

The Determination of Haemostasis Value in Relation to Potential Blood Leaf Leaves Filtrate (*Excoecaria cochinchinensis L*) as the External Medicine for Skin Wound of White Rat (*Rattus norvegicus*)

Siti Zaetun, Lalu Srigede

Program Study of Health Analyst, School of Health Polytechnic, Ministry of Health, Mataram

ABSTRACT

Background: Plants are currently used as medicine, i.e. medicinal plants. Medicinal plants consist of many chemical compounds, especially bioactive substances. One of them is Sambang Darah (*Excoecaria cochinchinensis*). Sambang Darah have so many benefits which usually applied in *vulnus combutio*. This study aimed to determine value of haemostasis after applied Sambang Darah leaves in white rat skins (*Rattus norvegicus*).

Subjects and Method: This was a true experimental study with post test only control group design. This study conducted at Mataram University, West Nusa Tenggara, Indonesia, from April to October 2017. A total sample of 16 white rats was selected for this study. The dependent variable in this study was the value of haemostasis and the independent variable was filtrate of Sambang Darah leaves. The data were analyzed using Man Whitney test.

Results: The average level of bleeding time was 3.76 minutes for the control group and 2.58 minutes for test group. The average level of clotting time were 2.20 minutes for control group and 1.36 minutes test group. The number

of platelet was 277,000/uL for the control group and 221,000/uL for test group. APTT level was 25.06 second for the control group and there was no clot in test group. PT level was 13.95 second for the control group and there was no clot in test group. TT levels was 18.2 second for the control group and there was no clot in test group. And the last, D Dimer level was 0.1 mg/l for the control group and there was no clot in test group. The statistical analysis showed $p \leq 0.001$.

Conclusion: Filtrat of sambang darah leaves can be applied as medicine for external wounds but it can not be applied in koagulasi test.

Keywords: Haemostasis value, blood leaf filtrate (*Excoecaria cochinchinensis L*)

Correspondence:

Siti Zaetun. Program Study of Health Analyst, School of Health Polytechnic, Ministry of Health, Mataram. Jl. Prabu Rangkasari, Dasan Cermen, Sandubaya, Mataram, West Nusa Tenggara. Email: miraigan@yahoo.co.id. Mobile: 081803630063.

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BACKGROUND

Indonesia is a country with considerable biodiversity. Of the 40,000 types of flora that grow in the world, 30,000 of them grow in Indonesia and at least 9,600 species are known to have medicinal properties, but only 300 species are used as traditional medicine industry and as raw materials for traditional

medicines (Sutopo, 2016). One of the medicinal plants is the bloody plant (*Excoecaria cochinchinensis L*). These blood-borne plants are usually planted in the yard as living fences or medicinal plants, in parks as ornamental plants or grow wild in forests and in fields, in open or slightly protected areas (Oktariza et al. 2013).

The use of plants as medicine is related to their chemical content, especially bioactive substances. The chemical content of blood-borne plants are tannins and flavonoids are the main compounds that play a role in the process of blood clotting. Tannins are astringent which have the ability to form complexes with macromolecules, especially proteins.

This ability can accelerate the process of blood clotting, the sap contains resins and compounds that are very toxic (Zega, 2013). It was also mentioned that blood-bearing plants also contain flavonoids. Flavonoid compounds can be used as a cure for cancer, lung disease, kidney disease, cancer, blood circulation and inhibit bleeding (Daniel, 2010). In addition, flavonoids can also be used for cytotoxic treatment, impaired liver function, inhibit bleeding, antioxidants, antihypertensive and anti-inflammatory (Puzi, et al. 20-15).

Other compounds contained in this plant are saponins. Saponins have a high level of toxicity against fungi, which helps in the process of wound healing (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 poisons (Liem et al. 2013). The many benefits of blood-bearing leaves, namely, can heal external wounds, overcome bleeding due to miscarriage, cope with bleeding due to irregular menstruation, treat blood vomiting, dysentery, and salivary thyroid disease, etc. (Oktariza, et al., 2013).

Plants that contain the same chemical content as blood-bearing plants are plant bananas. Plantain contains three dominant active compounds namely tannins, flavonoids and saponins. Raja plantain is efficacious in stopping bleeding (haemostasis). Some Authors from the University of Gadjah

Mada have tried the efficacy of plantain plants for healing cuts or burns on the skins of guinea pigs, dogs, dogs and sheep and tooth extraction wounds on guinea pigs. The results concluded that plantain sap was proven to not have a negative effect on wound healing in the skin, even there were beneficial things such as the presence of haemostasis that accelerated capillary stopping, did not show any swelling and histologically there was an amount of collagen fiber in the wound (Widyana, 2013).

Based on the chemical content contained in blood-bearing plants similar to those contained in plantains that are thought to have the potential to stop bleeding, the Authors are interested in conducting study on "Determination of haemostasis values in relation to the potential of blood-leaf leaf filtrate (*Excoecaria cochinchinensis L.*) as an external remedy for white rat (*Rattus norvegicus*) skin lesions".

This study is expected to provide scientific information about blood leaf leaves and as proof that the blood leaf filtrate (*Excoecaria cochinchinensis L.*) has the potential to stop bleeding.

SUBJECTS AND METHOD

1. Study Design

This was a randomized controlled trial. The study was conducted at the Faculty of Medicine, University of Mataram, West Nusa Tenggara, Indonesia, from April to October 2017.

2. Population dan Sample

The study population is a group of rats. The sample size was 16 male white rats grouped into 2 groups.

3. Study Variables

The dependent variable in this study was the length of time to stop bleeding from the incision in the skin of white rats. The independent variable in this study is the blood leaf filtrate.

4. Operational Definition of Variables

Blood leaf leaves are leaves of blood leaf plants used in this study, with the criteria that the leaves are green and the leaves are red below, quite old, fresh, and the length of leaf strands 9 to 12 cm and leaf width 2 to 4 cm.

Length of time to stop bleeding is a time when rat bleed then timing how long it takes to stop bleeding.

5. Procedures

The study began with acclimatization of white rats for 7 days for adaptation in the maintenance area to homogenize their way of life and food before conducting experiments to avoid animals trying to experience stress.

The making of blood leaf leaf filtrate was carried out as follows: (1) Weighed 20 sheets of blood leaf leaves and washed thoroughly, (2) Smoothed using a blender, (3) Squeezed and the liquid obtained is collected in a clean and dry container so that it is obtained \pm 1 ml of blood floating leaf filtrate.

Wounds in white white rat were carried out using the following ways: (1) Anesthetics were performed on white white rat that will be injured using ether, (2) Sheared white rat fur using a shaver in the area to be injured, (3) Draw a wound pattern on the back of the animal test to be injured, (4) Cleaned the area to be injured in test animals using 70% alcohol, (5) The wound was made according to a pattern using a razor blade with a wound length of 1 cm, with a wound depth of 1 mm and wound width of 1 cm.

A total of 16 white rat test animals were divided into 2 groups, namely the control group and the treatment group. In the control group, the body parts of rats treated was given 10% Providone iodine. In the treatment group the body parts of white rat that were given injury were given blood-bearing filtrate, and then the length of time to stop bleeding was measured.

6. Data Analysis

Data analysis was performed using the Mann Whitney test.

RESULTS

1. Study Preparation Phase

The average body weight of the white rat used in this study was 318 grams. An average of 20 sheets of blood-coated blood leaves with an average weight of 9 grams produces 1 ml of blood-leaf-leaf filtrate.

2. Treatment stage of experimental animals

a. Bleeding Time injury and examination.

The average Bleeding Time examination results of the control group was 3.76 minutes, while for the treatment group was 2.58 minutes. Bleeding time in the treatment group with blood-leaf leaf filtrate was faster or shorter compared to the control group.

b. Clotting Time Checking

Clotting Time examination was carried out at different times, considering the white rat were still in the healing stage of the wound in the back area. Blood samples were taken from the tail area. Clotting Time checking was done using the slide method. The average Clotting Time examination results were 2.20 minutes, while the treatment group was 1.36 minutes. Clotting time in the treatment group with blood soluble leaf filtrate was faster or shorter than the control group.

c. Platelet Count Calculations

Platelet counts were examined using the Rees Ecker method. The average number of platelet counts in the control group was 277,000 platelets per μ L of blood, while for the treatment group with blood leaf leaf filtrate was 221,000 platelets per μ L of blood.

3. Wound Healing Stage

Observation of wounds that have been smeared by blood leaf leaf filtrate was done every

day until the wounds appear to shrink (scar tissue). The giving of blood leaf filtrate was done 3 times a day.

4. White rat Surgery and APTT Examination

This stage is performed on the rat aortic vein with consideration of the amount of blood volume needed (\pm 5 to 6 ml) for examination of APTT, PT / PPT, TT, D-Dimer.

- a. The results of the APTT (Activated Partial Thromboplastin Time) examination of the control group were 25.06 seconds on average, while for the treatment group, there was no clot formation (no coagulation).
- b. The results of Partial Thrombo-plastin Time (PTT) examination in the control group were 13.95 seconds on average, while for the treatment group, there was no clot formation (no coagulation).
- c. The results of the Thrombin Time (TT) examination in the control group were 18.20 seconds on average, while for the treatment group, there was no clot formation (no coagulation). TT values were mostly still within normal limits.
- d. The average D-Dimer examination results in the control group were still within normal limits (<0.3 mg / L), while for the treatment group, there was no clot formation (no coagulation). TT values are mostly still within normal limits.

5. The results of bivariate analysis

This results of bivariate analysis are described in Table 1 and 2.

Table 1. Mann Whitney Test Results on Bleeding Time, Clotting Time, Calculate platelet count

Test	p
Bleeding Time	≤ 0.001
Clotting Time	≤ 0.001
Platelet count	0.025

The Mann Whitney test shows $p \leq 0.001$ which means that there were significant differences in the bleeding time and clotting time values in the control and the treatment group. The Mann Whitney test shows $p = 0.025$ which means that there was no significant differences in platelet count values in the control and the treatment group.

Table 2. Mann Whitney Test Results on APTT, PT, TT, and D-Dimer Examinations

Test	p
APTT	≤ 0.001
PT	≤ 0.001
TT	≤ 0.001
D-Dimer	≤ 0.001

The Mann Whitney test shows $p \leq 0.001$ which means that there were significant differences in the APTT, PT, TT, and D-Dimer values in the control and the treatment group.

DISCUSSION

In the study of determining the value of hemostasis in relation to the potential of blood leaf filtrate as an external drug in white rat skin lesions is motivated by the use of local potential which is sometimes overlooked by the community. One of the uses of blood leaf filtrate is as an external medicine.

The use of plants as medicine is related to their chemical content, especially bioactive substances. One of the medicinal plants is blood-spreading plant. The many benefits of blood-bearing leaves, among others, can heal external wounds, cope with bleeding due to miscarriage, winroll bleeding due to irregular menstruation, treat blood vomiting, dysentery, and pain in the throat/ salivary glands.

The chemical content of blood-borne plants are tannins and flavonoids. They are the main compounds that play a role in the

process of blood clotting (Daniel, 2010). Tannins are astringent which have the ability to form complexes with macromolecules, especially proteins. This ability can accelerate the process of blood clotting. Other compounds contained in this plant are saponins. Saponins have a high level of toxicity against fungi, thus helping in the wound healing process (Putri, 2012).

This study was conducted using white rat experimental animals with the consideration that rat skin is more likely to be injured compared to white rat. Besides considering the weight and body size of the experimental animals, Authors also considered the volume of blood samples needed so much that it was not possible to use white rat.

The initial treatment was carried out by acclimation activities for 2 weeks. This was done with the aim to provide rats the opportunity to adapt to the environment in the Laboratory and to increase the body weight of white rat to become ideal body weight. The weight of the 16 rats initially obtained was averaging 318 gr.

After the acclimation process was complete, it turned out that there was 1 mouse that died from stress. Furthermore, the experimental animals were treated as divided into 2 treatment groups. Group I wounded the skin on the left side of the back and immediately examined Bleeding Time after the blood stopped flowing the wound then given a drug iodine wound 10% and group II made a wound with an area of 1 cm and Bleeding Time examination, after the blood stopped flowing the wound then given blood leaf filtrate. Wounds that have been treated are observed for 1 week or until the wounds get smaller and scar tissue forms.

Provision of blood leaf filtrate is done 3 times a day until the wound dries. After treatment for 1 week, then before terminalisation, blood collection is carried out on the tail for clotting time and platelet co-

unt. In the process of terminalisation, surgery on white rat for blood collection in aortic blood vessels is done. Furthermore, making platelet poor plasma is made for examination of APTT (Activated Plasma Prothrombin Time), PT (Plasma Prothrombin Time), TT (Thrombin Time), and D-Dimer.

All actions and examinations were carried out at the University of Mataram's Faculty of Medicine except the D-Dimer examination was carried out at the Mataram City Hospital Laboratory. Examination for the Platelet Aggregation Test is carried out by sending samples to the Parahita Clinical Laboratory in Surabaya and showing the results of rejection or ambiguity due to instability during the trip. Samples for platelet aggregation examination are only valid for 2-4 hours. The results showed the average Bleeding time (BT) examination results in the control group was 3.76 minutes and the average BT in the treatment group giving blood-bearing leaf filtrate was 2.58 minutes. These results indicate the BT value in controls and treatments is still within normal limits. The BT value from treatment with blood-bearing leaves froze faster. This shows that blood-leaf filtrate has the potential as a wound drug in the event of bleeding. On examination of Clotting Time (CT), the average results of 2.20 minutes for the control group (time is still within normal limits) and the freezing time of the treatment group freeze faster (below normal time). This is likely due to substances contained in blood bearing leaves such as flavonoids which help accelerate blood clotting.

Based on the results of the platelet count, it appears that the platelet count in the control and treatment groups is still within normal limits but the platelet count in the treatment group is less than the control group. Possibly, this is caused by platelets in the blood given blood-leaf filtrate making platelets clustered so that it disturbs

when counting the number of cells. At the stage of wound healing on the first day, the wound has begun to dry and appears to constrict on the seventh day. This time is almost the same as the control group that was given the drug Iodine 10%.

The results of coagulation tests such as APTT, PT, TT and D-Dimer were performed after rat surgery. This is done because a large volume sample is needed so that it is not possible to do venous blood sampling in the tail. The results showed that the APTT, PT, TT, and D-Dimer values were still within normal limits for the control group. This is inversely proportional to the treatment group that does not show a freezing reaction. This shows that in the process of coagulation, the possibility of substances contained in blood leaf leaves are not able to bind to substances in the blood plasma so that coagulation is not formed.

The results showed that there were differences in bleeding time and clotting time between the blood of the control group and the treatment group. This is likely to occur because there is an influence from blood leaf filtrate because the average bleeding time which is overcome by giving blood leaf filtrate is shorter compared to the control. Statistical test results showed $p \leq 0.001$ which means there was a difference in the value of haemostasis in the control group and the treatment group. The value of BT haemostasis, CT tended to be shorter than the control group while the coagulation value did not produce results, so it was concluded that blood laden leaf filtrate could not be used for blood coagulation examination. Bloodstrip leaf filtrate can only be used for the treatment of external wounds.

AUTHOR CONTRIBUTION

Siti Zaetun and Lalu Srigele made blood leaf filtrate, measured bleeding time and clotting time, run data analysis, and wrote the article.

CONFLICT OF INTEREST

We declare that there was no conflict of interest.

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