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COMPARISON OF ANTIOXIDANT ACTIVITY BETWEEN SOCLETATION AND MACERATION EXTRACTION METHOD ON BINJAI LEAF EXTRACT (*Mangiferacaesia*)

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ABSTRACT

Background ***:****Wound is known to generate free radicals in inflammatory phase by the initiation of inflammatory cells such as polymorphonuclear. This cell is in function to destroy bacteria and viruses that are present in the wound. However, free radicals can also damage normal tissues when the amount of the accumulation is too much. Antioxidants are the ingredients that can eliminate free radicals throughchemical reaction so the formation of free radicals can be reduced. Binjai (Mangiferacaesia) is a plant that can produce natural antioxidants that are able to protect the body against the damage which caused by free radicals. The antioxidants in the leaves can be obtained by maceration and socletation extraction techniques.* **Purpose*:*** *To determine the difference between socletation and maceration extraction method towards antioxidant activity of binjai leaf ethanol extract as a preliminary study of binjai leaf antioxidant effect on wound healing.* **Methods*:*** *This research was pure experimental study(true experimental) with post-test only control group design, consisting of two treatment groups: maceration extraction methods group, socletation extraction methods group, and one control of ascorbic acidgroup. Measurement of antioxidant activity using DPPH method were measured by using a UV-Visspectrophotometry then IC50 values were calculated to determine the antioxidant activity.* **Results*:*** *Average IC50 values obtained fromthe socletation extract group was 50.791 ppm, while the maceration group was 60.135 ppm, and the control group was 13.825 ppm. Post Hoc LSD test result showed that the three groups had significant difference in each treatment.****Conclusion:****Based on this research, it can be concluded that binjai leaf extraction using socletation methods produce more powerful antioxidant activity compared to maceration method.*

Keywords: *antioxidants,binjai leaf (Mangifera caesia), IC50, maceration, socletation*

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INTRODUCTION

A total of 31,1% treatment actions performed on the field of dentistry is dental extraction.[1] Tooth extraction is the process of extracting the tooth against the socket.[2] The process of extracting the tooth from its socket will cause mild trauma to soft tissue in the location around tooth extraction and frequently can

cause complications after the extraction.[3] The wound has several phases: the hemostasis phase, the inflammatory phase, the proliferation phase, and the maturation phase.[4] Polymorphonuclear cells in the inflammatory phase will be stimulated to release macrophage cells as well as free radicals that act as bacteria and viruses destroyers that can invade the wound.[5] Free radicals are unstable compounds which have high energy. When the energy possessed by free radicals is too large, it will cause disruption in the wound healing phase including hemostasis disorders. This condition will lead to the necrosis and apoptosis of tissuecell.[6]

Compounds that can inhibit the oxidation process generated by free radicals are antioxidants.[7] Antioxidant compounds comprise of endogenous and exogenous antioxidants.[8] Antioxidant products are very easy to find, but the use of synthetic antioxidant in long term can rise the concerns of the side effects. Therefore, natural antioxidants can be used as an alternative.[9]

A typical Kalimantan plant that has the potential as an alternative antioxidant is Binjai (*Mangifera caesia).* The content of phenol contained in binjai leaves is identified to have antioxidant activity.[10] Testing of antioxidant activity using DPPH *(2,2-diphenyl-1-picrylhidrazyl)* method can be used to know the concentration of antioxidant activity. DPPH method is used because the solvent used is a polar solvent (96% ethanol concentration) which can dissolve synthetic radicals such as DPPH. The concentration is also the best concentration for obtaining antioxidant activity on Binjai leaf extract.[11] At the concentration of 96%, more antioxidants are produced compared to other solvent concentrations. The amount of compounds extracted in this extraction process can be affected by several things: the extraction method, the solvent, and the length of time of extraction.[12] Of the various types of extraction methods, maceration and socletation method are the commonly used types of conventional extraction method.[13]

Socletation method is an extraction method using a new solvent which is generally done with a special tool resulting in continuous extraction with a relatively constant amount of solvent in the presence of refrigerant.[14] Maceration is the process of extracting simplicia using the solvent by shaking or stirring it for several times at room temperature.[15]

Based on the background above, a research needs to be conducted to determine the effect of socletation and maceration extraction methods on antioxidant activity of binjai leaf ethanol extract as a preliminary study of antioxidant effect of binjai leaf to wound healing. The purpose of this study is to determine the value of antioxidant activity of binjai leaf extracted using socletation and maceration method. In this study, the preferred solvent was ethanol solvent with 96% concentration which has shown the most optimum value of antioxidant activity on binjai leaf.

MATERIALS AND METHOD

The implementation of the research began with asking the research permit and ethical clearance issued by the Ethics Committee of Faculty of Dentistry, Lambung Mangkurat UniversityNO.060/KEPKGFKGULM/EC/IX/2017. This research was a pure experimental study with post-test only control group design, divided into3 treatment groups consisted of socletation method group, maceration methods group and ascorbic acid as control group. Measurement of antioxidant activity was done by DPPH method using UV-Vis spectrophotometry then calculating the IC50 value to know the amount of antioxidant activity**.** This test took three repetitions (triplo) with 36n total sample used.

Sample Preparation and Extraction

The procedure of this study was started by washing the sample with flowing water, then sample was chopped and dried in the oven with temperature 50ºC for 4 hours. Leaves that had been dried were blended and filtered using mesh number 40. The

powder was obtained and then stored in a dry place and closed for the extraction process.

Preparation of Maceration Extract

100 grams of binjai leaf simplicia was extracted by macerating it in 96% ethanol solvent. The powder of simplicia was inserted into the maseration vessel. Then it was added with ethanol solvent until it swamped 1 cm higher than the simplicia. This mixture was stirred until blended and the maseration vessels were tightly closed and kept for 3x24 hours. The stirring was done every 24 hours using a magnetic stirrer with a speed of 50 rpm for 15 minutes. After 72 hours, the mixture was filtered and then concentrated with a rotary evaporator at a temperature of 40 ° C to obtain a viscous extract.

Preparation of Socletation Extract

Socket equipment was installed, then 100 grams sample were wrapped in filter paper, fastened with yarn, inserted into the socket tool and 96% ethanol solvent was put into the pumpkin socket. The extract socletated with a temperature of 60-80˚C until the dye cycle was colorless or less for 5 hours. The result of liquid extract from each test group was then concentrated with rotary evavorator at 40 ° C and followed by a waterbath.

Preparation of DPPH Solution 0,4mM (1,1-difenil-2-pikrilhidrazil)

3.94 mg DPPH (BM 394.32) was dissolved with ethanol p.a and put into a 25 ml measuring flask. The volume of ethanol p.a was sufficient to the boundary mark and then placed in a dark place

Preparation ofblanko Solution

0.5 ml of 0.4 mM DPPH solution was taken into the test tube and added with1.5 ml ethanol p.a. The extract was then covered with aluminum foil, homogenized with vortex and incubated in dark space. The absorbance of the solution was then measured at 516 nm wavelength

Preparation of control solution

As much as 10 mg ascorbic acid was dissolved in 10 ml ethanol to obtain 1000 ppm concentration. The solution was diluted in a 10 ml measuring flask by adding ethanol p.a and made in to various concentrations of 2 ppm, 4 ppm, 8 ppm, and 10 ppm. Each concentration was then inserted into the vial

Preparation of ethanol extract test solution 96%

10 mg extract of binjai leaves was dissolved in 10 ml ethanol to obtain the concentration of 1000 ppm. Then,it is diluted in 10 ml measuring flask with the addition of ethanol p.a. After that, it was made into various concentrations of 20 ppm, 40 ppm, 60 ppm, and 80 ppm. Each was inserted into the vial.

Determination of antioxidant activity

As much as 1.5 ml from each concentration of test solutions was inserted into the test tube. 0.5 ml of 0.4 mM DPPH solution was added and homogenized with vortex. Then, they were incubated in a dark room and kept for 20 minutes. This solution was then measured for its absorbance at 516nm wavelength.

Quantitative Analysis of Antioxidant Activity Using DPPH Method Determination of IC50 (Inhibitory Concentration)

Determination of IC50 value required the percentage of data inhibition. The absorbance measurements obtained from each sample were used to calculate the percentage of inhibition calculated using the following formula:

$$\text{Persen inhibisi} = \frac{[\text{Abs kontrol} - \text{Abs sampel}]}{\text{Abs kontrol}} \text{ x } 100\ \%$$

The sample concentration and inhibition percentage obtained were plotted respectively on the x and y axes in the linear regression equation. The equation was used to determine the IC50 value. Each

sample was expressed with y value of 50 and IC50 was from the x value which is going to be obtained.

RESULT

The curve of antioxidant activity measurement on binjai leaf consisted of 3 treatment groups (socletation method group, maceration method group and asorbic acid as positive control group) is as follow:

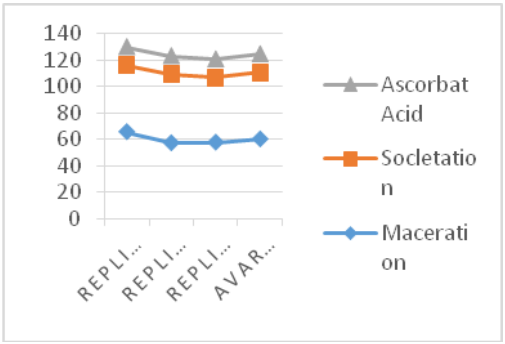

Figure1.Curve of antioxidant activity test result by DPPH method.

In figure 1, the comparison curve of antioxidant activity value between socletation and maceration method of binjai leaf extract showed that the value of antioxidant activity in both methods was 60,135 for maceration and 50,791 in socletation. The strength of antioxidants in binjai leaf extracts tested using DPPH is included in the active category because it is in the range of 50-100 ppm which means that binjai leaf extract has an active antioxidant. Meanwhile, the ascorbic acid used as a positive control in the study has an IC50 value of 13.825. This value is located less than 50 ppm indicating that the antioxidant activity in ascorbic acid is very active.[17]From the results of the antioxidant test, both treatment groups of socletation and maceration extraction methods did not show significant differences, where both are still in the same range of 50-100 ppm.

Data analysis was performed by the Saphiro-Wilk normality test and homogeneity test of Levene's test for equality of variances. The results of the Saphiro-Wilk normality test and the homogeneity test of the Levene's variant test are as follows:

Figure 2. The Result of Normality and Homogeneity test

	Normality test	Homogeneity test
Control	0,679	
Maceration	0,720	0,057
Socletation	0,260	

In the table above,the normality test of the control group and the treatment group data is p> 0.005 which indicates that the data obtained is normally distributed. On the homogeneity test, the significance value generated in p> 0.005. These results indicate that the data is homogeneous. One way ANOVA parametric analysis test method was then followed by Post-Hoc LSD (Least Significance Different).

One way ANOVA parametric test results obtained p value = 0.000 (p <0.05). These data shows that there were significant differences between the treatment groups tested. Result of Post Hoc LSD test obtained value of each treatment group where p = 0,000 (p <0,05) indicated that there was significant difference between control group, maceration group and socletation group.

DISCUSSION

Based on the result of the research, it indicates that there is difference in antioxidant activity value between extraction using socletation and maceration method. The value of antioxidant activity of both extraction methods is 60,135 for maceration and 50,791 in socletation. Differences in IC50 mean values of antioxidant activity in the treatment group of maceration and socletation methods indicate that the effect of extraction methods on the level of ability and antioxidant activity were tested upon binjai leaf samples. The treatment group which had higher antioxidant activity value in this study was found in socletation extraction method treatment group. Meanwhile, the ascorbic acid antioxidant used as a positive control on the research has an IC50 value of 13.825. This value is in the very active category.

Based on DPPH antioxidant activity table, socletation method and maceration method have no significant difference in range. After processing test, there are significant differences between the three groups. Differences in the value of antioxidant activity on socletation and maceration extraction methods indicate the effect of extraction methods based on the level of ability and antioxidant activity that tested on binjai leaf samples.

Socletation method is a heat extraction method, while maceration is a cold extraction method. It can be said that one of the factors that affect the value of antioxidant activity is the temperature in the extraction.[15] This temperature difference is suspected which results in differences in the value of antioxidant activity. In the influence of temperature, the heat temperature in socletation extraction method results in higher levels of antioxidant activity and ability.[16]

These results indicate that the antioxidant compounds in binjai leaves can be well extracted on the heat treatment. The heating process helps increase the solvent's ability to draw the amount of the insoluble compounds under room temperature conditions.[17]

Strong antioxidant activity is influenced by secondary metabolite content contained by plant extract. One example of secondary metabolite compounds owned by binjai plants is phenolic compounds.[18] Phenolic compounds have a powerful antioxidant effect.[19] In addition, based on the research, the highest contents in binjai leaf are flavonoids, tannins, and titerpenoid.[20]

High temperatures will increase solubility of phenol by using polar solvent.[21] By increasing the temperature, the diffusion is also greater, so the extraction process will also run faster.[22] However, the increase of the operating temperature also needs to be considered because it can cause damage to the processed material if being set too high.[23]

This may occur because the heat could release and activate low molecular weight of polymers molecule subunits which has high molecular weight. This condition is effective to increase the phenolic content in the plant. In addition, the heating process can break or open the tissue of the plant so that the active component that initially does not appear can be extracted.[24]

In this study, it is known that the method of extraction has an effect on the withdrawal of the secondary metabolite content which is preferably in hot temperature. This indicates that a type of heat-resistant secondary metabolite can increase antioxidant activity.[25]One of the antioxidant compounds that have antioxidant activity in binjai plants is tannin compound. Tanin has a large solubility properties and will increase through the heating process.[26]But it is not known exactly how much the percentage of tannin content and antioxidant compounds contained in binjai leaves after going through the extraction process.

Based on this research, it can be concluded that extraction using socletation method produces more powerful antioxidant activity compared to the extraction of binjai leaf using maceration method.

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