

## The bleeding and clotting time analysis of the stem extract of *Musa paradisiaca* var. *sapientum* (L.) Kunze on hemostatic response

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### ABSTRACT

A herbal medicine that can be used to accelerate bleeding is the Ambonese banana stem sap (*Musa paradisiaca* var. *Sapientum*) through secretion of Adenosin Diphosphate (ADP) and von Willebrand Factor (vWF) in the process of platelet adhesion on the injury vessels. The aim of this study to test the influence of Ambonese banana stem extract in a concentration of 25%, 50% and 100% against hemostatic response on mice. 24 animal study were randomly assigned into four groups. Group 1 was the control, group of 2 was given 25% extract concentration, group of 3 was given 50% extract concentration and group of 4 was given 100% extract concentration of Ambonese banana stem per-oral. Then all the groups were examined their bleeding and clotting time. The administration of Ambonese banana stem extract at the concentrations of 25%, 50 %, and 100% significantly shortened both bleeding time and clotting time compared to control group ( $p = 0.00$ ). The group with 100% extract of the Ambonese banana stem having the shortest bleeding time and clotting time. The conclusions of this study showed Ambonese banana stem extract has potential as the hemostatic agent by shortening bleeding and clotting time on mice.

**Keywords:** Ambonese banana stem extract, bleeding time, clotting time, hemostatic.

### 1. INTRODUCTION

The surgical procedures come with a risk of complications which may include pain, nerve injury, swelling, excessive bleeding (hemorrhage), and infections. Severe intraoperative or postoperative hemorrhage is one of the few life-threatening complications for which a dentist may have to initiate management.<sup>1,2</sup> The bleeding disorder, it doesn't clot fast enough. This results in too much bleeding or long-lasting bleeding, and become port de entry of infection.<sup>3,4</sup>

Hemostasis is a complex process that leads to the formation of a blood clot at the site of vessel injury and three phases can be distinguished by vascular spasm,

formation of a platelet plug and coagulation. Primary hemostasis starts immediately after damage of the vessel wall with vasoconstriction as a result of local contraction of vascular smooth muscle cells.<sup>5</sup> The von Willebrand factor (VWF) binds to the exposed subendothelial collagens. Platelets are tethered to the site of endothelial cell injury through the binding of VWF to the glycoprotein Ib receptor of the platelet.<sup>6</sup>

In major oral and maxillofacial surgical procedures, electrocautery and suture ligatures are most commonly used to control bleeding from small and major vessels. However, when the use of pressure is not effective, the use of electrosurgical instruments could endanger teeth or nerves, systemic and, or topical hemostatic agents may be needed.<sup>7,8</sup> The study shown the effectiveness of Plasma Rich in Growth Factors (PRGF) in controlling bleeding after dental extractions in patients with haemophilia. In this fascinating study there were no risks related to the use

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of blood derivatives, yet good haemostasis and healing of soft tissues were achieved. In other words, the post-extraction socket healed better and faster which can prevent further bleeding.<sup>9</sup>

Indonesia is a rich-tropical country in natural resources with the second highest biodiversity in the world after Brazil. Natural resources, such as plants and animals in Indonesia, are very varied, which can be used for improving natural products. The importance of biodiversity, e.g., in traditional medicine and agriculture, is deep-rooted in Indonesian society.<sup>10</sup> A plant that can be efficacious as a drug and has long been used to accelerate wound healing and tooth pain relief by the people of the village of Trunyan, Bali is Ambonese banana stem extract.<sup>11</sup> In banana plants, there are significant compounds, such as saponins, flavonoids, anthraquinones, and tannins contained in both its fruit and sap, as well as lectin compounds found in banana stems. Lectin plays a role in stimulating mitotic cells which has an effect on accelerating wound healing and bleeding cessation.<sup>12,13</sup> Meanwhile, tannins are astringent, stopping bleeding in the wound.<sup>14</sup> This study aimed to prove Ambonese banana stem extract (*Musa paradisiaca* var. *Sapientum*) administration can accelerate wound healing through hemostatic response.

## Materials and Methods

### Plant material and methods

Collected from the Plant Conservation Center Botanical Gardens Purwodadi, Pasuruan, Indonesia, Ambonese banana tree will be able to be harvested at age 12-13 months, with the height is about 2.5-3 m, and the stem diameter is about 17.3-18.9 cm wide. Determination was carried out by the Indonesian Institute of Sciences (LIPI) Purwodadi, Pasuruan, Indonesia number 1036/IPH.UPT.03.4/HM/IX to prove that the type of banana tree used is a type of Ambonese banana.

### Preparation of the sample

To get Ambonese banana stem's sap in large quantities, the stem need to be cut at the lower end part, then clean it to remove the impurities. After that, made it into small

pieces weighing 200 grams, add 200 ml of sterile distilled water and blend until smooth. The sap and water that have been mixed together are then filtered using a Buchner funnel connected with a vacuum pump (Gast, USA brand) with whatman filter paper number 1. The filtration results are dried using a freeze dryer so that it can be stored for a long time. The making of Ambonese banana stem extracts with concentration of 25%, 50% and 100% was carried out by dissolving dried preparations in sterile aquadest.

### Experimental design

The ethical approval to conduct the study was taken from the Research Ethics Committee, Faculty of Dentistry, Universitas Airlangga. This research used a randomized post-test only control group design. We used 24 male mice divided into 4 groups. The first group was the control group with the administration of vehicle. The second group was given Ambonese banana stem extract at a concentration of 25%. The third group was given Ambonese banana stem extract at a concentration of 50%. The fourth group was given Ambonese banana stem extract at a concentration of 100%. Those extracts were given for 3 consecutive days. Bleeding and clotting time examinations then were conducted in each group.

### Bleeding time experimental

The mice (*Mus musculus*), male, 20-30 gram of body weight were anesthetized with diethyl ether using inhalation method. Mice are put in a transparent box for easy viewing, which contains cotton with ether, and within a few seconds the activity of the mice begins to decline and then falls asleep. The mice's tail was cut 3 cm long from the tip of the tail, blood that came out after cutting was dropped on absorbed paper. The stopwatch was started to run along with the visible blood coming out of the mice that had been cut. Blood dripped on absorbent papers that should not touch the wound. The stopwatch was then stopped when blood did not drip anymore on the absorbent papers.<sup>15</sup> Mice are generally feed containing low fiber (5%), protein (20%) and fat (5-10%) in pellet.

### Clotting time experiment

Blood was taken from the heart of anesthetized mice by surgery using a scalpel, and then 1cc of blood was taken using a tuberculin syringe directly from the heart. The stopwatch was started to run along with the entry of blood on the syringe. The blood that had been taken was then inserted into capillary tube to measure the clotting time. Afterwards, the tubes were tilted slowly and re-stood up at 10-second intervals until the blood in the tubes did not flow again when they were tilted to a tilt angle of 90°. The stopwatch was stopped when the blood had not flowed even though the tubes were tilted at an angle of 90°. Surgical wounds then were stitched and oral antibiotics and analgesics were given.<sup>16</sup>

### Statistical analysis

All the data were tabulated and assessed using the

Statistical Package for Social Sciences (SPSS) version 20.0. Comparison of various time among four study groups were done using ANOVA test and post hoc Tukey test. A p-value  $\leq 0.05$  was considered to be statistically significant.

## Results

### Phytochemical components

The Saponin, flavonoid, tannin, anthraquinone and lectin levels were analyzed using UV-Visible Spectrophotometer by comparing the absorbance values of standard compounds with the extract of ambon banana stem sap. Detection of saponin compounds with a wavelength of 215 nm, flavonoids with wavelengths of 226 nm, tannins with wavelengths of 275 nm, anthraquinones with wavelengths of 285 nm and lectins with wavelengths of 228 nm (see table 1).

**Table 1. The concentration of each component in Ambonese banana stem extract**

<i>Components</i>	<i>Number of replicant</i>	<i>Concentration (mg/100mL)</i>
<i>Saponin</i>	3	1.30
<i>Flavonoid</i>	3	0.28
<i>Tannin</i>	3	1.50
<i>Antraquinone</i>	3	0.30
<i>Lectin</i>	3	0.11

### Hemostatic properties of plant extract

Bleeding time is a clinical laboratory test performed to evaluate platelet function. It involves the creation of a standardized incision and timing the cessation of bleeding. At sites of vascular injury, platelets are recruited to exposed subendothelial extracellular matrix components via specific platelet receptors involved in adhesion and aggregation. The herbal medicines contain a lot of different compounds which some of them have great complexities. Plants substances such as polysaccharides, mucilages and tannins may modulate and modify the effects of “active components”.

Results of the One-way ANOVA test showed that the administration of Ambonese banana stem extract at the concentrations of 25%, 50 %, and 100% significantly shortened both bleeding time and clotting time compared to control group ( $p = 0.00$ ). Ambonese banana stem extract at the concentrations of 100% even had the shortest bleeding time compared to the other groups. In other words, the higher the concentration is, the shorter the bleeding time is. Similarly, in the clotting time examination, the group given Ambonese banana stem extract at the concentration of 100% had the shortest clotting time compared to the other groups (see table 2).

Table 2. Bleeding and clotting time test

Groups	$\bar{X} \pm SD$	
	Bleeding Time (second)	Clotting Time (second)
1	149.5 <sup>a</sup> ± 33.2	183.3 <sup>a</sup> ± 19.5
2	75.2 <sup>b</sup> ± 23.2	128 <sup>b</sup> ± 25.8
3	53.2 <sup>c</sup> ± 7.9	94.7 <sup>c</sup> ± 17.1
4	35 <sup>d</sup> ± 8.53	86.33 <sup>c</sup> ± 13.2

The values with different superscript letters in a column are significantly different (p<0.05).

**Discussion**

The results of this research showed that bleeding time and clotting time in Group 1 (control) had a significant difference when compared with Group 2 (given Ambonese banana stem extract at the concentration of 25%), Group 3 (given Ambonese banana stem extract at the concentration of 50%) and Group 4 (given Ambonese banana stem extract at the concentration of 100%). In other words, the

administration of Ambonese banana stem extract at the concentrations of 25%, 50%, and 100% can accelerate both bleeding time and clotting time in mice. Moreover, it is also known that Group 1 needed the longest bleeding time compared to the other groups (see figure1). Ambonese banana stem extract actually contain various active compounds, namely lectins and tannins.<sup>17,18</sup>

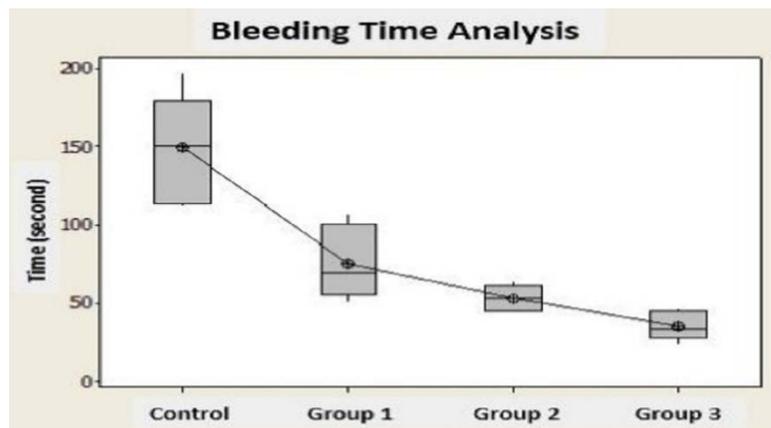


Figure 1. Examination of bleeding time by cutting the mice's tail.

C-type lectins are a subgroup of lectins, carbohydrate-recognizing proteins, which recognize and bind carbohydrates in a Ca<sup>2+</sup> ion-dependent manner. They play a crucial role in hemostasis, a complex system to stop bleeding, which encompasses coagulation, platelet activation, and thrombus formation.<sup>19</sup> The mechanism of lectin in accelerating the process of hemostasis is through some stages.<sup>20</sup> After an injury to a blood vessel, platelets

normally in a state of silence will turn into active and move towards an injured blood vessel to form a platelet plug at an initial phase. This phase is called platelet adhesion, which is the attachment of platelets to injured tissue.<sup>21-23</sup>

Tannic acid is a commercial compound which is similar to the plant polyphenol tannin, that stops bleeding from mucous membrane via vasoconstriction.<sup>2</sup> Tanin is another active compound contained in Ambonese banana

stems playing a role in increasing ADP secretion in blood. ADP compound is a vasoactive chemical substance released by the body during vascular injury. ADPs and other chemicals, including serotonin, then trigger platelets to deform irregularly and become sticky with each other. Thus, one platelet and another will be easier to bind. An increase ADP levels in the blood then will result in high platelet aggregation so that platelet plugs as a mechanism for body hemostasis will soon be formed and will accelerate the bleeding time indicated in this research by the rapid cessation of blood coming out of the mice that were cut off.<sup>24</sup>

Ambonese banana stem extract at the concentration of 100% is thought to have higher active compounds of tannin and lectin than concentrations of 25% and 50%. This high level of the active compounds, tannins and lectins, can increase platelet activity to adhesion and aggregate platelets when a vascular injury occurs. This is in accordance with the results of this research that showed

that the group of mice with Ambonese banana stem extract at the concentration of 100% had the shorten bleeding compared to the other groups.

In addition to accelerating the bleeding time, the administration of Ambonese banana stem extract at the concentrations of 25%, 50% and 100% can also accelerate the process of clotting time in mice (see figure 2). According to Ogle (2008), clotting time is an ability of blood to change from liquid to semi-solid. After the platelet plug is formed, the blood clotting cascade begins.<sup>7</sup> This process runs through extrinsic and intrinsic blood clotting pathways involving 13 blood clotting factors. After the process, a prothrombin activator is formed which has a task of converting prothrombin to thrombin. Next, thrombin converts soluble fibrinogen into solid fibrin polymer threads. These fibrin threads will later become nets that will capture plasma, blood cells, and platelets to become a clot.<sup>9,25</sup>

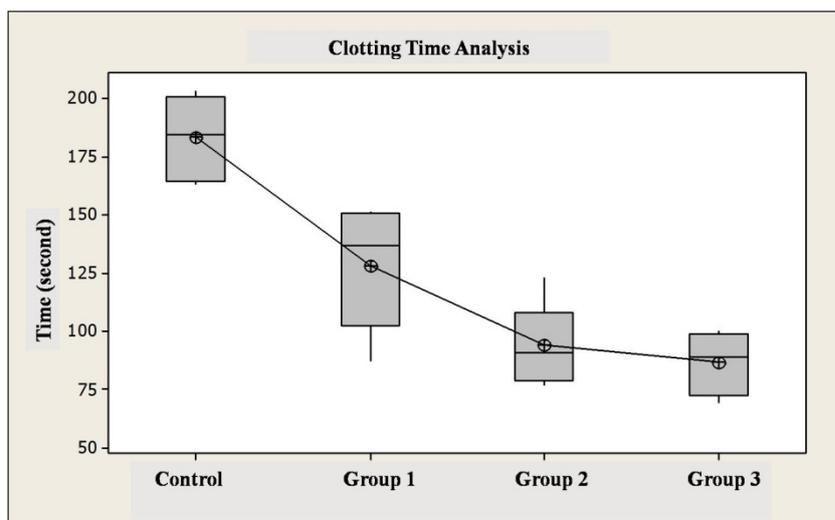


Figure 2. Examination of clotting time with modified Lee and White tube methods.

High platelet aggregation activities, furthermore, were found in the group with the administration of Ambonese banana stem extract since the effects of the active compounds, tannins and lectins, will be directly

proportional to the increased clotting time in mice. The solid fibrin threads formed then will attract more platelets carrying out more aggregation. As a result, the blood clotting process will occur faster. The results of this

research are the same as those reported by Bamidele, et al. (2010), examining the effects of methanol *ageratum conyzoides* leaf extract on hemostatic responses in mice. The research conducted by Bamidele, et al. (2010), finds that a decrease in bleeding time will compensate for a decrease in clotting time in mice.<sup>26</sup>

Based on the previous hypothesis, the higher the concentration of Ambonese banana stem extract is, the higher the platelet aggregation activity occurs. If more platelets are attached to one another, then the fibrin threads that have formed in the initial phase of blood clotting will attract more thrombocytes, and blood clots will also occur faster. But, the decrease in clotting time in the group with Ambonese banana extract at the concentration of 100% was not significantly different from the group with Ambonese banana extract at the concentration of 50%. Even though at the concentration of 100% had the fastest clotting time compared to the other groups. However, based on the results of Duncan's distance test, Ambonese banana extract at the concentration of 50% did not differ significantly compared to that at the concentration of 100%.

This may occur due to the influence of blood clotting factors working on blood clotting cascade. Before the formation of fibrin threads which will later capture blood cells and plasma, blood clotting cascade occurs first, affected by various factors including 13 intrinsic and extrinsic blood clotting factors, such as fibrinogen, prothrombin, thromboplastin tissue, calcium, and vitamins K. In this study, unfortunately, there was no observation on the blood clotting factors. Hence, it is assumed that there was less significant difference between the group with the administration of Ambonese banana stem extract at the 100% and the group with that at the concentration of 50%. This finding may be influenced by the blood clotting factors.

Finally, based on the results, it can be said that the study hypothesis has been proven. The administration of Ambonese banana stem extract has an effect on accelerating the hemostasis response in mice characterized by an increase in both bleeding time and clotting time. The active compounds of tannins and lectins working synergistically in mice can lead to an effect on accelerating both bleeding time and clotting time.

Indonesia is a rich-tropical country which plants are used as an alternative medicines. However, deeper research needs to be done to prove the efficacy of drugs from plants in order to be used by the community. Although this research has proven the benefits of Ambonese banana stem sap as hemostatic, the platelet activity on the wound needs to be known to support this study.

The use of plant-derived medicines continues to be developed through biomolecular research so that they can be used as a standardized medicine. Ambonese banana stem has the potential to be developed into hemostatics through several studies that have been done before, and even has been patented for cancer therapy. Topical and hemostatic drugs are also capable for tooth extraction or scaling and root planning in gum disease that are needed for patients with blood disorders, such as hemophilia, which are still common in Indonesia, even in the world.

### **Conclusions**

Ambonese banana stem extract (*Musa paradisiaca* var. *Sapientum*) have a potential as hemostatic agent by shortening bleeding and clotting time on mice.

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## تحليل زمن النزف والتخثر لمستخلص ساق *Musa paradisiaca var. sapientum* (L.) Kunze على الاستجابة المرقنة

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### ملخص

أحد الأدوية العشبية التي يمكن استخدامها لتسريع النزيف هو عصارة جذع الموز الأمبونية (*Musa paradisiaca var. sapientum*) من خلال إفراز Adenosin Diphosphate (ADP) وعامل von Willebrand (vWF) في عملية التصاق الصفائح الدموية على الأوعية المصابة. تهدف هذه الدراسة إلى اختبار تأثير مستخلص جذع الموز الأمبوني بتركيز 25% و 50% و 100% استجابة مرقى على الفئران. تم تقسيم 24 دراسة حيوانية بشكل عشوائي إلى أربع مجموعات. المجموعة الأولى كانت المجموعة الضابطة، المجموعة الثانية أعطيت 25% تركيز المستخلص، المجموعة الثالثة أعطيت تركيز المستخلص 50% والمجموعة الرابعة أعطيت تركيز 100% من مستخلص جذع الموز الأمبوني عن طريق الفم. تم فحص جميع المجموعات وقت النزيف والتجلط. أدى إعطاء مستخلص جذع الموز الأمبوني بتركيزات 25% و 50% و 100% إلى تقصير وقت النزف ووقت التخثر بشكل كبير مقارنة بمجموعة التحكم ( $p = 0.00$ ). أظهرت استنتاجات هذه الدراسة أن مستخلص جذع الموز الأمبوني لديه القدرة على أن يكون عامل مرقى عن طريق تقصير وقت النزف والتخثر على الفئران.

الكلمات الدالة: مستخلص جذع الموز الأمبوني، زمن النزف، زمن التخثر، مرقى.

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