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Antibacterial Activity of *Myrmecodia pendens* (Ant Nest Plant) against Multidrug Resistance Bacteria from Clinical Samples

Muhammad Evy Prastiyanto[1], Aditya Rahman Ernanto[1], Sri Darmawati[1*], Endang Tri Wahyuni Maharani[2], Arif Mulyanto[3], Rizal Maarif Rukmana[4], Wahyu Aristyaning Putri[5], Lia Kusmita[6], Shiv Kumar Sah[7], Daniel Geleta[8]

[1]Department of Medical Laboratory Technology, Faculty of Health and Nursing Science, Universitas Muhammadiyah Semarang, Semarang, Indonesia
[2]Department of Chemistry Education, Universitas Muhammadiyah Semarang, Semarang, Indonesia
[3]Department of Medical Laboratory Technology, Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto, Indonesia
[4]Research Center for Pharmaceutical Ingredients and Traditional Medicine, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, 16911, Indonesia
[5]Department of Tropical Biology, Universitas Gadjah Mada, Yogyakarta, 55281
[6]STIFAR Yayasan Pharmasi Semarang, Jl. Letjend Sarwo Edhie Wibobo Km 1, Semarang, Indonesia
[7]Department of Pharmacy, Maharajgunj Medical Campus (MMC), Institute of Medicine (IOM), Tribhuvan University, Kathmandu, Bagmati, Nepal
[8]Department of Medical Laboratory Sciences, Jimma University, Jimma, Oromia, Ethiopia

ABSTRACT

The emergence of multidrug-resistant (MDR) bacteria causes a substantial barrier to treatment, demanding the development of new antibacterial medicines. *Myrmecodia pendens* (ant nest plant), an indigenous medicinal species, is esteemed for its potential medicinal properties. This study aimed to examine the antibacterial efficacy of *M. pendens* extracts against MDR bacterial strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii*, and *Pseudomonas aeruginosa*) obtained from clinical samples. Extracts were prepared via maceration using ethanol, chloroform, and n-hexane solvents. Antibacterial efficacy was evaluated using the agar diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The ethanol solvent extract exhibited the most significant antibacterial activity, particularly against *P. aeruginosa*, with an inhibition zone of 23.15 ± 0.21 mm at 100 mg/mL. The MIC and MBC values were 3.13 mg/mL and 12.5 mg/mL, respectively. The chloroform and n-hexane extracts showed lower, but comparable, inhibitory effects against the tested strains. These findings provide compelling evidence that *M. pendens* extracts, especially the ethanol extracts, represent a promising source for developing alternative antibacterial agents to combat MDR infections.

Keywords: Antibacterial; Ant Nest; *Myrmecodia pendens*; *Pseudomonas aeruginosa*

INTRODUCTION

The increasing prevalence of multidrug-resistant (MDR) bacteria poses a significant global health threat, including in Indonesia (Prastiyanto et al., 2024a). MDR bacteria constitute a critical global health threat, undermining the effectiveness of antibiotics and leading to increased morbidity, mortality, and healthcare costs (van Duin & Paterson, 2020). Antibiotic resistance is widespread in major bacterial pathogens, such as *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp., with alarming resistance levels reported globally (Idris & Nadzir, 2023).

***Corresponding author: Sri Darmawati**
Email: ciciekdarma@unimus.ac.id

Antimicrobial resistance (AMR) was directly responsible for 1.27 million deaths in 2019 in the world, with 4.95 million deaths associated with drug-resistant infections (Murray et al., 2022). A significant portion of these deaths occurred in low- and middle-income countries, in which surveillance and antibiotic stewardship are often limited (Murray et al., 2022). Recent studies in Indonesia reported a 35% prevalence of MDR bacterial isolates in clinical samples, with *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus* spp. being the most common pathogens (Prastiyanto et al., 2024b).

While antibiotics have transformed modern medicine and saved countless lives, their overuse and misuse have accelerated the emergence of MDR bacteria (Mancuso et al., 2021). The advantages of current antibiotic therapy include

rapid action and broad efficacy; however, these are increasingly offset by disadvantages, such as declining effectiveness, side effects, and the limited pipeline of new antibiotics. Novel approaches, such as natural products from plants (Prastiyanto, 2021; Prastiyanto et al., 2021; Prastiyanto et al., 2022a), mushrooms (Prastiyanto et al., 2020), lactic acid bacteria (Lestari et al., 2019), and marine-sourced drugs ((Prastiyanto et al., 2022b; Prastiyanto et al., 2024c; Prastiyanto et al., 2024d) are being explored.

Natural products, especially those derived from medicinal plants, remain underexplored as sources of new antibacterial compounds effective against MDR bacteria. In particular, *Myrmecodia pendens* (ant nest-plant), a traditional medicinal plant from Indonesia, has shown promising antibacterial activity in preliminary studies. Still, its efficacy against MDR clinical isolates has not been fully established. *M. pendens*, native to Southeast Asia and traditionally used in folk medicine, has shown promising antibacterial properties against pathogens like *Shigella dysenteriae* and *Enterococcus faecalis*, which are implicated in diarrheal and oral infections, respectively (Kuswandani et al., 2019). This research aims to address the gap by systematically evaluating the antibacterial activity of *M. pendens* extracts against MDR bacteria isolated from clinical samples. The findings will provide scientific evidence to support the traditional use of *M. pendens* and may inform the development of new treatments for MDR infections.

MATERIALS AND METHODS

Collection of ant nest plant (*Myrmecodia pendens*) specimens

The subjects of this investigation were *Myrmecodia pendens* (Figure 1). *M. pendens* were collected from Teluk Bintuni Regency, West Papua, Indonesia (2°02'56.9"S 133°43'43.7"E). The identification and classification of *M. pendens* were performed in Generasi Biologi Indonesia.

Ant nest plant extraction

The extraction of *Myrmecodia pendens* was performed using maceration procedure with ethanol, chloroform, and n-hexane. The freshly harvested *Myrmecodia pendens* was cleansed, chopped into small fragments, and sun-dried (Prastiyanto et al., 2022a). Subsequently, desiccated *M. pendens* were pulverized in a blender to provide a fine powder. The plant powder was immersed in the solvent for three consecutive 24-hour periods, with the solvent being replenished each time. The mixture was then filtered through Whatman filter paper number 1, concentrated via a rotary evaporator at temperatures below 40 °C, and subsequently processed in a water bath to get a viscous extract. The resulting extract was afterward collected and kept at ambient temperature (Prastiyanto et al., 2021) (Figure 1).

Preparation of test microorganisms

MDR bacteria were acquired from patients at K.R.M.T. Wongsonegoro Regional General Hospital, Semarang, Indonesia. This study was approved by the Hospital Ethics Committee, with Approval No. 161/Kom.EtikRSWN/VIII/2025. Clinical specimens were obtained using established techniques, inoculated onto blood agar and MacConkey agar (both from Merck, Darmstadt, Germany), and incubated overnight at 37±2 °C. Bacterial colonies were recognized based on colony type, margin, elevation, size, shape, and color. All isolates were identified, and their resistance patterns were analyzed using BD Phoenix apparatus. The MDR microorganisms utilized in this study are presented in Table I.

Subsequently, they were inoculated onto oblique HIA media, cultured at 37 °C for 24 hours, and preserved in the refrigerator as a bacterial stock. The colonies were suspended in a test tube containing 0.95% NaCl (physiological saline) and subsequently homogenized using an inoculating needle. The suspension turbidity was standardized to the McFarland 0.5 solution (1.5×10^8 cells/mL).

Antibacterial efficacy assessments

The antibacterial properties of ant nest plant extracts were assessed using diffusion and microdilution techniques (Prastiyanto & Wardoyo, 2025)

Diffusion technique

MDR bacteria were incubated on blood agar plates for 24 hours at 35 ± 2 °C. All MDR bacteria were calibrated to a 0.5 McFarland standard (1.5×10^8 colony-forming units [CFU]/mL). Each MDR bacterium was cultivated on MHA media utilizing a sterile cotton swab. After 5 minutes, the medium was perforated using a cork borer with a diameter of 0.5 cm. Three concentration variants were prepared: 100, 75, and 50 mg/mL. The resultant extract was solubilized in dimethyl sulfoxide (DMSO). Each well was administered 100 µL of the extract at different concentrations,

Figure 1. Ant nest plant (*Myrmecodia pendens*) extractions with different solvents

Table I. The organisms employed for in vitro antibacterial evaluation in this study

Species	Source
Staphylococcus aureus	blood
Escherichia coli	urine
Klebsiella pneumoniae	Sputum
Citrobacter freundii	feces
Pseudomonas aeruginosa	wound

subsequently incubated at 35±2 °C for 16-20 hours (Prastiyanto et al., 2020). Gentamicin (10 µg) was used as the positive control for *S. aureus* and *E. coli*, piperacillin/tazobactam (100 µg /10 µg) for *K. pneumoniae, C. freundii*, and *P. aeruginosa*. Dimethyl sulfoxide (DMSO**)** was used as the negative control to assess the impact of the solvent on bacterial growth.

Dilution method

Assessment of minimal inhibitory concentration (MIC)

MIC was determined using Mueller-Hinton broth (MHB) (Prastiyanto et al., 2022b). In this study, 100 µL of MHB was dispensed into each well of a microwell plate. One hundred µL of ant nest plant extract (200 mg/mL) was introduced into the first well, followed by sequential dilutions extending to the twelfth well. Following the dilution of the ant nest plant extracts, 10 µL (0.5 McFarland; 1.5 × 10^8 CFU/mL) was introduced to each well, excluding the negative control, and incubated at 35±2 °C for 18-20 hours. Subsequently, 10 µL of MDR bacteria was introduced into each well and cultured for 18-20 hours at 35±2°C. A sodium salt solution of resazurin (Sigma Aldrich ref R7017) was aseptically produced at a concentration of 0.01-0.02 g per 100 mL in sterile distilled water (Dian et al., 2019). The MIC value was ascertained by monitoring the color change on the microplate wells and contrasting it with the controls.

Assessment of minimum bactericidal concentration (MBC)

The MBC value was determined by subculturing each well onto blood agar plates for 16 to 20 hours at 35±2 °C. The value was established by monitoring the proliferation of bacterial colonies on a blood agar plate. The minimum concentration of mangrove fruit extract, at which MDR bacteria cannot proliferate, was indicated by the MBC value of the extract (Prastiyanto & Wardoyo, 2025).

RESULTS

This study represents a significant contribution by systematically comparing the antibacterial activity of *Myrmecodia pendens* extracts prepared with three diverse polarity

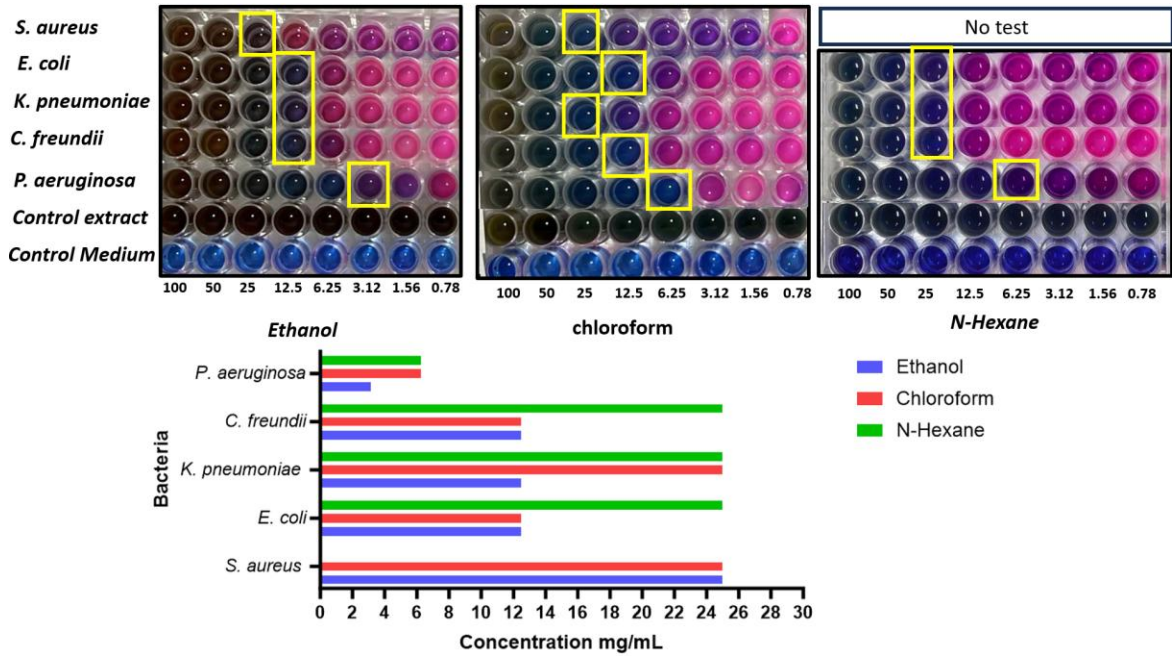

Figure 2. MIC for the extracts from ant nest plant (*Myrmecodia pendens*)

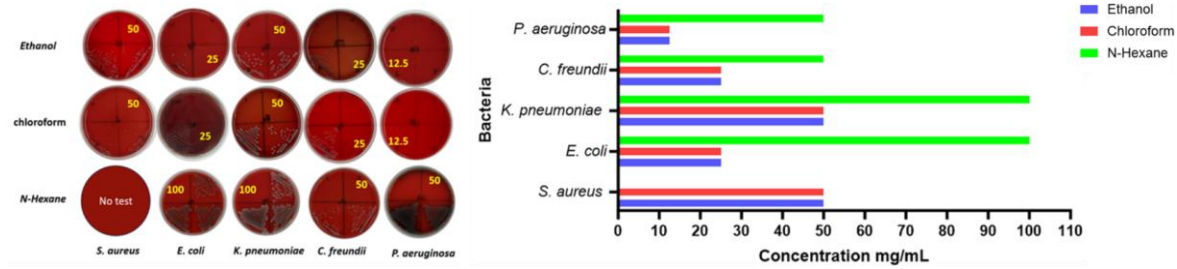

Figure 3. MBC for the ethanol extracts from ant nest plant (*Myrmecodia pendens*)

solvents (ethanol, chloroform, and n-hexane) against a panel of five multidrug-resistant clinical isolates, a direct comparison that has been limited in previous research. Our findings demonstrate that the ethanol extract of *M. pendens* possesses the most potent antibacterial activity against all tested MDR isolates, with a maximal inhibition zone of 23.15 ±0.21 mm against *P. aeruginosa*. This is consistent with existing literature suggesting that polar solvents like ethanol and methanol are highly effective in extracting bioactive compounds, such as flavonoids and phenolics, known for their antibacterial efficacy.

The ethanol extract demonstrated the strongest antibacterial effect, particularly against *P. aeruginosa*, with an inhibition zone measuring 23.15 ± 0.21 mm at 100 mg/mL concentration (Table II). The MIC and MBC for this extract against *P. aeruginosa* were 3.13 mg/mL and 12.5 mg/mL, respectively (Figures 2 and 3). The chloroform extract showed the second-highest antibacterial activity, producing an inhibition zone of 18.73 ± 0.17 mm at the same concentration, with MIC and MBC values of 6.25 mg/mL for *P. aeruginosa*. The n-hexane extract exhibited a smaller inhibition zone of 15.25 ± 0.17 mm, with MIC and MBC values of 6.25 mg/mL and 50 mg/mL, respectively, against *P. aeruginosa*.

Comparable inhibitory effects were observed for all three extracts against the other MDR bacterial strains tested. Specifically, *P. aeruginosa* was the most susceptible strain to all extracts, while *S. aureus* showed resistance to the n-hexane extract (0.00 mm). Negative results were also recorded for some extracts against certain bacterial strains, indicating variability in susceptibility. These findings suggest that while *M. pendens* extracts have potential antibacterial properties, their efficacy depends on both the solvent used for extraction and the bacterial species targeted.

Table II. A measurement of the diameters of the inhibitory zones found in the extract of the ant nest plant (*Myrmecodia pendens*)

Solvent/ antibiotic	Concentrations (mg/mL)	MDR-Bacteria test				
		S. aureus	***E. coli***	***K. pneumoniae***	***C. freundii***	***P. aeruginosa***
Ethanol	50	14.70±0.16	7.23±0.17	11.28±0.23	7.03±0.10	17.38±0.22
	75	14.68±0.21	7.70±0.18	12.72±071	11.58±0.17	17.83±0.17
	100	16.50±0.32	10.13±0.30	14.78±017	15.48±0.34	**23.15±0.21**
Chloroform	50	11.25±0.21	8.83±0.17	6.23±0.22	9.73±0.17	11.58±0.10
	75	14.83±0.17	11.25±0.21	6.75±0.17	11.25±0.21	18.25±0.21
	100	18.20±0.18	14.08±0.22	12.18±0.15	11.60±0.07	**18.73±0.17**
n-hexane	50	0.00±0.00	10.28±0.21	6.18±0.17	7.38±0.16	11.70±0.18
	75	0.00±0.00	10.53±0.15	6.70±0.18	6.58±0.25	14.85±0.13
	100	0.00±0.00	11.35±0.26	10.75±0.17	12.40±0.29	**15.25±017**
Gentamicin	10 µg	21.10±0.00	16.05±0.00	0.00±0.00	13.70±0.00	00±0.00
Erythromycin	15 µg	24.90±0.00	-	-	-	-
Streptomycin	10 µg	10.90±0.00	-	-	-	-
Ofloxacin	5 µg	02.70±0.00	-	-	-	-
Oxacillin	5 µg	02.10±0.00	-	-	-	-
Levofloxacin	5 µg	05.00±0.00	-	-	-	-
Meropenem	10 µg	-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Sulfamethoxazole/ trimethoprim	25 µg	-	23.55±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Piperacillin/ tazobactam	100 µg /10 µg	-	20.90±0.00	16.25±0.00	21.60±0.00	20.20±0.00
Ampicillin-sulbactam	20 µg	-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Ciprofloxacin	5 µg	-	0.00±0.00	0.00±0.00	22.15±0.00	0.00±0.00

DISCUSSION

Previous studies on the antibacterial properties of *Myrmecodia* species have primarily focused on their traditional uses and phytochemical compositions, with limited exploration of their efficacy against multidrug-resistant (MDR) clinical isolates. Moreover, research often lacked comprehensive comparative analyses of different solvent extracts and their specific activities against a broad spectrum of MDR pathogens. This gap highlights the need for systematic investigations into the antibacterial potential of *M. pendens*, particularly in light of the growing threat of antibiotic resistance.

This study demonstrates that ethanol extracts of *M. pendens* possess potent antibacterial activity against a range of MDR clinical isolates, including *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *C. freundii*. The ethanol extract showed the largest inhibition zone (23.15 ± 0.21 mm at 100 mg/mL), with an MIC of 3.13 mg/mL and MBC of 12.5 mg/mL against *P. aeruginosa*, outperforming both chloroform and n-hexane extracts, which showed lower activity at the same concentrations.

The results align with previous research indicating that ethanol is an effective solvent for extracting antibacterial compounds from *M. pendens*, likely due to its ability to dissolve a broad range of phytochemicals, such as flavonoids, tannins, alkaloids, and triterpenoids (Kuswandani et al., 2019). In that study, ethanol extracts and fraction combinations (particularly hexane–ethyl acetate) exhibited significant activity against *Enterococcus faecalis*, with MICs as low as 0.049 mg/mL and MBCs of 12.5 mg/mL. These findings suggest that antibacterial efficacy is closely linked to solvent polarity and the resulting phytochemical profile. This reinforces the broad-spectrum potential of *M. pendens* extracts, as observed in the present study against multiple MDR pathogens. The absence of a noteworthy difference between *M. pendens* and *M. tuberosa* is attributed to similar phytochemical contents, particularly terpenoids ansd phenolics. Other reviews and experimental papers have shown that ethanol and ethyl acetate extracts of *M. pendens* are generally more effective than water or n-hexane extracts, with inhibition zones for various pathogens (e.g., *E. coli*, *S. aureus*, *K.* and *pneumoniae*) ranging from 10 to

21 mm, depending on concentration and bacterial species (Lisnanti et al., 2024). The observed high sensitivity of *P. aeruginosa* may be attributed to specific phytochemicals extracted by ethanol, whose mechanisms could effectively overcome the efflux pump systems characteristic of this Gram-negative bacterium (Buommino and Abrosca, 2020). Conversely, the low activity exhibited by the n-hexane extract against most strains suggests that the primary antibacterial components in *M. pendens* are relatively polar, hence poorly extracted by the non-polar n-hexane solvent.

Despite these encouraging results, certain limitations must be noted. The in vitro nature of the assays may not entirely replicate the complex interactions occurring *in vivo*, including bioavailability and toxicity profiles. Additionally, the study did not isolate and characterize the specific bioactive compounds responsible for the antibacterial effects, which limits the understanding of the underlying mechanisms. These factors may influence the translational potential of our findings and should be addressed in future research.

Future investigations should focus on bioassay-guided fractionation to isolate and characterize the active constituents of *M. pendens* extracts. *In vivo* studies assessing pharmacokinetics, toxicity, and therapeutic efficacy are also essential to validate the clinical applicability of these extracts. Furthermore, exploring synergistic effects with existing antibiotics could provide valuable insights into overcoming MDR bacterial infections more effectively.

CONCLUSION

This study provides compelling evidence that *Myrmecodia pendens* extracts, particularly the ethanol extracts, possess significant antibacterial activity against a panel of five MDR bacteria from clinical samples. These findings support the plant's potential as a novel and sustainable source for developing new antibacterial agents to fight antibiotic resistance. Future investigations are highly recommended to perform bioassay-guided fractionation to isolate and identify the particular active compounds and to conduct *in vivo* studies to validate their clinical efficacy and safety

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CONFLICT OF INTEREST

The contributors assert the absence of any conflict of interest.

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