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Research Article

Effect of Sappan Wood Ethanol Extract in CRP Level and Phagocytic Index Between Group of Mice Infected with *S. aureus* and *E. coli*

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ABSTRACT

S.aureus and *E.coli* are pathogenic bacterial that cause many infectious disease in the world. Immunomodulator is needed to prepare the immune system to be able against the infection. Some parameters usually used to assess the immunomodulatory activity such as C-Reactive Protein (CRP) and phagocytic index. This study aims to investigate the difference effect of sappan[1]wood extract in CRP level and phagocytic index between group mice infected with *S. aureus* and *E. coli.* Two treatment groups of mice were prepared for *S. aureus* and *E. coli* test. Each group subjected to 7 treatments i.e. (1) Normal mice[1](CMC-Na 1 %- Merck® 217277), [1](2) Negative Control (bacterial infection + CMC-Na 1 %),[11](3) Positive Control (bacterial infection + Imboost force®[1]treatment, PT SOHO Industri Pharmasi), (4) Bacterial infection and EESW treatment 25 mg/kg BW, (5) 50 mg/kg BW, (6) 100 mg/kg BW, and (7) 200 mg/kg BW. Mice blood was taken to detect the CRP and phagocytic index after treatment. The T test showed that there was a significant difference between CRP levels ($p<0.05$) and phagocytic index ($p<0.05$) of *S. aureus* and *E. coli* group. EESW 200 mg/kg BW reduced CRP level to 11 mg/dL (*S. aureus)* and 6 mg/dL in (*E. coli).* EESW 200 mg/kg BW increased phagocytosis to 1.54 folds (*S. aureus*) and 4.62 folds (*E. coli*). Sappan wood ethanol extract effect to CRP level and phagocytic index in mice group infected with *E. coli* is better than *S. aureus* infection group.

Keywords: CRP; *E.coli*; Phagocytic index; Sappan wood; *S. aureus*

INTRODUCTION

Infectious diseases are disorders caused by pathogenic microorganisms that can be transmitted between humans and increase the potential for disease and death. Bacteria cause many infectious disease in the world. Infection of bacteria has a high impact in health problem (Susmitha et.al., 2022). Although bacterial infections are easier to treat than viral infections, bacterial resistance is one of the problems that has not been resolved (Doron, S & Gorbach, 2020). Immunity needs to be increased to prevent infection and the pathogenesis of infectious diseases (Reyes-Silveyra & Mikler, 2016). Immunomodulators are active ingredients that can optimize the regulation of the immune system to increase immunity (Gond et al., 2022).

S. aureus and *E. coli* are pathogenic bacteria that causes various human disease. *S. aureus* is a Gram-positive and most dangerous among *Staphylococcus* species. This bacteria secrete enterotoxin that cause human skin infection, pneumonia, hearth valve, and bone infections (Ibrahim, 2020). *E.coli* (Gram-negative bacteria) cause some infectious diseases including gastroenteritis, pneumonia, bacteremia, and peritonitis (Mueller et al., 2023). The basic difference between both bacteria is in cell wall structure of cell wall. There are differences metabolic physiological and immune response between gram-positive and negative. Serum metabolite level (ketone body and 3-hydroxybutyrate) of *S. aureus* significantly increases. It

caused by some bacterial exo-metabolomes and high fatty acid oxidation. It indicates high virulence and severe changes in liver cellular response. Different from *S. aureus* infection, *E. coli* induces immuno response such as Toll Like Receptor 4 (TLR4) and reduces metabolic activity (Hoerr et al., 2012).

Immune system has two ways to response infections including innate and adaptive immunity. Innate immunity is first immune response to defence infection. There are many paramater that used in innate immunity activity including C-Reactive Protein (CRP) and phagocytosis activity. CRP is protein in acute phase that most synthesized in hepatocytes. CRP increases infection and inflammation until 1.000 folds (Sproston & Ashworth, 2018). CRP can become a marker in *S. aureus* (Mölkänen et al., 2016) and *E. coli* infection (Narayan Swamy et al., 2022). Phagocytosis is a removal mechanism the pathogen by ingestion the pathogene molecule. During infection and inflammation, phagocytosis response increase caused by bacterial product, cytokines and inflammatory mediator. Reduction of phagocytosis usually show the reduction of immune defense that seen by phagocytic index (Gordon, 2016).

Sappan wood usually used as herbal medicine. Sappan wood has antibacterial activity in *S. aureus* (Hemthanon & Ungcharoenwiwat, 2022) and *E. coli* (Widigdyo et al., 2017). Sappan wood has immunomodulatory activity (Sunitha et al., 2015). It contains phytochemical substances including brazilin (major component), brazilide A, brazilein, protosappanin A, B, C, D, dan E etc (Muti et al., 2021). Brazilin (specific color of Sappan wood) has antioxidant effect (IC50 57,2 µM) and potential effect on anti-inflammation (Vij et al., 2023). Brazilin also has anti-inflammatory effect, inhibits LPS (Lippo Poly Saccharide), prostaglandin E2, TNF-α and IL-1β (Arjin et al., 2021). This research aims to study the effect of Sappan wood ethanol extract in CRP level and phagocytic index between groups of mice infected with *S. aureus* and *E. coli* .

METHODS

Mice *(Mus musculus)* Balb/C strain (Male, 25 days, 20-40 g, n=70) was used in this research. The protocol of this research has ethics approve from Faculty of Dental Medicine Health Research Ethical Clearance Commission (Certificate number 217/HRECC.FODM/V/2022). Semi-quantitative phytochemical screening was carried out to detect the presence of secondary metabolite in sappan wood extract. The parameter measured in this research were CRP level and phagocytic index using post-test only control group design. The mice divided into 2 groups: *S. aureus* and *E. coli* infected mice. Each group consists of 7 treatments including (1) Normal mice (CMC-Na 1 %- Merck® 217277), (2) Negative Control (bacterial infection + CMC-Na 1 %), (3) Positive Control (bacterial infection + Imboost force® treatment, PT. SOHO Industri Pharmasi), (4) Bacterial infection and EESW treatment 25 mg/kg BW, (5) 50 mg/kg BW, (6) 100 mg/kg BW, and (7) 200 mg/kg BW. Each treatment group consist of 5 mice as replication data.

Preparation of Sappan Wood Extract

Sappan wood (500 g sappan wood powder from Materia Medica, Batu) was macerated using using 3.750 mL of 96 % ethanol (PT Brataco, Bandung). Maceration and re-maceration process was carried out for 5 day while stirring occasionally 3 times a day The obtained filtrate was concentrated at 65 ^{0}C using rotary evaporator (IKA Scientific®, No. 0010012324, Germany). We made EESW filtrate in 4 concentrations including 25, 50, 100, and 200 mg/kg BW.

Phytochemical Screening of Sappan Wood Extract

Phytochemical screening of Sappan wood ethanol extract was carried out using qualitative assay to determine the presence of flavonoid, polyphenols, alkaloid, tannin, saponin, and steroid compounds. Flavonoid was detected using Mg powder (PT Multi Medika Laboratory) and concentrated with HCl. The alkaloid was tested using Dragendorf reagent (Matelab Trifa Solusindo). Tannin was tested using iron (III) chloride 10 % (Merck KGaA, Germany). Saponin was tested using HCl 1 N (Merck KGaA, Germany). Polyphenol was tested using $FeCl_3$ 1 % (Merck KGaA, Germany). Steroid was tested HCl and H_2SO_4 (Merck KGaA, Germany). The CRP test kit (Glory Diagnostic®, Spain).

S.aureus and *E.coli* Preparation

The bacterial suspension (*S. aureus* and *E. coli)* was prepared at a concentration of 10 CFU/mL. Bacterial suspension (1 mL) was added with sterile distillated water (9 mL) then diluted by taking 1 mL of suspension from tube 1 and added with 9 mL of distilled water to reach the concentration of 10^9

CFU/mL.

Mice Preparation and Infection

Mice was given pellets that contains fiber, protein, fat, minerals, and water *ad libitum*. Mice was infected by *S. aureus* and *E. coli* (10^9 FU/mL by oral. After 24 hours, CRP level was tested to confirm effect of infection. Each group of mice were subjected to treatments procedure for 7 days. Each day the mice blood was collected from jugular vein. The blood serum was separated using centrifuge.

CRP Test

The presence of CRP in sample were detected using qualitative test. About 50 mL CRP latex were drop on the slide followed by 50 mL serum sample. CRP test was positive if agglutination existed and vice versa. Sample with positive CRP will be followed semi-quantitatively CRP test using serum, NaCl and CRP latex by dilution method. Titter of CRP was calculated by multiplying the CRP constant value (6 mg/L) with the highest dilution number.

Phagocytosis Activity Test

Carbon clearance method was used to determine phagocytosis activity using UV-Vis Spectrophotometer (Thermo-Scientific®, USA) at 650 nm wavelengt (Nirmal et al., 2015). Constant carbon elimination speed value was measured followed by calculation of phagocytic index using formulas from several previous studies (Reze & Rosidah, 2020) (Rahman et al., 2016).

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening of Sappan Wood Ethanol Extract

The result of phytochemical screening showed that Sappan wood ethanol extract contain flavonoids, polyphenols, alkaloids, saponins, and tannins, but no steroid and terpenoid were detected. The phytochemical compounds detected in sappan wood extract was showed in Table 1.

This research uses ethanol which is a polar solvent. Flavonoids, polyphenols, saponins and tannins are polar compounds and can dissolve in ethanol (Stern et al., 2022). Alkaloid can dissolve in semi-polar solvents, so they are also found in the ethanol extract of Sappan wood. Other compounds such as steroids and terpenoids in this study are not soluble in ethanol because the compounds are non-polar. This fits with the like dissolves like theory.

Flavonoids, polyphenols, alkaloids, saponins, and tannins have immunomodulator effect. Flavonoids modulate some immune activity such as inhibit TLR mediated inflammation, inhibit ROS, and inhibit release of inflammatory cytokine (Bernard et al., 2015). Flavonoids inhibit the secretion of lysozymes, β-glucuronidase and arachidonic acid. It causes reduces inflammatory reactions. It also induces anti-inflammatory cytokines such as TNFα, IL-1β, and IL-8 (Al-Khayri et al., 2022). Flavonoid stimulates NK cells, dendritic cell maturation reduction, promote macrophage conversion from M1 to M2. Flavonoid also induce Treg cell proliferation, increase CTL and B cell activation (Han et al., 2022). Alkaloids regulate proliferation of thymic and splenic lymphocytes. It also increase anti-inflammatory cytokine secretion (Jiang et al., 2021). Polyphenols pro inflammatory cytokine suppression, natural killer cells and macrophages mediation, and improve commensal bacteria (Mamun et al., 2024). Tannins reduce Th-derived cytokines level (IL-17, IFN-γ, and IL-4) by oral administration (Piazza et al., 2022).

Table 1. Phytochemical Screening of Sappan Wood Ethanol Extract

Compound	Result	Observation
Positive		
Flavonoid	+	Orange precipitation
Polyphenol	+	Dark blue solution
Alkaloid	+	Orange precipitation
Saponin	+	Presence of foam
Tanin	+	Bluish green color
Negative		
Steroid	-	Discoloration
Terpenoid	-	No brownish-red

Effect of Sappan Wood Ethanol Effect on CRP Levels between Group Mice Infected with S. aureus and E. coli

The first parameter that used to assess immunomodulator activity in this research is CRP. The result of CRP level assessment listed in Table 2. Statistical analysis showed that there were significant differences among CRP level of 7 treatments of both *S. aureus* (p=0.000) and *E. coli* (p=0.002) infection groups. CRP level is zero in health mice, increased in negative control (infected mice) and low in positive control (drug treatment after infection). CRP level of EESW 25 kg/kg BB still same with negative control (p>0.05) in both *S. aureus* and *E. coli* infection, but not EESW 50, 100, and 200 mg/kg BW. The CRP level of EESW 100 and 200 mg/kg BW were not different with positive control (p>0.05). T test showed that there was significant different between CRP level of *S.aureus* and *E. coli* infection groups (p<0.05). CRP level between *S. aureus* and *E. coli* treatment group was showed on graph in Figure 1.

Data showed that EESW has potential in immunomodulator and anti-inflammatory activity through CRP reduction effectively on 100 and 200 mg/kg BW in both *S. aureus* and *E. coli*. Immunomodulatory effect of Sappan wood ethanol extract on CRP level reduction in *E. coli* infection was better than *S. aureus*. EESW 100 mg/kg BW reduces CRP level until 12 mg/dL in *E.coli* infection and 22 mg/dL in *S.aureus* infection. EESW 200 mg/kg BW reduces CRP level until 6 mg/dL in *E. coli* infection and 11 mg/dL in *S.aureus* infection.

CRP is synthesized primarily in hepatocytes and other cell such as lymphocyte, monocyte, muscle, endothelial cell etc (Sproston & Ashworth, 2018). It binds to damage tissue, antigen and to pathogenic organism in a calcium-dependent manner. CRP activates complement, binds to Fc receptor (FcR) and activates opsonin for various pathogens. CRP and FcR induces proinflammatory cytokine and enhance the inflammatory response. CRP was measured to monitor various inflammatory state and response of immune system (Du Clos, 2000). CRP increased in infection and inflammation (Sproston & Ashworth, 2018). CRP level is also as a predictive factor of Sepsis mortality (Lubis et al., 2020).

CRP level of *S. aureus* (Gram-positive bacteria) infection was higher than *E.coli* (Gram-negative bacteria). CRP level of *S. aureus* infection was 8,7 mg/dL (87 mg/L) (Sproston & Ashworth, 2018), whereas *E. coli* infection was 6,2 mg/dL (62 mg/L) (Park et al., 2005). Based on CRP level interpretation, the CRP level of *S. aureus* and *E. coli* was moderate elevation between 1.0–10.0 mg/dL or 10–100 mg/L (Nehring et al., 2023). Acute inflammatory response of gram-negative versus gram positive bacterial study showed that pro-inflammatory cytokine serum concentration in Gram-positive bacteria is higher than negative bacteria. *S. aureus* induces lipopolysaccharide more than *E. coli*. The levels of Interleukin 1Ra (IL-1Ra), IL-10, IL-8, and TNFα in patients with sepsis with Gram-negative bacteria infection were not different from those with sepsis with Gram-positive bacterial infection, but the concentrations were higher in patients with Gram-positive bacterial infections. However, plasma concentrations of IL-18 and IL-1β were significantly higher in Gram-positive infection patients (Feezor et al., 2003). CRP level after *Echinacea purpura* (positive control) decreased CRP level in *S. aureus* and *E. coli* infection successively 6 mg/L and 4 mg/L (minor elevation). *Echinacea purpura* has returned CRP level at the normal condition (0 mg/L) after oxidative stress. It also may afford protection against oxidative stress in tissues and modulate immune responses (Ezz, 2011).

Table 2. CRP Level of *S. aureus* and *E. coli* – Infected Mice after Treatment

Treatment Group	Mean of CRP level (mg/L) ± SD	
	S. aureus	***E. coli***
1. Normal (Health Mice)	$0^{a} \pm 0$	$0^{a} \pm 0$
2. Negative Control (Infected Mice)	$86^{b} \pm 8$	$42^{b} \pm 3$
3. Positive Control (Infected Mice + *Echinacea* Immune booster)	$6^{a} \pm 1$	$4^{c} \pm 2$
4. Infected Mice + EESW 25 mg/kg BW	$77^{b} \pm 5$	$38^{b} \pm 2$
5. Infected Mice + EESW 50 mg/kg BW	$43^{d} \pm 3$	$21^{d} \pm 0$
6. Infected Mice + EESW 100 mg/kg BW	$22^{a} \pm 3$	$12^{c} \pm 3$
7. Infected Mice + EESW 200 mg/kg BW	$11^{a} \pm 3$	$6^{c} \pm 0$

Note: Different notations (a, b, c, and d) showed the different effects.

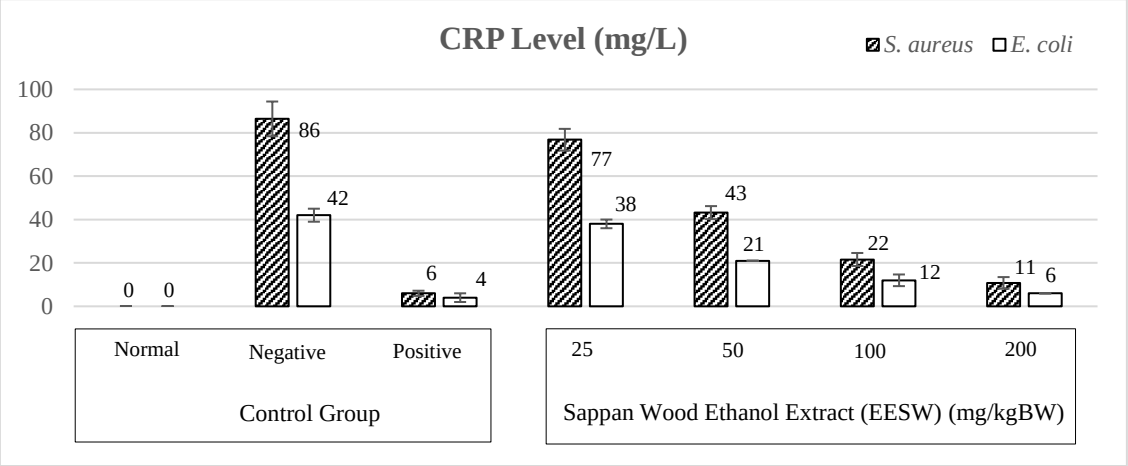

Figure 1. CRP Level Comparison Between *S. aureus* and *E. coli* Treatment Group.

EESW 50, 100, and 200 mg/kg BW could reduce CRP level. EESW has immunomodulator and anti-inflammatory activity through CRP reduction in both *S. aureus* and *E. coli* effectively on 100 and 200 mg/kg BW. Immunomodulatory effect of EESW CRP level reduction in *E. coli* infection was better than *S. aureus*. EESW 100 mg/kg BW reduce CRP level until 12 mg/dL in *E. coli* infection and 22 mg/dL in *S. aureus* infection. EESW 200 mg/kg BW reduce CRP level until 6 mg/dL in *E. coli* infection and 11 mg/dL in *S. aureus* infection. Based on these data, EESW has similar activity with positive control in CRP level reduction. EESW reduce CRP level in moderate elevation in *S. aureus* infection and until minor elevation in *E. coli* infection. This result was associated with proinflammatory and CRP level in both infections. CRP level and proinflammatory cytokine of *S. aureus* (Gram-positive bacteria) infection was higher than *E. coli* (Gram-negative bacteria). In previous study showed that Sappan wood ethanol extract 100 mg/kg BW also has antioxidant and hepatoprotective activity in Diabetic Rats (Holidah et al., 2022). Previous study showed that Sappan wood (*Caesalpinia sappan* L. extract reduces necrosis, inflammatory cell infiltration, and myocardial interstitial edema of rats induced by isoproterenol at 100 and 200 mg/kg BW (Nugraheni & Saputri, 2017).

Effect of Sappan Wood Ethanol Extract on Phagocytosis Index Between Group of Mice Infected with *S. aureus* and *E. coli*

Phagocytosis is one of innate immune response that promotes pathogen elimination and limit pathogen growth. Phagocytosis is indicated by phagocytic index. The phagocytic index (α) is the average number of bacteria ingested per macrophage. The phagocytic index data are shown in Figure 2.

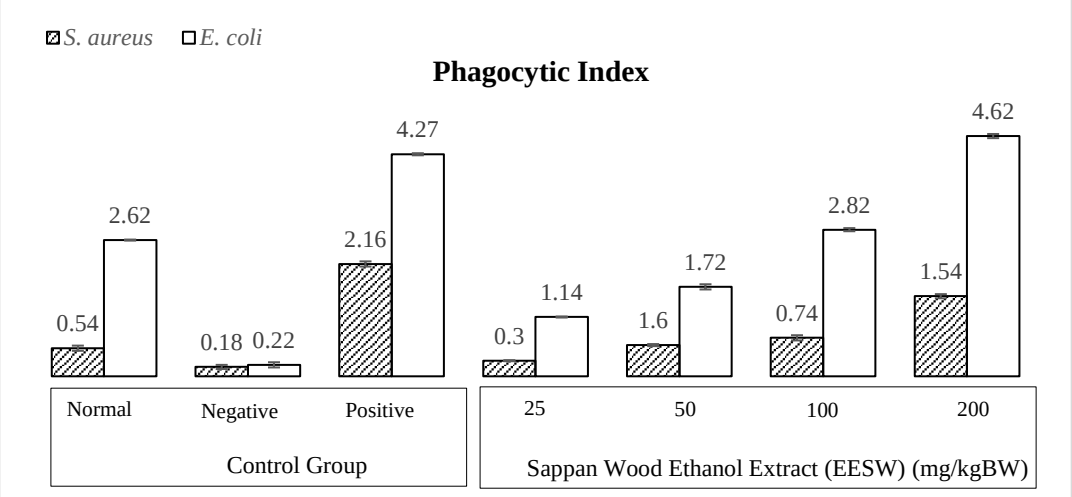

Figure 2. Phagocytic Index After Treatment on Mice Infected *S. aureus* and *E. coli*.

The statistical analysis showed that there were significant differences among treatments in *S. aureus* (p= 0.000) and *E. coli* (p= 0.000). The phagocytic index of *E. coli* was higher than *S. aureus* infection. Phagocytic index of negative control was lower than normal control, but the phagocytic index of positive control was higher than normal. The phagocytic index of the infection group with EESW 25, 50, 100, and 200 mg/kg BW treatment were higher than negative control. EESW 100 mg/kg BW increased phagocytic index to 0.74 folds (*S. aureus*) and 2.82 folds (*E. coli*). EESW 200 mg/kg BW increased phagocytic index to 1.54 folds (*S. aureus*) and 4.62 folds (*E. coli*).

Phagocytosis is an immunological process that can engulf and eliminate particles larger than 0.5 µm in diameter, including foreigner substances, microorganisms, and apoptotic cells (Uribe-Quero & Rosales, 2017). The phagocytic index is the average number of bacteria ingested per macrophage (Jäger et al., 2021). Phagocytic number showed the number of bacteria ingested per macrophage. In this research showed the low phagocytic index of *S. aureus* and *E. coli* infection successively until 0.18 and 0.22 folds. This phagocytic index was lower than phagocytic index of normal control 0.54 and 2.62 folds. This data indicated that *S. aureus* and *E. coli* infection decrease phagocytosis. In the previous study showed that *S. aureus* secretes the 16 kD Extracellular fibrinogen binding protein (Efb) that effectively blocks phagocytosis in mouse peritonitis model (Ko et al., 2013). Previous studies showed that neutrophils in infected patients were significantly lower compared with healthy adults. After 24 hours after differentiation and maturation during infection, neutrophils will spontaneously apoptosis so that the phagocytes produced in the bone marrow are released in an immature condition. This causes the phagocytic index in infectious conditions to quickly fall (Yang et al., 2021).

Phagocytic activity shown through the phagocytic index indicates the condition of the immune system when infection occurs. The phagocytic index in *S. aureus* and *E. coli* infection groups with immune booster was 2.16 and 4.27 times respectively. This phagocyte index was better than the negative control which did not receive immune booster treatment with *Echinacea purpurea* L. Previous research showed that Sappan wood increased phagocytosis and the release of TNF-a, IL-6, and IL-1b (pro-inflammatory cytokines) (Sudeep et al., 2023). The phagocytic index of Sappan wood was higher than the negative control (see Figure 2). Increasing EESW concentrations correlate with higher phagocytic index. EESW 100 mg/kg BW increased the phagocytic index up to 0.74-fold in *S. aureus* infections and 2.82-fold in *E. coli*. EESW 200 mg/kg BW increased the phagocytic index up to 1.54-fold in *S. aureus* infection and 4.62-fold in *E. coli*. This difference may correspond to the phagocytic activity against bacteria.

Previous research showed that neutrophil phagocytosis against *E. coli* (46.91–83.09 %) was higher than *S. aureus* infection (33.92–69.48 %) (Yang et al., 2021). *Caesalpinia sappan* L. ethanol extract 25 mg/kg BW increased the phagocytic index in infected mice by more than 80-fold (Mathew, 2015). *Caesalpinia sappan* L also increased Interleukine-10 in candidiasis. IL-10 increases phagocytosis and neutrophil recruitment thereby mediating inflammation (Wahyu et al., 2021). *Caesalpinia sappan* L ethanol extract 100 and 200 mg/kg BW increased the number of Kupffer cells (liver macrophages) (Erick Khristian, Ratu Safitri, Mohammad Ghozali, 2022).

CONCLUSION

Sappan wood ethanol extract has immunomodulatory effect marked by CRP reduction and phagocytosis enhancement. Immunomodulatory effect of Sappan wood ethanol extract in group of mice infected with *E. coli* was better than the group of mice infected with *S. aureus*. Sappan wood reduced CRP in moderate elevation in *S. aureus* mice group and minor elevation in *E. coli* mice group. Sappan wood also increased phagocytic index in *E. coli* mice group better than *S. aureus* mice group. This result associated with bacterial virulence and innate immune response of body against bacterial infection.

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CONFLICT OF INTEREST

All authors have not conflict of interest in this research

REFERENCES

Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules*, 27(9). https://doi.org/10.3390/molecules27092901

Arjin, C., Hongsibsong, S., Pringproa, K., Seel-Audom, M., Ruksiriwanich, W., Sutan, K., Sommano, S. R., & Sringarm, K. (2021). Effect of ethanolic caesalpinia sappan fraction on in vitro antiviral activity against porcine reproductive and respiratory syndrome virus. *Veterinary Sciences*, 8(6). https://doi.org/10.3390/vetsci8060106

Bernard, M., Furlong, S. J., Power Coombs, M. R., & Hoskin, D. W. (2015). Differential Inhibition of T Lymphocyte Proliferation and Cytokine Synthesis by [6]-Gingerol, [8]-Gingerol, and [10]-Gingerol. *Phytotherapy Research*, 29(11), 1707–1713. https://doi.org/10.1002/ptr.5414

Doron, S; Gorbach, S. (2020). Bacterial Infections : Overview. *Bacteriology Amsterdam, Amsterdam, The Netherlands*, *3*(January).

Du Clos, T. W. (2000). Function of C-reactive protein. *Annals of Medicine*, 32(4), 274–278. https://doi.org/10.3109/07853890009011772

Erick Khristian, Ratu Safitri, Mohammad Ghozali, M. H. B. (2022). *Effect of Chronic Toxicity Studies of Sappan Wood Extract on The Kupffer Cells Number in Rats (Rattus novergicus)* (pp. 695–700).

Ezz, M. K. (2011). The ameliorative effect of Echinacea purpurea against gamma radiation induced oxidative stress and immune responses in male rats. *Australian Journal of Basic and Applied Sciences*, 5(10), 506–512.

Feezor, R. J., Oberholzer, C., Baker, H. V., Novick, D., Rubinstein, M., Moldawer, L. L., Pribble, J., Souza, S., Dinarello, C. A., Ertel, W., & Oberholzer, A. (2003). Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. *Infection and Immunity*, *71*(10), 5803–5813. https://doi.org/10.1128/IAI.71.10.5803-5813.2003

Gond, S. P., Sahu, S., Rawat, S., Rajendiran, A., & Singh, A. (2022). Immunomodulatory Natural Product: Review. *Asian Journal of Pharmaceutical and Clinical Research*, 15(5), 5–9. https://doi.org/10.22159/ajpcr.2022.v15i5.44374

Gordon, S. (2016). Phagocytosis: An Immunobiologic Process. *Immunity*, *44*(3), 463–475. https://doi.org/10.1016/j.immuni.2016.02.026

Han, L., Fu, Q., Deng, C., Luo, L., Xiang, T., & Zhao, H. (2022). Immunomodulatory potential of flavonoids for the treatment of autoimmune diseases and tumour. *Scandinavian Journal of Immunology*, 95(1), 1–19. https://doi.org/10.1111/sji.13106

Hemthanon, T., & Ungcharoenwiwat, P. (2022). Antibacterial activity, stability, and hemolytic activity of heartwood extract from Caesalpinia sappan for application on nonwoven fabric. *Electronic Journal of Biotechnology*, *55*, 9–17. https://doi.org/10.1016/j.ejbt.2021.10.002

Hoerr, V., Zbytnuik, L., Leger, C., Tam, P. P. C., Kubes, P., & Vogel, H. J. (2012). Gram-negative and gram-positive bacterial infections give rise to a different metabolic response in a mouse model. *Journal of Proteome Research*, 11(6), 3231–3245. https://doi.org/10.1021/pr201274r

Holidah, D., Dewi, I. P., Siregar, I. P. A., & Aftiningsih, D. (2022). Hepatoprotective Effect of Caesalpinia sappan L. Ethanolic Extract on Alloxan Induced Diabetic Rats. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *8*(1), 1–9. https://doi.org/10.22487/j24428744.2022.v8.i1.15601

Ibrahim, O. O. (2020). *Cronicon EC Microbiology EC Microbiology Staphylococcus aureus a Gram-positive Coccid Bacterium Causing Microbial Infections, and Toxins Symptoms Including Food Poisoning*. *October*, 61–76.

Jäger, A. V., Arias, P., Tribulatti, M. V., Brocco, M. A., Pepe, M. V., & Kierbel, A. (2021). The inflammatory response induced by Pseudomonas aeruginosa in macrophages enhances apoptotic cell removal. *Scientific Reports*, 11(1), 1–13. https://doi.org/10.1038/s41598-021-81557-1

Jiang, L., Zhang, G., Li, Y., Shi, G., & Li, M. (2021). Potential Application of Plant-Based Functional Foods in the Development of Immune Boosters. *Frontiers in Pharmacology*, 12(April), 1–18. https://doi.org/10.3389/fphar.2021.637782

Ko, Y. P., Kuipers, A., Freitag, C. M., Jongerius, I., Medina, E., van Rooijen, W. J., Spaan, A. N., van Kessel, K. P. M., Höök, M., & Rooijakkers, S. H. M. (2013). Phagocytosis Escape by a Staphylococcus aureus Protein That Connects Complement and Coagulation Proteins at the Bacterial Surface. *PLoS Pathogens*, 9(12), 1–13. https://doi.org/10.1371/journal.ppat.1003816

Lubis, M., Lubis, A. D., & Nasution, B. B. (2020). The usefulness of c-reactive protein, procalcitonin, and PELOD-2 score as a predictive factor of mortality in sepsis. *Indonesian Biomedical Journal*, 12(2), 102–108.

https://doi.org/10.18585/inabj.v12i2.1073

Mamun, M. A. Al, Rakib, A., Mandal, M., Kumar, S., Singla, B., & Singh, U. P. (2024). Polyphenols: Role in Modulating Immune Function and Obesity. *Biomolecules*, 14(2). https://doi.org/10.3390/biom14020221

Mathew, J. (2015). Immunomodulatory Activity of Caesalpinia sappan L. Extracts on Peritoneal Macrophage of Albino Mice. *International Journal of Science and Research (IJSR)*, *4*(12), 449–452. https://doi.org/10.21275/v4i12.nov151953

Matthew Mueller; Christopher R. Tainter. (2023). Escherichia Coli Infection Medscape. *NCBI Bookshelf*, 1–14. https://www.ncbi.nlm.nih.gov/books/NBK564298/

Mölkänen, T., Ruotsalainen, E., Rintala, E. M., & Järvinen, A. (2016). Predictive Value of C-Reactive Protein (CRP) in identifying fatal outcome and deep infections in staphylococcus aureus bacteremia. *PLoS ONE*, 11(5), 1–14. https://doi.org/10.1371/journal.pone.0155644

Muti, A. F., Pradana, D. L. C., & Rahmi, E. P. (2021). Extract of Caesalpinia sappan L. heartwood as food treatment anti-diabetic: A narrative review. *IOP Conference Series: Earth and Environmental Science*, 755(1). https://doi.org/10.1088/1755-1315/755/1/012042

Narayan Swamy, S. N., Jakanur, R. K., & Sangeetha, S. R. (2022). Significance of C-reactive Protein Levels in Categorizing Upper and Lower Urinary Tract Infection in Adult Patients. *Cureus*, 14(6). https://doi.org/10.7759/cureus.26432

Nehring, S.M; Goyal, Amandeep; Patel, B. C. (2023). C reactive protein (Japanese). *NCBI Bookshelf. A Service of the National Library of Medicine, National Institutes of Health*.

Nirmal, N. P., Rajput, M. S., Prasad, R. G. S. V., & Ahmad, M. (2015). Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. *Asian Pacific Journal of Tropical Medicine*, 8(6), 421–430. https://doi.org/10.1016/j.apjtm.2015.05.014

Nugraheni, K., & Saputri, F. C. (2017). The effect of secang extract (Caesalpinia sappan linn) on the weight and histology appearance of white male rats' hearts induced by isoproterenol. *International Journal of Applied Pharmaceutics*, 9, 59–61. https://doi.org/10.22159/ijap.2017.v9s1.35_41

Park, W. B., Lee, K. D., Lee, C. S., Jang, H. C., Kim, H. Bin, Lee, H. S., Oh, M. D., & Choe, K. W. (2005). Production of C-reactive protein in Escherichia coli-infected patients with liver dysfunction due to liver cirrhosis. *Diagnostic Microbiology and Infectious Disease*, 51(4), 227–230. https://doi.org/10.1016/j.diagmicrobio.2004.11.014

Piazza, S., Fumagalli, M., Martinelli, G., Pozzoli, C., Maranta, N., Angarano, M., Sangiovanni, E., & Dell'Agli, M. (2022). Hydrolyzable Tannins in the Management of Th1, Th2 and Th17 Inflammatory-Related Diseases. *Molecules*, 27(21), 1–25. https://doi.org/10.3390/molecules27217593

Rahman, H., Aldi, Y., & Mayanti, E. (2016). Aktivitas Imunomodulator dan Jumlah Sel Leukosit dari Ekstrak Kulit Buah Naga Merah (*Hylocereus Lemairei* (Hook.) Britton & Rose) Pada Mencit Putih Jantan. *Jurnal Farmasi Higea*, 8(1), 44–58.

Reyes-Silveyra, J., & Mikler, A. R. (2016). Modeling immune response and its effect on infectious disease outbreak dynamics. *Theoretical Biology and Medical Modelling*, 13(1), 1–21. https://doi.org/10.1186/s12976-016-0033-6

Reze Fikrih Utama, Rosidah, Y. (2020). Immunomodulator Activity of Puguntano (Picria felterrae Lour.) Extract in White Male Mice by Carbon Clearance Method. *Indonesian Journal of Pharmaceutical and Clinical Research (IDJPCR)*, 03(02), 19–24.

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology*, 9(Apr), 1–11. https://doi.org/10.3389/fimmu.2018.00754

Stem, T., Littoralis, S., Methanol, H., Mahardhika, C., Yuni, D., Hidayati, N., Yudani, T., & Raras, M. (2022). *Asian Journal of Health Research*. 1, 19–24.

Sudeep, H. V., Gouthamchandra, K., Ramanaiah, I., Raj, A., Naveen, P., & Shyamprasad, K. (2023). A standardized extract of Echinacea purpurea containing higher chicoric acid content enhances immune function in murine macrophages and cyclophosphamide-induced immunosuppression mice. *Pharmaceutical Biology*, 61(1), 1211–1221. https://doi.org/10.1080/13880209.2023.2244000

Sunitha, V. S., Sunil, M. A., Radhakrishnan, E. K., & Mathew, J. (2015). Immunomodulatory Activity of Caesalpinia sappan L. Extracts on Peritoneal Macrophage of Albino Mice. *International Journal of Science and Research (IJSR)*, 4(12), 449–452. https://doi.org/10.21275/v4i12.nov151953

Susmitha, N., Swamy, P.N., P. Venkatesh. (2022). Review on Infectious Diseases. *International Journal of Indigenous Herbs and Drugs*, *07*(01),

18–21.

Uribe-Quero, E., & Rosales, C. (2017). Control of phagocytosis by microbial pathogens. *Frontiers in Immunology*, 8(Oct), 1–23. https://doi.org/10.3389/fimmu.2017.01368

Vij, T., Anil, P. P., Shams, R., Dash, K. K., Kalsi, R., Pandey, V. K., Harsányi, E., Kovács, B., & Shaikh, A. M. (2023). A Comprehensive Review on Bioactive Compounds Found in Caesalpinia sappan. *Molecules*, 28(17). https://doi.org/10.3390/molecules28176247

Wahyu, S., Hamid, F., Hatta, M., Djawad, K., & Bukhari, A. (2021). Effectiveness of Giving Secang Wood Extract (Caesalpinia Sappan L) Against IL-6 And IL-10 Levels in Balb / C Mice With Vulvovaginalis Candidiasis. *Indian Journal of Forensic Medicine & Toxicology*, *15*(2), 1609–1614. https://doi.org/10.37506/ijfmt.v15i2.14567

Widigdyo, A., Widodo, E., & Djunaidi, I. H. (2017). Extract of Caesalpinia sappan L. as Antibacterial Feed Additive on Intestinal Microflora of Laying Quail. *The Journal of Experimental Life Sciences*, 7(1), 7–10. https://doi.org/10.21776/ub.jels.2016.007.01.02

Yang, F., Zhang, F., Yang, L., Li, H., & Zhou, Y. (2021). Establishment of the reference intervals of whole blood neutrophil phagocytosis by flow cytometry. *Journal of Clinical Laboratory Analysis*, 35(8), 1–8. https://doi.org/10.1002/jcla.23884.