FLAVONOID LEVEL TEST ON ETHANOL EXTRACT OF BINJAI LEAF (Mangifera Caesia)

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FLAVONOID LEVEL TEST ON ETHANOL EXTRACT OF BINJAI LEAF (*Mangifera Caesia*) (Research report)

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BACKGROUND

Tooth extraction is one of the most common actions in the field of dentistry. It is an act of removing teeth from the alveolar bone socket which causes injury to the tissue. Optimal wound healing can be achieved if there are no complications in the form of deficiencies or excess components of wound healing, especially collagen and epithelial cells.[1,2,3,4]

According to the scientific research by Sabirin et al. (2013), flavonoids can reduce inflammatory cell infiltration, increase the number and maturation of collagen protein, epithelialization, fibroblast counts, neurovascularization, accelerate wound contraction, increase insulin receptor expression like growth factor-1 (IGF- 1) as mediator of fibroblast cell proliferation and collagen synthesis and can improve the initial healing of wounds that are shown by the regulation of vascular endothelial growth factor (VEGF) and formation of collagen type III.[4]

Flavonoids display antioxidants and anti-inflammatory effect in the tissue. The antioxidant effects of flavonoids can prevent tissue damage by superoxide radicals released by neutrophil cells, while their antiinflammatory effects can inhibit the release of degradative enzymes from

neutrophils which can inhibit collagen cross-linking. [4,5]

Flavonoids are compounds consisting of 15 carbon atoms which are generally scattered in plants. Producing red or blue color for flowers, flavonoids are plant pigments which also produce yellow pigment which attract pollinators.[6]

According to a scientific research by Rosyidah et al (2011), Binjai is regarded as one of flavonoid containing plants. Although it is a typical species of *mangifera* found in South Kalimantan Island, Binjai is still rarely used as research material. People of Hulu Tabalong, South Kalimantan and surrounding areas employ Binjai as a cure for diabetes assuming the presence of secondary metabolites component such as alkaloids, triterpenoids, flavonoids, saponins and tannins.[7,8,9]

Utilization of Binjai leaf as traditional medicine can be managed by isolating the flavonoid compounds contained therein. Extraction is the activity of separating a soluble chemical from an insoluble material with a liquid solvent. The component of extracted compounds can be influenced by many factors, such as the extraction method, type of solvent, solvent concentration and temperature used for extraction.[10]

The results of the Winata's research (2011) prove that there are differences in antioxidant activity which are influenced by the concentration of solvents used. Nevertheless, increasing the concentration of solvent during extraction may not necessarily increase the total level of flavonoids.[11,12]

The purpose of the study is to determine the concentration of ethanol that extract flavonoid levels in Binjai leaf optimally.

MATERIAL AND METHOD

This study applied pure experimental research (true experimental) method with a post-test-only design with control group design. Binjai leaf sample was carried by simple random sampling consisting of 6 experiment groups and 1 control group, namely: 50% ethanol solvent group, 60% ethanol solvent group, 70% ethanol solvent group, 80% ethanol solvent group, ethanol solvent group 90%, 95% ethanol solvent group, and 95% n-hexane solvent as control.

The number of samples in this study was obtained from the calculations with Federer formula, which is 28 samples of Binjai leaf. The Binjai leaf used was obtained from Mandiangin Village, Karang Intan District, Banjar Regency, Martapura, South Kalimantan.

The research began with cleaning process by washing the sample with water until clean. And then the sample was chopped to small and dried for ± 72 hours in the shade and avoid direct sunlight. The dried sample was blended to obtain dry simplicia. The extraction of dry simplicia by using maceration method 50%, 60%, 70%, 80%, 90% and 95% ethanol solvents and 95% n-hexane as a control.

As much as 50 grams of dry – filled simplicia are fortified with each solvent on the erlenmeyer tube with 1:10 or 1 cm above the simplicia. The mixture is stirred until smooth and tightly closed and soaked for 72 hours. Every 24 hours, stirring process using the Magnetic stirrer was done at 40 rpm (rotation per minute) for 15 minutes. After 72 hours, the mixture was filtered and enhanced with rotary evaporator on temperatures 40-500 C to acquire thick extract.

Maximum wavelength was confirmed by weighing 2 mg quarcetin which then dissolved with 95% ethanol to 100 ml. A total of 0.5 ml of the solution was taken and then reacted with 2 ml of distilled water and 0.15 ml of 5% $NaNO_2$, then left it for 6 minutes. After that, the solution was added to 0.15 ml of $AlCl_3$ 10% and put still for 6 minutes. The solution was then reacted with 2 ml of 4% NaOH and diluted to a total volume of 5 ml and waited for 15 minutes. The solution was absorbed using a UV-Vis spectrophotometer at a wavelength of 250-600 nm.

The quarcetin standard was prepared in volumetric flash (10 ml). Then, the standard solution was put in each flash with 0.01 g, 0.02 g, 0.03 g, 0.04 g, and 0.05 g respectively. Adding the solution with aquades to 10 ml, the absorbance was then measured at maximum wavelength like previous procedure and standard curve was made between absorbance (A) with the concentration of quarcetin (φ).

The standard solution was made by weighing 2 mg of the sample then dissolved with ethanol 50%, 60%, 70%, 80%, 90%, 95% and n-hexane 95% to 100 ml and the concentration of 20 ppm was obtained. A total of 0.5 ml of each extract solution was reacted with 2 ml of distilled water and 0.15 ml of 5% $NaNO_2$ and left for 6 minutes. 0.15 ml of 10% $AlCl_3$ was added in the solution and left for 6 minutes. The solution was reacted with 2 ml of 4% NaOH and diluted to a total

volume of 5 ml and left for 15 minutes. After that, the absorbance of the extract solution was measured with maximum wavelength using a UV-Vis spectrophotometer. Total flavonoid level was determined based on the calculation results from the quarcetin calibration curve equation.

RESULTS

Based on the research that has been done, the average value of total Flavonoids level from Binjai extract is as follow.

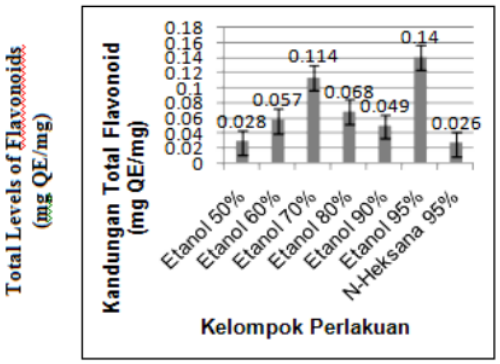

Fig.1. Diagram result of flavonoid component in Binjai leaf extract.

Figure 1 show the highest total flavonoids found in 95% ethanol extract and the lowest total flavonoids found in 95% n-hexane extract. The average total flavonoid of Binjai leaf extract from the highest to the lowest is in the 95% ethanol extract, 70% ethanol extract, 80% ethanol extract, 60% ethanol extract, 90% ethanol extract, 50% ethanol extract and 95% n-hexane extract.

Aquired data was analyzed by Shapiro-Wilk normality test and Levene's variance of homogeneity test. The results of the Shapiro-Wilk normality test in each group demonstrated normally distributed data with p value 0.05. Meanwhile, the homogeneity test results denoted homogeneous variant with p value 0.142 ($p > 0.05$). It can be concluded that the data is normal and homogeneous.

The statistical analyses used were *One way ANOVA parametric test*, $p = 0.00$ ($p < 0.05$). There were significant differences in the total flavonoid extract of Binjai leaf between experiment groups. The Post Hoc LSD test was conducted to find out which groups had significant differences. The test results showed that there were no significant differences between groups of 50% ethanol extract with 95% n-hexane extract group ($p = 0.636$), whereas the other groups showed significant differences with a significance value less than 0.05.

DISCUSSION

The polarity of each solvent can affect the total amount of flavonoids obtained. According to Sudarmadji et al. (1989), level of polarity will determine the results of extraction and antioxidant activity contained in the extract. Polar solvents will dissolve polar components, while non-polar solvents will dissolve non-polar compounds. This is in accordance with the principle of dissolving a substance *like dissolve like*.[13]

The use of solvents with different polarity causes shifting in absorption peak of a compound. In other words, the solvent polarity affects λ max of a compound. Solvent polarity affects λ max because the polarity of molecules usually changes if an electron moves from one orbital to other. [13]

Polarity of a solvent can be determined based on the chemical properties of dielectric constant which is a measure of the polarity of a solvent. Solvents with large dielectric constants will further dissolve polar compounds, whereas solvents with small dielectric constants will dissolve non-polar compounds. Ethanol solvents 50%, 60%, 70%, 80%, 90%, 95% and n-hexane 95% respectively have dielectric constants of 55, 50, 45, 40, 35, 30 and 2.[13,14,15,16]

The maximum total of flavonoids is found in 95% ethanol extract group of Binjai leaf. It is contradicting the hypothesis in this study, possibly because the similarity between 95% ethanol and flavonoids polarity compared to other solvents thus enable more flavonoids to be extracted. This is consistent with Pine et al's research. (2011) which mentioned 96% ethanol as a compatible solvent to obtain a favorable amount of flavonoids than 70% ethanol and water. [17]

Ethanol solvents 95% and 96% have a dielectric constant that is not much different so they can extract the compound optimally. Based on initial theory of ideal solvent, ethanol or its water mixture is frequently used as extracting solvent which process the highest extractive ability for all compounds with low molecular weights such as alcohol, saponins and flavonoids. Ethanol mixture with water in a ratio of 7: 3 is most suitable for the raw material in the form of simplicia roots, stems or woody parts of plants. Ratio of 1:1 is very useful for chlorophyll, resin or polymer compounds that usually shows no meaningful activity but often cause pharmaceutical problems.[13,17,18]

The significant difference from the statistical results is probably generated by the difference of

polarity possessed by ethanol at each concentration according to its dielectric constant so that it has different abilities in dissolving flavonoids.[13]

Difference between n-hexane extract 95% group with ethanol extract 50% group is 0.636 ($p > 0.05$) which indicate that the difference is not significant. This is probably induced by the difference in polarity possessed by 50% ethanol and 95% n-hexane compare to flavonoids polarity in binjai leaf thus the flavonoids levels obtained are decreased. This state showed that there is no significant difference between the ethanol extract 50% group and n-hexane extract 95% group. The highest solubility of flavonoids is not always presented in polar extracts, but depends on the structure of the flavonoids compounds themselves.[13,14] It can be concluded that discerned that the optimal solvents concentration to dissolve maximum amount of flavonoids in Binjai leaf is 95% ethanol solvents.

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