The Effect of *Bajakah Tampala* Stem (*Spatholobus littoralis* Hassk) Extract on Clotting Time In Vitro

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ABSTRACT

Wounds that are not treated immediately will have a risk for the patient; the risk is infection. The risk of this infection can be reduced by stopping the bleeding as soon as possible. One way to accelerate the bleeding to stop is by giving hemostatic agents. One source of this haemostatic agent can come from plants, namely *Bajakah Tampala* (*Spatholobus littoralis* Hassk.), which contains flavonoids, saponins, and tannins. This study examines the effect of extract *bajakah tampala* (*Spatholobus littoralis* Hassk.) on clotting time. This study used an experimental method, namely true experimental laboratories with Post Test-Only Group Design research on 18 men aged 19-25 years, which were divided into 8 groups, namely group 1 (normal group), group II, III, and IV (administration of DMSO and aquadest at concentrations of 10%, 5%, and 2.5%), group V (Tranexamic acid), group VI; VII; and VIII (extracts of tampala plough rods 10%; 5%; 2.5%). This research uses the glass object method. Data on clotting time *in vitro* will then be analysed using one way ANOVA test. The result of one-way ANOVA is 0.000 (<0.05), which indicated an effect between the administration of *Bajakah tampala* stem extract on blood clotting. The increase in the concentration of Bajakah tampala stem extract is not directly proportional to the clotting time.

Keywords: *Haemostasis, Bleeding, Object Glass, Bajakah tampala, Clotting time.*

INTRODUCTION

Wounds are one of the most common problems now. Based on the Riskesdas data in 2018, the national injury prevalence rate has increased by 1% compared to the previous 5 years. If a wound is not treated immediately, it can lead to infection. Because of this, the patient's condition could be dangerous [1]. One way to prevent infection is to accelerate blood clotting, reducing the possibility of infection [2]. Hemostatic agents are one example of a material that can accelerate the blood clotting process [3]. Hemostatic agents can come from humans, animals, and plants [4].

One plant that can potentially be used as hemostatic agents is *Bajakah tampala*, or the Latin name *Spatholobus littoralis* Hassk. *Bajakah tampala* usually grows in Kalimantan, especially Central Kalimantan and has often been used as traditional medicine. Bajakah tampala stems contain secondary metabolites in the form of flavonoids, saponins, terpenoids, tannins, phenols and steroids.

Clotting time is the time required for a blood sample to clot and will measure the activity of blood clotting factors [1]. There are several ways to check the clotting time: the tube method (lee and white) and the glass object method. The tube method is inserting a sample of blood into a test tube and rotating it at 45° to form a blood clot [5]. While the way to do the glass object method is by dripping blood on a glass object, and then the surface of the blood is lifted using a lancet needle to form a fibrin thread [6].

2. MATERIAL AND METHODS

2.1 Ethical Clearance

Ethical clearance for this study was approved by the institutional ethical committee University of Surabaya. All subjects gave their informed consent for inclusion before they participated in the study.

2.2 Methods

This research methods is true experimental laboratory research. This study will assess the effect of the extract bajakah tampala stem with concentrations of 10%, 5%, and 2.5% as the independent variable, while the dependent variable in this study is clotting time. This research took place at the Palang Merah Indonesia East Kotawaringin Regency

2.3 Population

This study used the population at the East Kotawaringin Regency. The people who want to donate

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their blood at the palang merah indonesia east kotawaringin regency. The sample used in this study is a man aged 19-25, weight >45 kg, blood systolic100 until 140, blood diastolic 80-100, doesn't consume heart drugs, coagulant drugs, suffering diseases like cancer, infection, heart disease and autoimmune, also doesn't have any pathological blood disease

2.4 Plant Materials

The stem of Bajakah tampala (*Spatholobus littoralis* Hassk.) was collected from a village called Tumbang Samba in East Borneo Province. This plant was collected by original foreign inside the forest at that village.

2.5 Extract Bajakah Tampala

Laboratorium prepared the extract at Stikes Karya Putra Bangsa using maceration methods. 70 gr simplicia of Bajakah Tampala will be soaked at 420 mL ethanol. This extraction will be left for 24 hours, and this extraction will be filtered. This progress will be repeated 3 times. After getting the filtrate, the filtrate will be evaporated at 40oC-50oC until got the extract. This extract will be divided into 3 groups. There are 10%,5%, and 2,5% concentrations between extract bajakah tampala and Dimethyl sulfoxide (DMSO) also aquadest.

2.6 Blood samples

The blood used in this study is venous blood. Venous blood was obtained from *Palang Merah Indonesia* (PMI) East Kotawaringin Regency, East Borneo. All protocols to collect the blood were followed the procedure from *Palang Merah Indonesia*. After *Palang Merah Indonesia* collected the blood, the researcher collected 10 mL of blood for the study.

2.7 In Vitro Study

The in vitro effect of *bajakah tampala* on the clotting time was evaluated using object glass. The volume of venous blood in each group is 1 ml. This venous blood will be dripped on the test tube and added 10 µl from each group except the normal group. Group I was a normal group, group II, III, and IV were negative control groups with the addition of dimethyl sulfoxide (DMSO) and aquadest at concentrations of 10%, 5%, and 2.5%, and group V was a positive control group with the addition of tranexamic acid. Meanwhile, groups VI, VII, and VIII were the treatment groups with adding bajakah tampala stem extract with concentrations of 10%, 5%, and 2.5%. After this solutin mixed, it will taken as much 50 µl to be dripped on a object glass. Every 30 seconds, the researcher will lifting up the blood surface using a lancet until the fibrin threads form. When the fibrin threads form, the researcher will record it.

2.8 Statistical Analysis

Results are presented as a clotting time (seconds) and will be analysed using the SPSS application. The data will be tested for normality with the Shapiro-Wilk test and then continued with the homogeneity test. The data that has been declared to be normal and homogeneous are then tested with one-way ANOVA with a 95% confidence level and will be continued with the LSD test to see the comparison between each group.

Table 1 The Result of Clotting Time

Groups	Mean ± SD (s)	Maksimum (s)	Minimum (s)
Normal	951,67±32,971	1230	660
DMSO and Aquadest 10%	950,00±30,870	1140	660
DMSO and Aquadest 5%	956,67±34,461	1260	660
DMSO and Aquadest 2,5%	956,67±34,461	1260	660
Tranexamic Acid	868,33±33,473	1050	570
Bajakah Tampala Stem Extract 10%	806,67±33,156	990	540
Bajakah Tampala Stem Extract 5%	768,33±28,299	900	510
Bajakah Tampala Stem Extract 2,5%	835,00±32,832	1020	510

3. RESULTS

Based on the results of the study in table 1 describes the average time each group needed to form a blood clot. In table 1 its describe that the normal group and the negative control group had an average time that was not much different, while the 5% tampala bajakah extract group had the fastest blood clotting time compared to the other groups.

Results were expressed as mean ± SD and analysed with one-way ANOVA followed by LSD Test. But before that a normality test and homogeneity test were carried out. Based on Table 2, it is known that the group adding *bajakah tampala* stem extract has significant differences compared to other groups. The normal group, the negative control group, and the positive control group did not have a significant difference. The *bajakah tampala* stem extract 5% group also had a significant difference when compared to the 10% and 2.5% *bajakah tampala* stem extract group.

4. DISCUSSION

In the results of this study, it was found that there was a significant difference in blood clotting time in each treatment group when compared to the normal group, negative control group, and positive control group ($p < 0.05$); this indicates that there is an effect of giving the *bajakah tampala* stem extract against blood clotting time. This shows that the stem extract of *bajakah tampala* contains secondary metabolites that are proven to accelerate clotting time. The metabolites secondary of *bajakah tampala* stem extract include phenols, tannins, saponins, flavonoids, terpenoids, and steroids [7]. Metabolites secondary that have an effect are tannins, saponins, and flavonoids.

Flavonoids, tannins and saponins are active compounds that are able to accelerate blood clotting by accelerating the synthesis of thromboxane A2 which is involved in the blood when blood flows out through the walls of injured blood vessels and precipitates protein and calcium in the blood quickly so that the platelet surface becomes sticky and quickly triggers platelet

Table 2 The Result of Clotting Time

Groups	Normal	DMSO and Aquadest 10%	DMSO and Aquadest 5%	DMSO and Aquadest 2,5%	Tranexamic Acid	*Bajakah Tampala* Stem Extract 10%	*Bajakah Tampala* Stem Extract 5%	*Bajakah Tampala* Stem Extract 2,5%
Normal	-	0,971	0,914	0,914	0,073	0,002	0,000	0,013
DMSO and Aquadest 10%	-	-	0,885	0,885	0,079	0,002	0,000	0,014
DMSO and Aquadest 5%	-	-	-	1,000	0,058	0,001	0,000	0,009
DMSO and Aquadest 2,5%	-	-	-	-	0,058	0,001	0,000	0,009
Tranexamic Acid	-	-	-	-	-	0,184	0,032	0,471
***Bajakah Tampala* Stem Extract 10%**	-	-	-	-	-	-	0,407	0,540
***Bajakah Tampala* Stem Extract 5%**	-	-	-	-	-	-	-	0,151
***Bajakah Tampala* Stem Extract 2,5%**	-	-	-	-	-	-	-	-

aggregation, where platelets will bind to other platelets so that blood clots form faster [8].

In this study, the increase in the concentration of the *bajakah tampala* stem extract was not directly effect the clotting time. It can be seen that the *bajakah tampala* stem extract with a concentration of 5% has a better effect when compared to the *bajakah tampala* stem extract with a concentration of 10%. This can occur due to several possibilities, including the amount of metabolites secondary contained in each different concentration and the concentration level of the extract of *bajakah tampala* stem (*Spatholobus littoralis* Hassk) which is too high also allows it to be less able to accelerate clotting time. This is presumably because the molecules in the extract bind to each other so that the molecules inside are larger than the other concentration. At higher extract concentrations, saturation may also occur, this causes the compounds contained in the extract not dissolve completely so that the effects given will also be different [9]. It is also possible that the primary content in the extract of *bajakah tampala* stems also has some influence on blood clotting, this is influenced by where the plant comes from because the nutrient content of the soil also affects this.

In the negative control group, there was no significant time difference compared to the normal and positive control groups. This negative control group consisted of dimethyl sulfoxide (DMSO) and also aquadest. The addition of dimethyl sulfoxide to blood samples can cause hemolysis [10]. While aquadest does not affect blood clotting because aquadest does not contain active compounds that can affect blood clotting. Due to the smaller ratio between DMSO and aquadest, the effect of DMSO is not too influential compared to aquadest. So that the negative control group does not cause a bias due to the addition of DMSO and aquadest.

In the positive control group, tranexamic acid was added. In the data analysis of the positive control group, there was no significant difference compared to the normal and negative control groups. However, the positive control group had a better effect in accelerating blood clotting because tranexamic acid is a competitive inhibitor of plasminogen activator and plasmin inhibitor. Plasmin itself plays a role in destroying fibrinogen, fibrin, and other clotting factors. So tranexamic acid in this study had a better effect than the normal and negative control groups. This means tranexamic acid can help treat heavy bleeding due to fibrinolysis [11].

5. CONCLUSION

In conclusion, the study focused on the potential use of Bajakah Tampala (*Spatholobus littoralis* Hassk.) as a haemostatic agent to reduce the risk of infection in wounds by accelerating clotting time. The research employed an experimental method involving 18 male participants aged 19-25, divided into different groups based on the administration of DMSO, aquadest, Tranexamic acid, and varying concentrations of Bajakah Tampala stem extract.

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