Antioxidant activity test on ambonese banana stem sap (Musa parasidiaca var. sapientum)

by Hendrik Setiabudi

Research Report

Antioxidant activity test on ambonese banana stem sap (*Musa parasidiaca var. sapientum)*

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ABSTRACT

***Background**: Polymorphonuclear cells (PMN) release oxygen free radicals or reactive oxygen species (ROS) during inflammation. As a result, ROS level is higher than antioxidant level in our body during oxidative stress leading to prolong inflammation or continuous tissue damage. Indonesia, on the other hand, is a country with various herbal medicines. For instance, ambonese banana (Musa parasidiaca var. sapientum) is often used as herbal medicine. Ambonese banana, moreover, has flavonoid, polyphenol, tannin, and saponin as antioxidants to reduce free radicals by transferring their hydrogen atom. Medicine used to reduce the impact of free radicals is known as antioxidant. Antioxidant is proved to accelerate wound healing. **Purpose:** This research aims to analyze the effects of the antioxidant activity of Ambonese banana stem sap extract. **Method**: Antioxidant activities in this research were examined with 1,1-Diphenyl-2-picryl-hidrazyl (DPPH) method by reacting with stable radical compounds. Spectrophotometry with a wavelength of 517 nm was used to measure absorption results shown in purple. The absorption results then were calculated by IC_{50} reduction activity. **Result**: There were significant differences of Ambonese banana stem sap antioxidant activity ($p<0.05$) at the concentrations of 15%, 30%, and 60 %. All concentrations have greater absorbance scores than IC_{50} (>50%). **Conclusion:** Ambonese banana stem sap extract has antioxidant activities.*

***Keywords**: herbal medicine, antioxidant, wound healing, inflammation*

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INTRODUCTION

Wound is tissue damage, caused by physical factors and accompanied by a disturbance of normal tissue continuity structure. Based on the damage of the tissue, wound can be divided into two, namely open wound and closed wound. However, healing process of those wounds is basically similar, but the speed of the process depend on infection suffered, surgical intervention obtained, and medicines used. In tooth extraction process, the healing process involves several stages, namely

hemostasis stage (the formation of blood clots), inflammation process (leukocyte infiltration), proliferation stage (the formation of connective tissue), granulation and epithelialization stages, and remodeling stage. Thus, local therapy can be conducted to reduce the systemic effects and stop excessive bleeding, so the healing process will not be disturbed.[1,2]

Inflammation, moreover, is a complex reaction to the causative agents of injury, such as microbial and cell damage. The inflammatory response is closely related to the healing process since it can destroy the causative agents of inflammatory lesion and cause a chain of events aimed to heal or repair damaged tissue.[3] In tooth extraction, reactive oxygen species (ROS) is increased by phagocytic cells, namely monocytes, macrophages, and neutrophils (PMN). Reactive oxygen compounds will involve oxidants in various pathological processes in the body.[4,5]

In the medical field, oxidants and free radicals are often confounded since both have similar properties. Activities of both compounds often cause the same effects. Medicines that can reduce the activity of free radicals are known as antioxidants. Free radicals or ROS at their "physiological concentrations" can serve as a regulator of cell growth, differentiation, adhesion between cells, cell senescence, and apoptosis. Nevertheless, if ROS with a high concentration or more than antioxidants in the body is obtained, ROS will be destructive. Consequently, ROS can oxidize fat and protein, as well as damage DNA by mediating DNA fragmentation.

Prolonged exposure of ROS is suspected as the cause of chronic inflammation and tissue damage. Increased ROS on inflamed tissue even can cause endothelial dysfunction and tissue damage. Increasingly with age, the levels of antiox idants in the body will be reduced, so the healing response becomes slower. ROS can be neutralized by antioxidants, such as catalase, superoxide dismutase, glutathione and non-enzymes (Vitamins C, E, A, and pyruvate).[6] Antioxidant activity, furthermore, can be examined with 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method using a spectrophotometer with a wavelength λ = 517 nm. A material is considered to have antioxidant activity when the percentage of the antioxidant activity was greater than or equal to 50%[7,8]

Fresh banana stem extract, moreover, can shorten bleeding and clotting time due to the activation of clotting factors and response of endothelial glycoprotein-Ib (GPIB). Glycoprotein plays a role in platelet adhesion to the endothelium so that activated platelets will release the contents of granules during healing process.[9,10] Banana stem sap contains polyphenols, flavonoids, saponins, anthraquinone, and tannin, which can capture free radicals to inhibit cell damage. Previous researches showed that the effective concentration of banana stem sap in healing wounds is 15%, 30%, and 60%. Moreover, the results of biocompatibility, anti-inflammatory, and analgesic tests showed that banana stem sap at concentration up to 100% is relatively not toxic to fibroblasts, and has properties as anti-inflammatory and analgesics. [11,12] This research aims to analyze the

effects of the antioxidant activity of Ambonese banana stem sap extract, so it can be developed into medical herbs that have medicinal properties.

MATERIALS AND METHOD

This research is experimental laboratory research with post-test only control group design. The treatment group was banana stem sap extract with the concentrations of 15%, 30%, and 60%. Making banana stem sap extract was conducted through several stages. The central part of banana stems was cut into small pieces, weighing 200 grams. Those pieces were put into a blender and added with 200 cc of sterile distilled water. Those pieces were blended for five minutes until smooth. It was filtrated using Whatman filter paper no. 1. Since the result obtained was at a concentration of 100%, it then was diluted to obtain concentrations of 15%, 30%, and 60%. To make it at the concentration of 15%, 15 ml of 100% banana stem extract was dissolved in 100 ml of water. Meanwhile, to make it at the concentration of 30%, 30 ml of 100% banana stem extract was dissolved in 100 ml of water. And, for the concentration of 60%, 60 ml of 100% banana stem extract was dissolved in 100 ml of water.

Preparations for positive control group were prepared using 200 mg of powdered vitamin C (L-ascorbic acid) 200 mg dissolved in 200 ml of distilled water. DPPH reagent then was prepared by mixing 4 mg of powdered DPPH with ethanol as much as 100 ml. For the blank solution, DPPH reagent was mixed with distilled water at a ratio of 2: 1. Finally, each group (concentrations of 15%, 30%, and 60%, positive control, and blank) was replicated seven times. Thus, the total of samples was 35 samples.

To examine antioxidant activity, DPPH method was used. For the treatment groups (concentrations of 15%, 30%, and 60%), 2 ml of each group was taken and mixed with 1 ml of DPPH reagent in each test tube. Meanwhile, for the positive control group, 2 ml of vitamin C solution was mixed with 1 mL of DPPH reagent in each test tube. And, for a blank solution, 2 ml of distilled water was mixed with 1 mL of DPPH reagent.

After all samples were homogeneous, those samples were put into cuvette, and then measured, using a spectrophotometer with a wavelength λ = 517 nm. Absorbance score derived from the results of the spectrophotometer measurement was calculated, using the following antioxidant activity formula: [7]

$$\frac{Abs\ Blanko - Abs\ Sampel}{Abs\ Blangko} \times 100\%$$

The absorbance score of each sample was measured using a UV-VIS spectrophotometry. Absorbance scores obtained were then calculated using the percentage formula for antioxidant

activity. A material can be indicated to be active as an antioxidants if the percentage of its antioxidant activity is more or equal to 50% or so-called inhibitor concentration 50 (IC50). IC50 is used to determine which concentration can reduce 50% of free radicals. IC50 is the standard for determining antioxidant activity.[8] One way Anova was was conducted, followed by Tukey's Post Hoc test to determine the difference of antioxidant activity in those groups.

RESULTS

Examination of banana stem sap extract was conducted using spectrophotometry. The results showed chemical compounds contained in the banana stem sap have antioxidant activities and play a role in the healing process (Table 1). Based on the observation and calculation results of the reduction activities conducted on 35 samples, the absorbance scores obtained in all research groups are as follow (Table 2). The calculation of the absorbance scores in each study group was conducted. Next, the percentage of the reduction activity was measured to analyze the ability of antioxidant activity (Table 3).

The highest percentage of the reduction activity was obtained at the concentration of 60% for 73.17%, while the percentage of the reduction activity was found at the concentration of 15% for 64.04%. The results of one way anova test showed $p<0.05$, which means that Ho was accepted. Thus, it indicates that there were no statistically significant differences among the research groups. Therefore, Post-Hoc Tukey test was performed to determine significant difference in each group (Table 4). The results of Post Hoc Tukey test were used to determine which group pair has significant difference with a significance of $p<0.05$. In Table 4 shows that among all groups, a significant difference was marked with an asterisk.

DISCUSSION

Wound caused by medical intervention will lead to an inflammatory response as wound healing process. The release of inflammatory mediators, such as bradykinin, histamine, and free radicals from leukocytes, can increase vascular permeability. A high number of free radicals then can cause damage to cell damage, reduce the cell's ability to adapt to the environment that would cause cell death, and inhibit the wound healing process.

Ascorbic acid or vitamin C is a six- lactone carbon atom synthesized from glucose contained in the liver. The chemical name of ascorbic acid is 2-oxo-L-threo-hexono-1.4-lactone-2.3-enediol. The main form of ascorbic acid is called L-ascorbic acid and dehydroascorbic. Vitamin C atom donates H + or H oxidized by ROS that produces neutral tricarbonyl ascorbate free radicals. The hydrogen atom donor then can reduce free radicals • OH and ROO •. In addition, Vitamin C is a compound that has a very active antioxidant activity compared with other oligoresveratrol

compounds. Oligoresveratrol compound is derived polyphenolic compounds that have antioxidant activity.[12] In this research, vitamin C has antioxidant activity amounted to 55.20%, so according to the standard IC50, vitamin C has an ability as an antioxidant.

Based on the results, the average of antioxidant activity in banana stem sap extract at the concentration of 15% was 64.04%, 69.63% at the concentration of 30%, and 73.17% at the concentration of 60%. It means that those scores are in accordance with the appropriate standard of IC50. Therefore, it can be concluded that the concentrations of the banana stem sap extract have antioxidant activity. Antioxidant activity can be caused by flavonoids contained in green banana stem sap. These compounds act as free radical catchers because of containing hydroxyl group. Thus, as reducing agents, flavonoids can act as hydrogen donors against free radicals. Donor hydrogen released by flavonoids then will bind with • OH to produce neutral H2O.

Atomic hydrogen can also neutralize ROO• which is the result of the reaction R• with O2.[13] This group will neutralize free radical characters from DPPH. If all the electrons in the free radical of DPPH become in pairs, then the color of the solution will change from dark purple to yellow light, and the absorbance at 517nm wavelength will be lost. Flavonoids can actually increase the production of SOD, GPX, and CAT. These enzymes play a role in reducing free radicals in the body. Flavonoids can also bind Cu 2+, which also plays a role in the formation of free radicals, • OH.[14]

Phenol compound can chemically be defined as the presence of at least one aromatic ring carrying one (phenol) or more (polyphenols) hydroxyl groups. Polyphenols are a group of chemical substances found in plants. This substance has a distinctive sign that has a lot of phenol group in the molecule. Derivative polyphenols as antioxidants can stabilize free radicals to complete the lack of electron free radicals and inhibit the chain reaction of free radical formation. The mechanism of polyphenol compounds as antioxidants is to donate the hydrogen of the hydroxyl groups. Polyphenols are components that contribute to the activity of antioxidants in fruits and sayuran.[15] Fenton reaction occurs when H2O2 binds to Fe^{2+}, which will generate • OH. Polyphenols have the ability to bind Fe^{2+} so that free radicals • OH will be reduced. Donor hydrogen atoms of polyphenols can also neutralize • OH into H_2O, and can neutralize ROO • which is the result of the reaction R • with O_2.[16]

Tannins are secondary metabolites of active compounds, which are known to have some of the properties, such as astringent, anti-diarrhea, anti-bacterial and antioxidant. Tannins are components of organic substances that are very complex, consisting of phenolic compounds which are difficult to separate and crystallize, but precipitate proteins out of the solution and fuse with the proteins.[17] Tannins are divided into two groups: the hydrolyzed tannins and condensed tannins. Tannins have complex biological roles ranging from precipitating proteins to chelating metals.

Tannins can also serve as a biological antioxidant with the ability to bind metal. Metal compounds, such as Fe^{2+} and Cu^{2+} can react with H_2O_2 through Fenton reaction that produces reactive • OH, as a result, by binding with the metals, • OH levels in the body can be in reduced.[18,19]

In general, compounds classified as a class of polyphenols and carotenoids have antioxidant properties. Some researches show that saponin also acts as an antioxidant. Saponins have antioxidant properties that reduce superoxide to form intermediate hydroperoxide cells and prevent biomolecular damage from free radicals. [20] Saponins increase the production of SOD that plays a role in reducing ROS and H_2O_2, and has an ability to bind so that inhibits the Fenton reaction from generating • OH. Saponins also bind $O2^{-\bullet}$. As a result, ROS binding with nitric oxide (NO) can generate more reactive $ONOO^-$.[21, 22]

In this study a solution of Vitamin C as the positive control (+) has antioxidant activity smaller than the extract of banana stem sap used due to the crude extract. The crude extract actually contains some compounds that can be collected, such as flavonoids, saponins and polyphenols, and tannins that have antioxidant properties. Meanwhile, vitamin C is a single compound. Some of the compounds contained in the crude extract of banana stem sap even have a different damping mechanism of ROS, thereby reducing a wide range of ROS, such as ·OH, ROO·, $O_2^{-\bullet}$, and $ONOO^-$. On the other hand, vitamin C just donates a hydrogen atom, and can only reduce some kinds of ROS, such as ·OH, ROO·.[23,24]

In conclusion, antioxidant activities found in all of the banana stem sap concentrations indicate that the banana stem sap has the potential to be developed as a biomaterial medicine for wound healing. The optimum result obtained in this research was at the concentration of 60% with the antioxidant activity of 73.17%.

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Table 1. The test results of chemical compound

Compounds	Results
Polyphenols	+
Tanin	+
Saponins	+
Flavonoids	+

Table 2. The average of absorbance score with UV-VIS spectrophotometry

Treatment	$\sum \pm$ SD
Control +	0.441 ± 0.03
Concentration of 15 %	0.355 ± 0.35

Concentration of 30%	0.299 ± 0.01
Concentration of 60 %	0.265 ± 0.01
Blank	0.987 ± 0

Table 3. The average of the percentage of reduction activity of IC50 as antioxidant

Treatment	∑ ± SD
Control +	55.20 % ± 3.06
Concentration of 15 %	64.04 % ± 4.16
Concentration of 30%	69.63 % ± 0.58
Concentration of 60 %	73.17% ± 0.43

Table 4. The results of Post Hoc Tukey test

	C(+)	K15%	K30%	K60%
C(+)		0.000*	0.000*	0.000*
K15%			0.000*	0.000*
K30%				0.000*
K60%				

Note: (C (+): positive control; K15%: at the concentration of 15%; K30%: at the concentration of 30%; and K60%: at the concentration of 60%).

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