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In Vitro Screening of the Antibacterial Activity of *Bidens pilosa* Flower Extract against Methicillin-Resistant *Staphylococcus aureus* Isolated from Wounds of Patients at National Polytechnic University Institute Laboratory

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of wound infections worldwide, associated with delayed healing, prolonged hospital stays, and increased treatment costs. The growing resistance of MRSA to conventional antibiotics highlights the urgent need for alternative therapies. This study evaluated the *in vitro* antibacterial activity of *Bidens pilosa* flower extracts against MRSA isolates from wound infections. A laboratory-based experimental design was employed at the National Polytechnic University Institute Laboratory. The fresh plant was collected, washed and dried away from sunlight. It was then crushed and blended to powder form and extraction was done. Extracts were prepared using aqueous, 50% ethanol, and 80% ethanol solvents, and their antibacterial activity was assessed through agar well diffusion and disk diffusion methods, inhibition zone measurements, and minimum inhibitory concentration (MIC) determination. Data was analyzed using SPSS version 23 and presented in tables and figures. Inferential statistics was computed using the t-test and the ANOVA test to assess for differences in mean values. Statistical significance was considered when p value was less than or equal to 0.05. Results showed that the 80% ethanol extract exhibited the strongest antimicrobial activity, producing a mean inhibition zone of 9.8 ± 1.7 mm, significantly higher than the 50% ethanol extract (6.8 ± 1.2 mm) and the aqueous extract (3.7 ± 0.5 mm) ($F = 18.5$, $p = 0.003$). Antibacterial activity decreased progressively with dilution, confirming a dose-dependent relationship.

At ½ dilution, only the 80% ethanol extract retained activity (3.6 ± 0.6 mm), while aqueous and 50% ethanol extracts lost activity completely (0.0 ± 0.0 mm) ($F = 54.3$, $p < 0.001$). At ¼ dilution, the 80% ethanol extract still showed inhibition (2.1 ± 0.3 mm), and at ⅛ dilution, activity persisted (1.1 ± 0.1 mm) ($F = 121.6$, $p < 0.001$). MIC testing confirmed that the 80% ethanol extract had a measurable MIC of 12.5 mg/mL, while aqueous and 50% ethanol extracts showed no detectable MIC. Ethanol extracts of *Bidens pilosa*, particularly at 80% concentration, demonstrate significant antibacterial activity against MRSA, validating its traditional use in wound care. These findings provide preliminary scientific evidence supporting the potential of *Bidens pilosa* as a locally available, affordable alternative for managing resistant wound infections in resource-limited settings.

Subject Areas

Clinical Medicine

Keywords

Bidens pilosa, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Wound Infections, Antibacterial Activity

1. Introduction

Antimicrobial resistance (AMR) has become one of the greatest threats to global public health, compromising the effective treatment of bacterial infections and increasing morbidity, mortality, healthcare costs, and the burden on healthcare systems, particularly in low- and middle-income countries [1]. Among the pathogens driving this crisis, *Staphylococcus aureus* is one of the most clinically significant due to its ability to colonize the skin and mucosal surfaces and cause infections involving nearly every organ system. The emergence of methicillin-resistant *S. aureus* (MRSA) shortly after the introduction of methicillin marked a major setback in antimicrobial therapy. Resistance is primarily mediated by the mecA gene, which encodes the altered penicillin-binding protein PBP2a, rendering MRSA resistant to methicillin and most β-lactam antibiotics and substantially limiting available treatment options [2]-[4]. Consequently, MRSA has become a major cause of healthcare-associated and community-acquired infections worldwide, necessitating the use of more expensive and less accessible antibiotics such as vancomycin, linezolid, and daptomycin [3] [5].

Wound infections caused by MRSA represent a significant clinical challenge because of the organism's capacity to adhere to damaged tissues, produce numerous virulence factors, and establish biofilms that protect bacterial cells from host immune responses and antimicrobial agents [6]-[8]. Biofilm formation contributes to persistent inflammation, delayed wound healing, chronic infection, prolonged hospitalization, and increased healthcare costs [7] [8]. If inadequately

managed, MRSA wound infections may progress to severe complications including cellulitis, necrotizing fasciitis, osteomyelitis, sepsis, and death, particularly among immunocompromised individuals and patients with underlying comorbidities [9]. The situation is especially concerning in developing countries, where inadequate diagnostic facilities, weak antimicrobial stewardship, poor infection prevention and control practices, and unrestricted access to antibiotics accelerate the emergence and spread of resistant strains [5] [10].

In Cameroon, MRSA has emerged as an important cause of wound and healthcare-associated infections, with several studies reporting increasing isolation rates from wounds, surgical sites, and burns [10] [11]. Although national strategies aligned with the World Health Organization Global Action Plan on Antimicrobial Resistance have been implemented to strengthen infection prevention, antimicrobial stewardship, and rational antibiotic use, significant challenges remain. Weak laboratory capacity, inadequate surveillance systems, limited access to advanced antimicrobial agents, poor enforcement of prescription regulations, and widespread self-medication continue to undermine effective management of resistant infections [1] [10] [11]. Consequently, empirical antibiotic therapy remains common, often resulting in treatment failure and further selection of resistant organisms, thereby highlighting the urgent need for alternative, affordable, and locally available therapeutic options.

Medicinal plants have long served as important sources of antimicrobial agents and remain the primary form of healthcare for a large proportion of populations in developing countries [12]. Their therapeutic potential is largely attributed to bioactive secondary metabolites including flavonoids, alkaloids, tannins, terpenoids, phenolic compounds, and essential oils, which possess antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities through multiple mechanisms of action [13]. Among these plants, *Bidens pilosa* has attracted considerable scientific interest because of its widespread traditional use in the management of wounds, skin infections, inflammatory disorders, and other infectious diseases [14]. Phytochemical investigations have demonstrated that the plant, particularly its flowers, contains abundant flavonoids and phenolic compounds capable of disrupting bacterial membranes, inhibiting energy metabolism, interfering with quorum sensing, and suppressing biofilm formation in *S. aureus*, suggesting its potential as a natural anti-MRSA agent [15]-[17].

Despite the growing body of evidence supporting the antimicrobial properties of *Bidens pilosa*, studies evaluating its activity against clinically isolated MRSA strains remain limited and inconsistent. Most previous investigations have focused on laboratory reference strains rather than clinical isolates, while differences in plant origin, extraction methods, solvent systems, and plant parts used have contributed to variability in reported antimicrobial efficacy [18]. Furthermore, scientific evidence validating the activity of *Bidens pilosa* flower extract against MRSA isolated from wound infections in Cameroon is scarce. This knowledge gap limits the incorporation of this readily available medicinal plant into evidence-

based wound management strategies. Therefore, the present study was designed to evaluate the *in vitro* antibacterial activity of *Bidens pilosa* flower extract against MRSA isolated from wound infections, with the aim of generating evidence that may support the development of affordable, locally sourced complementary therapies for the management of resistant wound infections in Cameroon.

2. Methodology

This laboratory-based experimental study was conducted over a three-week period (10 February–6 March 2026) at the National Polytechnic University Institute (NPUI) Laboratory, Bamenda, Cameroon, to evaluate the *in vitro* antibacterial activity of *Bidens pilosa* flower extracts against MRSA isolated from wound infections. Fifty wound swab samples were collected aseptically from consenting patients with clinically diagnosed wound infections who had not received antibiotics within the preceding seven days. Samples were cultured on Nutrient Agar, Blood Agar, and Mannitol Salt Agar, and isolates were identified using colony morphology, Gram staining, catalase, coagulase, and oxidase tests. MRSA was confirmed using the cefoxitin disc diffusion method following standard microbiological procedures. Fresh *Bidens pilosa* flowers were collected, washed with clean water, shade-dried, pulverized, and extracted using distilled water and ethanol (80% and 50%), after which crude extracts were serially diluted (1:2, 1:4 and 1:8) and sterilized. The antibacterial activity of the extracts was assessed using agar well diffusion and disc diffusion techniques against standardized MRSA inocula (0.5 McFarland standard), with inhibition zones measured in millimeters. Minimum inhibitory concentration (MIC) was determined using serial dilution methods. Data quality was ensured through adherence to standard laboratory protocols, quality control procedures, and replicate testing. Statistical analysis was performed using SPSS version 23, with descriptive statistics summarizing the findings and comparisons of mean inhibition zones conducted using Student's t-test and one-way analysis of variance (ANOVA), considering $p \leq 0.05$ as statistically significant. Ethical approval and administrative authorization were obtained from the relevant institutional and regional authorities, and written informed consent was obtained from all participants prior to sample collection.

3. Results

3.1. Antimicrobial Activity of *Bidens pilosa* Flower Extracts against MRSA

The antimicrobial activity of *Bidens pilosa* flower crude extracts against methicillin-resistant MRSA varied significantly according to the extraction solvent used. The 80% crude ethanol extract demonstrated the highest antibacterial activity, producing a mean zone of inhibition of 9.8 ± 1.7 mm, followed by the 50% ccrude ethanol extract (6.8 ± 1.2 mm), while the aqueous crude extract exhibited the lowest activity (3.7 ± 0.5 mm). The differing superscript letters indicate that the mean inhibition zones were significantly different among the three extraction solvents. The one-way

ANOVA revealed a statistically significant difference in antimicrobial activity between the extracts (F = 18.5, p = 0.003), indicating that the choice of extraction solvent significantly influenced the efficacy of *B. pilosa* flower extracts against MRSA. The superior performance of the 80% ethanol extract suggests that ethanol at higher concentrations was more effective in extracting bioactive phytochemicals responsible for the antibacterial activity as shown in Figure 1 and Table 1 below.

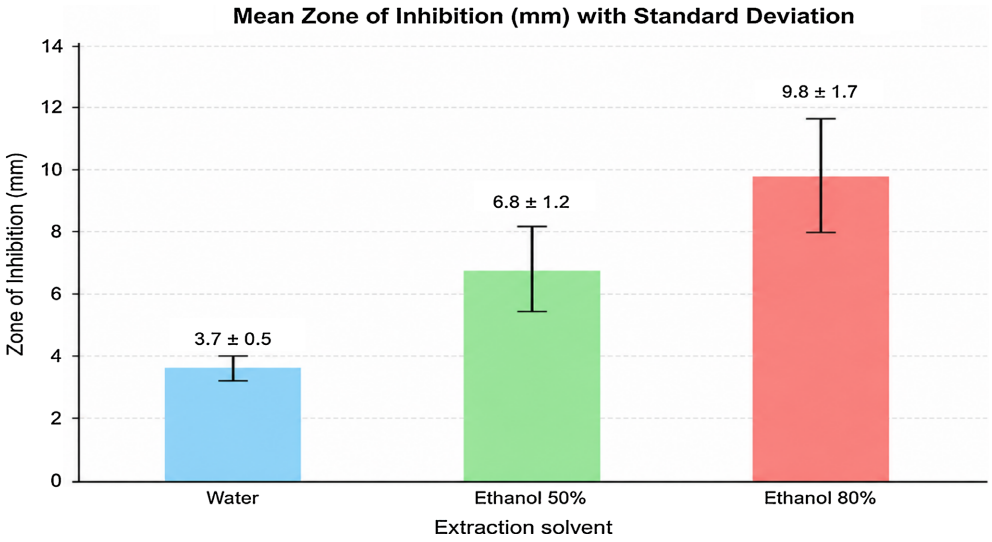

Figure 1. Mean ± SD zone of inhibition of antimicrobial activity of *Bidens pilosa* flower crude extracts against MRSA.

Table 1. Antimicrobial activity of *Bidens pilosa* flower crude extracts against MRSA.

Extraction solvent	Mean ± SD zone of inhibition (mm)
Water	3.7 ± 0.5[c]
Ethanol 50%	6.8 ± 1.2[b]
Ethanol 80%	9.8 ± 1.7[a]
F value	**18.5**
p value	**0.003***

*Statistically significant at 0.05 significance level.

3.2. Antibacterial Effectiveness of Different Concentrations of *Bidens pilosa* Extracts against MRSA

The antimicrobial activity of *Bidens pilosa* flower extracts against MRSA decreased progressively with increasing dilution of the extracts. At the crude concentration, the 80% ethanol extract exhibited the highest mean zone of inhibition (9.8 ± 1.7 mm), followed by the 50% ethanol extract (6.8 ± 1.2 mm) and the aqueous extract (3.7 ± 0.5 mm). Statistical analysis showed a significant difference among the extraction solvents at the crude concentration (F = 18.5, p = 0.003), indicating that solvent type significantly influenced antibacterial efficacy. At the ½ dilution, only the 80% ethanol extract retained antimicrobial activity, producing a mean inhibition

zone of 3.6 ± 0.6 mm, whereas both the aqueous and 50% ethanol extracts showed no detectable inhibition (0.0 ± 0.0 mm). The difference among the extracts was highly significant ($F = 54.3$, $p < 0.001$). Similarly, at the ¼ dilution, the 80% ethanol extract continued to demonstrate antibacterial activity with a mean inhibition zone of 2.1 ± 0.3 mm, while the aqueous and 50% ethanol extracts remained inactive. This difference was highly significant ($F = 147.8$, $p < 0.001$). At the ⅛ dilution, antimicrobial activity was still observed only in the 80% ethanol extract, although the inhibition zone decreased further to 1.1 ± 0.1 mm. No inhibition was recorded for the aqueous and 50% ethanol extracts. The variation among the extracts remained highly significant ($F = 121.6$, $p < 0.001$) (See Table 2 and Figure 2).

Table 2. Antibacterial effectiveness of different concentrations of *Bidens pilosa* extracts against MRSA.

Extraction solvent	Mean ± SD zone of inhibition in mm			
	Crude extract	1/2	1/4	1/8
Water	3.7 ± 0.5^{c}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Ethanol 50%	6.8 ± 1.2^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Ethanol 80%	9.8 ± 1.7^{a}	3.6 ± 0.6^{a}	2.1 ± 0.3^{a}	1.1 ± 0.1^{a}
F-value	**18.5**	**54.3**	**147.8**	**121.6**
p value	**0.003***	**<0.001***	**<0.001***	**<0.001***

*Statistically significant at 0.05 significance level.

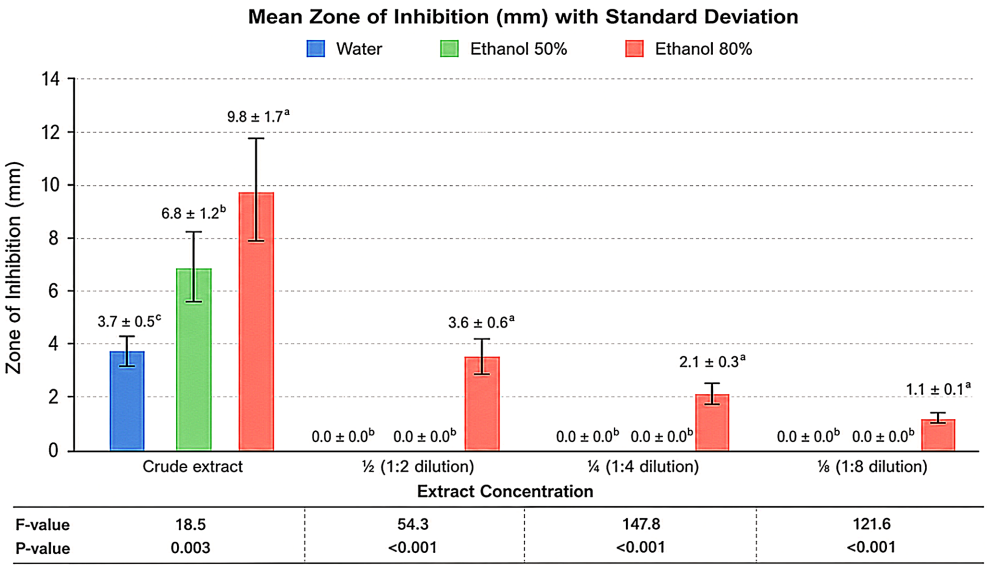

Figure 2. Mean ± SD zone of inhibition of antibacterial effectiveness of different concentrations of *Bidens pilosa* extracts against MRSA.

3.3. Minimum Inhibitory Concentration (MIC) of *Bidens pilosa* Flower Extract against MRSA Isolates

The minimum inhibitory concentration (MIC) results of *Bidens pilosa* flower ex-

tracts against MRSA isolates demonstrate marked variation depending on the extraction solvent used. The aqueous extract and 50% ethanol extract did not exhibit any detectable inhibitory activity within the tested concentration range, as indicated by "ND" (not detected), suggesting that their antimicrobial effects were insufficient to establish an MIC under the experimental conditions. In contrast, the 80% ethanol extract demonstrated measurable antimicrobial activity, with an estimated MIC of 12.5 mg/mL, derived from the lowest tested concentration (⅛ dilution) that still produced observable inhibition of MRSA growth. This finding indicates that the 80% ethanol extract possesses the highest antibacterial potency among the tested preparations, likely due to its greater efficiency in extracting bioactive phytochemical constituents responsible for antimicrobial effects. This is shown in Table 3 below.

Table 3. Minimum inhibitory concentration (MIC) of *Bidens pilosa* flower extract against MRSA isolates.

Extraction solvent	MIC (mg/mL) against MRSA
Water	ND*
Ethanol 50%	ND*
Ethanol 80%	12.5mg/mL

*ND = Not detected within the tested concentration range.

4. Discussion

The present study demonstrated that the antibacterial activity of *Bidens pilosa* flower extracts against MRSA was significantly influenced by the extraction solvent, with the 80% ethanol extract exhibiting the greatest antimicrobial activity, followed by the 50% ethanol extract, while the aqueous extract showed the least activity. The statistically significant difference observed among the extracts ($p = 0.003$) suggests that solvent polarity plays a critical role in extracting the bioactive compounds responsible for antibacterial activity. These findings are consistent with previous studies which reported that hydroethanolic and ethanolic extracts of *B. pilosa* possess stronger antibacterial properties than aqueous extracts because ethanol efficiently extracts flavonoids, phenolic compounds, tannins, and polyacetylenes that are responsible for antimicrobial effects [15] [16] [19]. The superior performance of the 80% ethanol extract may therefore be attributed to its ability to solubilize a wider range of phytochemicals with potent antibacterial activity compared with water or lower ethanol concentrations [20].

The progressive decline in antibacterial activity with increasing dilution of the extracts further demonstrates that the antimicrobial efficacy of *B. pilosa* is concentration dependent. While the crude 80% ethanol extract produced the largest inhibition zone, its activity gradually decreased at the ½, ¼, and ⅛ dilutions, although measurable inhibition persisted even at the lowest concentration tested. In contrast, both the aqueous and 50% ethanol extracts lost detectable

activity immediately after dilution. These findings indicate that the concentration of bioactive metabolites directly influences the antibacterial effectiveness of the extract. Similar concentration-dependent antimicrobial activity has been reported in studies evaluating medicinal plant extracts against *S. aureus* and MRSA, where higher extract concentrations produced significantly larger inhibition zones than diluted preparations [21] [22]. The persistence of inhibitory activity in the 80% ethanol extract even after serial dilution further supports its potential as the most effective extraction solvent for isolating antibacterial compounds from *B. pilosa* flowers.

The observed superiority of the ethanol extract may be explained by the phytochemical composition of *B. pilosa.* Previous phytochemical investigations have shown that the plant contains abundant flavonoids, phenolic acids, tannins, and polyacetylenes, many of which exhibit antibacterial properties through disruption of bacterial cell membranes, inhibition of protein synthesis, interference with nucleic acid replication, and suppression of quorum sensing mechanisms involved in biofilm formation [13] [15] [17]. These mechanisms are particularly relevant in MRSA, whose ability to form biofilms contributes to persistent wound infections and reduced susceptibility to conventional antibiotics [7]. Ethanol extraction has consistently been shown to recover higher concentrations of these bioactive constituents than aqueous extraction, thereby enhancing antimicrobial potency [19] [23].

The MIC findings further support the greater efficacy of the 80% ethanol extract. Unlike the aqueous and 50% ethanol extracts, which failed to demonstrate detectable MIC values within the tested concentration range, the 80% ethanol extract inhibited MRSA growth at an MIC of 12.5 mg/mL. This indicates that the extract possesses measurable bacteriostatic activity even at relatively low concentrations. Comparable MIC values have been reported for ethanolic extracts of *B. pilosa* and other medicinal plants tested against Gram-positive bacteria, with ethanol-derived extracts consistently exhibiting lower MIC values than aqueous extracts [13] [24]. The inability of the aqueous extract to achieve an MIC may reflect inadequate extraction of hydrophobic phytochemicals that contribute substantially to antibacterial activity.

5. Conclusion

This study demonstrated that *Bidens pilosa* flower extract possesses *in vitro* antibacterial activity against MRSA, with the magnitude of activity significantly influenced by the extraction solvent. The 80% ethanol extract exhibited the greatest antibacterial efficacy, producing the largest zones of inhibition and the lowest measurable MIC, whereas the aqueous and 50% ethanol extracts showed limited or no inhibitory activity at lower concentrations. These findings suggest that *B. pilosa* flowers contain bioactive compounds with potential anti-MRSA properties and highlight the plant as a promising source of affordable, locally available antimicrobial agents for further investigation.

6. Limitations

This study had some limitations. First, the antibacterial activity was evaluated only under *in vitro* laboratory conditions, which may not accurately predict the therapeutic efficacy of the extracts in living organisms. Second, the study used a relatively small number of MRSA isolates from a single geographical location, limiting the generalizability of the findings. Finally, phytochemical characterization, toxicity assessment, and molecular identification of the active compounds were not performed; therefore, the specific constituents responsible for the observed antibacterial activity and their safety profiles remain to be established through further research.

Conflicts of Interest

The authors declare no conflicts of interest.

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