Antioxidant activity test of ethyl acetate fraction of binjai (Mangifera caesia) leaf ethanol extract

by Deby Kania Tri Putri

Submission date: 23-May-2023 03:47PM (UTC+0700)
Submission ID: 2099929329
File name: KKHAIR_1.PDF (151.52K)
Word count: 2952
Character count: 15476

INTRODUCTION

Interrupted wound healing after tooth extraction frequently occurs even in cases of healthy patients due to several factors which inhibit the process. There are approximately 11 million patients who experience post-operative conditions such as pain, swelling and bruising on a daily basis. It has been reported that in 1.0-11.5% of cases patients experience inconsistent wound healing.1 A wound is classified as physical damage or anatomical injury caused by microbes, a chemical reaction, temperature, mechanical trauma or surgery resulting in continuous tissue breakdown.[2] The healing process itself occurs in three phases, namely: an inflammatory phase, a proliferation or epithelization phase and a remodelling or maturation phase. A fibroblast is a cell that plays a critical role in wound healing. Fibroblasts will proliferate and produce collagen to repair damaged tissues in the inflamed wound tissue site.[3]

Wound healing requires antioxidants, substances which protect cells, protein, tissue and body organs from free radicals in order to accelerate the process. The excessive presence of reactive oxygen during the wound healing process can induce a reduction in the collagen production matrix produced by fibroblast proliferation. Therefore, antioxidants are required to inhibit the oxidation

Dental Journal (Majalah Kedokteran Gigi) p-ISSN: 1978-3728; e-ISSN: 2442-9740. Accredited No. 32a/E/KPT/2017.

DOI: 10.20473/j.djmkg.v51.i4.p164–168

process. Antioxidants work by donating a hydrogen atom to radical substances with the result that they become more stable.[4,5]

Antioxidants present in natural materials can be obtained through extraction. In this study, the researcher chose to adopt a socletation method using ethanol solution 96% because Denis, (2017) claimed that it promotes greater antioxidant activity than maceration. After binjai (*Mangifera caesia*) leaf ethanol extract has been obtained, the fractionation process is completed.[6] Fractionation involves separation of an antioxidant compound based on its degree of solvent polarity in order to produce a fraction with similar secondary metabolite properties.[7] According to research conducted by Rohman et al, (2010), ethyl acetate fraction produces stronger antioxidant activity compared to methanol and chloroform fraction.[8] Phang et al, (2011), also compared methanol, n-hexane and aquadest to ethyl acetate, concluding that ethyl acetate fraction promotes stronger antioxidant activity.[9]

Binjai part of the mangifera genus and ancardiaceae family, represents one of the herbs indigenous to South Kalimantan which act as antioxidants. The resistance of binjai to pests and disease is evident from the widespread natural presence of its wild variety throughout Sumatra and the Malayan Peninsula. A cultivated form was subsequently produced in locations such as Bali, Philippines, Thailand and certain areas on Java where the local populations value binjai as part of the daily diet and an important element of the treatment for diabetes.[10,11]

Antioxidant activity analysis of binjai leaf ethanol extract fraction can be performed using a 1.1- diphenyl-2-picryhidrazyl (DPPH) method. The score of antioxidant activity lower than IC50 recorded by a specific compound leads it to be considered highly active.[12] The advantages of the DPPH method can be more easily realized because the radical compounds employed are more stable than those of other methods. DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, with the result that the molecules do not dimerise like most other free radicals.[13] The activity of bioactive substances contained in the ethyl acetate fraction of binjai leaf extract has been tested by means of secondary metabolite qualitative test using colour performance with tube method.[14] The objective of this study was to analyze the antioxidant capacity of ethyl acetate fraction in binjai leaf ethanol extract.

MATERIALS AND METHODS

The research reported here was granted ethical clearance by document No. 031/KEPKG-FKGULM/EC/IX/2017 issued by the Faculty of Dentistry, Universitas Lambung Mangkurat. This research represented a true experiment incorporating a post-test only and control group design. The research sample consisted of binjai leaves selected through simple random sampling using two groups of unpaired comparative numerical formula. The three samples used ascorbic acid as ethyl acetate fraction within a positive control and three samples of binjai leaf ethanol extract as a treatment group.

The separation process of ethanolic extract from binjai leaf used soxhlet extraction. The binjai leaf samples were washed and dried in a room free from direct sunlight for four days. After the leaves had been dried, they were chopped and blended to the consistency of a powder which was subsequently sieved through a size 40 mesh until homogeneous and weighed to obtain 91.61 grams of simplicia powder. Binjai simplicia was extracted by socletation method using 96% ethanol solution for five hours. The liquid extract was then concentrated and evaporated using a 40°C rotary evaporator and water bath for seven hours in order to produce 11.92 grams of thick extract.[6]

Fractionation was conducted using a separating funnel by suspending the thick binjai leaf extract in aquadest at a ratio of 1:2. First, fractionation was conducted by adding 100ml of n-hexane solution prior to agitation for one minute and settling until it separated into two layers. The bottom layer constituted n-hexane fraction, while the top layer consisted of aquades fraction. Ethyl acetate fraction was obtained by adding 100ml ethyl acetate to the aquadest fraction, shaking it for one minute and then allowing it to stand until the ethyl acetate fraction separated from the aquadest fraction. The ethyl acetate fraction was then concentrated, evaporated and weighed using a rotary evaporator and water bath. The ethyl acetate fraction obtained amounted to 0.62 grams.[5]

In order to analyze quantitative antioxidant activity with DPPH assay on ethyl acetate fraction of binjai leaf ethanol extract, the solution sample was made by carefully measuring a 10 mg sample dissolved into 96% p.a. ethanol until its volume reached 10 ml. The researcher extracted 200, 300, 400 and 500 µl samples from the solution each of which was then inserted into a 10 ml volumetric flask. 96% p.a. ethanol was added until the boundary mark was reached in order to obtain concentrations of 20, 30, 40 and 50 ppm. One ml of DPPH 0.4 mm solution was added to each 4 ml sample of solution concentrate which were deposited in a dark room for 20 minutes. The solution was found to have absorbed a maximum wavelength of 516 nm.[6]

In order to measure quantitative antioxidant activity with DPPH assay on ascorbic acid, the comparative solution was made by carefully weighing 10 mg of ascorbic acid which was then dissolved into 96% p.a ethanol until it reached a volume of 10 ml. From the solution, the researcher took 40, 80, 120 and 160 ul samples each of which was then placed in a 10 ml volume flask and each 96% p.a. ethanol was added as far as the boundary mark in order to obtain concentrations of 4, 8, 12 and 16 ppm. One millimeter of

DPPH 0.4 mm solution was added to each 4 ml of standard ascorbic acid solution, before being left in a dark room for 20 minutes. The solution was read its absorbance on maximum wavelength 516 nm.[12]

Secondary metabolite qualitative testing on ethyl acetate fraction of binjai leaf extract involved several tests. In the alkaline reagent test 0.5 mg of flavonoids were dissolved in 100 ml of solvent.1 ml of the sample was then added to some drops of NaOH solution. If a yellow color appears and subsequently fades when added to a dilute acid mixture, this represents a positive test of the presence of flavonoids. 0.5 mg of lead acetate test was first dissolved in 100 ml of its solvent before 1 ml was taken as a sample. 1 ml 10% Pb acetate was subsequently added to the sample and agitated. If the solution colour changes to yellowish brown, this signifies that it contains flavonoids.[15]

In a terpenoids test using a Libermann Burchard test, a 0.5 mg sample was dissolved in chloroform and then strained. The filtrate obtained was added to several drops of concentrated sulfuric acid before being shaken. If a brown ring formed, terpenoid was present. [15]

In a tannin test using a gelatin test, a 0.5 mg sample was dissolved in 100 ml of its solvent, 2 ml samples were added to gelatin solution 1% containing NaCl. If white sediment formed, this confirmed the presence of tannin.[15]

In a saponin test using a Froth method, a 0.5 mg sample was dissolved in 100 ml of its solvent, with 2 ml samples being taken and agitated in 2 ml water. If the foams are stable, it shows positive result for saponin.[15]

In an alkaloids test using a Dragendroff test a 0.5 mg sample was dissolved in 100 ml of its solvent. A 1 ml sample was added to 1 ml Dragendroff reagent (bismuth potassium iodide). If a red sediment was formed, this signified a positive result for alkaloid. A Mayer test used a 0.5 mg sample dissolved into 100 ml of its solvent. A 1 ml sample was added to 1 ml of Mayer reagent (mercuric-potassium iodide). If yellow sediment was formed, this indicated the presence of alkaloids.[15]

In a steroid test using a Libermann Burchard test a 0.5mg sample was dissolved in chloroform, before being strained. The resulting filtrate was added to anhydride acetic acid, heated and then allowed to cool. Concentrated sulfuric acid was carefully added to the tube wall. If a brown ring formed, this confirmed the presence of steroids.[15]

In a phenol test using an iron (III) chloride test a 0.5 mg sample was dissolved in 100 ml of its solvent. A 1 ml sample was added to 1 ml of FeCl3 3%. A blue black sediment indicated the presence of phenol.[16]

Following the tally result for antioxidant activity of ethyl acetate fraction on binjai leaf, the data obtained was subjected to a normality test in the form of a Shapiro-Wilk test. The data was then evaluated by means of an independent-test which confirmed it to be normally distributed ($p>0.05$). If the data obtained was not normally distributed ($p<0.05$), a nonparametric test, namely a Wilcoxon test, was conducted instead.

RESULTS

Based on the research results, the average calculation of the ethyl acetate fraction of binjai leaf ethanol extract and ascorbic acid and the score of comparison on antioxidant activity based on the IC_{50} parameter were obtained, as shown in Figure 1. It can be concluded that the average IC_{50} in ascorbic acid was 13.812. This score was obtained from the calculation of ascorbic acid solution absorbance made by using the raw relationship curve between the ascorbic acid concentrate and percentage inhibition. The linear regression equation of $y= 3.997x - 5.141$ was obtained with a correlation coefficient of $r= 0.996$, in which x is the antioxidant score and y represents the absorbance score. Meanwhile, the antioxidant activity of ethyl acetate fraction of binjai leaf ethanol extract produces an IC_{50} average score of 38.526 ppm obtained from a linear regression calculation of $y= 1.395x - 3.720$ with a correlation coefficient $r= 0.999$.

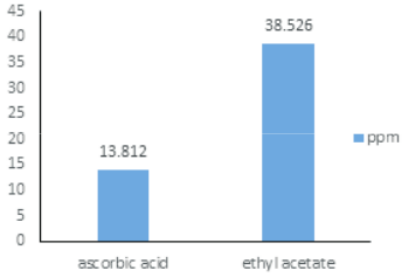

Figure 1. The comparative scores of antioxidant activity on ascorbic acid and ethyl acetate fraction of injai leaf ethanol extract based on IC_{50}.

Table 1. The result of secondary metabolites qualitative testing on ethyl acetate fraction of binjai leaf ethanol extract.

Compounds and reagents classes	Result	Explanation
Flavonoid: Alkaline + HCL Pb acetate	+	Fading yellow and brownish yellow
Saponin: Foams	–	No foams
Alkaloid: Mayer Dragendroff	–	No sediment
Tannin: Gelatin	+	White sediment
Steroid: Libermann Burchard's	–	No brown ring was formed
Terpenoid: Libermann Burchard's	–	No brown ring was formed
Phenol: Iron (III) chloride	+	Black blue sediment

The antioxidant activity scores of the ethyl acetate fraction of binjai leaf ethanol extract and ascorbic acid can be seen in Table 1. This data shows that there were secondary metabolite substances such as flavonoid, tannin, and phenol contained in the ethyl acetate fraction of binjai leaf ethanol extract.

A statistical analysis was conducted with normality testing of both groups using a Shapiro-Wilk. All values showed normal distribution since the score obtained was p>0.005. Statistical analysis of the data obtained was followed by independent t-test. The data analysis results had a significance score of p=0.00 (p<0.005). It can be concluded that there was a significant difference between the treatment group of ethyl acetate fraction and the control group of ascorbic acid.

DISCUSSION

Based on the research conducted, it is argued that antioxidant activity in the ascorbic acid and the ethyl acetate fraction of binjai leaf ethanol extract demonstrate a significant difference. The difference obtained was caused by the antioxidant activity score in ascorbic acid being more active compared to that of ethyl acetate fraction which was 13.812 ppm. Meanwhile, the antioxidant activity score in the ethyl acetate fraction of binjai leaf ethanol extract was 38.526 ppm. The parameters used to establish the antioxidant activity in the samples determined the score of (Inhibitory concentration) IC_{50}. IC_{50} is a concentration which causes the loss of 50% of DPPH activity. The score of IC_{50} is considered a valid measurement of the efficiency of the antioxidants of both pure and extract compounds.[17] The smaller the absorbance score, the bigger the inhibition percentage score. Therefore, lower IC_{50} scores show that the antioxidant activity compound is more active or stronger.[18] Anggresani et al., (2017) discussed the level of antioxidant strength based on IC_{50} parameters: less than 50 ppm is said to be highly active, 50-100 ppm is said to be active, 101-250 ppm is said to be average and 250-500 ppm is said to be weak. Based on the research conducted, it is argued that antioxidant activity in the ascorbic acid and ethyl acetate fraction of binjai leaf ethanol extract has significant difference.[19] The difference obtained is caused by the antioxidant activity of ascorbic acid being more active compared to that of the ethyl acetate fraction which was 13.812 ppm. Meanwhile, the antioxidant activity score on ethyl acetate fraction of binjai leaf ethanol extract was 38.526 ppm.

The difference between the antioxidant score activity in ascorbic acid, which was more active compared to ethyl acetate fraction, is in accordance with the findings of Martiningsih et al., (2016) who conducted antioxidant activity testing on eggplant ethanol extract which was comparable to ascorbic acid. The research concluded that ascorbic acid activity was very strong compared to that of eggplant ethanol extract because the antioxidant activity is determined by the compounds extracted which depend on the solution used, namely ethanol. This causes the extracted compound to form part of the polyphenol class because the solvent is polar. Meanwhile, other antioxidant compounds such as beta carotene and vitamin C are not being extracted because vitamin C does not dissolve in ethanol.[20]

Ascorbic acid, as a comparative solvent, is a non-enzymatic antioxidant which functions by catching free radical compounds to avoid chained oxidation reaction so that they will not react with other components.[12,21] The stronger antioxidant activity in ascorbic acid compared to the ethyl acetate fraction in binjai leaf ethanol extract is also caused by the ascorbic acid itself. Ascorbic acid is a pure antioxidant compound which means it only demonstrates antioxidant activity while present in natural ingredient extracts. These are complex compounds which have many activities such as antioxidant anti-inflammatory and anti-cancer among others.[22] Moreover, Munte et al (2015) stated that ascorbic acid demonstrates highly active antioxidant activity because during its chemical reaction, ascorbic acid holds two hydroxyl atom groups making it easier to donate hydrogen in order to suppress free radicals.[23]

Meanwhile, antioxidant working mechanism on ethyl acetate fraction to supress DPPH radicals is caused by the existance of certain compound which able to give hydrogen radicals to unpaired DPPH radicals. This results on reaction to compounds which able to supress free radicals, causing the one-electron binding to the electron which donates it and forms diphenylpicrylhidrazin. The formation of reduced DPPH into DPPH-H radical results on colour decay from purple to yellow.[24]

Secondary metabolite qualitative testing in this research was aimed at determining the secondary metabolite compounds which were found in the ethyl acetate fraction of Binjai leaf ethanol extraction. This qualitative testing involved flavonoids testing, terpenoid testing, tannin testing, saponin testing, alkaloids testing, steroid testing and phenol testing. After these tests had been conducted, positive test results were obtained for flavonoids, tannin and phenol, confirming that such compounds are semi-polar. These test results were also in accordance with those of Tanaya et al, (2015) which stated that flavonoid and tannin exist in ethyl acetate fraction because those compounds are semi-polar.[25] Tursiman et al, (2012) mentioned the semi-polar compounds which will be extracted from ethyl acetate solvent based on qualitative testing conducted on ethyl acetate fraction of kandis, contains phenol compounds which acts as antioxidant.[26] It can be concluded that even though there is a difference between the ethyl acetate fraction and ascorbic acid, based on IC_{50} parameter the score of ethyl acetate fraction of binjai leaf ethanol extract and ascorbic acid both have highly active antioxidant activity in preventing free radical since their score were lower than 50 ppm.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Faculty of Dentistry, Universitas Lambung Mangkurat and the Phytochemical Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat for permitting access to laboratory facilities and providing support during the experimental research.

Antioxidant activity test of ethyl acetate fraction of binjai (Mangifera caesia) leaf ethanol extract

ORIGINALITY REPORT

5%	2%	2%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universidad San Francisco de Quito Student Paper	1%
2	Submitted to SVKM International School Student Paper	1%
3	Submitted to Texas State University- San Marcos Student Paper	1%
4	www.ncbi.nlm.nih.gov Internet Source	<1%
5	icoen.org Internet Source	<1%
6	Rini Devijanti Ridwan, Yuliati, Sidarningsih, Fitri Mar'atush Sholihah, Mohammed Ahmed Qasim Aljunaid, Dur Mohammad Lashari. "A study of the mucoadhesive patches loaded with mangosteen peel extract in periodontitis", Journal of Taibah University Medical Sciences, 2021 Publication	<1%

7	1library.net Internet Source	<1%
8	Maharani Laillyza Apriasari, Selviana Rizky Pramitha, Dewi Puspitasari, Diah Savitri Ernawati. "Anti-Inflammatory Effect of Musa acuminata Stem", European Journal of Dentistry, 2020 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		