plant from the Asteraceae family that grows widely in tropical and sub-tropical regions. It is known as Thickhead, Redflower rag leaf, Fireweed, and locally referred to as Terapaibi in Manipur, India. It has been traditionally used for treating a variety of ailments. In Manipur, a paste made from its leaves is commonly applied to minor wounds by the local population [16,17]. However, there has been less scientific evidence to prove the wound healing potential of *C. crepidioides*. There are several reports on antibacterial, antioxidant properties [17], anti-inflammatory [18], and antidiabetic properties [19]. However, the antibacterial activity of *C. crepidioides* leaf extract against the bacterial strains isolated from the infected wound has not yet been reported. Hence, this study aims to analyze the bacterial profile of infected wounds, access their antibiotic susceptibility patterns, and evaluate the in vitro antibacterial activity of *C. crepidioides* leaf extract against clinical wound isolates.

MATERIALS AND METHODS

Plant collection and extraction

Crassocephalum crepidioides leaves were collected from various regions of Kakching District, Manipur, India, and was authenticated by Dr. P. Palani, Centre for Advanced Studies in Botany, University of Madras, Chennai, with the voucher specimen number MUBL116. The leaves were extracted following the method described in the previous study by Devi *et al*. [17]. Briefly, the plant material was washed with distilled water, shade dried, and finally ground using an electric grinder. 25 g of powdered plant material was mixed with 250 ml of sterile distilled water and heated in a hot plate at 60° for 2 hours with intermittent shaking. The mixture was then filtered first through muslin clot and subsequently through Whatman No.1 filter paper. The yield percentage was calculated using the formula, weight of the dried extract/weight of the initial dried sample × 100 [20].

Study area and time frame

This cross-sectional study was conducted over a span of 7 months, from November 2023 to June 2024. Wound samples showing signs of infection received at Laboratory of Sathyabama General Hospital, Chennai, were included in the study. Wounds from cuts, burns, abrasions, and surgical wounds with signs of infection before administration of antibiotics were considered in this study. However, samples from wounds of different aetiologies such as leg ulcers, diabetic foot ulcers, and pressure ulcers were excluded. Ethical approval for this study was granted by the Institutional Human Ethical Committee of the Sathyabama Institute of Science and Technology, India, under certificate number 330/IRB-IBSEC/SIST Dated 18th October 2023.

Collection and identification of bacterial wound isolates

A total of 69 wound swab samples were collected from the laboratory of Sathyabama General Hospital and transported within an hour to the Microbiology Laboratory of Sathyabama Dental College and Hospital, Tamil Nadu, India. Subsequently, each specimen was cultured on various agar media, including nutrient agar plates, blood agar, MacConkey agar, mannitol salt agar, and eosin methylene blue. Preliminary bacterial identification was carried out based on colony morphology. Further characterization was performed through Gram staining

and Biochemical tests, followed by identification using the automated VITEK MS (Biomerieux).

Inoculum preparation

A single colony of each organism from 24 hours old culture plate was picked and inoculated separately into sterile nutrient broth. The cultures were then incubated at 37°C for 3 hours, after which the turbidity of the bacterial suspension was adjusted to a density of 1.5×10^8 CFU ml^{-1}, equivalent to the 0.5 McFarland standard [21].

Antibiotic susceptibility test

The antibacterial susceptibility of wound isolates was assessed using the Kirby–Bauer disc diffusion method [21]. A lawn culture of the isolates was prepared on sterile Muller–Hinton agar (MHA) plates. Antibiotics discs for Gram-Positive bacteria included Amikacin (AK 10 µg), Amoxicillin (AMX 10 µg), Bacitracin (B 10 units), Cephalothin (CEP 30 µg), Erythromycin (E 15 µg), Novobiocin (NV 30 µg), Oxytetracycline (O 30 µg), and Vancomycin (VA 30 µg). For Gram-negative bacteria, the antibiotics tested were Amikacin (AK 10 µg), Carbericillin (CB 100 µg), Ciprofloxacin (CIP 10 µg), Co-Trimazine (CM 25 µg), Kanamycin (K 30 µg), Nitrofurantoin (NIT 300 µg), Streptomycin (S 10 µg), and Tetracycline (TE 30 µg). Antibiotics used in the study were purchased from HiMedia, Maharashtra, India. After incubating the plates at 37°C for 24 hours, zone diameters were measured in mm and the results were interpreted as sensitive, intermediate, or resistant according to the Clinical Laboratory Standard Institute guidelines [22]. Organisms exhibiting resistance to three or more antibiotics were classified as multidrug-resistant (MDR) [23]. Extensively drug-resistant (XDR) organisms were defined as those resistant to all antimicrobial agents except for two or fewer antimicrobial categories, and pan drug-resistant organisms were the organisms that were resistant to all antimicrobial agents [24,25].

Antibacterial activity assay

The culture was evenly spread onto MHA using a sterile cotton swab. On each plate, equidistant wells, 8 mm in diameter, were created using a gel puncher, positioned 2 mm from the plate edge. Aseptically, 100 µl of the plant extract (500 mg/ml) was introduced into the respective wells. Ciprofloxacin (5 µg) served as the standard, while distilled water was used as the negative control. The plates were left undisturbed for 40 minutes to allow pre-diffusion, then, incubated at 37°C for 24 hours [26]. The assay was conducted in triplicates.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The agar well diffusion method was carried out to determine the MIC of *C. crepidioides* leaf extract against bacteria isolated from wounds. Lawn culture of the bacterial isolates was made onto the Mueller–Hinton agar plates using sterile cotton swabs. Different concentrations (6.25, 12.5, 25, 50, 100, 200, and 400 mg/ml) of the extract were prepared using double-fold serial dilutions. Wells were then created into the agar plates, and 100 µl from various concentrations was transferred into the respective wells. The plates were left undisturbed for 30 minutes at room temperature to allow proper diffusion, followed by incubation at 37°C for 24 hours. Distilled water was used as the negative control, while ciprofloxacin (5 µg) was used as the standard antibiotic. After incubation, zones of inhibition were measured, and the lowest concentration of the extract that inhibited the growth of microorganisms was recorded as the MIC [27,28].

MBC was performed by touching the inhibition zone of MIC plates of the four lowest concentrations of the plant extract that showed no visible bacterial growth and subculture onto the Nutrient agar plates. The sub cultured plates were then incubated at 37°C for 24 hours, after which bacterial growth on these plates was assessed. The concentration of the plant extract that did not produce any bacterial growth on freshly inoculated plates was recorded as the MBC [28].

Statistical analysis

Data were expressed as mean ± SD, analyzed using One-way ANOVA, and the statistical difference was evaluated by Dunnett *t* tests using SPSS software version 25 (p-value ≤ 0.05 is considered statistically significant).

RESULTS

Yield of plant extract and properties

The percentage yield of the hot aqueous extract of *C. crepidioides* was found to be 16.13%. The obtained extract was brown in color, and amorphous powder in nature.

Identification of bacterial wound isolates

A total of 20 pure bacterial species were isolated from 69 wound samples. The distribution of these isolates was as follows: 5 (25%) GPC and 15 (75%), GNB as shown in Figure 1. The identified organisms included 5 *Staphylococcus species* (25%), 5 *E. coli* (25%), 3 *Klebsiella* spp. (15%), 2 *Proteus spp*. (10%), 1 *P. aeruginosa* (5%), 1 *A. baumannii* (5%), 1 *E. hormaechei* (5%), and 2 *Providencia species* (10%) (Fig. 2).

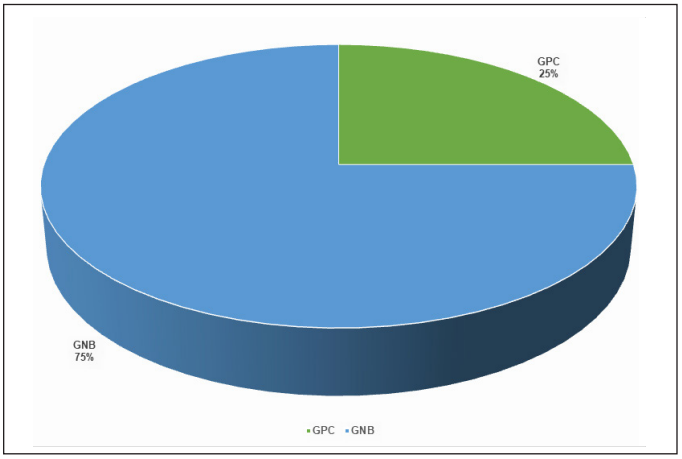

Figure 1. Incidence of Gram-positive cocci and Gram-negative bacilli causing wound infections (n = 20).

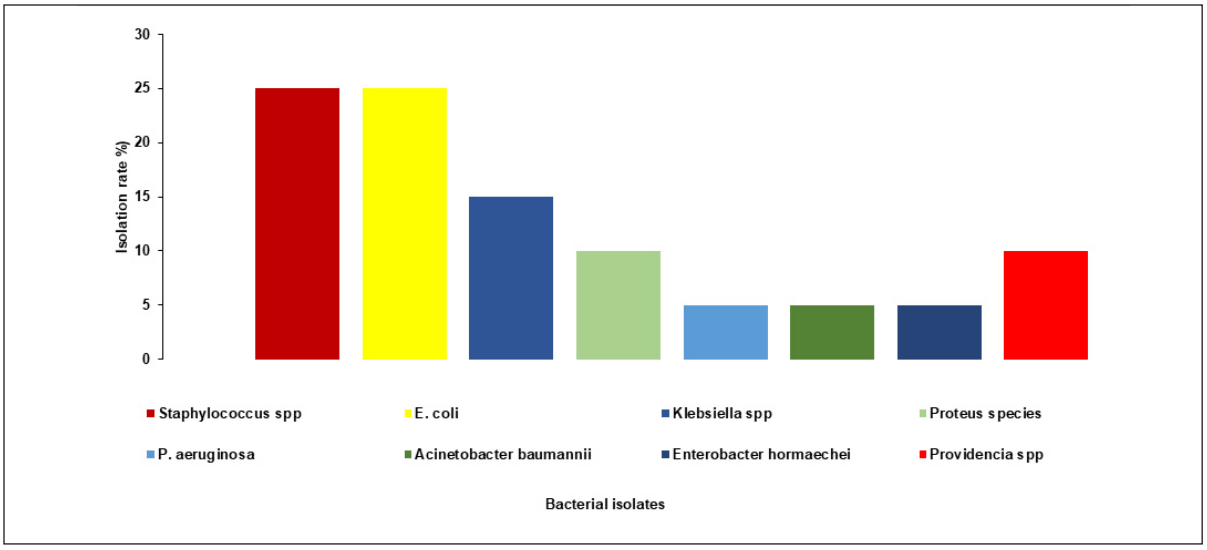

Figure 2. Distribution of bacterial isolates from wound infection.

Table 1. Susceptibility pattern of Gram-positive cocci against standard antibiotics.

Bacterial isolates	Antibiotics							
	AK	AMX	B	CEP	E	NV	O	VA
Staphylococcus aureus	S	R	S	R	R	S	S	S
Staphylococcus epidermidis	S	R	S	R	R	S	S	S
Staphylococcus spp.1	S	R	S	R	R	S	S	S
Staphylococcus spp.2	S	R	S	R	R	S	S	S
Staphylococcus spp.3	S	S	S	S	S	R	S	S

The diameters of the inhibition zones were interpreted following CLSI guidelines, categorizing the tested isolates as Susceptible (S), Intermediate (I), or Resistant (R) to the antibiotics evaluated. AK = amikacin; AMX = amoxicillin; B = bacitracin; CEP = cephalothin; E = erythromycin; NV = novobiocin; O = oxytetracycline; VA = vancomycin.

Antibiotic susceptibility test

The results revealed that GPC exhibited 100 % sensitivity to Amoxicillin, Bacitracin, Oxytetracycline, and Vancomycin, however, showed 80% resistance to Novobiocin, Amoxicillin, Cephalothin, and Erythromycin. Conversely, GNB exhibited high levels of resistance, including 86.7% to Ciprofloxacin, 80% to Carbericillin and Nitrofurantoin, 66.7% to Streptomycin and Tetracycline, 60% resistance to Co-Trimazine; however, they showed 73.3% sensitivity to Amikacin and 53.3% sensitivity to Kanamycin.

Staphylococcus spp. isolates exhibited 100% sensitivity to Amikacin, Bacitracin, Oxytetracycline, and Vancomycin, followed by 80% sensitivity to Novobiocin. However, they exhibited 80% resistance to Amoxicillin, Cephalothin, and Erythromycin (Table 1). The susceptibility pattern of GNB against standard antibiotics is shown in Table 2. *P. aeruginosa* exhibited 100% sensitivity to Amikacin, Carbericillin, Ciprofloxacin, Co-Trimazine, Streptomycin, Tetracycline, and 100% resistance to Nitrofurantoin and intermediate to Kanamycin. *Proteus species* were highly resistant (100%) to Ciprofloxacin, Co-Trimazine, Nitrofurantoin, and Tetracycline, followed by 50% resistance to Amikacin, Carbericillin, and Streptomycin. *E. hormaechei* demonstrated 100% resistance to Amikacin, Carbericillin, Ciprofloxacin, Co-Trimazine, Kanamycin, Nitrofurantoin, and Tetracycline, while showing 100% intermediate to S. *A. baumannii* exhibited 100% resistance to Amikacin, Carbericillin, Ciprofloxacin, Co-Trimazine, Kanamycin, Nitrofurantoin, and Streptomycin, and 100% sensitive to Tetracycline. *Providencia* species isolates exhibited 100% resistance to Nitrofurantoin and Tetracycline, followed by 50% resistance to Amikacin, Carbericillin, Ciprofloxacin, and Kanamycin. These strains also showed 100% sensitivity to Co-Trimazine and Streptomycin. *E. coli* demonstrated 100% resistance to Carbericillin and Ciprofloxacin, 80% resistance to Co-Trimazine and Tetracycline, however, exhibited 100% sensitivity to Amikacin, 80% sensitivity to Kanamycin, 60% sensitivity to Nitrofurantoin and S. *Klebsiella* spp. showed 100% sensitivity to Amikacin, Kanamycin, and Streptomycin, followed by 66.6% sensitivity to Co-Trimazine and Tetracycline. These strains showed 100% resistance to Carbericillin, Ciprofloxacin, and Nitrofurantoin.

Among the 20 bacterial strains, 13 (65%) were classified as MDR, 4 (20%) as XDR, and the remaining 3 isolates (15%) were sensitive to most of the antibiotics tested. Figure 3 illustrates the prevalence of MDR and XDR among both GP and GN isolates. Among the five *Staphylococcus* species, four were found to be MDR. Of the two *Providencia* species, *Providencia stuartii* was XDR. *A. baumannii* and *E. hormaechei* were identified as XDR. One of the two *Proteus* species was found to be XDR, while the other was MDR. All the *E. coli* and *Klebsiella* species isolates were found to be MDR.

Table 2. Susceptibility pattern of Gram-negative bacilli against standard antibiotics.

Bacterial isolates	Antibiotics							
	AK	CB	CIP	CM	K	NIT	S	TE
P. aeruginosa	S	S	S	S	I	R	S	S
Proteus spp. 1	R	S	R	R	R	R	R	R
Proteus spp.2	S	R	R	R	I	R	S	R
Enterobacter hormaechei	R	R	R	R	R	R	I	R
Acinetobacter baumannii	R	R	R	R	R	R	R	S
Providencia rettgeri	S	S	S	S	S	R	S	R
Providencia stuartii	R	R	R	S	R	R	S	R
E. coli 1	S	R	R	S	S	R	I	R
E. coli 2	S	R	R	R	S	S	S	S
E. coli 3	S	R	R	R	S	S	R	R
E. coli 4	S	R	R	R	I	R	S	R
E. coli 5	S	R	R	R	S	S	S	R
Klebsiella spp.1	S	R	R	R	S	R	S	R
Klebsiella spp.2	S	R	R	S	S	R	S	S
Klebsiella spp.3	S	R	R	S	S	R	S	S

The diameters of the inhibition zones were interpreted following CLSI guidelines, categorizing the tested isolates as Susceptible (S), Intermediate (I), or Resistant (R) to the antibiotics evaluated. AK = Amikacin; CB = carbenicillin; CIP = ciprofloxacin; CM = co-trimazine; K = kanamycin; NIT = nitrofurantoin; S = streptomycin; TE = tetracycline.

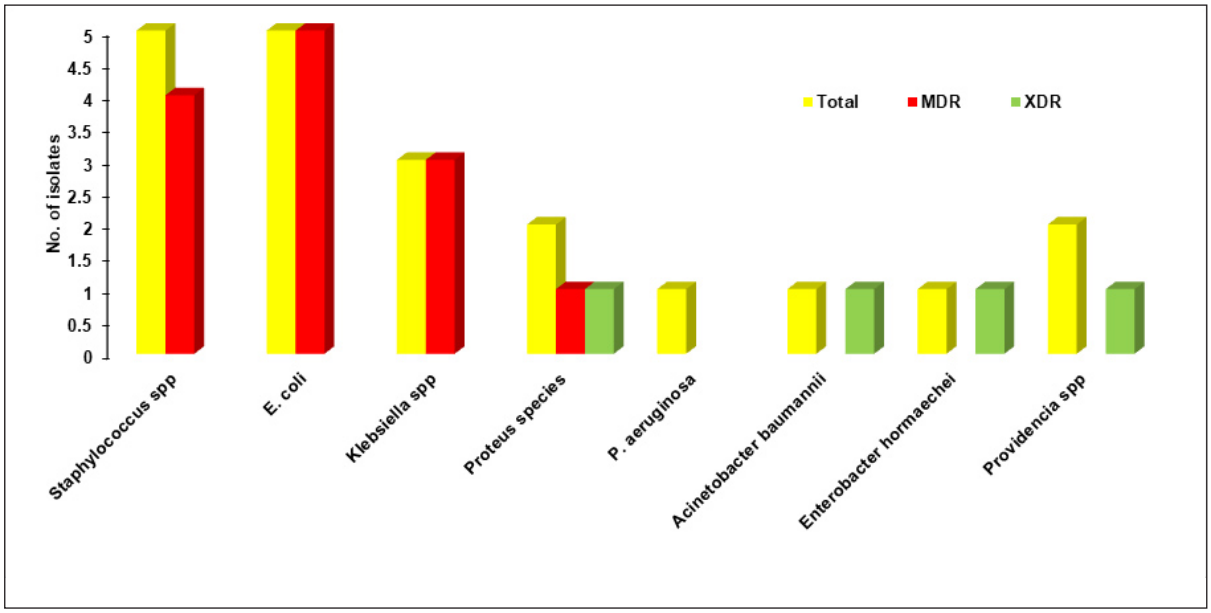

Figure 3. Incidence of MDR and XDR of the Gram-positive and Gram-negative isolates.

Effect of *C. crepidioides* leaf extract against the wound isolates

Crassocephalum crepidioides leaf extract was found to be effective against all the *Staphylococcus* spp. isolates (17 ± 1 mm–25.83 ± 1.04 mm), *E. hormaechei* (11.16 ± 0.76 mm), *A. baumannii* (13 ± 0.5 mm), *Providencia rettgeri* (14 ± 1 mm), *P. stuartii* (15 ± 0.5 mm), two *E. coli* isolates (12 ± 0.5 mm and 10.83 ± 0.28 mm), and one *Klebsiella* spp. (11.0.5 mm) which is shown in Figure 4. On the other hand, *P. aeruginosa*, *Proteus* spp., three *E. coli* isolates, and two *Klebsiella* spp. isolates were resistant to it. It showed superior efficacy against GPC compared to GNB. Notably, it exhibited sensitivity against MDR and XDR bacterial isolates from the infected wound, thus signaling its broad-spectrum antibacterial potential.

MIC and MBC

Crassocephalum crepidioides leaf extract exhibited antibacterial activity against 12 bacterial wound isolates at a concentration of 50 mg/ml. Therefore, the MIC of *C.*

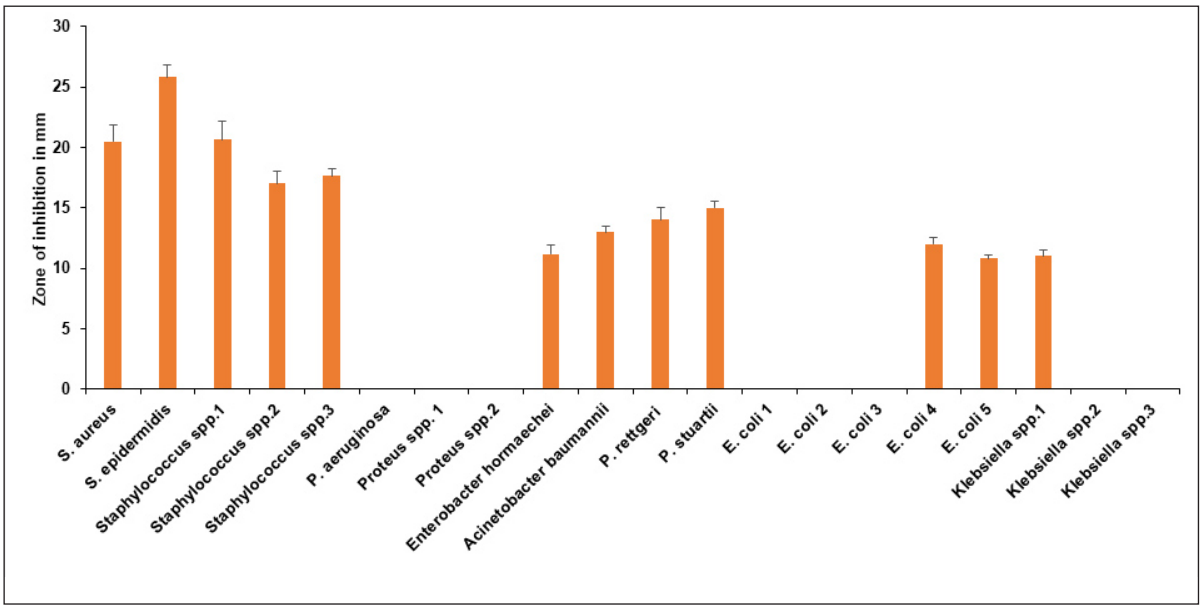

Figure 4. Zone of inhibition in mm of *C. crepidioides* leaf extract against the bacterial isolates.

Table 3. MIC of *C. crepidioides* leaf extract against the bacteria isolated from wounds.

Bacterial isolates	Zone of inhibition in mm							MIC of *C. crepidioides* leaf extract (mg/ml)	Ciprofloxacin (5 µg)
	0.625 mg/ml	1.25 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	40 mg/ml		
S. aureus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 0.5	14.5 ± 0.76	16.7 ± 1.53	19.2 ± 0.76	5 ± 0.00.	13 ± 0.87
S. epidermidis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	13.3 ± 0.29*	15.5 ± 0.5	16.3 ± 0.58	18.5 ± 1.32	5 ± 0.00	33 ± 1*
Staphylococcus spp.1	0.0 ± 0.0	0.0 ± 0.0	11.8 ± 0.76*	14.2 ± 1.04	16.5 ± 0.29	18 ± 0.5	19.5 ± 0.87	2.5 ± 0.00	18.17 ± 0.76*
Staphylococcus spp.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.8 ± 0.76	13.5 ± 0.58	14.8 ± 0.29	16.7 ± 1.04	5 ± 0.00	11.5 ± 0.5
Staphylococcus spp.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.1 ± 1.04*	13.8 ± 0.29	15.5 ± 0.87	17 ± 1	5 ± 0.00	32.7 ± 0.29*
E. hormaechei	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10 ± 0.5*	40 ± 0.00	0.0 ± 0.0*
A. baumannii	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11 ± 0.5*	12.1 ± 0.29	12.5 ± 0.5	13.1 ± 0.29	5 ± 0.00	0.0 ± 0.0*
P. rettgeri	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.8 ± 0.29*	12 ± 1	20 ± 0.00	26.2 ± 0.76*
P. stuartii	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	12.2 ± 0.29*	13 ± 0.5	13.5 ± 0.5	14.2 ± 0.58	5 ± 0.00	27 ± 0.5*
E. coli 4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.7 ± 0.29*	40 ± 0.00	0.0 ± 0.0*
E. coli 5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9 ± 0.5*	40 ± 0.00	0.0 ± 0.0*
Klebsiella spp.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.2 ± 0.58*	40 ± 0.00	0.0 ± 0.0*

Data were expressed as mean ± SD, $n = 3$. Zone of inhibition of Ciprofloxacin and MIC of *C. crepidioides* leaf extract were compared using Dunnett t-tests. * denotes statistically significant (p value ≤ 0.05) according to Dunnett t tests.

crepidioides leaf extract was determined utilizing the agar well diffusion method against 12 bacterial wound isolates. The MIC of *C. crepidioides* leaf extract was found to be between 2.5 and 5 mg/ml against all *Staphylococcus* spp., with the inhibition zones of 9.1 ± 1.04–13.3 ± 0.29 mm, and 5 mg/ml for *P. stuartii* (12.2 ± 0.29) and *A. baumannii* (11 ± 0.5). MIC values against *P. rettgeri* was 20 mg/ml with the inhibition zone of 10.8 ± 0.29 mm, 40 mg/ml for *E. coli* 4 (10.7 ± 0.29), *E. coli* 5 (9 ± 0.5), *E. hormaechei* (10 ± 0.5), and *Klebsiella* spp. 1 (10.2 ± 0.58) (Table 3). MBC of *C. crepidioides* leaf extract was found to be between 5 and 10 mg/ml against all *Staphylococcus* spp. MBC value was 10 mg/ml for *P. stuartii* and *A. baumannii*, and 40 mg/ml against *P. rettgeri*, *E. coli 4, E. hormaechei,* and *Klebsiella* spp.1 (Table 4).

DISCUSSION

Wound infections can prolong hospital stays and increase mortality rates by 70%–80% [29]. The administration of antibiotics is usually initiated empirically, which may help microorganisms become resistant to antibiotics [6].

A total of 20 bacterial species were isolated from 69 pus samples. The distribution of these isolates was as follows: 5 (25%) GPC and 15 (75%) GNB. The higher prevalence of GN

Table 4. MBC of *C. crepidioides* leaf extract against the bacteria isolated from wounds.

Bacterial isolates	MBC of *C. crepidioides* leaf extract (mg/ml)
S. aureus	10 ± 0.00
S. epidermidis	10 ± 0.00
Staphylococcus spp.1	5 ± 0.00
Staphylococcus spp.2	10 ± 0.00
Staphylococcus spp.3	10 ± 0.00
E. hormaechei	40 ± 0.00
A. baumannii	10 ± 0.00
P. rettgeri	40 ± 0.00
P. stuartii	10 ± 0.00
E. coli 4	40 ± 0.00
E. coli 5	>40
Klebsiella spp.1	40 ± 0.00

bacteria could be linked to their elevated antibiotic resistance. These results are in agreement with previous studies [13].

Gram-negative bacteria exhibit high resistance to antibiotics, such as β-lactams and quinolones, due to their outer membrane, which acts as a barrier preventing most of the antibiotics from reaching their targets [30]. Hydrophilic antibiotics can enter through porins, while hydrophobic drugs enter via diffusion pathways. In contrast, vancomycin cannot cross the outer membrane because of its structure. Resistance can develop when the outer membrane is altered, such as through changes in porin function. However, Gram-positive bacteria lack an outer membrane and instead have a thick peptidoglycan layer, facilitating easier antibiotic penetration and resulting in minimal resistance [31,32].

In the present investigation, *Staphylococcus* species and *E. coli* were the most common bacteria isolated from wound infection samples, consistent with previous studies [4,33–35]. The high incidence of *S. aureus* infections is often linked to endogenous sources or contamination of surgical instruments, as it can easily enter wounds when the skin barrier is breached [4]. The study by Atef *et al*. [13] also noted a high incidence of *P. aeruginosa*. These variations may be ascribed to factors such as the type of wound, the site of infection, and the use of prophylactic antibiotics during treatment [13]. According to WHO statistics, approximately 50% of *E. coli*, *K. pneumoniae*, *S. aureus,* and *P. aeruginosa* were resistant to most antibiotics, including cephalosporins which could be linked to misuse of antibiotics and prolonged hospital stays [36].

In our study, *Staphylococcus* isolates demonstrated high sensitivity to Amikacin, Bacitracin, Oxytetracycline, and Vancomycin (100%) followed by Novobiocin (80%); however, they exhibited resistance to Amoxicillin, Cephalothin, and Erythromycin (80%). These findings align with the previous studies indicating that *Staphylococcus* isolates are highly sensitive to Amikacin (100%) and Vancomycin (100%) [37–39]. The same organism showing notable resistance to Amoxicillin and Cephalothin demonstrates results consistent with studies conducted by Nobel *et al*. [40] and Ahmed *et al*. [41]. Over 90% of *S. aureus* were reported to be resistant to Amoxicillin and Ceftazidime [42]. The high sensitivity of gram-positive bacteria to Amikacin and Vancomycin could be attributed to their infrequent use, likely due to less availability, high cost, and potential toxicity [4]. Aminoglycosides represent the only class of ribosome-targeting antibiotics that exhibit bactericidal activity, due to their unique ability to cause mRNA misreading during translation [13].

Amoxicillin, a β-lactam drug was introduced in the 1970s for treating bacterial infections in humans [43]. *S. aureus* resistance to β-lactam primarily results from β-lactamase production, which deactivates the antibiotic and the acquisition of the *mecA* gene [44–46]. In addition, mutations in penicillin-binding proteins (PBPs) lead to the formation of PBP 2a, which aids in cell wall synthesis and further contributes to resistance [47].

Pseudomonas aeruginosa demonstrated 100% sensitivity to Amikacin, Carbericillin, Ciprofloxacin, Co-Trimazine, Streptomycin, and Tetracycline, while exhibiting 100% resistance to Nitrofurantoin and intermediate sensitivity to Kanamycin. Earlier investigations by Bhalchandra *et al*. [48] and Mama *et al*. [4] similarly found that *P. aeruginosa* strains exhibited 100% susceptibility to Ciprofloxacin.. Currently, Ciprofloxacin (oral) and gentamycin (injectable) are considered the most potent antibiotics for managing *P. aeruginosa*-related wound infections, outperforming other commonly used antimicrobial agents [4]. The increasing prevalence of *P. aeruginosa* in wound infections among hospitalized patients in developing regions raises significant concern, largely attributed to poor hygiene conditions, the use of low-quality antiseptics and low-quality medicinal solutions [49].

Proteus spp. demonstrated complete resistance (100%) to Ciprofloxacin, Co-Trimazine, Nitrofurantoin, and Tetracycline in our study, along with 50% resistance to Amikacin, CB, and Streptomycin. Previous study reported that *Proteus mirabilis* showed 100% resistance to Tetracycline [13]. Another study also reported that *Proteus* species were resistant to Tetracycline (73.9%) and sensitive to Ciprofloxacin (83%) [4].

Acinetobacter baumannii exhibited complete resistance (100%) to Amikacin, Carbericillin, Ciprofloxacin, Co-Trimazine, Kanamycin, Nitrofurantoin, and Streptomycin, while showing 100% sensitivity to Tetracycline. These findings align with the previous study which showed 70.6% resistance of *A. baumannii* to Amikacin [50]. In contrast, Puca *et al*. reported that *A. baumannii* was highly sensitive to Amikacin, with a sensitivity rate of 96.7% [6]. Sheeba *et al*. observed the highest percentage of resistant strains among *A. baumannii* isolates [51].

It was found that *E. coli* exhibited high resistance (100%) to Carbericillin and Ciprofloxacin, followed by Co-Trimazine and Tetracycline (80%), while exhibiting 100% sensitivity to Amikacin, 80% to Kanamycin, and 60% to Nitrofurantoin and Streptomycin. The resistance of *E. coli* to Ciprofloxacin and Tetracycline aligns with findings from other studies [4,13,39,52]. It was reported that Amikacin was highly effective against *E. coli* [53,54].

Klebsiella species exhibited 100% sensitivity to Amikacin, Kanamycin, and Streptomycin, followed by 66.6% sensitivity to Co-Trimazine and Tetracycline; however, 100% resistant to Carbericillin, Ciprofloxacin, and Nitrofurantoin. These findings contrast with those reported by Atef *et al*. [13] and Gomatheswari and Jeyamurugan [53].

Among the 20 bacterial strains isolated in the present study, 13(65 %) were classified as MDR and 4 (20%) were XDR. Of the 17 MDR and XDR isolates, 4 (24 %) were gram-positive bacteria, while 13 (76 %) were Gram-negative bacteria. The elevated resistance observed in these isolates may be attributed to factors such as self-medication, limited diagnostic services, and lack of proper antibiotic guidelines [4]. The global spread of antimicrobial resistance is driven by antibiotic misuse, with mobile genetic elements and horizontal gene transfer playing a significant role [55]. Most pathogens isolated from infected wounds demonstrated resistance to commercial antibiotics (Tables 1 and 2).

The *C. crepidioides* leaf extract demonstrated varying levels of antibacterial activity against bacterial isolates from the infected wound. It showed superior efficacy against GPC compared to GNB. Notably, it exhibited sensitivity against *Staphylococcus* spp. isolates (16.16 ± 0.76 mm–25.83 ± 1.04 mm), *E. hormaechei* (11.16 ± 0.76 mm), *A. baumannii* (13 ± 0.5 mm), *P. rettgeri* (14 ± 1 mm), *P. stuartii* (15 ± 0.5 mm), two *E. coli* isolates (12 ± 0.5 mm and 10.83 ± 0.28 mm), and one *Klebsiella* spp. (11.0.5 mm), with a MIC between 2.5 and 40 mg/ml. The previous study done by Omotayo *et al*. [56] confirmed the strong antibacterial activity of *C. crepidioides* leaf extract against *S. aureus* (18 mm), *E. coli* (16 mm), and *K. pneumoniae* (21 mm) at 150 mg/ml. Another study also reported that *C. crepidioides* leaf extract was found to be sensitive against *S. aureus and P. aeruginosa* [57]. The use of plant extract to treat wound infections is supported by Atef *et al*. [13] who reported the effectiveness of aqueous extract of *Moringa oleifera* against *Staphylococcus* spp. (13–30 mm), *E. coli* (13–19 mm), *Klebsiella* spp. (12–26 mm), *P. aeruginosa* (14–20), and *Proteus mirabilis* (15 mm) at 500 mg/ml isolated from the wound infections. Aqueous extract of *Alchornea cordifolia* had strong antibacterial activity against the MDR *S. aureus* isolated from post-operative wound infections with an inhibition size of 21.4 mm [22].

According to previous studies, *C. crepidioides* leaf extract contains bioactive constituents, including phenol, tannin, flavonoids, terpenoids, and saponin [17,56]. The possible antibacterial mechanisms of some secondary metabolites can be described as follows: Tannins can disrupt the structural integrity of bacterial cell walls and membranes, resulting in cellular damage and death. They can interfere with bacterial enzymes, impairing essential metabolic processes and limiting bacterial growth [58]. Furthermore, Tannins may inhibit microbial growth by interfering the cell wall synthesis, and cell envelope transport proteins [59]. Flavonoids can disrupt microbial cell membranes, inhibit energy metabolism, and interfere with nucleic acid synthesis [60]. They can interact with extracellular proteins in the bacterial cell wall, forming complex bonds that weaken its structure, eventually leading to cell wall breakdown and cell lysis [61]. Alkaloids, a diverse group of compounds, exhibit antimicrobial effects by inhibiting enzyme activity and other cellular mechanisms [62]. Saponins have been reported to exhibit antibacterial activity, likely due to their ability to form complexes with extracellular proteins, soluble proteins, and bacterial cell wall [14].

The differences in sensitivity observed among the tested pathogens may be due to the intrinsic tolerance of the microorganisms, as well as the specific types and combinations of phytochemicals present in the crude extract. The structural differences in bacterial cell walls also play a role; Gram-positive bacteria have a single-layered cell wall, whereas Gram-negative bacteria possess a more complex multi-layered cell wall. This structural complexity in Gram-negative bacteria may impede the penetration of active compounds, contributing to the differences in inhibition zones [63].

The present study highlights the potential of *C. crepidioides* leaf extract as effective antibacterial agents against the MDR and XDR organisms causing wound infection, emphasizing the significant role of plant extracts in treating bacterial wound infections, thereby preventing the delay of the wound healing process. These findings provide a robust scientific foundation for the ethnopharmacological use of *C. crepidioides* in the management of wound infections and underscore the potential of plant-derived extracts as effective antibacterial agents in clinical settings.

LIMITATIONS OF THE STUDY

This study did not include the isolation of anaerobic bacteria and fungi, as such procedures are not routinely performed in our laboratory. In addition, the small sample size represents another limitation of the study as the analysis was performed during a short period of time. Other herbal extracts with established antibacterial properties will be incorporated in future studies as control treatments to further contextualize the antibacterial efficacy of *C. crepidioides*. The wound-healing effect of *C. crepidioides* leaf extract on *in vitro* and *in vivo* condition needs to be done.

CONCLUSION

In the current research, 20 pure bacterial species were isolated from 69 wound samples, of which 13 (65%) were classified as MDR and 4 (20%) as XDR. The most common bacterial isolates were *Staphylococcus species* and *E. coli* followed by *Klebsiella* spp., *Proteus spp.*, *P. aeruginosa*, *A. baumannii*, *E. hormaechei*, and *Providencia species*. Most pathogens isolated from infected wounds exhibited resistance to commercial antibiotics. Given the rapid emergence of antibiotic resistance, the use of herbal products as a novel, non-toxic, and environmentally sustainable alternative for managing virulent diseases is essential. *C. crepidioides* plant is traditionally used for minor wounds. *In vitro* antibacterial activity assay revealed that *C. crepidioides* leaf extract produced an inhibitory effect against MDR and XDR bacterial wound isolates. These findings provide a robust scientific foundation for the ethnopharmacological use of *C. crepidioides* in the management of wound infections and underscore the potential of plant-derived extracts as effective antibacterial agents in clinical settings. Further investigation on the wound-healing effect of *C. crepidioides* on *in vitro* and *in vivo* conditions is recommended.

AUTHOR CONTRIBUTIONS

All the authors made significant contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/ guidelines.

FUNDING

There is no funding to report.

CONFLICT OF INTEREST

The authors reports no financial or any other conflicts of interest in this work.

ETHICS STATEMENT

Ethical approvals are given in the 'Material and Method' section.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Devi YA, Gnanasekaran P, Devi HJ, Siva D, Seshadri S, Ravindrran MB, Partheeban S, Bhuvaneswari S. Antibiogram and antibacterial activity of *Crassocephalum crepidioides* (Thickhead) leaf extract against the wound isolates. J Appl Pharm Sci. 2025;15(09):041-051. DOI: 10.7324/JAPS.2025.250483