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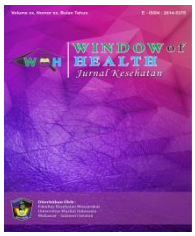

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In vitro Hemostasis Activity Analysis of Ethanol Extract of Sappan Wood Administration Based on Clotting Time Lee and White Method

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ABSTRACT

Hemostasis is a combination of events following vascular injury, including vasoconstriction, platelet aggregation, thrombus formation, recanalization, and debridement. Clotting time is an assessment to determine the time required for blood to clot in vitro. This study aimed to determine the effect of sappan wood ethanol extract on the outcome of the clotting time test using the Lee and White method. This study is an experimental study with several samples of 32 and divided into 3 groups of Lee and White method clotting time examination. Group C as a control group (1 mL of venous blood only), P1 and P2 as much as 1 mL of venous blood by adding 0.3 grams and 0.4 grams of sappan wood ethanol extract respectively. Data analysis used was One way Anova with Post hoc LSD test. One way Anova test revealed significant differences between research groups (<0.05). Post hoc LSD results showed a significant difference between P1 and P2 compared to group C. It can be summarized that there is a difference in the results of the Lee and White method clotting time examination between blood with and without sappan wood ethanol extract.

Key Word : Sappan wood; clotting time; Lee and White

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INTRODUCTION

Cardiovascular disease is the first cause of death in the world, with an estimated 17.9 million people dying in 2021. Atherosclerosis is one of the entities in heart disease. Lesions due to dilated atreosclerosis cause apoptosis and atherosclerotic plaques. Medications for atherosclerosis include antiplatelet drugs and anticoagulants such as heparin.[1,2] The use of heparin is not recommended as it may cause hemostasis disorders such as excessive hemorrhage.[3,4]

Hemostasis is a combination of events following vascular injury, including vasoconstriction, platelet aggregation, thrombus formation, recanalization, and healing. The process of hemostasis begins with the formation of a platelet plug at the site of injury that has undergone vascular disintegration. This process is the primary hemostasis mechanism[5]. Secondary hemostasis includes the interaction of plasma coagulation factors, consisting of intrinsic and extrinsic pathways that combine into a common path with the result of fibrin that reinforces the platelet plug. Platelet disorders can result from various congenital or acquired disorders and affect surface receptors that play a role in the platelet aggregation and adhesion process. Both quantitative and qualitative platelet dysfunction, including those caused by antiplatelet drugs, can interfere with primary hemostasis.[6,7]

Traditional medicine has been widely used by the people of Indonesia. One of the traditional medicines traditionally used as medicine is sappan wood (*Caesalpinia sappan L.*), classified as an herbal plant that grows naturally in secondary forests. Sappan wood contains phenolic compounds such as flavonoids, which have antioxidant activity to capture free radicals. [8,9] Antioxidant compounds from natural materials or plants have advantages compared to synthetic materials because of the speed of degradation of residues.[10] Studies that were conducted by [11] proved that 100 grams of sappan wood powder macerated in 1000 ml of 96% ethanol contained 6.02% flavonoids and 2.43% anthocyanins. Flavonoids are one type of antioxidant that can inhibit platelet attachment, aggregation, and secretion. The ability of flavonoids to inhibit platelet aggregation is due to the flavonoids being able to inhibit arachidonic acid metabolism by cyclooxygenase.[11] Based on research conducted by [12], brazilin in sappan wood extract is also capable of being an antithrombotic agent. [12]

Clotting time is an assessment to determine the time required for blood to clot in-vitro, the unit used is minutes..[13] One of the methods that can be used in testing clotting time is the Lee and White method (tube method). The study aimed to determine the effect of ethanol extract of sappan wood (*Caesalpinia sappan L.*) on the results of Lee and White method clotting time examination.

METHOD

The type of research used was experimental research. The research was conducted in August 2023 at the Chemistry and Hematology Laboratory of the Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang. Population and samples were obtained from the calculation of Federer's formula with a total of 27 samples. The tools used in this study were blender, stopwatch, test tube, spatula, scale, filter paper, rotary evaporator, funnel, erlemeyer, measuring cup, water bath, and

the materials used were sappan wood, 96% ethanol, and venous blood. The procedure in this study goes through two stages. The first is the extraction of sappan wood using the maceration method. Sappan wood obtained from the Peterongan Semarang market is prepared and then put in the oven for 1 week at a temperature of 55º C, which is then crushed with a blender. After pulverization, the sappan wood powder was filtered with a 60 mesh sieve and then macerated. 300 grams of sappan wood powder was macerated in 3000 ml of 96% ethanol (1:10) for 3x24 hours at room temperature and stirred once every 24 hours. Then, the macerate was filtered to separate the pulp and filtrate, and then the mixture was evaporated using a rotary evaporator at 40°C until the extract evaporated. Then, the evaporated extract was put in a water bath until it thickened into a paste. The thick extract obtained was stored in a brown bottle before being used for further tests.[11,14]

The weight of sappan wood ethanol extract used based on preliminary tests is 0.3 grams and 0.4 grams. The second stage is the sappan wood ethanol extract that has become a paste carried out the Lee and White Method Clotting Time test with 0.3 grams of sappan wood ethanol extract (P1), 0.4 grams (P2) and without sappan wood ethanol extract (C). The sample group without sappan wood extract was carried out by inserting venous blood into 3 test tubes of 1 mL each.[13,15,16] Data from the Lee and White method clotting time test were tested for normality with Saphiro Wilk and tested for differences between groups using the one way Anova test and continued with the LSD Post hoc test.

RESULT

The results of the study regarding the average results of the Lee and White method clotting time examination can be seen in table 1.

Table 1. The results of the Lee and White method clotting time test (minutes)

	Average ± SD	Minimum	Maximum
C	10.84 ± 0.34	10.50	11.50
P1	13.37 ± 0.58	12.83	14.17
P2	15.11 ± 0.45	14.50	16.00

Based on Table 1, it can be seen that the lowest average result of the Lee and White method clotting time examination is from the group without administration of sappan wood ethanol extract. While the highest average is from group P2, which is the group with 0.4 grams of sappan wood ethanol extract.

The results of the data normality test using the Saphiro Wilk test showed a normal data distribution with a significance value of 0.07 ($p>0.05$), so the test for differences between groups was carried out with the one way Anova test and the LSD Post hoc test. The results of the one way Anova test showed a significance value of 0.00 ($p<0.05$) indicating that there was a significant difference in the results of the Lee and White method clotting time examination. Post hoc LSD test results are shown in Figure 1.

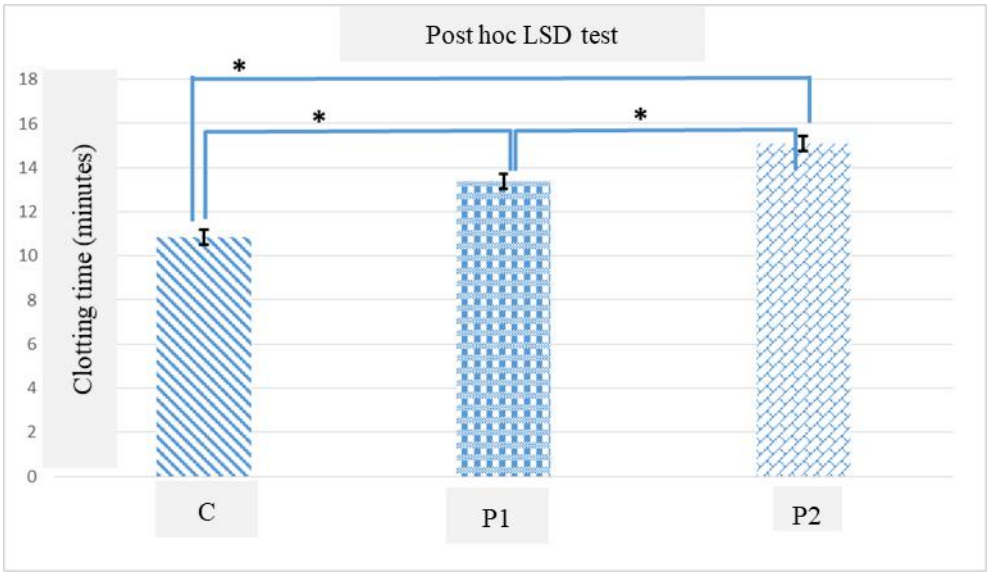

Figure 1. Post hoc LSD test results (Note: * significant)

Figure 1 shows that there is a significant difference in the results of the Lee and White method clotting time examination between each treatment group ($p<0.05$).

DISCUSSION

Hemostasis is the body's mechanism to stop bleeding spontaneously so as not to lose too much blood when there is an injury to the blood vessels so that the blood remains fluid and flows smoothly. Hemostasis disorders can occur in people with cardiovascular diseases such as atherosclerosis, myocardial infarction and ischemic stroke due to the presence of plaque or thrombus.[17] One of the hemostasis disorders in cardiovascular disease can be in the form of impaired platelet function. The Lee and White clotting time test is one of the examination parameters to test platelet function. [18]

The group without the addition of sappan wood ethanol extract showed results that were still within the normal range of values. The normal condition occurs because there is no anticoagulant agent added so that it becomes a standardized control for the treatment group. The use of sappan wood ethanol extract in this study showed a prolongation of blood clotting time using the Lee and White method. Herbal plants such as sappan wood can be used as an alternative medicine in platelet function disorders seen through clotting time examination. The average results of the Lee and White method clotting time examination in each group experienced lengthening. The group without the administration of sappan wood ethanol extract became a comparison to determine the effect of giving sappan wood ethanol extract. Sappan wood contains flavonoids, brazilin, alkaloids, saponins, tannins, phenyl propane, and terpenoids. [19]

Sappan wood contains phenolic compounds such as flavonoids, which have antioxidant activity as an antidote to free radicals. One of the flavonoid groups in sappan wood is anthocyanins. Anthocyanin compounds are glycosides of anthocyanidin compounds and are part of flavonoid secondary metabolites. The results of the study are in line with research [12] which shows that sappan wood can be an anti-platelet agent that can extend clotting time.[12] Flavonoids are one type of antioxidant that can inhibit platelet

attachment, aggregation, and secretion. The ability of flavonoids to inhibit platelet aggregation is due to the flavonoids being able to inhibit arachidonic acid metabolism by cyclooxygenase.[20]

The average results of the clotting time test in group P2 showed the highest prolongation of time. The greater the administration of sappan wood ethanol extract, the longer the time it takes for blood to clot. Based on one way Anova test, there is a significant difference between treatment groups. The clotting time results of the treatment groups were compared with each other, especially with the untreated group. Group T with the lowest clotting time results was significantly different from both groups P1 and P2. This indicates that the highest ethanol extract content significantly affects the hemostasis activity carried out in vitro through the Lee and White method clotting time examination. This is supported by research [21] which states that the antioxidant content in sappan wood extract has better antioxidant activity to increase the value of total antioxidant units in the body when compared to vitamins C and E. [21]

CONCLUSION AND SUGGESTION

Sappan wood ethanol extract can affect the results of the Lee and White method clotting time examination. The results showed a significant difference between the groups without administration of sappan wood ethanol extract, P1, and P2, indicating that the dose of ethanol extract that most affected the clotting time examination was 0.4 grams. This is due to the greater antioxidant content when compared to the group with 0.3 grams. It is suggested that future researchers conduct in vivo studies to investigate the effect of sappan wood ethanol extract on various indicators of hemostasis.

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REFERENCES

1. Lopez, Edgardo Olvera, Brian D. Ballard, A. J. Cardiovascular disease: About cardiovascular disease. in Cardiovascular Disease 1–12 (NCBI, 2022).

2. Maharani, A. et al. Cardiovascular disease risk factor prevalence and estimated 10-year cardiovascular risk scores in Indonesia: The SMARThealth Extend study. PLoS One 14, 1–13 (2019).

3. Berger, J. S. Aspirin for Primary Prevention - Time to Rethink Our Approach. JAMA Netw. Open 5, E2210144 (2022).

4. Undas, A., Brummel-Ziedins, K. & Mann, K. G. Why does aspirin decrease the risk of venous thromboembolism? On old and novel antithrombotic effects of acetyl salicylic acid. J. Thromb. Haemost. 12, 1776–1787 (2014).

5. Zaidi, A. & Green, L. Physiology of haemostasis. Anaesth. Intensive Care Med. 20, 152–158 (2019).

6. Lapelusa, A., Dave, H. D. & Federation, R. Physiology , Hemostasis. 1–9 (2023).

7. Zaidi, A. & Green, L. Review Article: Physiology of Haemostasis. Anaesthesis Intensive Care

Med. 20, 152–158 (2019).

8. Vij, T. et al. A Comprehensive Review on Bioactive Compounds Found in Caesalpinia sappan. Molecules 28, (2023).

9. Lioe, H. N., Adawiyah, D. R. & Anggraeni, R. Isolation and characterization of the major natural dyestuff component of brazilwood (Caesalpinia sappan L.). Int. Food Res. J. 19, 537–542 (2012).

10. Nirmal, N. P. & Panichayupakaranant, P. Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich Caesalpinia sappan extract. Pharm. Biol. 53, 1339–1343 (2015).

11. Nomer, N. M. G. R., Duniaji, A. S. & Nocianitri, K. A. Kandungan Senyawa Flavonoid dan Antosianin Ekstrak Kayu Secang (Caesalpinia sappan L.) serta Aktivitas Antibakteri Terhadap Vibrio cholerae. J. Ilmu dan Teknol. Pangan 8, 216 (2019).

12. Chang, Y. et al. Brazilin isolated from Caesalpinia sappan L. acts as a novel collagen receptor agonist in human platelets. J. Biomed. Sci. 20, 4 (2013).

13. Ciesla, B. Basic Hematologica Principle. Hematology in Practice (2018).

14. Abubakar, A. R. & Haque, M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J. Pharm. Bioallied Sci. 12, 1–10 (2020).

15. De Brito Sousa, J. D. et al. Accuracy of the lee–white clotting time performed in the hospital routine to detect coagulopathy in bothrops atrox envenomation. Am. J. Trop. Med. Hyg. 98, 1547–1551 (2018).

16. Suseel, A., Abraham, S. V, Paul, S., Tomy, M. M. L. & Rafi, A. M. Comparing modified Lee and White method against 20-minute whole blood clotting test as bedside coagulation screening test in snake envenomation victims. J. Venom. Anim. Toxins Incl. Trop. Dis. 29, e20220088 (2023).

17. Alkarithi, G., Duval, C., Shi, Y., Macrae, F. L. & Ariëns, R. A. S. Thrombus Structural Composition in Cardiovascular Disease. Arterioscler. Thromb. Vasc. Biol. 41, 2370–2383 (2021).

18. Little, P. J., Askew, C. D., Xu, S. & Kamato, D. Endothelial dysfunction and cardiovascular disease: History and analysis of the clinical utility of the relationship. Biomedicines 9, 1–9 (2021).

19. Nirmal, N. P., Rajput, M. S., Prasad, R. G. S. V. & Ahmad, M. Brazilin from Caesalpinia sappan heartwood and its pharmacological activities: A review. Asian Pac. J. Trop. Med. 8, 421–430 (2015).

20. Mu'nisa, A., Hala, Y. & Muflihunna, A. Analysis of Phenols and Antioxidants Infused Sappan. Int. J. Sci. Dev. Res. 2, 89–93 (2017).

21. Taveepanich, S. et al. Iron chelating, antioxidant, and anti-inflammatory properties of brazilin from Caesalpinia sappan Linn. Heliyon 10, e38213 (2024).