

The Effect of Sambang Darah Leaf Filtrate (*Excoecaria Cochinchinensis* L) to Reduce The Bleeding Time in Skin Wounds of White Rats (*Rattus Norvegicus*)

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ABSTRACT

Hemostasis is the process of stopping bleeding from a damaged blood vessel. When bleeding occurs, the body will naturally respond with a hemostatic mechanism to stop the bleeding. Indonesian people have long used plants in medicine, one of which is sambang darah (*Excoecaria cochinchinensis* L) plant. Sambang darah has a chemical compound that is thought to stop bleeding. The purpose of this study was to determine the potential of sambang darah filtrate to reduce the bleeding time in skin wounds of white rats (*Rattus norvegicus*). This study is a pre-experimental study with a static group comparison approach. The number of experimental units in this study were 10 white rats which were divided into treatment group and control group. Data analysis used the Independent Sample T Test. The results showed that the average of bleeding time in the skin wounds of white rats without drops of sambang darah leaf filtrate was 252 seconds. While the average of bleeding time in the skin wounds of white rats with drops of sambang darah leaf filtrate is 163 seconds. The conclusion of this study is sambang darah (*Excoecaria cochinchinensis* L) leaf filtrate have a potential to reduce the bleeding time in the skin wounds of white rats (*Rattus norvegicus*).

INTRODUCTION

Indonesia is 15th ranks as the largest country in the world. Indonesia has abundant natural wealth, that caused by several factors such as climate, diversity of soil types, and environmental factors (Setiawan, 2022). Indonesia has the largest biodiversity in the world (mega biodiversity country) with around 15,000 plants that have the potential for medicine. Indonesian people have long known and used medicinal plants as an effort to overcome health problems. Plants contain various types of bioactive compounds which are efficacious as medicine (Pradhan et al., 2013). The use of medicinal plants as raw material for medicine is widely used because they have relatively small side effects and are cheaper between to chemical drugs.

One of the uses of medicinal plants is to stop bleeding. When the surface of the body is injured, the body will bleed, a normal body when injured after a while the blood will stop flowing, whereas in large wounds it can cause profuse bleeding resulting in blood loss and even causing death. The body has a mechanism to block and improve the circulatory system, one of which is hemostasis mechanism (Sidrotullah, 2021). Hemostasis is the process of

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stopping bleeding which is divided into 3 stages, namely constricting blood vessels, forming platelet plugs, and blood coagulation. The process of hemostasis is the initial stage of wound healing (Kainde et al., 2016).

One of the medicinal plants is the sambang darah (*Excoecaria cochinchinensis* L). Sambang darah plant is usually planted in the yard as a living fence or medicinal plant, in parks as an ornamental plant or grows wild in the forest and in the fields, in open or slightly protected places (Oktariza et al., 2013). The use of plants as medicine is related to their chemical content, especially bioactive substances. The chemical constituents of the sambang darah plant are tannins, bahnigic acid, exocarol triterpenoids, silosterol. The sap contains resins and compounds which are highly toxic (Zega, 2013). It was also stated that blood sambang plants also contain flavonoids and saponins (Oktariza et al., 2013).

Tannins and flavonoids are the main compounds that play a role in the blood clotting process. The pharmacological effects of tannins are astringent, healing, antiseptic, antioxidant, vasoconstrictor, hemostatic, antidiarrheal, microbial antipathogen, anticancer and antidiabetic (Gaib et al., 2019). Sambang darah plants also contain flavonoids. Flavonoid compounds can be used as a cure for cancer, lung disease, kidney disease, cancer, improving blood circulation and inhibiting bleeding. In addition, flavonoids can also be used for the treatment of cytotoxicity, impaired liver function, inhibition of bleeding, antioxidants, antihypertensives and anti-inflammatories (Puzi et al., 2015).

The chemical compounds also contained in sambang darah plants are saponins. Saponins have a high level of toxicity against fungi, thus helping in the wound healing process (Susilawati, 2015). At low concentrations, saponins can cause hemolysis of red blood cells. Whereas in the form of a very dilute solution, saponins are toxic to cold-blooded animals and are commonly used as fish poison.

Based on the description of the effectiveness of sambang darah in stopping the bleeding and supported by empirical experience from the community, the researcher intends to look at the effectiveness of sambang darah (*Excoecaria cochinchinensis* L) leaves in stopping bleeding which was tested on white rats (*Rattus norvegicus*) which were used as test animals.

METHOD

This research is a *Pre-Experiment* with a *Static Group Comparison* approach. The number of experimental units in this study were 10 white rats (*Rattus norvegicus*). Samples were taken using a non-random purposive sampling technique, with the criteria of a sambang darah plant that is quite old and fresh with green upper leaves and red lower leaves with a leaf blade length of 9-12 cm and a leaf blade width of 2-4 cm. While the experimental animals used white rats with the following criteria: male sex, 3-4 months old, 300-400 gram weight, no anatomical abnormalities and had been acclimatized for seven days to water, food and laboratory conditions. The tools needed in this study included rat cages, rat hair clippers, scissors, test tubes, razor blades, knives, Pasteur pipettes, rulers, digital scales, blenders, and stopwatches. The materials needed include sambang darah leaves, white rat (*Rattus norvegicus*), animal feed, cotton, tissue, 70% alcohol, and water. Preparation of sambang darah (*Excoecaria cochinchinensis* L) leaves filtrate is done by weighing as much as 9 grams of sambang darah leaves and washing them clean, then pulverizing them using a blender, and squeezing them using sterile gauze and the liquid obtained is collected in a clean and dry container. Wounds were made in the white rats (*Rattus norvegicus*) by first cleaning the back area of the experimental animal to be injured, then shaving the animal's hair using a

hair clipper in the area to be injured, cleaning it using 70% alcohol in the area to be injured, then a wound was made by pinching the back of the experimental animal then using a razor a wound was made with an incision length of 1 cm, width of 1 cm, and depth of wound 3 mm. The treatment of white rats (*Rattus norvegicus*) was that the wound was dripped with sambang darah leaf filtrate until it covered the wound, then the blood was observed and wiped with filter paper every 30 seconds, then the bleeding time was calculated in the wound until the wound stopped bleeding by using a stopwatch, after which the wound is covered with sterile gauze and given a plaster. The obtained data were analyzed using a statistical test, namely the Independent Sample T Test with a confidence level of 95% ($P\alpha$ 0.05).

RESULT AND DISCUSSION

A total of 10 experimental animals were divided into two groups, such as the control group (T1) which consisted of 5 experimental white rats (*Rattus norvegicus*), and the experimental group (T2) which consisted of 5 experimental white rats (*Rattus norvegicus*). The results of measuring the bleeding time for each treatment can be seen in table 1.

Table 1. Results of Measurement of Bleeding Time

No.	Sample	Length of Bleeding Time	
		T1 (second)	T2 (second)
1	1	261	181
2	2	302	201
3	3	193	127
4	4	256	147
5	5	247	159
Oldest Time		302	201
Fastest Time		193	127

Table 1 shows that the average length of time to stop bleeding in the skin wounds of white rats (*Rattus norvegicus*) for the control group is 252 seconds, the average length of time to stop bleeding in the skin wounds of white rats (*Rattus norvegicus*) after dropping sambang darah leaf filtrate (*Euphorbia cochinchinensis* L.) is 163 seconds. The longest bleeding cessation time in the control group was 302 seconds and in the treatment group was 201 seconds. The fastest bleeding cessation time in the control group was 193 seconds and in the treatment group was 127 seconds.

To find out whether there is an effect or not of giving sambang darah leaf (*Euphorbia cochinchinensis* L.) filtrate in the process of stopping bleeding, a statistical test is carried out, namely the parametric *Independent Sample T test*. In table 2, we can see the result of the statistical test.

Table 2. Result of Independent Sample T Test

No	Group	Mean	P	Explanation
1	T1	04:11.800±00:39.060	0,003	Sig. $P \leq 0,05$
2	T2	02:43.000±00:28.879		

The results of the Independent Sample T Test showed that P value is 0.003, it means that the data measured the length of time it took to stop bleeding in the skin wounds of white rats (*Rattus norvegicus*) that were not dripped with sambang darah (*Euphorbia cochinchinensis* L) leaf filtrate and those that were dripped with sambang darah (*Euphorbia cochinchinensis* L) is significant.

Based on the results of the study, it was shown that the use of sambang darah (*Excoecaria cochinchinensis* L) leaf filtrate in the process to stopping bleeding on the skin wounds of white rats (*Rattus norvegicus*) had significant results because of the time to stop bleeding using sambang darah leaf filtrate and without using filtrate sambang darah have different measurement results, the process of hemostasis plug using sambang darah leaf filtrate is faster than without using blood sambang leaf filtrate. This because the white rat carries out a hemostatic process assisted by a hemostatic substance contained in the leaves of the sambang darah plant.

In this study, the average length of time to stop bleeding in skin wounds of white rats without dripping sambang darah leaf filtrate was 252 seconds. The time to stop bleeding in each sample is different, this is due to the length of the wound, the width of the wound and the depth of the wound made. Besides that, it was also influenced by the different conditions of each white rat experimental animal. However, the results of the measurement of bleeding time in the four samples were still within the normal range of bleeding time, namely 120-420 seconds.

The average length of time to stop bleeding on the skin wounds of white rats dripped with sambang darah leaf filtrate is 163 seconds. Bleeding cessation time is faster because sambang darah leaf filtrate contains active compounds of secondary metabolites including tannins, flavonoids and saponins. Tannins and flavonoids are the main compounds that play a role in the blood clotting process, while saponins have a hemostatic effect by reducing the blood clotting period (Gaib et al., 2019).

Tannins which are astringent have the ability to form complexes with macromolecules, especially proteins, so that they can accelerate the blood clotting process. The mechanism of action of tannins as a vasoconstrictor is through its astringent effect. Tannins accelerate the release of proteins from cells and precipitate these proteins on the cell surface, also reduce capillary secretion and permeability, contract the space between cells, harden the capillary endothelium and then form a protective layer of skin so that the superficial cells tighten and shrink. This situation will result in local vasoconstriction of the capillaries (Sidrotullah, 2021). Tannins can also precipitate blood proteins, namely albumin. This protein deposition process will induce the synthesis of thromboxane A2 to increase platelet aggregation, thereby accelerating the formation of temporary platelet plugs in injured blood vessels (Tedjasulaksana & Regina, 2013). More and more blood proteins are precipitated by tannins, causing reduced blood albumin and resulting in increased synthesis of thromboxane A2 and making it easier for platelets to release ADP, ADP and thromboxane A2.

In table 1 it can be seen that the wound without being given sambang darah (*Excoecaria cochinchinensis* L) leaf filtrate when compared to the wound with being given sambang darah (*Excoecaria cochinchinensis* L) leaf filtrate is different. This is because the wound is not given drugs or active compounds that can stop bleeding. The active compounds that are also contained in blood sambang plants (*Excoecaria cochinchinensis* L) are flavonoids that can also inhibit bleeding. Flavonoids are known to have a hemostatic effect

by accelerating the increase in the number of platelets. The mechanism of flavonoids in stopping bleeding is by means of vasoconstriction. Platelets normally convert plasma membrane arachidonic acid to thromboxane. Thromboxane A₂ is synthesized in platelets and is a strong vasoconstrictor that can stimulate platelet aggregation, so that platelet plugs can form (Tedjasulaksana & Regina, 2013).

In addition to tannins and flavonoids, saponins also have a hemostatic effect by reducing blood clotting time. Saponins contained in sambang darah plants also act as antimicrobials which inhibit the growth of these microbes so that they can accelerate wound healing. Saponins can accelerate the formation of collagen connective tissue by stimulating TGF- β to secrete collagen (Sembiring et al., 2021).

Based on the statistical test results in table 2, it can be seen that the measurement data for the length of time the bleeding stops in the skin wounds of white rats (*Rattus norvegicus*) is significant ($P = 0.003 < 0.05$). This means that H_a is accepted and H_o is rejected so it is concluded that there is significant potential from the filtrate of sambang darah leaves (*Excoecaria cochinchinensis* L) in the process of stopping bleeding in the skin wounds of white rats (*Rattus norvegicus*). This study proves that the filtrate of sambang darah leaves (*Excoecaria cochinchinensis* L) can be used as a traditional medicine to stop bleeding at a low price and easy to obtain.

CONCLUSION

This study proves that sambang darah (*Excoecaria cochinchinensis* L) leaf filtrate can shorten the bleeding time on the skin wounds of white rats (*Rattus norvegicus*) which were observed based on the results of decreased bleeding time after treatment.

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