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Optimizing Sappan Wood Extract to Boost Superoxide Dismutase Activity and Phagocytosis in *Escherichia coli* Infected Mice

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ABSTRACT
Antibiotic resistance, particularly in *E. coli* ESBL, is sparked by self-medication practices encouraged by infectious infections in Indonesia. It is anticipated that the application of immunomodulators like sappanwood will defeat resistant bacterial infections. Although sappanwood extract's antioxidant, antibacterial, and immunomodulatory properties have been demonstrated in earlier research, nothing is known about how it affects *E. coli* ESBL infections. The purpose of this study is to determine whether or not sappanwood extract can boost the immune response in mice harboring antibiotic-resistant *E. coli*. Methods: Fifty male rats were employed in the investigation, split into five groups. For ten days, ethanol extract of sappanwood (EEKS) at doses of 280 ppm, 560 ppm, and 840 ppm were administered to each of the three treatment groups (K1, K2, and K3), while the positive control group (K+) received standard brazilin. The negative control group (K-) received sterile distilled water. Following the injection of *E. coli* ESBL into groups K+, K1, K2, and K3, measurements of SOD activity, phagocytosis capacity (KF), and phagocytosis index (IF) were performed. Results: When compared to the negative control group, the EEKS treatment group had higher KF of 86.4%, IF of 81%, and SOD levels of 64.3%. Conclusion: The ethanol extract of sappanwood significantly stimulated the immune system by raising the phagocytosis index and capacity as well as SOD enzyme activity.

Keywords: Sappanwood, *Escherichia coli* ESBL, Superoxide dismutase, Phagocytosis

INTRODUCTION
Among the top 10 most prevalent diseases in Indonesia are infectious diseases. The public's affordable option for medical care is to self-medicate with freely obtained medications from pharmacies or drug stores without a prescription (Harun, H., et.al., 2021). Self-medication is a major issue for world health. 64% of antibiotic purchases in Southeast Asia were done without a prescription, according to WHO data from 2015. Self-medication is still very common in Indonesia. As per Riskesdas (2013), self-medication was practiced by 24.4% of the populace, and 35.2% of homes had medications kept for this purpose. Unreasonable drug use was demonstrated by the fact that 81.9% of hard drugs and 86.1% of antibiotics were stored without a prescription (Kesehatan, 2013).

Antibiotic resistance results from the overuse of antibiotics, which makes germs resistant to the medications and renders them ineffective. According to the Dr. Soetomo Hospital in Surabaya and Dr. Kariadi Hospital in Semarang AMRIN (Antimicrobial Resistant in Indonesia) study, 90% of pediatric patients treated for more than five days were given empirical antibiotics (Anugrahsari et al., 2022). Antibiotic use with no indication is between 45% and 76% in Surabaya and between 56% and 76% in Semarang. This investigation also discovered multiresistant bacteria like ESBL and MRSA (Sarker et al., 2019). Both the US Centers for Disease

Control and Prevention and the World Health Organization have noted a rise in ESBL infections, or diseases caused by bacteria like *Escherichia coli* (Bezabih et al., 2021; Falgenhauer et al., 2019).

Antibiotic resistance raises the likelihood of cross-resistance, lengthens hospital stays, and increases infection rates. Additionally, it increases the number of community members who contract multiresistant bacterial diseases (Bezabih et al., 2021). Antimicrobials, bacteriophages, immune system regulation, and antibiotic administration are strategies used to treat bacterial infections (Abbas et al., 2021). Using naturally occurring antimicrobials to treat bacterial illnesses, such as sappanwood, which is widely available in Indonesia (Septama et al., 2018). Immunomodulators for immune system regulation (Kesharwani et al., 2022). Right now, the most practical course of action is the combination of sappanwood immunomodulators and natural antimicrobials.

Immunomodulators are compounds that have the ability to control, inhibit, or activate the innate and adaptive immune systems (Nair et al., 2019; Shahbazi & Bolhassani, 2016). Infections recover more quickly when the immune system is functioning better. Plants like sappanwood can be utilized as adjuvants in the treatment of infections because they activate both specific (T cells, B cells, and cytokine production) and nonspecific (macrophages, NK cells) immunological capabilities (Gordon & Plüddemann, 2018). Since sappanwood effectively combats antibiotic resistance, allergic reactions, immunosuppressive effects, and the negative effects of antibiotics on viral infections, it was selected as an immunomodulator (Mueller et al., 2016; Wan et al., 2019).

Although sappanwood extract's antioxidant, antibacterial, and immunomodulatory qualities have been demonstrated in earlier research (Febriyanti et al., 2018; Israel Pérez-Torres et al., 2021), nothing is known about how it affects Escherichia coli ESBL infection. The purpose of this work is to maximize the activity of the enzyme superoxide dismutase (SOD) and phagocytosis in mice infected with Escherichia coli by using sappanwood extract (Caesalpinia sappan L.). Escherichia coli is a pathogenic bacterium that can cause a variety of infections, so boosting the immune system is a key component of treatment. The flavonoid concentration of sappanwood may boost SOD activity, which shields cells from oxidative damage and improves immune cell phagocytosis (Hosseinzade et al., 2019). By boosting SOD activity and phagocytosis, this study seeks to demonstrate sappanwood's potential as a natural immunomodulator that effectively treats bacterial infections.

RESEARCH METHOD

Fifty male Swiss Webster mice, all 10 weeks old, were included in this completely randomized laboratory experiment. The mice were split into five groups: the positive control group (K+) received brazilin at a dose of 0.18 ppm/day, the normal group (K-) received sterile distilled water, and the three treatment groups (K1–K3) received ethanol extract of sappanwood at varying doses (280 ppm/day, K2–560 ppm/day, and K3–840 ppm/day). After ten days of treatment, groups K+, K1, K2, and K3 received intraperitoneal injections of an Escherichia coli ESBL suspension (1.5 x 105 cfu/mL). Three mice were removed for analysis from each group on days twelve and fourteen. To measure the phagocytic capacity, phagocytic index, and superoxide dismutase (SOD) enzyme activity, peritoneal macrophages were extracted. The one-way anova statistical test was then used to examine the data.

RESULT AND DISCUSSION

Findings from the phagocytosis index and capacity analysis. According to the study's findings, phagocytosis capability and index

are significantly increased by the administration of Secang Wood Ethanol Extract (EEKS) and standard brazilin.

Table 1. Shows the data analysis of the values of phagocytosis ability in groups (K-) through (P3)

Treatment Group	N	Phagocytosis Capacity					
		Average (x̄)	SD	Min-Max	p *Shapiro-Wilk*	p Levene	p Anova
K-	9	5,00	-	-	-		
K+	9	21,50	± 0,05[a]	0,23 – 0,40	0,973		
P1	9	24,33	± 0,06[a]	0,25 – 0,45	0,805	0,897	0,001*
P2	9	27,44	± 0,07[a]	0,27 – 0,49	0,527		
P3	9	36,72	± 0,06[b]	0,33 – 0,52	0,902		

Note: Shapiro-Wilk: significance level ≥ 0.05 normally distributed data
(*) significant at α = 0.05 (Oneway Anova Kruskal Wallis)
[abc] same superscript indicates no difference between groups (multiple comparisons LSD)

Table 2. Data analysis showing the values of the phagocytosis index in groups (K-) to (P3)

Treatment Group	N	Phagocytosis Index					
		Average (x̄)	SD	Min-Max	p *Shapiro-Wilk*	p Levene	p Anova
K-	9	6,00	-	-	-		
K+	9	33,06	± 0,04[a]	0,04 – 0,15	0,137		
P1	9	23,50	± 0,03[b]	0,00 – 0,09	0,887	0,261	0,001*
P2	9	23,56	± 0,04[a]	0,02 – 0,12	0,063		
P3	9	28,89	± 0,03[a]	0,03 – 0,11	0,437		

Note: Shapiro-Wilk: significance level ≥ 0.05 normally distributed data
(*) significant at α = 0.05 (Oneway Anova Kruskal Wallis)
[abc] same superscript indicates no difference between groups (multiple comparisons LSD

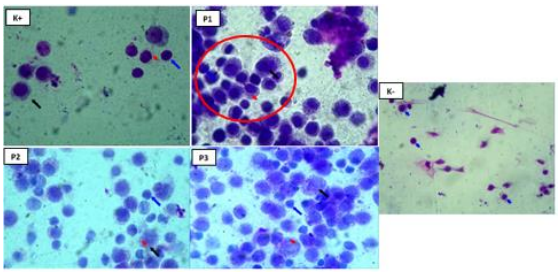

Figure 1. Phagocytosis activity

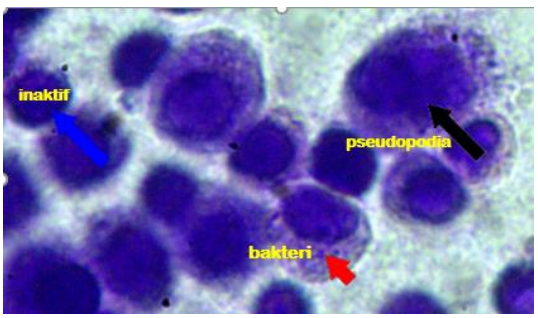

Figure 2. Macrophages with pseudopodia and phagocytosed bacteria

The research results, as indicated by tables 1 and 2, figures 1 and 2, and an increase in the phagocytosis index and capacity value, can be explained by taking into account a number of factors, including the relationship between dose and immune response, the function of active substances like brazilin and Secang Wood Ethanol Extract (EEKS), and the mechanism of phagocytosis. Phagocytosis and the Index of Phagocytosis. The process by which phagocytic cells—such as neutrophils and macrophages—engulf and eliminate microbes, foreign particles, or injured cells is known as phagocytosis (Schütze et al.,

2021). The number of particles or microbes that a phagocyte can consume is known as its phagocytic capacity, and the number of particles ingested per phagocyte is known as its phagocytosis index. These two metrics are used to quantify phagocytic ability. In this study, groups treated with brazilin and EEKS were compared to negative (K-) and positive (K+) control groups. According to the findings, standard brazilin enhanced phagocytosis index by 83.4% and phagocytosis capacity by 76.7%. In the meantime, there was an 81% increase in the phagocytosis index and an 86.4% increase in phagocytosis capacity in the group that received EEKS (P3). Brazilin and Ethanol Extract from Secang Wood (EEKS). Sappan wood contains an active substance called brazilin (Caesalpinia sappan). Numerous biological actions of this chemical exist, such as immunomodulatory and anti-inflammatory properties. Agents known as immunomodulators modify the immune system by either raising or lowering its level of activity.

According to the study's findings, giving regular brazilin was able to boost phagocytosis in terms of both capacity and index. Brazilin may contribute to the activation of phagocyte cells, enhancing their capacity to identify and assimilate pathogens. Brazilin may thereby strengthen the body's defenses against infection and other harmful substances. Additionally, EEKS shown a noteworthy impact on enhancing phagocytosis. Indeed, the phagocytosis capacity in group P3, which received the highest dose of EEKS, was 86.4%, surpassing that of regular brazilin. This suggests that the active ingredients in EEKS are capable of boosting phagocyte function. The results on day 14 with the highest phagocytic capability indicate that higher dosages of EEKS seem to elicit a stronger reaction.

The Phagocytosis Effect and the Dose Relationship. There is a dose-response association shown by the rise in phagocytosis capacity and index in response to an increase in EEKS dose. The effect of immunomodulatory drugs on boosting immune system activity, particularly phagocytosis, is generally stronger the larger the amount administered. It should be remembered, nevertheless, that because the body has a limit to how much external substance it can tolerate, raising the dosage may not necessarily result in increased effectiveness.

Clinical Importance. The creation of immunomodulatory drugs based on organic materials, such sappanwood, to strengthen the immune system has significant ramifications for this study. Brazilin and EEKS may be used to assist the body fight infections, particularly in people with impaired immunity or circumstances that call for immune system stimulation, as suggested by the rise in phagocytosis capacity and index. Overall, the study's findings reveal that phagocytosis can be improved by both regular brazilin and EEKS, with the latter having more promise, particularly at higher dosages. This supports the theory that sappanwood's natural chemicals can be utilized as an alternative to boost immunity by using the phagocytosis mechanism.

Results of the Superoxide Dismutase (SOD) analysis

Table 3. Superoxide dismutase levels in groups (K-) to (P3) data analysis

Treatment Group	N	*Superoxide Dismutase (SOD)*					
		Average ($\bar{x}$)	SD	Min-Max	p *Shapiro-Wilk*	p Levene	p Anova
K-	9	10,83	± 24,22[a]	42,95 – 126,29	0,698		
K+	9	34,39	± 85,19[b]	152,95 – 428,19	0,222	0,001	0,001*
P1	9	10,28	± 39,99[a]	42,48 – 152,48	0,130		

P2	9	29,17	± 126,59[b]	90,10 – 428,19	0,208
P3	9	30,33	± 71,44[b]	96,76 – 344,86	0,460

Note: Shapiro-Wilk: significance level ≥ 0.05 normally distributed data
(*) significant at α = 0.05 (Oneway Anova Kruskal Wallis)
[abc] same superscript indicates no difference between groups (multiple comparisons LSD)

The study's findings demonstrated the substantial antioxidant benefits of Secang Wood Ethanol Extract (EEKS) and conventional brazilin, which raised the body's levels of Superoxide Dismutase (SOD). Antioxidants can be indicated by SOD levels (Mouffouk et al., 2021). One enzyme that is crucial to the body's defense against oxidative stress is superoxide dismutase (SOD) (Li et al., 2019). Superoxide free radicals are broken down by SOD into oxygen and hydrogen peroxide, which are further transformed into water and oxygen by other enzymes such glutathione peroxidase and catalase (Gil et al., 2017). Hence, elevated SOD levels suggest that the body is more capable of combating oxidative stress, shielding cells from free radical damage, and fending off degenerative processes (Gupta et al., 2021).

The findings of the investigation into the impact of regular brazilin. According to the study, regular brazilin raised SOD levels by 68.5% when compared to the K-negative negative control group. This demonstrates that: Brazilin possesses potent antioxidant qualities. Brazilline has a significant role in promoting the body's antioxidant enzymes to function more actively, as seen by the 68.5% increase in SOD levels. This effect of boosting SOD is critical in the fight against free radicals, which are linked to chronic diseases like cancer, diabetes, and heart disease as well as oxidative stress-related cell aging.

The findings of the investigation into the effects of Secang Wood ethanol extract, or EEKS. SOD levels increased 64.3% in the EEKS treatment group, particularly in group P3, which got the highest dose, in comparison to the negative control group (K-). This suggests that, like brazilin, EEKS also has a considerable antioxidant capability, but at a somewhat lesser rate of growth. There is a dose-response relationship in the antioxidant impact of EEKS, which rises with higher doses administered. The increase in SOD levels that is seen increases with increasing doses of EEKS.

Brazilin and EEKS are compared. SOD levels increased more in Brazilin than in EEKS, with a difference of 68.5% against 64.3%. Although the difference was not statistically significant, this suggests that brazilin may be more effective in generating increases in SOD. Significant promise was seen by EEKS in raising SOD levels, particularly at high dosages. This implies that, when taken in the right amounts, EEKS can be a useful antioxidant agent.

The connection between elevated SOD levels and EEKS dosage. The study's findings also demonstrated a correlation between the rise in EEKS dosage and SOD levels. The level of SOD released increases with increasing dosage. This demonstrates a dose-response relationship in which EEKS's antioxidant efficacy increases with dose. This suggests that the effectiveness of EEKS is dose-dependent, meaning that the proper amount of EEKS will have the greatest antioxidant effects.

Brazilin and EEKS's antioxidant properties. Both EEKS and brazilin have strong antioxidant properties. Both have the ability to raise the SOD enzyme's activity, which helps to reduce the harm that free radicals do to cells. Brazilin's 68.5% increase in SOD levels and EEKS's 64.3% increase in SOD levels demonstrate how these two nutrients help fortify the body's defenses against oxidative damage. The therapy or prevention of oxidative stress-related diseases, such as degenerative

illnesses, chronic inflammation, and metabolic abnormalities, may benefit greatly from this antioxidant impact. Clinical Implications and Scientific Discussion. Because brazilin and EEKS raise SOD levels, it is possible to employ these substances as natural antioxidants in treatment or preventative medicine for conditions involving oxidative stress and free radicals. The following are some crucial points about the study's findings: Effectiveness of Antioxidants: Brazilin and EEKS supplementation can offer robust defense against oxidative stress, as evidenced by a notable rise in SOD levels. Brazilin and EEKS have been shown to raise SOD levels, suggesting that they may be employed as natural antioxidants in treatment or preventive medicine for conditions involving oxidative stress and free radicals. Important details about the study's findings include the following: Antioxidant Effectiveness: Brazilin and EEKS use can offer robust protection against oxidative stress, as evidenced by a notable rise in SOD levels.

CONCLUSION AND RECOMMENDATION

The purpose of this work is to maximize the activity of the enzyme superoxide dismutase (SOD) and phagocytosis in mice infected with Escherichia coli by using sappan wood extract (Caesalpinia sappan L.). While EEKS exhibits more promise, particularly in improving phagocytosis capacity, standard brazilin is effective in increasing phagocytosis capacity and index. When compared to brazilin, EEKS at the maximum dose increased phagocytosis more than the former, particularly on day 14, when the phagocytosis capacity value peaked. When EEKS was administered, the effects increased in proportion to the dose, resulting in an increase in phagocytic activity as measured by both capacity and index. All things considered, the study's findings suggest that EEKS may be a useful immunomodulatory drug, particularly when it comes to boosting the immune system's response via the phagocytosis mechanism. Standard Brazilin exhibited a 68.5% increase in SOD levels, suggesting a highly promising antioxidant potential. Additionally effective, EEKS raised SOD levels by 64.3%, and its efficacy improved with dose. Through an increase in the SOD enzyme's activity, both drugs demonstrated notable antioxidant properties that counteract free radical damage. Higher doses had stronger antioxidant effects, according to the dose-response relationship in EEKS. Overall, this study showed that EEKS and brazilin can significantly improve the body's defenses against oxidative stress, which can help avoid diseases linked to free radicals.

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