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**Potential of Sappan Wood Ethanol Extracts for Cardioprotection in an ESBL
Escherichia coli -Infected Mice Model**

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ABSTRACT

Background: One of the top causes of death in the world is still cardiovascular disease. Increased oxidative stress and immune system dysregulation are two pathways that contribute to its development. Blood vessel inflammation may be made worse by an imbalance between the body's ability to produce free radicals and its antioxidant capacity. **Object:** The purpose of this work is to clarify how sappan wood ethanol extract (SWEE) protects mice against oxidative stress and immunological responses, which are risk factors for cardiovascular disease caused by *Escherichia coli* ESBL. **Method:** Five groups (K⁻, K⁺, K1, K2, K3) were created from a total of forty-five male *Swiss Webster* mice. For ten days, the K⁻ group received sterile distilled water, K⁺ received standard brazilin, and K1, K2, and K3 received SWEE at doses of 280 ppm, 560 ppm, and 840 ppm, respectively. On day 11, *Escherichia coli* ESBL (1.5×10^3 cfu/mL) was injected intraperitoneally into all groups except K⁻. On days 12 and 14, superoxide dismutase (SOD) activity and interferon-gamma (IFN- γ) levels were assessed. **Result:** SWEE administration reduced IFN- γ levels by 78.1% and increased SOD activity by 64.3% compared to the negative control group. **Conclusion:** SWEE exhibited dual effects, namely as an immunosuppressive agent by reducing IFN- γ levels and as an immunostimulant by increasing SOD activity.

Keywords: Sappan Wood, Ethanol Extract, Oxidative Stress, Interferon-gamma (IFN- γ), Superoxide dismutase (SOD)

BACKGROUND

Cardiovascular disease continues to be the world's leading cause of mortality and one of the leading sources of disease burden (Shaito et al., 2022). Increased oxidative stress and immune system dysregulation are two major pathogenic pathways causing cardiovascular diseases (Yan et al., 2023). Atherosclerosis and other heart problems are caused by endothelial cell damage, persistent inflammation, and vascular dysfunction brought on by an imbalance between the production of free radicals and the endogenous antioxidant system's capacity (Gusev & Sarapultsev, 2023).

This circumstance implies that immune response modulation and oxidative stress control are important preventative strategies that slow the development of cardiovascular disease (Kong et al., 2022).

Many pharmacological strategies, including the use of synthetic antioxidants and anti-inflammatory medications, have been developed to address oxidative stress and inflammation, which are linked to cardiovascular disease (Blagov et al., 2024; Zhang et al., 2023). However, these synthetic medicines' long-term efficacy and adverse effects continue to be barriers to their adoption (Gasmi et al., 2023);

Porwal et al., 2021; Zebeaman et al., 2023). Natural substances with immunomodulatory and antioxidant qualities are becoming more popular as alternative preventive treatments as biological research progresses. One possible natural source is sappan wood (*Caesalpinia sappan* L.), which contains the active ingredient brazilin, which has been shown to have immunomodulatory, anti-inflammatory, and antioxidant properties in several animal models (Krihariyani et al., 2020).

While sappan wood has been shown to have anti-inflammatory and antioxidant properties in several studies, there is currently little scientific data assessing the protective effects of sappan wood ethanol extract against oxidative stress and immune responses in an Extended-Spectrum Beta-Lactamase (ESBL)-producing bacterial infection model (Krihariyani et al., 2024). Systemic oxidative stress and an increase in pro-inflammatory immune mediators like interferon-gamma (IFN- γ), which contribute to vascular tissue damage, are known to be triggered by ESBL bacterial infection (Nunes et al., 2021). Therefore, more investigation is required to clarify the preventive mechanisms of SWEE in the context of inflammation and infection that resemble situations that increase the risk of cardiovascular disease (Pattananandecha et al., 2022)(Vij et al., 2023).

The purpose of this study is to assess how sappan wood ethanol extract protects mice against oxidative stress and immunological reactions brought on by *Escherichia coli* ESBL. The study focuses on assessing superoxide dismutase (SOD) enzyme activity as a measure of antioxidant status and IFN- γ levels as a sign of pro-inflammatory immune responses (Firdawati et al., 2023; Nurullita et al., 2023). It is anticipated that the findings of this study will contribute scientifically to the development of the usage of natural chemicals, particularly SWEE containing brazilin, as potential

preventive agents against inflammation and oxidative stress that contribute to the pathophysiology of cardiovascular disease (Asih et al., 2023; S. et al., 2022). These discoveries may also serve as the foundation for the creation of natural preventive treatments that are safer and more long-lasting.

RESEARCH METHODS

Materials and Method

An imbalance between the body's ability to produce free radicals and its antioxidant capacity leads to oxidative stress, which can cause endothelial cell damage and hasten the onset of cardiovascular disease. Superoxide radicals are neutralized by the enzyme superoxide dismutase (SOD), and pro-inflammatory immunological activity is reflected in interferon-gamma (IFN- γ) (Sasongko et al., 2024). Brazilin, a substance with immunomodulatory and antioxidant properties that may shield tissues from oxidative stress and vascular inflammation, is present in the ethanol extract of sappanwood.

Description of Materials or Research Subjects

Fifty male *Swiss Webster* mice weighing 20–25 grams and aged 8–10 week were utilized in this investigation. Malang Murine Farm, a partner supplier of experimental animals, provided all of the mice. Five mice per cage were housed in polypropylene plastic cages filled with clean, dry wood shavings. A temperature of $22 \pm 2^{\circ}\text{C}$, a humidity of 50–60%, and steady lighting with a regulated day-night cycle (12 hours light and 12 hours dark) were all maintained in the maintenance environment. Standard pellet meal and unlimited access to water were provided to the animals.

Sappan wood ethanol extract derived from *Caesalpinia sappan* L. wood powder extracted with 70% ethanol served as the primary research material. The comparison was made with a pure standard

brazilin compound. Extended Spectrum Beta-Lactamase (ESBL) bacteria from *Escherichia coli* were employed as agents to trigger immunological reactions and oxidative stress.

Research Design

With a post-test only control group design, this study employed a true experimental design. Each of the five treatment groups—K⁻ (negative control), which received sterile distilled water without *Escherichia coli* ESBL induction; K⁺ (positive control), which received standard brazilin and *Escherichia coli* ESBL induction; K1, which received SWEE at a dose of 280 ppm; K2, which received SWEE at a dose of 560 ppm; and K3, which received SWEE at a dose of 840 ppm. For ten days in a row, the therapy was administered. All groups, with the exception of K⁻, received an intraperitoneal injection of *Escherichia coli* ESBL at a dose of 1.5×10^1 cfu/mL on the eleventh day. On days 12 and 14 following induction, SOD enzyme activity and IFN- γ levels were assessed.

Research Procedure

To make Sappan Wood Ethanol Extract, 70% ethanol was used to macerate secang wood powder. The resulting thick extract was then filtered and evaporated. To reach the required concentrations (280 ppm, 560 ppm, and 840 ppm), the extract was diluted in sterile distilled water. Prior to treatment, the test animals were acclimated for seven days. *Escherichia coli* ESBL was induced in accordance with the group design following the treatment period. The mice were morally killed 72 hours after induction in order to get blood from the orbital vein. Centrifugation was used to separate the serum so that SOD activity and IFN- γ levels could be measured.

Instruments and Equipment

A digital animal scale, a micropipette, a microcentrifuge, a vortex

mixer, a UV-Vis spectrophotometer, a microplate reader, an automatic micropipette, and a polypropylene cage with a sterile wood shavings base were among the primary tools utilized. A commercial ELISA kit (Enzyme-Linked Immunosorbent Assay) was used to assess IFN- γ in accordance with the manufacturer's instructions, and a Superoxide Dismutase Assay Kit based on nitroblue tetrazolium (NBT) reduction reaction inhibition was used to measure SOD activity.

Data Collection Methods

Using a microplate reader, absorbance measurements at particular wavelengths were used to collect data on IFN- γ and SOD activity. Every sample was examined three times. A standard curve was used to convert the data to concentrations, which were then expressed in pg/mL for IFN- γ and U/mL for SOD.

Data Analysis

SPSS software version 25.0 was used to analyze the data. The Shapiro-Wilk test was used to assess normality, while Levene's test was used to assess homogeneity. One-way ANOVA was used to examine group differences, and Tukey's post hoc test was used if significant differences were discovered. At a significance level of $p < 0.05$, the results are displayed as mean \pm standard deviation (SD).

Research Ethics

The Animal Research Ethics Committee of the Faculty of Dentistry at Airlangga University has granted ethical permission for all animal research methods; the ethics approval number is 873/HRECC.FODM/XII/2022.

RESULT AND DISCUSSION

Interferon-Gamma (IFN- γ) Levels

IFN- γ levels were measured to assess the immune response of mice following administration of sappan wood ethanol

extract and induction of *Escherichia coli* ESBL. The analysis showed a significant difference between treatment groups ($p =$

0.001). The mean IFN- γ levels are shown in Table 1.

Table 1.

Interferon-Gamma (IFN- γ) levels in each treatment group

Treatment Group	N	Interferon gamma (IFN- γ)					
		Average (\bar{x})	SD	Min-Maks	p Shapiro-Wilk	p Levene	p Anova
K-	9	38,00	$\pm 3,70^a$	7,82 – 20,62	0,960		
K+	9	11,67	$\pm 2,21^b$	3,06 – 9,57	0,004		
P1	9	28,00	$\pm 1,40^c$	8,05 – 12,32	0,731	0,051	0,001*
P2	9	29,00	$\pm 2,14^c$	6,98 – 12,53	0,097		
P3	9	8,33	$\pm 0,85^b$	1,90 – 4,68	0,572		

Note:

Shapiro-Wilk: significance level ≥ 0.05 for normally distributed data.

(*) significant at $\alpha = 0.05$ (One-way ANOVA, Kruskal-Wallis).

The same superscript abc indicates no difference between groups (multiple comparisons LSD).

Superoxide Dismutase (SOD) Activity
SOD enzyme activity was measured to evaluate the endogenous antioxidant status of mice after treatment. The analysis

showed a significant difference between treatment groups ($p = 0.001$). The mean SOD activity values are presented in Table 2.

Table 2.

Superoxide Dismutase (SOD) activity in each treatment group

Treatment Group	N	Superoxide Dismutase (SOD)					
		Average (\bar{x})	SD	Min-Maks	p Shapiro-Wilk	p Levene	p Anova
K-	9	10,83	$\pm 24,22^a$	42,95 – 126,29	0,698		
K+	9	34,39	$\pm 85,19^b$	152,95 – 428,19	0,222		
P1	9	10,28	$\pm 39,99^a$	42,48 – 152,48	0,130	0,001	0,001*
P2	9	29,17	$\pm 126,59^b$	90,10 – 428,19	0,208		
P3	9	30,33	$\pm 71,44^b$	96,76 – 344,86	0,460		

Note:

Shapiro-Wilk: significance level ≥ 0.05 for normally distributed data.

(*) significant at $\alpha = 0.05$ (One-way ANOVA, Kruskal-Wallis).

The same superscript abc indicates no difference between groups (multiple comparisons LSD).

Discussion

Interpretation of Results:

The findings of this study show that giving mice infected with *Escherichia coli* ESBL sappan wood ethanol extract can dramatically lower interferon-gamma (IFN- γ) levels and boost superoxide dismutase (SOD) enzyme activity. These results corroborate the original theory that SWEE protects against immunological dysregulation and oxidative stress, both of which contribute to the inflammatory process in the heart. The capacity of the SWEE to inhibit immune cells' pro-inflammatory activity is demonstrated by the drop in IFN- γ levels, whilst its function in bolstering the body's natural antioxidant system is demonstrated by the rise in SOD activity (Liu et al., 2023).

These findings are consistent with other studies showing that sappanwood's brazilin and brazilein chemicals have strong antioxidant activity and immunomodulatory properties that can lower the generation of pro-inflammatory cytokines. Additionally, a number of studies demonstrate that brazilin can prevent the activation of the NF- κ B pathway, which is essential for controlling the production of inflammatory genes. Additionally, the rise in SOD activity is consistent with earlier findings that sappanwood's polyphenolic chemicals can scavenge free radicals and stop oxidative stress-related cell damage (Gadelha et al., 2025; Simao et al., 2024).

Overall, the study's findings support the notion that SWEE holds promise as a natural agent with dual effects—anti-inflammatory and antioxidant—relevant to the prevention and management of cardiovascular disorders linked to oxidative stress. These findings have significance for the development of SWEE as a potential natural-based supportive therapy to preserve the immune system's equilibrium and the body's oxidative defenses (Sun et al., 2024).

RESEARCH IMPLICATIONS

The findings of this study have significant theoretical and practical ramifications for the health sector, especially with regard to the prevention and management of cardiovascular illnesses brought on by inflammation and oxidative stress. This study theoretically supports the idea that natural bioactive substances, including brazilin in sappan wood ethanol extract, can function as potent antioxidants and immunomodulators. Additionally, by raising SOD enzyme activity, these findings improve scientific knowledge of how polyphenolic substances reduce pro-inflammatory immune responses and strengthen the body's natural antioxidant defense system.

Practically speaking, the findings of this study can serve as the foundation for creating SWEE as a phytopharmaceutical base or natural supplement to promote cardiovascular health and lower the risk of oxidative stress-related tissue damage. Public health policies that emphasize the use of natural, safe, and sustainable ingredients can benefit from the application of SWEE in the manufacture of preventive health products.

Additionally, the growth of Indonesian medicinal plants as alternative supporting therapies may benefit from the establishment of research strategies based on local biological resources. The application of these research findings in the field of public health can support preventive and promotional initiatives to lessen the burden of degenerative diseases using a quantifiable, natural strategy supported by scientific data.

RESEARCH LIMITATIONS

When evaluating the findings, it is important to take into account the many limitations of this study. The degree of external validity and the generalizability of the findings to a larger population may be impacted by the use of mice as test animals

with a very small sample size. Additionally, the relatively brief duration of the extract's administration might not accurately represent the long-term impacts of sappan wood ethanol extract on oxidative stress and immune system regulation.

From a methodological standpoint, the parameters examined in this study were restricted to superoxide dismutase (SOD) enzyme activity and interferon-gamma (IFN- γ) levels, which did not fully represent the breadth of biological mechanisms involved. Despite meticulously regulated rearing circumstances, biological heterogeneity among test animals may potentially lead to variations in outcomes. To get more representative results, it is advised that future studies increase the sample size and lengthen the treatment period. A more complete picture of the extract's mechanism of action may also be obtained by adding additional biomarkers, such as catalase activity, TNF- α , or malondialdehyde (MDA). To validate the safety and efficacy of sappanwood ethanol extract as a viable natural medicine option for the prevention of cardiovascular diseases, more study is required, including toxicity testing and preliminary human clinical studies.

CONCLUSION

This study demonstrates that giving mice sappan wood ethanol extract (SWEE) protects them from oxidative stress and immune system dysregulation brought on by *Escherichia coli* ESBL. When compared to the negative control group, SWEE dramatically boosted superoxide dismutase (SOD) activity by 64.3% and decreased interferon-gamma (IFN- γ) levels by 78.1%. These findings suggest that SWEE functions as an immunomodulatory drug in two ways: immunosuppressive by lowering the inflammatory response and immunoprotective by boosting antioxidant activity.

From a scientific standpoint, this study validates the potential of sappanwood's active ingredient, brazilin, as a natural candidate for the prevention or early treatment of cardiovascular illnesses linked to inflammation and oxidative stress. These results can be used as a foundation for future investigations into the application of SWEE in the development of secure and efficient natural preventative treatments

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