

Antibacterial Potential of Sarang Semut Herbal Extract (*Myrmecodia pendans*) from Timor Against *Staphylococcus aureus*: An In Vitro Study

Abdul Majid¹, Frans Salesman¹, Emanuel Gerald Alan Rahmat² & Nikmah²

¹ Program Ilmu Kesehatan, Universitas Citra Bangsa, Kupang, Indonesia

² Politeknik Kesehatan Kementerian Kesehatan, Kupang, Indonesia

Correspondence: Frans Salesman, Universitas Citra Bangsa, Jalan Manafe Kayu Putih, Kota Kupang. E-mail: Franssalesman@gmail.com

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Abstract

Background: The resistance of *Staphylococcus aureus* bacteria to antibiotics is still a problem in clinical medicine. The potential of Ant-nest plant extract (*Myrmecodia pendans*) from the island of Timor as an antibacterial needs to be tested to be used as a new alternative.

Objective: This study aims to determine the antibacterial potential and dose level of *Myrmecodia pendans* extract from Timor as bacteriostatic and bactericidal bacteria against *Staphylococcus aureus* in vitro.

Method: This study is an experimental study, using five doses of *Myrmecodia pendans* extract from Timor, namely positive control (cephalexin drug) and negative control (200 µl ethanol 95% + 50 µl DMSO), concentration 62.5 mg/ml (P1), concentration 125 mg/ml (P2), concentration 250 mg/ml (P3), concentration 500 mg/ml (P4), and concentration 1000 mg/ml (P5), with five replications each. Samples of *Myrmecodia pendans*, taken from Nonbaun Village, Central Fatuleu District, Kupang Regency, Mauleum Village, East Amanuban District, and Oenae Village, Kie District, South Central Timor Regency. The Kupang City Food and Drug Control Center obtained the test bacteria. Data were obtained and analyzed using the Standard *Plate Count* (SPC) method. The SPC value of each treatment was compared with the SPC value of the control treatment.

Results: The results of the analysis showed that the positive control treatment (0), negative control, and concentration treatment of 62.5 mg/ml (P1) had too much bacterial growth value (TBUD). Conversely, treatment with concentrations of 125 mg/ml (P2) and 250 mg/ml (P3) has a Minimum Inhibition Concentration (MIC) value of 9.4×10^4 CFU / ml and 6.7×10^4 CFU / ml, respectively. Treatment with concentrations of 500 mg/ml (P4) and 1000 mg/ml (P5) can both kill bacteria with a *Minimum Bactericidal Concentration* (MBC) value of 0.

Conclusion: *Myrmecodia pendans* extract from Timor Island has bacteriostatic potential (inhibits) the growth of *Staphylococcus aureus* bacteria at a concentration of at least 125 mg/ml and potentially bactericidal (deadly) at a concentration of at least 500 mg/ml.

Keywords: antibacterial, sarang semut herbal extract, vitro study

1. Introduction

Staphylococcus aureus is a bacterium that can multiply in various media, ferment carbohydrates, and produce pigments from white to yellow (Álvarez et al., 2019). Some strains of these bacteria are part of the normal flora of human skin and mucous membranes. Some other types can cause abscesses in various purulent infections, even causing fatal septicemia (Larsen et al., 2022). These pathogenic bacteria often hemolysis the blood, clot plasma, and produce various extracellular enzymes and toxins (Larsen et al., 2022; Guo et al., 2020).

Staphylococcus aureus enterotoxin can rapidly develop resistance to various antibacterials and become a problem in therapy (Álvarez et al., 2019; 2). *Staphylococcus aureus enterotoxin* has beneficial coagulation properties that distinguish it from other species. *Staphylococcus enterotoxin* is the primary pathogen of infection in humans (Guo et al., 2020; Chaudhury et al., 2016). Every tissue and organ of the body can be infected by these bacteria and cause disease with characteristic signs of inflammation, necrosis, and abscess formation (AlSheikh et al., 2020). *Staphylococcus aureus* is one of the pathogens that cause gastrointestinal infections by producing toxins that result

in fluid secretion and interfere with cell function or nerve function (Dirgantara, Insanu, & Fidrianny, 2022).

Staphylococcus aureus is sensitive to various antimicrobials such as penicillin G, ampicillin, tetracycline, naphthillin, methicillin, and oxacillin (Soviati et al., 2022). In addition, *Staphylococcus aureus* can carry genes resistant to tetracycline, erythromycin, aminoglycosides, and methylene, resulting in new strains. New strains, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), produce beta-lactamase, so these bacteria have different sensitivities to different antimicrobial drugs (Dirgantara, Insanu, & Fidrianny, 2022; Khairiah et al., 2019).

Staphylococcus aureus is a pathogen that has infected 30% of the world's human population (; even most hospital infections are known to be resistant to all antibiotics (Chaudhury et al., 2016; Kementerian Kesehatan RI, 2014). *Staphylococcus aureus* will form mutants immune to specific antibiotic attacks (Baron, Peterson, & Finegold, 1994). If that happens, patients with pneumonia and postoperative infection due to mutant *Staphylococcus aureus* bacterial infection can no longer be treated with antibiotics (Khairiah et al., 2019). In addition to vancomycin, the antibiotic drug relied upon to date for *Staphylococcus aureus* bacterial infection is *Cephalexin*. (Guo et al., 2020)

Study results show that aspects of therapy with antibiotics available are limited and less reliable, so it is necessary to research the effects of medicinal plants as an alternative (AlSheikh et al., 2020). Plants that contain several active compounds, including tannins, essential oils, phenols, alkaloids, flavonoids, and others, are proven to inhibit bacterial growth or, at specific concentrations, can kill gram-positive and harmful bacteria (Dirgantara et al., 2022).

Based on the problem description above, it is necessary to research the potential of medicinal plants as an alternative. One of the medicinal plants commonly used by people in Timor NTT empirically and potentially as an antibacterial is Sarang Semut (*Myrmecodia pendans*). The anthill is a plant with sharp bulbs of the *Rubiaceae* family and is found abundantly in Timor.

The potential of Sarang Semut (*Myrmecodia pendans*) as an alternative treatment is proven to be accurate ($p < 0.05$) and can inhibit the growth of *Streptococcus sanguinis* bacteria (batesiotatis) in dental therapy (Soviati et al., 2022). The results of other studies in vitro showed that fractionation of anthill plants (*Myrmecodia pendans*) was able to reduce *TNF-α* ($p = 0.011$) and histopathological improvement in cases of osteoarthritis cartilage ($p = 0.034$) (Hidajat et al., 2018). The results of other experiments showed that anthills (*Myrmecodia pendans*) as a food additive also produced a high antioxidant potential of 85.90%, even offered to have antibacterial activity against five types of bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 25175, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028) with an inhibitory zone of 10-13.5 mm (Khairiah et al., 2019). Anthill plant extracts have been widely studied for pharmacological activities such as antioxidants, cytotoxic, and anti-cancer (Dirgantara et al., 2022).

Sarang semut (*Myrmecodia pendans*) is a plant with sharp bulbs of the *Rubiaceae* family, found in Timor, East Nusa Tenggara. Seeing the potential benefits of anthill plants (*Myrmecodia pendans*) as an empirical alternative treatment, it is necessary to conduct research that explicitly examines these plants' antibacterial potential. This study aims to determine the antibacterial potential and dose level of *Myrmecodia pendans* extract from Timor, which can inhibit the growth (bacteriostatic) and deadly (bactericidal) of *Staphylococcus aureus* bacteria.

2. Research Method

This research design is a type of experimental research consisting of 5 (five) concentrations of *Myrmecodia pendans* extract (P1, P2, P3, P4, P5) and control (C) every five replications (Figure 1).

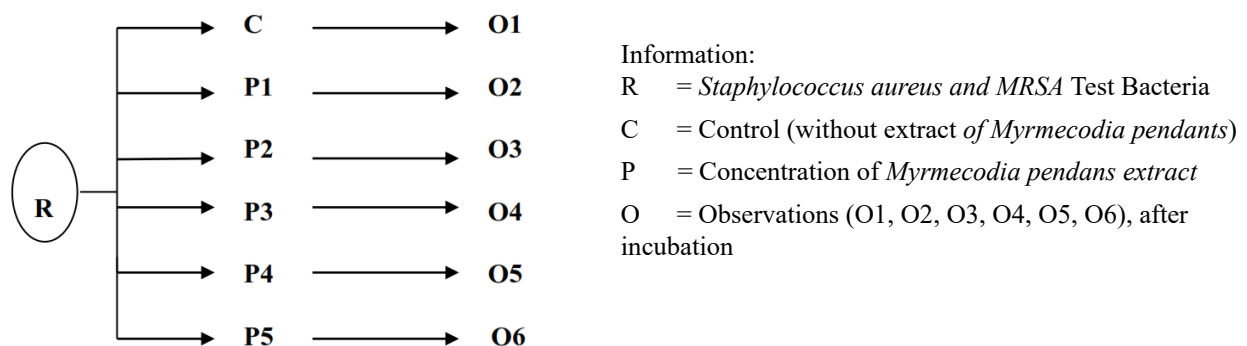


Figure 1. Research Design

The samples in this study were *Myrmecodia pendans* and test bacteria (*Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA)). *Myrmecodia pendans* were obtained from Baun Village, West Amarasi District, Kupang Regency, East Nusa Tenggara. *Staphylococcus aureus* was obtained from the Center for Drug Control and Kupang City, and MRSA was obtained from isolates of burn patients.

2. Materials

Myrmecodia pendans, Ethanol 96%, Barium Sulphate (BaSO₄), NaCl 0.85%, Medium (Tryptic Soy Broth), Medium Nutrient Broth, Autoclave, Oven, Incubator, Colony Counter, Vortex, Analytical Scales, Vacuum Evaporator, Syringe disposable 5 ml, *Staphylococcus aureus* and *Methicillin Resistant Staphylococcus aureus* (MRSA) Bacteria

2.1 Time and Location

This study was conducted from March to June 2016. Simplisia and antibacterial activity tests of *Myrmecodia pendans* were carried out at the Biological Laboratory, Faculty of Teacher Training and Education, University of Muhammadiyah Kupang. *Myrmecodia pendans* extract, preparation, testing, and purification of test bacteria (*Staphylococcus aureus* and MRSA) were carried out at the Chemistry Laboratory of the Faculty of Teacher Training and Education, Nusa Cendana University Kupang.

2.2 Procedures, Data Collection and Analysis

2.2.1 Extract Preparation

The preparation of *Myrmecodia pendans* extract refers to (Harborne et al., 1996; Kementerian Kesehatan RI, 2014), namely: *Myrmecodia pendans* herbs (roots, stems, leaves) are dried by aeration (not with direct sunlight). After drying, a fine powder is made using a grinding machine. A fine powder of 2 kg was soaked in 96% ethanol solvent for 4 x 24 hours. Every 24 hours, it is filtered, and then ethanol is added. The filtrate is accommodated in sterile Erlenmeyer tubes. Twelve Liters of filtrate are evaporated with a vacuum evaporator to obtain a viscous extract. The thick extract is dried in a freeze-dryer.

2.2.2 Test Bacterial Culture

Test bacterial cultures were propagated on nutrient media sequentially and incubated at 37 °C for 24 hours after planting.

2.2.3 Bacterial Test Suspension Preparation

The results of the test bacterial culture were taken in as many as 4-5 colonies, then suspended in a tube containing 5 ml of 0.85% sodium chloride (NaCl) solution. After that, the turbidity of NaCl-containing test bacteria was adjusted to the standard turbidity of barium sulfate (BaSO₄) 0.5 (Mc. Farland 0.5). After the turbidity is appropriate, the test bacteria on liquid media can be grown on the test media (Baron et al., 1994).

2.2.4 Extract Activity Test Against *Staphylococcus aureus* and MRSA

Test the effect of *Myrmecodia pendans* extract of *Staphylococcus aureus* bacteria and MRSA in vitro, carried out by tube dilution method referring to (Baron et al., 1994). 800 mg thick herb extract of *Myrmecodia pendans* dissolved in 160 ml of 0.2% ethanol solvent and 40 ml of DMSO. Furthermore, dilution was carried out with TSB liquid media to obtain a series of sample concentrations of 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and control 0 mg/ml. Each extract concentration was taken 1 ml, and 0.1 ml of 24-hour age test bacteria suspension was added so that each concentration contained 150 million test bacteria/ml (1.5×10^8 CFU / ml). Then vortex or homogenised then incubated for 24 hours, temperature 37 °C.

After incubation, the dilution is repeated: A dilution of 10⁻³ is taken in 0.1 ml on agar media by the Pour method. Let stand to dry at room temperature, then incubate for 24 hours, temperature 37°C. After incubation, the number of colonies of *Staphylococcus aureus* and MRSA was calculated and compared with controls at the lowest concentrations, indicating bacteriostatic or bactericidal activity. The data obtained were analyzed using Standard Plate Count (SPC).

3. Results

3.1 Growth of *Staphylococcus aureus* Bacteria

The results of calculating the number of *Staphylococcus aureus* bacteria growing (viable count) on agar media as antibacterial activity of *Myrmecodia pendans* herbal extract from Timor can be seen in Table 1.

Table 1. Number of Live *Staphylococcus aureus* Bacteria growing on Viable count (CFU/ml)

Replication	The concentration of herbal extract of <i>Myrmecodia pendans</i> (Dilution 10 ⁻³)					
	Control (+) dan Control (-)	P1	P2	P3	P4	P5
1	0 TMTC	TMTC	8,4x10 ⁴	9,6x10 ⁴	0	0
2	0 TMTC	TMTC	7,2x10 ⁴	7,6x10 ⁴	0	0
3	0 TMTC	TMTC	9,2x10 ⁴	3,2x10 ⁴	0	0
4	0 TMTC	TMTC	1,0x10 ⁵	8,4x10 ⁴	0	0
5	0 TMTC	TMTC	1,2x10 ⁵	4,8x10 ⁴	0	0
Mean	0 TMTC	TMTC	9,4x10 ⁴	6,7x10 ⁴	0	0

*TMTC = Too Many To Count.

The data in Table 1 above show that concentrations of 62.5, 125 and 250 mg/ml show an average number of bacteria that are too much to count (TMTC), and concentrations of 500 and 1000 mg/ml show an average number of live bacterial colonies of 1.7 x 10⁵ CFU/ml (concentration of 500 mg/ml) and 1.3 x 10⁵ CFU/ml (concentration of 1000 mg/ml), It can be concluded that at this concentration, the growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria is inhibited because the bacteria planted earlier in *this* study were 1.5 x 10⁸ CFU/ml (McFarland Table 0.5). The test results showed that no concentration killed the growth of *Methicillin-Resistant Staphylococcus aureus*.

3.2 Growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria

The results of observation and calculation of the number of growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria (viable count) on agar media as an activity of herbal extracts of *Myrmecodia pendans* from Timor can be seen in Table 2.

Table 2. Number of Methicillin Resistant *Staphylococcus Aureus Bacteria* (MRSA) Live (CFU/ml)

Replication	The concentration of herbal extract of <i>Myrmecodia pendans</i> (Dilution 10 ⁻³)					
	Control (+) dan Control (-)	Control (+) dan Control (-)	Control (+) dan Control (-)	Control (+) dan Control (-)	Control (+) dan Control (-)	Control (+) dan Control (-)
1	0 TMTC	TMTC	TMTC	TMTC	1,5x10 ⁵	1,6x10 ⁵
2	0 TMTC	TMTC	TMTC	TMTC	1,4x10 ⁵	9,2x10 ⁴
3	0 TMTC	TMTC	TMTC	TMTC	1,4x10 ⁵	6,8x10 ⁴
4	0 TMTC	TMTC	TMTC	TMTC	9,2x10 ⁴	1,1x10 ⁵
5	0 TMTC	TMTC	TMTC	TMTC	1,9x10 ⁵	6,4x10 ⁴
Mean	0 TMTC	TMTC	TMTC	TMTC	1,7x10 ⁵	1,3x10 ⁵

*TMTC = Too Many To Count.

Table 2 data above, shows that concentrations of 62.5, 125 and 250 mg/ml on average show the number of bacteria too much to count (TMTC), and concentrations of 500 and 1000 mg/ml show an average number of live bacterial colonies of 1.7 x 10⁵ CFU/ml (concentration 500 mg/ml) and 1.3 x 10⁵ CFU/ml (concentration 1000 mg/ml) so it is considered at these concentrations the growth of *Methicillin-Resistant Staphylococcus aureus* bacteria (MRSA) was inhibited because bacteria grown earlier in *this* study were 1.5 x 10⁸ CFU/ml (McFarland Table 0.5), while the concentration that killed the growth of *Methicillin-Resistant Staphylococcus aureus* was absent.

4. Discussion

Staphylococcus aureus is a pathogenic bacteria that causes gastrointestinal infections with a toxin that results in fluid secretion and disrupts cell or nerve function (Baron et al., 1994). In contrast, Methicillin Resistant *Staphylococcus aureus* (MRSA) is a *Staphylococcus* bacterium resistant to most antibiotics (Martinez, 2023).

The results showed that *the Myrmecodia pendens' herbal extract at 125 mg/ml reduced* the number of *S. aureus* bacteria. These *bacteria died at the concentration of Myrmecodia pendens herbal extract of 500 mg/ml*, while MRSA bacteria could only be reduced in number at a concentration of 500 mg/ml. The results show that this study's treatment cannot kill MRSA bacteria. The results of this study are supported by previous studies that found that a concentration of 3.12% ethanol extract of *Myrmecodia pendens* only has a Minimal Inhibition Concentration (MIC) against the growth of *Shigella dysenteriae* bacteria. Similarly, Hakim (2011) found that ethanol extract of *Myrmecodia pendens* at 31.25 mg/ml had a Minimal Inhibition Concentration (MIC) against the growth of *Salmonella typhi* bacteria. Other study results also found that the effect of *Myrmecodia pendens* herbal extract, at a concentration of 50%, can inhibit the growth of *Escherichia coli* bacteria and *Candida albicans* (Roslizawaty, Ramadani, & Fakhurrrazi, 2013; Yuliani & Ismail, 2023).

The results of testing the activity of herbal extracts of *Myrmecodia pendans* against MRSA growth found that at a concentration of 500 mg/ml, there was no bacteriostatic activity. No concentration in this study could kill these bacteria as it is known that MRSA bacteria are resistant bacteria, generally in beta-lactam drugs in the penam group, especially methicillin. In contrast, these bacteria are still slightly sensitive to penicillin, amoxicillin, ampicillin, and others (Lowy, 2003). MRSA's resistance to test extracts in this study is most likely due to the influence of adaptation and evolution of these bacteria, stating that the pathogenic nature of *S. aureus* is caused by the influence of bacterial adaptation and evolution so that bacteria become more virulent and more resistant to antibiotics (Wong et al., 2022).

Increasing the concentration of *Myrmecodia pendans* herbal extract will increase penetration of cell walls and cytoplasmic membranes of *Staphylococcus aureus* and MRSA bacterial cells and increase interaction with biological receptors. The increase in the concentration of such extracts is directly proportional to the antibacterial activity of the herbal extract of *Myrmecodia pendans*. The results of research data analysis of the number of *Staphylococcus aureus* bacteria grown (viable count) in the media show that the herbal extract of *Myrmecodia pendans* from Timor has the potential to inhibit (bacteriostatic) the growth of *Staphylococcus aureus* bacteria and MRSA bacteria. However, in this study, no concentration could kill MRSA bacteria. Moreover, on the contrary, only a concentration of 500 mg/ml can kill *Staphylococcus aureus* bacteria, *so it can be ascertained for the treatment process of diseases in humans caused by Staphylococcus aureus* bacteria, a concentration of 500 mg/ml of *Myrmecodia pendans* herbal extract those containing flavonoid compounds and tannins can be used. Conversely, the treatment of diseases due to MRSA still requires greater concentration.

5. Conclusion

The herbal extract of *Myrmecodia pendans* from Timor has the potential as bacteriostatic in the growth of *Staphylococcus aureus* bacteria (Minimum Inhibition Concentration 125 mg/ml) and against Methicillin-Resistant *Staphylococcus aureus* (Minimum Inhibition Concentration 500 mg/ml). While the bactericidal potential in *Staphylococcus aureus* bacteria is (Minimum Inhibitory Concentration 500 mg/ml), there is no lethal potential (bactericidal) against Methicillin-Resistant *S. aureus*.

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Data Availability Statement

The data that support the findings of this study are available on request.

Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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