DENTINO
JURNAL KEDOKTERAN GIGI
Vol IV. No 1. Maret 2019

ANTIOXIDANT ACTIVITY OF BINJAI LEAVES(*Mangifera caesia*) ETHANOL EXTRACTS (Research report)

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ABSTRACT
Background: *Binjai is one of* Mangifera *species which commonly found in South Kalimantan. Binjai leaves are known to contain flavonoids compounds, that have an effect as antioxidant that can accelerate wound healing process after tooth extraction. This study was conducted to examine the variation of solvent concentration towards antioxidant activity of Binjai's leaves extract using maceration method. Antioxidant activity is tested with DPPH (2,2-diphenyl-1-picrylhydrazyl) and examined using Spectrophotometer UV-Vis.* ***Purpose:*** *This study aims to determine solvent concentration which exxpres optimal antioxidant activity of Binjai leaves extract.* ***Methods:*** *This study is a true experimental with post-test only control group design. The sampling technique of Binjai leaves was determined by simple random sampling and 7 samples was opted for each treatment.* ***Results:*** *The study revealed that 96% ethanol extract of Binjai leaves obtained IC_{50} (Inhibitory Concentration) as much of 16.14 ppm (very active), 70% ethanol extract of the Binjai leaves obtained 37.94 ppm (very active), and 50% ethanol extract of Binjai leaves obtained 58.07 ppm (active). Data analysis was conducted using One Way ANOVA parametric test and LSD post hoc test which demonstrated a significant difference among 96%, 70% and 50% of ethanol extracts of Binjai leaves with p = 0.000 (p <0.05).* ***Conclusion:*** *Binjai leaves extracted using 96% ethanol solvent possesses higher level of antioxidant activiy compared to 70% and 50% ethanol concentration.*

Keywords*: Antioxidants, Binjai leaves, DPPH, maceration.*

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INTRODUCTION

Dental extraction is a common procedure performed in the field of dentistry, particularly oral and maxillofacial surgery. Dental extraction can result in a large open wound that mostly experience complete healing without any complications.[1] Tooth extraction could lead to alveolar defect and periodontal damage, mainly to the adjacent attached gingiva. According to Halimah (2013), the prevalence of post-extraction complications is 16.8%. These complications are attributed with several factors affecting one or more stages of wound recovery and thus leading to impairment of wound healing process in particular cases such as pathologic wound inflammation and chronic wound due to ischemia, diabetes mellitus and venous stasis disease.[1,2]

Data regarding various materials capable in accelerating oral wound healing process and reducing post-tooth removal complications are emerging recently. As a part of post-extraction wound care, a topical antibiotic is usually prescribed in order to achieve rapid wound healing. Additional symptomatic drugs are also prescribed, including analgesics and anti-inflammatory drugs to reduce pain and swelling.[4]

People, especially in Indonesia, tend to use plants as a source of herbal products. Recently, studies have been conducted to investigate the

benefits of herbal products as they are found to be effective and commonly available. Herbal plants can induce healing and regeneration of tissue damage through various mechanisms. The use of plants for medical purpose (phytomedicine) does not only cost-effective but also safe.[5]

Plants are one of the main natural resources, and one of the species of *Mangifera* genus that typically grow in the land of South Kalimantan is binjai (*Mangifera caesia*) characterized by mango-like tree producing fragrant and sweet and sour taste. Binjai trees are spread over Sumatera, Kalimantan, and Malayan gulf, and were brought and cultivated in the Philippines, Thailand, and a part of Java. According to Ministry of Health Indonesia (2007), seeds, leaves, and stalks of *Mangifera sp.* contain large amount of flavonoids, whereas its leaves and barks are rich of saponins. The seeds and barks of *Mangifera sp* are also reported rich of tannins.[6,7,8]

Every part of green plants contains high amount of flavonoids, one of the biggest natural phenol group compounds that possess antioxidant properties. Flavonoid is a polyphenolic compound known to show protective activity against free radicals, inhibit oxidative and hydrolytic enzymes as well as produce anti-inflammatory properties.[9,10]

Rohman and Riyanto (2008) showed that phenol compounds, particularly flavonoids, produce antioxidant properties. Free radicals and reactive oxygen species (ROS) produced within the normal limits are important for protective response towards bacterial and fungal infections as well as for regulation of cellular growth. The excessive level of free radicals and ROS in a human body will lead to the occurence of oxidative stress. The increase of free radicals causing toxic effects in human body can be neutralized by antioxidants. Antioxidants are a group of compounds capable in inhibiting ROS and free radicals within human body. Antioxidants will transfer one or more electrons to the free radical to form a normal molecule and stop tissue damage caused by free radicals.[11,12]

Natural antioxidants are necessary to inhibit and prevent oxidative reaction caused by free radicals. DPPH *(2,2-diphenyl-1-picrylhidrazyl)* test is one of the currently available methods to test the antioxidant activity of a compound. DPPH test uses synthetic radical compound dissolved in a polar solvent such as methanol and ethanol and can be detected using ultraviolet-visible spectrophotometry. [13,14]

The content of a plant extract can be optimized by performing extraction in different solvent concentrations. Solvent is an important external chemical factor that affects the quality of a plant-based extract. According to Faturrachman (2014), antioxidant properties of 70% soursop leaves extract are stronger compared to the same products with solvent concentrations of 50% and 96%. The antioxidant activity strength of 70% soursop leaves extract is probably due to the secondary metabolite contents extracted during the extraction process. This study showed that soursop leaves extracted in 70% ethanol possesses higher level of antioxidant activity compared to 50% and 96% ethanol extraction. The difference in extracted secondary metabolites during the process using three different concentrations is caused by different polarity between the 50%, 70% and 95% extracts. The smaller the concentration of organic solvent, the smaller the cost. Increasing solvent concentration does not simply increase the antioxidant activities. This evidence leads to careful consideration in choosing proper solvent concentration used for extraction.[13,14,15]

MATERIALS AND METHODS

A true experimental study was conducted using *post-test only with control groups design*. Sample collection was conducted using simple random sampling technique which was divided into 4 different experiment group according to the concentration of binjai extract used, including 96% binjai extract, 70% binjai extract, 50% binjai extract, and aquadest as the negative control in which each comprised of 7 samples. The material used in the experiment was binjai leaves simplicia, DPPH *(2,2-diphenyl-1-picrylhydrazyl)* reagent, ethanol solvent in different concentrations (50%, 70%, and 96%, respectively), pro-analysis ethanol, and aquadest.

Preparation of Raw Materials

Collected leaves of binjai plant were washed out prior to extraction using clean water to remove the dirt. It was subsequently mashed up and dried by aerating the leaves without exposing it to sunlight for as long as 3x24 hours. The dry leaves were blended into powder and the simplicia powder was obtained and subsequently sifted using mesh 40. The powder was then put into an oven, dried using the temperature of 38^{o} C until dry and then stored in a clean and dry container that protected it from sunlight exposure for further extraction.

Preparation of Extraction

Ten grams of simplicia powder were extracted through maceration process in 50%, 70%, and 96% ethanol concentrations. The

simplicia powder was poured into a maceration container and ethanol solvent was added until it reached a level of 1 cm higher than the surface of the simplicia. The mixture was then mixed until blended and the container was then covered tightly and incubated for 3x24 hours. Stirring of the mixture using magnetic stirrer as fast as 50 rpm for 15 minutes was done every 24 hours. This mixture was then extracted and concentrated using rotary evaporator at 45-50^{o} C until a thick extract was produced.

Testing of Antioxidant Activity Using Quantitative DPPH Method

Preparing a 0.4 mM (2,2-diphenyl-1-picrylhydrazyl) solution. A total of 4 mg DPPH (MW 394.32) was dissolved in pro-analysis ethanol solvent and was poured into a 25 mL volumetric flask. The ethanol solvent was added until it reached sufficient volume and the solution was then stored in the dark.

Measuring DPPH maximum absorption wavelength. Around 1 ml of 0.4 mM DPPH solution was placed into a test tube and mixed with 4 ml of pro-analysis ethanol and covered with aluminium foil, homogenised in a vortex and poured into a cuvette and measured using UV-Vis spectrophotometry on 500-600 nm wavelength.

Determining operating time of the solution. The determination of operating time was performed by adding 1 ml of 0.4 mM DPPH solution and 4 ml of 100 ppm experimented solution. The mixture was then measured for its absorption at the maximum wavelength that was previously obtained within 5-minute intervals until it reached stable absorbance.

Preparing a reference solution. Approximately 1 ml of 0.4 mM DPPH solution was poured into a pipette and then into a test tube and mixed with 4 ml of pro-analysis ethanol. Furthermore, the mixture was covered using aluminium foil. Then, the solution was homogenised in a vortex and subsequently incubated in a dark room and stored for as long as the operating time obtained previously. The absorbance of this solution was measured at the maximum wavelength.

Making 50%, 70%, and 96% binjai extract solution. Ten mg binjai extracts (50%, 70% and 96%) were dissolved in 10 ml pro-analysis ethanol solvent creating a concentration of 1000 ppm. The solution was diluted in a 10 ml volumetric flask by adding more ethanol and was divided into different concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm and each was placed in a vial.

Measuring antioxidant activity. Around 4 ml of each solution was poured into a test tube. One ml of 0.4 DPPH solution was added into each tube and was homogenised in a vortex. The mixture was incubated in a dark room and stored for as long as the assigned operating time. The absorbance of this solution was measured at the maximum wavelength.

Quantitative Analysis of Antioxidant Activity Using DPPH Method

Data regarding percent of inhibition from the experiment are essential in order to quantify the IC_{50} value. The percent of inhibition can be calculated using the following formula:

$$\% \text{ immersion of DPPH} = \frac{[Ak - As]}{Ak} \times 100\ \%$$

Each of the sample concentration and percent of inhibition obtained were plotted on the x and y axis on a linear regression equation. The equation was used to determine the IC_{50} value of each sample and was expressed with a y value of 50 and the x value to be obtained as the IC_{50}.

RESULTS

The antioxidant activity is considered as an ability of any particular compounds or extracts to inhibit oxidative reaction that can be expressed as percent (%) of inhibition. The value that is commonly used to interpret the results from antioxidant activity test using DPPH methods is EC_{50} (efficient concentration) or commonly expressed as IC_{50}, a concentration that causes loss of DPPH activity for as much as 50%.

Testing the antioxidant activity is conducted by measuring DPPH radical absorption using UV-Vis spectrophotometry. The level of antioxidant activity of binjai leaves extract is expressed as a percent of inhibition. The mean percents of inhibition of the binjai leaves extract obtained in this study is shown in Figure 1.

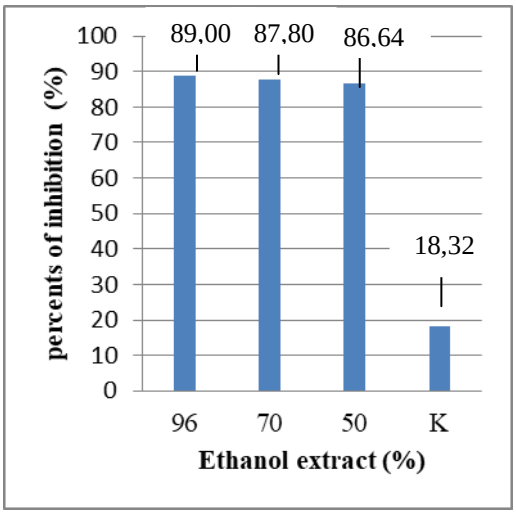

Figure 1. Mean score of percent of inhibition of binjai leaves (*Mangifera caesi*) extract's antioxidant activity

The percent of inhibition value cannot be directly used as the main parameter in determining antioxidant activity of a sample. Given the fact that it only shows the response produced by each concentration of the solution, thus it does not reflect the best antioxidant activity of all the studied samples. The main useful parameter employed to determine antioxidant level of a compound is the IC_{50} value.

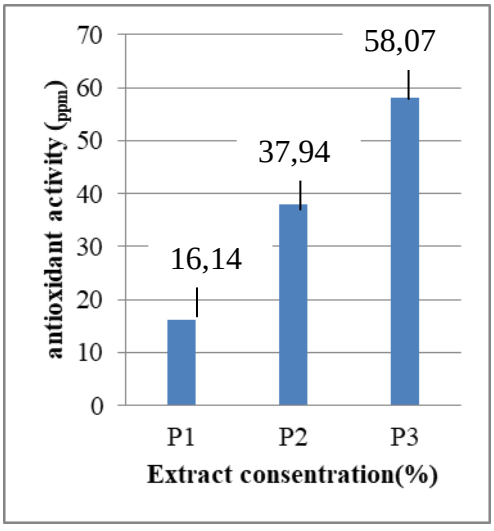

Figure 2. Mean antioxidant activity of binjai leaves (*Mangifera caesia*) extract.

Testing of the antioxidant activity level was performed on three different concentrations of binjai leaves extract (96%, 70% and 50%) that resulted in IC_{50} values of 16.14, 37.94, and 58.07 respectively. As the negative control, aquadest did not show any IC_{50} owing to the fact that aquadest could not be further diluted (concentration series); hence, quantification of antioxidant activity of aquadest was not possible. Jun (2008) reported that according to the level of antioxidant towards inhibition of free radicals, 96% and 70% binjai leaves's ethanol extracts showed a very strong antioxidant activity, while the 50% binjai leaves's ethanol extract showed a strong antioxidant activity.

DISCUSION

Antioxidant activity of binjai leaves extract macerated in 96% ethanol solvent is higher compared to the 70% and 50% ethanol concentrations. The high level of antioxidant activity found in the extract is associated with its secondary metabolite contents extracted during the maceration process. Different amount of secondary metabolite extracted using three different concentrations of binjai leaves extract is probably caused by the presence of different polarity between the solvent used in 96%, 70% and 50% ethanol concentration. According to Palleros (1993), the main principle of a solution is that a material will easily be dissolved in a solvent that possesses the same polarity and it affects its physical and chemical attributes of the compound.[8]

Polarity is commonly defined as a separation of positive pole from negative pole in a molecule resulted from specific configuration initiation of atomic constituents. This phenomenon leads to molecule retraction by another molecule that possesses the same polarity between both the phenolic compounds and its solvent concentration. The phenol component is one of secondary metabolites that can be extracted by organic solvent which is semi polar or have moderate polarity. Ethanol is classified as semi polar solvent which enabling them to penetrate cellular wall and dissolving active substances of the cells after entering intracellular space. The higher the concentration of a solvent, the richer the secondary metabolites extracted. These findings are in concordance with a study by Denny (2012) which reported that different solvent concentration is associated with the metabolite contents extracted during the extraction process.[8,15]

A study conducted by Paulinus (2015) showed that Mangifera sp. possess antimicrobial,

antidiabetic, and antioxidant activities using solvent concentration of 70%; this expresses that various compounds found within Mangifera sp. are responsible for the level of medicinal property of this plant.[15]

In this study, we found that the 96% binjai leaves ethanol extract showed higher antioxidant activity compared to the 70% and 50% binjai leaves ethanol extracts. Its ability to inhibit the DPPH radicals is probably associated with its chemical constituents such as flavonoid. Flavonoid is a phenolic compound that has more than one hydroxyl group. One of flavonoid classes contains hydroxyl group is flavonols. Rohman and Riyanto (2008) demonstrated that phenolic and polyphenolic compounds possess radical scavenging activity which enable them to inhibit oxidative reaction by transferring one of its electron to free radical that converts it into a stable non-radical molecule.[13]

One of the limitations in this study is the inability to obtain any data regarding antioxidant potential of aquadest as negative control hence statistical analysis could not be performed. This refers to aquadest properties where dilution (concentration series), a process required for assessment of antioxidant activity, could never be performed. It can be concluded binjai leaves extracted using 96% ethanol solvent possesses higher level of antioxidant activity compared to 70% and 50% ethanol concentration.

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