FLAVONOID LEVEL ANALYSIS OF BINJAI LEAF EXTRACT (Mangifera caesia) IN ETHANOL, METHANOL, AND N-HEXANE SOLVENTS

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(Research report)

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INTRODUCTION

Wounds are injuries of body tissues due to physical force, causing disruption in normal tissue continuity thus supposed to be treated. One example of the most frequent injury to be complained in the field of dentistry is a post-extraction wound. Friedman (2007) declared that there are 11 million patients per day who experience complaints such as pain, swelling and postoperative bruising after third molar extraction. Wound care aims to stop bleeding and clean the wound area from foreign objects, dead cells and bacteria for the healing process. In general, wound healing process will occur physiologically. However, problem may arise along the process which will cause pain and discomfort.[1]

Several methods can promote the acceleration of wound healing process, one of the them is by the use of herbal medicines.[1] Herbal remedies are traditional medicines derived from natural ingredients which are provided by nature in the form of plants and have been known and used by Indonesian people. Generally, traditional medicine is more easily accepted by the community because it has been familiarized among them. In addition, this medicine is cheaper and easier to obtain.[2]

Binjai (*Mangifera caesia*) is categorized in *Mangifera* genus and *Anarcadiaceae* family. This

plant can be discovered in South Kalimantan and consumed by people who live in the region. Some experts believe that Kalimantan is the the origin place of this plant. Afterwards, it was brought and cultivated in Bali, Java, Philippines and Thailand. The society of Hulu Tabalong, South Kalimantan and surrounding areas use binjai plant roots as a diabetes medicine. A study by Mustikasari and Ariyani (2008) mentioned that binjai plants are proven to contain secondary metabolites, one of them is flavonoid.[3,4]

Flavonoids are the largest natural phenol compounds found in all green plants. Antioxidant activity from plants is caused by the presence of polyphenol compounds which are known having capability as free radical scavenger, hydrolysis and oxidative enzyme inhibitors and also work as anti-inflammatory agent.[5] Kurniasari (2006) stated that a number of medicinal plants contains flavonoids which possess antioxidant, antibacterial, antivirus, anti-inflammatory, anti-allergic and anticancer effects.[5] The process of wound healing can be accelerated by the antioxidant, antibacterial and anti-inflammatory effect of flavonoids compound by inhibiting the production of sodium oxide which has a role in causing tissue damage, preventing secondary infections in wound area, reducing acute inflammatory exudates and stimulating T lymphocytes and macrophages activation.[1]

Flavonoids act as antioxidants by donating hydrogen atoms to bind metals not only in the form of glucosides, but also the free forms called aglycones.[6] Antioxidants demonstrate an effect as osteoblast stimulator and osteoclasts inhibitor.[6] They can affect bone formation by stimulating alkaline phosphatase activity. With the result that, the osteoblast activity increases.[5] Flavonoids are found in the form of glycosides which are more soluble in polar solvents.[6]

Polar solvents are able to extract compounds such as quaternary alkaloids, phenolic components, carotenoids, tannins, sugars, amino acids and glycosides.[7] Active polar substances such as flavonoids can be dissolved by methanol and ethanol solvents which later affect the type of medicine preparations.[7,8]

According to the explanation above, the researcher is interested in conducting a study to compare the total flavonoid content of binjai leaf extract in ethanol solvents and methanol solvents as a preliminary study for medicine preparations of wound healing process.

MATERIALS AND METHODS

This study is a pure experimental study with post-test only control group design conducted in August 2015 at Biochemistry Laboratory of Medical Faculty, Lambung Mangkurat University, Banjarbaru. This study used simple random sampling, consisting of 3 treatments. The number of repetitions for each treatment was determined using Federer formula. Each group required 9 times of repetition, with minimal total of sample number required was 27 samples. The sample in this study was binjai leaves taken from Desa Mandiangin, Kecamatan Karang Intan, Kabupaten Banjar, South Kalimantan.

The data obtained from this study was measured by observing the total number of flavonoids through UV-Vis spectrophotometer method to analyze ethanol, methanol and n-hexane groups using the following formula:

$y = ax + b$

Description:

y = absorbance value
x = flavonoid levels
a, b = constant

Data analysis was performed by testing data normality using Shapiro-Wilk[5] test and its homogeneity using Levene's test. The data was evaluated based on the type of variable measurement scale. It was obtained that variable data was categorized as numerical comparative unpaired more than 2 groups and then analyzed using Oneway ANOVA to test hypothesis with 95% confidence level ($\alpha = 0.05$). The significant differences between each of the test groups can be attained. The analysis continued with Post Hoc LSD (Least Significant Difference) test to compare the significant difference among test groups.

RESULT

According to the study result of total flavonoid content comparison of binjai leaf extract in ethanol and methanol solvents as a preliminary study, the preparation of medicine preparations for wound healing process revealed that the total flavonoid content of ethanol, methanol and n-hexane groups have different values.

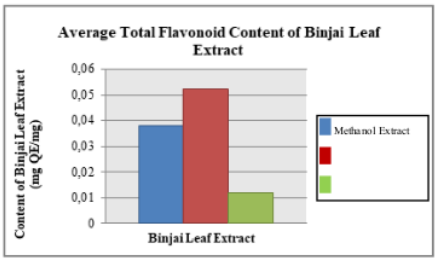

Figure 1. Diagram of Average Total Flavonoid Content in Binjai Leaf Extract

The results of data analysis in this study indicate that there are significant differences among the solvents. According to the diagram (figure 1), highest total flavonoids is denoted in methanol group with an average of 0.0522 mgQE / mg, it is followed by ethanol with an average of 0.0382 mgQE / mg and the lowest are in n-hexane group with an average of 0.0121 mgQE / mg.

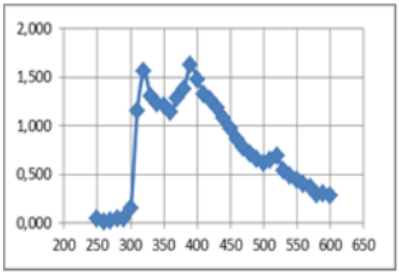

Figure 2. Wavelength Graph of Binjai Leaf Extract

The results also reveal that flavonoids found in binjai leaf were classified in chalcone and auron. This is based on the maximum wavelength result (figure 2) which is at 250 nm (band II) and 390 nm (band I).

DISCUSSION

According to the results of this study, methanol solvent has higher effectivity in binding flavonoids compared to ethanol and n-hexane solvents. Markom (2007) declared that this is caused by different polarity of each solvents, that is 5.2 for ethanol solvent, 5.6 for methanol solvent and 0.1 for n-hexane solvent.[7]

It is also mentioned that total flavanoid content is directly proportional to the polarity levels of solvents where the highest to the lowest values are methanol, ethanol and n-hexane respectively. According to the Rijkestatement (2005), the higher the level of polarity of a solvent, the higher the flavonoids that can be extracted. It is related with the number of unsubstituted hydroxyl groups. Hence, flavonoids can be extracted using polar solvents.

The results also claimed that the types of flavonoids contained in binjai leaf were chalcone and auron. This can be observed through the maximum wavelength which is 250 nm (band II) and 390 nm (band I). According to an article written by Neldawati (2013), it is known that flavonoids in the form of chalcone have a maximum absorption band area in the range of 230-270 nm (band II) and 340-390 nm (band I). Whereas, flavonoids in the form of auron have a maximum absorption band area in the range of 230-270 nm (band II) and 380-430 nm (band I).[8]

Chalcone-type flavanoids are secondary metabolites of several flavonoids found, especially in plants whereas auron is a derivate of chalcone that has cyclization. Inspite of the spread in a variety of plant families, chalcone and auron are among the type of flavonoids which are rarely found because of their limited number compared to other flavonoid compounds. This resulted the compound to be classified into minor category because the small percentage in plants and structure variety.[9,10,11] A number of chalcone compounds and their derivatives have been known to have biological activities such as anticancer, anti-inflammatory, antioxidant, antitumor, antimicrobial, antifungal, antibacterial, antimutagenic and hypo-allergenic.[9,11]

Based on the conducted research, it can be concluded that there are differences in the total flavanoid content of binjai leaf dissolved in ethanol solvent and methanol solvent.

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