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Research Article

Effect of Katuk Leaves (*Sauropus androgynus*) on Haematology Profile of Infected Rats with Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a formidable pathogen, causing severe infections in humans and animals, often leading to local and systemic inflammation. In light of this, it becomes imperative to explore novel therapeutic avenues. One such promising approach is the utilization of herbal-derived antioxidants, with katuk leaves (*Sauropus androgynus*) being a prime example. This study aimed to evaluate the efficacy of katuk leaves alcoholic extract (KLE) against systemic MRSA infection in rat models.

Materials and methods: This study used 36 male Sprague-Dawley rats. They were divided into six groups including, healthy rats (Group C), infected rats without treatment (Group K), infected rats + 100 mg vancomycin per kg BW (Group V), infected rats + 1,000 mg/kg BW of KLE (Group T1), infected rats + 2,000 mg/kg BW of KLE (Group T2), and infected rats + 4,000 mg/kg BW of KLE (Group T4). The therapy was given twice daily for seven days. On the final day, the blood and sera were collected and tested against total erythrocytes, leucocytes, indices of erythrocytes, differential leucocyte count, and C-reactive protein (CRP).

Results: The findings showed that the administration of 4,000 mg/kg BW of KLE potentially leads to more favorable changes in haematological parameters compared to the healthy group, particularly for hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets, monocytes, neutrophils, and CRP. Additionally, the 4,000 mg/kg BW of KLE increases the ratio of lymphocytes/neutrophils compared to the other groups.

Conclusion: The KLE has the critical benefit of being a systemic antibacterial agent against MRSA at dosage 4,000 mg/kg BW, especially in improving the haematological profile and CRP in rat models.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to cause severe infections in both humans and animals. In 2005, the rate of MRSA infections was reported to be 31.8 per 100,000 individuals in the United States[1]. The impact of MRSA goes beyond systemic infections and extends to conditions such as pneumonia, hepatitis, meningitis, encephalitis, and, in some cases, fatalities[2]. Owing to the threat of escalating antibiotic resistance, the use of antibiotics is restricted[3,4], underscoring the critical need to develop alternative therapies against MRSA.

One promising avenue in the search for alternative therapies against MRSA involves the use of herbal-derived antioxidants. These antioxidants have shown the potential to inhibit bacterial colonization, reduce oxidative stress, and aid in cellular repair[5]. Among these, katuk leaves (*Sauropus androgynus*) stand out as a unique herbal product, rich in alkaloids, terpenoids, flavonoids, tannins, and saponins[6]. These biochemical compounds have demonstrated antimicrobial properties and the potential to act as inhibitors of membrane synthesis and membrane

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osmolarity[7]. The objective of this study is to investigate the efficacy of katuk leaves' alcoholic extract against systemic MRSA infection in rat models, aiming to gather evidence on the potential of herbal-derived antioxidants as alternative therapies against MRSA.

2. Materials and Methods

2.1. Ethical approval

The ethical clearance committee from the Faculty of Veterinary Medicine, UWKS approved the animal utilization in this study. The registration number was KKE-34. The committee performed the monitoring during the experiment. The study was conducted from January 2022 until August 2022 in the Laboratory of Pharmacology, Faculty of Veterinary Medicine, University of Wijaya Kusuma Surabaya, Indonesia.

2.2. Herbal preparation

The katuk leaves from Mojokerto, East Java, were dried and extracted using 70% alcohol according to established research procedures[8]. The maceration process was repeated four times, and all the supernatant was evaporated using an evaporator from Switzerland until a thick extract was obtained. The resulting katuk leaf extract (KLE) was then stored in a fridge at 4°C.

2.3. Research procedure

Before conducting the artificial infection, a clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) was obtained from the Laboratory of Bacteriology, Faculty of Health, University of Muhammadiyah Sidoarjo. The isolate was then enriched using mannitol salt agar (MSA) and transferred into liquid media. The turbidity of the bacterial suspension was standardised using a densitometer with 1.0 McFarland standard[9].

Thirty-six male Sprague Dawley rats (3 months old, 250-gram weight) from the Laboratory of Animal Models, Faculty of Health, University of Muhammadiyah Sidoarjo were acclimated for seven days and separated into six groups. The rats were maintained at 12/12 hours of light/dark, 25°C temperature, with *ad libitum* access to food and water. All groups were intraperitoneally infected using 50µL of MRSA suspension at 1.0 McFarland standard[8]. The therapy was administered 24 hours after the artificial infection.

The groups were as follows,healthy rats (Group C), Group K: infected rats without treatment (Group K), Group V: infected rats + 100 mg vancomycin per kg BW (Group V), Group T1: infected rats + 1,000 mg/kg BW of KLE (Group T1), Group T2: infected rats + 2,000 mg/kg BW of KLE (Group T2), and Group T4: infected rats + 4,000 mg/kg BW of KLE (Group T4). The therapy was given twice daily (6 am and 6 pm) for seven days using a gastric probe.

After seven days of infection, blood samples were collected from the tail vein (2 ml), and subsequent serum was obtained from the blood samples. The rats were then euthanised using lethal doses of dissociative anaesthetic (150 mg/kg BW ketamine [Netherlands] and 10 mg/kg BW xylazine [Netherlands]). A routine haematological test was conducted using an automated haematology analyser (Boule, Sweden) to test for total red blood cells (RBCs), total white blood cells (WBCs), haemoglobin (Hb), packed cell volume (PCV), RBC indices, WBC differential count, total platelets, and the lymphocytes/neutrophils (L/N) ratio. The serology was tested against C-reactive protein (CRP)[10].

2.4. Statistical analysis

The collected data was analyzed using SPSS (USA) version 26. The analysis was performed using one-way ANOVA and a post hoc test using LSD. The statistical test used a probability equal to or less than 0.05. The statistical result was reported as the mean ± standard deviation.

3. Results

The research findings indicate that simulated MRSA infection in mouse models resulted in anaemia on day 7. This anaemia was characterized by a significant reduction in RBCs, Hb, and PCV in Group K compared to Group C ($p < 0.05$). Additionally, untreated infection decreased platelet counts compared to the other groups ($p < 0.05$). Treatment with 100 mg/kg of vancomycin, 1,000 mg/kg BW of KLE, and 2,000 mg/kg KLE produced similar results in RBCs, Hb, MCV, MCH, MCHC, and platelets ($p > 0.05$). However, the group treated with 4,000 mg/kg BW of KLE demonstrated the most significant improvement in Hb, PCV, MCV, MCH, MCHC, and platelets, which were comparable to those of the healthy group ($p > 0.05$) and distinct from the infected groups ($p < 0.05$, Table 1).

Table 1. RBC indices and platelets count of rats systemically infected by MRSA after 7 days of treatment

Parameters	Group (mean ± standard deviation)					
	C	K+	V	T1	T2	T4
RBCs (× 10^3 cells/µL)	5.54±0.02^a	4.86±0.17^b	5.40±0.42^c	5.30±0.17^c	5.38±0.26^d	5.37±0.12^c
Hb g/dL)	14.05±0.24^a	10.38±0.21^b	10.90±0.16^c	11.09±0.16^c	11.44±0.24^c	13.41±1.22^a
PCV (%)	41.42±0.37^a	33.32±0.05^b	37.89±0.24^c	37.58±0.05^c	37.98±0.16^d	39.06±0.57^a
MCV (fL)	72.56±1.03^a	68.55±4.22^b	70.14±0.09^c	70.92±0.12^c	70.57±0.08^a	72.70±2.51^a
MCH (Pg)	25.68±0.07^a	21.36±1.00^b	20.18±0.23^c	29.52±0.4^c	21.26±0.73^a	24.96±2.50^a
MCHC (%)	35.12±0.81^a	31.21±1.52^b	28.76±3.04^c	29.52±1.05^c	30.13±1.14^c	34.32±3.10^a
Platelets (× 10^3 cells/µL)	4.23±0.18^a	3.38±0.29^b	4.07±0.81^a	4.00±0.19^a	4.16±0.81^a	4.27±0.12^a

RBCs: Red blood cells, Hb: Haptoglobin, PCV: packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration

Table 2. White blood cells and differential leucocyte count of rats systemically infected by MRSA after 7 days of treatment

Parameters	Group (mean ± standard deviation)					
	C	K+	V	T1	T2	T4
White blood cells (× 10^3 cells/µL)	6.35±0.05[a]	8.73±0.14[b]	6.89±0.42[c]	7.29±0.17[d]	7.17±0.26[d]	7.03±0.19[c]
Neutrophils (× 10^3 cells/µL)	1.13±0.21[a]	2.09±0.21[b]	1.72±0.16[c]	1.47±0.16[d]	1.35±0.24[c]	1.14±0.12[a]
Lymphocytes (× 10^3 cells/µL)	4.45±0.02[a]	5.94±0.05[b]	4.55±0.05[a]	5.44±0.05[d]	5.5±0.16[d]	5.41±0.48[d]
Monocytes (× 10^3 cells/µL)	0.24±0.2[a]	0.57±0.22[b]	0.53±0.09[c]	0.26±0.12[a]	0.21±0.08[a]	0.27±0.19[a]
Ration L/N	3.98±0.42[a]	2.85±0.25[b]	2.65±0.23[c]	3.72±0.4[a]	4.17±0.73[a]	4.77±0.58[a]
CRP (mg/dL)	49.12±0.04[a]	92.20±4.49[b]	55.60±3.04[c]	58±1.05[c]	57.6±1.14[c]	50.40±1.14[a]

CRP: C-reactive protein, Ratio L/N: Lymphocyte/Neutrophils

Furthermore, there was an elevation in WBCs, neutrophils, lymphocytes, monocytes, and CRP in the untreated infected group compared to the healthy group ($p < 0.05$). Group V exhibited significant differences in the reduction of WBCs, neutrophils, lymphocytes, and CRP compared to Group K+ ($p < 0.05$). Nonetheless, Group V still displayed higher levels of WBCs, neutrophils, monocytes, and CRP compared to Group C ($p \leq 0.05$), except for the L/N ratio, which showed a lower level. Additionally, Groups T1 and T2 did not exhibit differences in WBCs, lymphocytes, monocytes, L/N ratio, and CRP. Group T4, treated with 4,000 mg/kg BW of KLE, demonstrated the most favorable outcome regarding neutrophils and monocytes compared to Group C ($p > 0.05$). However, Group T4 still exhibited higher levels of WBCs, lymphocytes, L/N ratio, and CRP compared to Group C ($p < 0.05$, Table 2).

4. Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant pathogenic bacterium in public health. This bacterium can induce severe organ damage through infection. Bacteremia, the presence of bacteria in the bloodstream, further exacerbates organ damage within the host's circulatory system[11]. MRSA releases various virulence factors throughout infection, including enzymes, leucocidin, adhesins, and toxins[12]. These virulence factors play a pivotal role in supporting MRSA's capacity to form biofilms, propagate, and ultimately result in fatal outcomes.

The pathogenesis of MRSA within the host's body can be identified through a haematological profile. Anaemia is evident in the infected group, while in the untreated group, anaemia is a response to the haemorrhage caused by MRSA[13]. Furthermore, the presence of MRSA results in an elevation in white blood cell count (leukocytosis)[9], leading to an increase in C-reactive protein (CRP). CRP is a critical acute-phase protein in the pathogenesis of the disease. Thus, prompt treatment of MRSA infection is essential to mitigate extensive organ damage[14].

This study has shown that vancomycin, the primary treatment for MRSA infection, has significant effects. Rats treated with vancomycin exhibited elevated RBCs, Hb, PCV, and platelets and decreased WBCs, neutrophils, lymphocytes, and CRP levels. The decrease in CRP levels, an acute-phase protein synthesized by the liver and a biomarker for inflammation and disease prognosis, indicates a reduction in inflammation[15]. The increased presence of lymphocytes, which play a crucial role in attracting other healing factors during infection[16], further supports the effectiveness of vancomycin in treating MRSA. These findings provide strong evidence for the use of vancomycin in MRSA treatment.

In this particular investigation, the therapeutic potential of katuk leaves was explored in the context of combating artificially induced MRSA infection in rat models. Previous research has affirmed the presence of essential biochemical constituents in these leaves, including alkaloids, flavonoids, phenolics, saponins, and tannins[17,18]. Notably, the phenolic content within the leaves predominates, and its inhibitory effect on MRSA growth in both liquid and solid cultures has been well-documented[6]. The efficacy of katuk leaf extract (KLE) was validated in this study through observed improvements in the haematology profile of rat models with experimentally induced MRSA infection. Additionally, the administration of 4,000 mg/kg BW of KLE exhibited the potential to ameliorate the L/N ratio in rat models, reflective of heightened circulatory lymphocyte levels.

The elevation of lymphocytes plays a pivotal role in enhancing the specific immune system's ability to combat infection and oxidative stress[19]. The presence of KLE as a source of exogenous antioxidants in the bloodstream contributes to preserving the integrity of host cell membranes and inhibiting bacterial attachment. The reduced occurrence of bacterial attachment within the KLE group mitigates the risk of more severe infection and pathogenesis.

5. Conclusion

Katuk leaves alcoholic extract (KLE) demonstrates essential benefits as a systemic antibacterial agent against MRSA. The effective dose of KLE is 4,000 mg/kg BW. Blood indicators can assess KLE potency against MRSA infection. Future research should focus on advanced histopathology studies, immunohistochemistry, and toxicity of KLE during MRSA infection.

Declarations

Competing interests

The author declare that they have no competing interests.

Authors' contributions

Yos Adi Prakoso, Puput Ade Wahyuningtyas, Paskalis

Guntur Widya Mahendra, and Oscar Maulana Pribadi contributed to the design and conducting the experimental procedure. Yos Adi Prakoso conducted writing the draft of the manuscript. Puput Ade Wahyuningtyas, Paskalis Guntur Widya Mahendra, and Oscar Maulana Pribadi agreed on the submitted version of this manuscript. The analyzed data and final version of the article are approved by all authors.

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Ethical considerations

The authors carefully examined all ethical issues concerning plagiarism, approval to publish, errors in farbrication, double publication, and submission.

Availability of data and materials

All data and findings related to research are prepared for publication in this journal.

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References

1. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, and Ray S. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA. 2007; 298(15): 1763-1771. DOI: 10.1001/jama.298.15.1763
2. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): prevalence and antimicrobial sensitivity pattern among patients-A multicenter study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019; 2019: 8321834. DOI: 10.1155/2019/8321834
3. Qasemi A, Bayat Z, Akbari N, Babazadeh D. Bacterial Resistance of *Acinetobacter baumannii:* A Global Concern. Res Biotechnol Environ Sci. 2022; 1(2): 36-42. DOI: 10.58803/RBES.2022.1.2.01
4. Eslammanesh T, Rezaei M, Dahmardeh N, Anoosha A. Investigation of the Antimicrobial Activity of Nine Medicinal Plants on Standard Bacteria. Res Biotechnol Environ Sci. 2024; 3(2): 29-38. DOI: 10.58803/rbes.v3i2.47
5. Yashin A, Yashin Y, Xia X, and Nemzer B. Antioxidant activity of spices and their impact on human health: A review. Antioxidants. 2017; 6(3): 70. DOI: 10.3390/antiox6030070
6. Anju T, Rai N, and Kumar A. *Sauropus androgynus* (L.) Merr.: A multipurpose plant with multiple uses in traditional ethnic culinary and ethnomedicinal preparations. J Ethn Foods. 2022; 9: 10. DOI: 10.1186/s42779-022-00125-8
7. Rahayu A, Rini CS, Prakoso YA, Palgunadi BU, and Munandar MA. Effects of *Sauropus androgynus* extract and its combination with ampicillin against methicillin-resistant *Staphylococcus aureus*: An *in vitro* study. Int J One Health. 2020; 6(2): 128-133. Available at: https://www.onehealthjournal.org/Vol.6/No.2/6.html
8. Prakoso YA, and Kurniasih. The effects of *Aloe vera* cream on the expression of CD4+ and CD8+ lymphocytes in skin wound healing. J Trop Med. 2018; 2018: 6218303. DOI: 10.1155/2018/6218303
9. Prakoso YA, and Wijayanti AD. Efficacy of celery (*Apium graveolens* L.) alcoholic extract against systemic methicillin-resistant *Staphylococcus aureus* infection in rat models. Vet World. 2022; 15(4): 898-905. DOI: 10.14202/vetworld.2022.898-905
10. Kanaparthy A, Kanaparthy R, and Niranjan N. Evaluation of serum C-reactive protein levels in subjects with aggressive and chronic periodontitis and comparison with healthy controls. Dent Res J. 2012; 9(3): 261-265. DOI: 10.4103/1735-3327.99810
11. Hassoun A, Linden PK, and Friedman B. Incidence, prevalence, and management of MRSA Bacteremia across patient populations-A review of recent developments in MRSA management and treatment. Crit Care. 2017; 21: 211. DOI: 10.1186/s13054-017-1801-3
12. Cheung GYC, Bae JS, and Otto M. Pathogenicity and virulence of *staphylococcus aureus*. Virulence. 2021; 12(1): 547-569. DOI: 10.1080/21505594.2021.1878688
13. Verdecia J, Hernandez J, Izzo C, Sottile E, and Isache C. Methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis complicated by warm autoimmune haemolytic anaemia secondary to antimicrobial therapy. BMJ Case Rep. 2019; 12(8): e229114. DOI: 10.1136/bcr-2018-229114
14. Sproston NR, and Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front immunol. 2018; 9: 754. DOI: 10.3389/fimmu.2018.00754
15. Anush MM, Ashok VK, Sarma RI, and Pillai SK. Role of C-reactive protein as an indicator for determining the outcome of sepsis. Indian J Crit Care Med. 2019; 23(1): 11-14. DOI: 10.5005/jp-journals-10071-23105
16. Rimmelé T, Payen D, Cantaluppi V, Marshall J, Gomez H, Gomez A, et al. Immune cell phenotype and function in sepsis. Shock. 2016; 45(3): 282-291. DOI: 10.1097/SHK.0000000000000495
17. Prakoso YA, Kurniasih, Wijayanti AD, and Kristianingrum YP. Activity of *Aloe vera*, *Sauropus androgynus* and Sauropus androgynus alcoholic extracts against methicillin–resistant *Staphylococcus aureus*. World Vet J. 2019; 9(4): 302-310. Available at: https://wvj.scienceline.com/attachments/article/59/WVJ%209(4)%20302-310,%20December%2025,%202019.pdf
18. Purba RAP, and Paengkoum P. Exploring the phytochemical profiles and antioxidant, antidiabetic, and antihemolytic properties of *Sauropus androgynus* dried leaf extracts for ruminant health and production. Molecules. 2022; 27(23): 8580. DOI: 10.3390/molecules27238580
19. Kabak M, Çil B, and Hocanl I. Relationship between leukocyte, neutrophil, lymphocyte, platelet counts, and neutrophil to lymphocyte ratio and polymerase chain reaction positivity. Int Immunopharmacol. 2021; 93: 107390. DOI: 10.1016/j.intimp.2021.107390