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THE COMPARISON OF TOTAL FLAVONOID CONTENT IN RAMANIA (*Bouea macrophylla* Griffith) BARK AND LEAF EXTRACT USING MACERATION METHOD

Sofyan Erwandi¹, Irham Taufiqurrahman², Bayu Indra Sukmana³

Faculty of Dentistry Lambung Mangkurat University Banjarmasin

Department of Oral Surgery, Faculty of Dentistry, Lambung Mangkurat University Banjarmasin

ABSTRACT

Background: The wound is defined as a disorder of the functional anatomical structure on the human body. Traditional medicines, specifically herbs, have been used for a long time and used for the research on wound healing process. There are many medicinal plants that can be used to accelerate wound healing, such as Ramania (*Bouea macrophylla* Griffith). Ramania has numerous contents and one of them is flavonoid that functions as antibacterial and antioxidant. Flavonoids are able to accelerate the growth of new cells and stimulate the fibroblasts formation, thus accelerating the wound healing process. The result of flavonoid compound extraction is influenced by many factors such as part of the plant, solvent and method which is used for the extraction. **Purpose:** To analyze the difference of total flavonoid contents in ramania (*Bouea macrophylla* Griffith) bark and leaf extract using maceration method with 95% ethanol solvent. **Methods:** This study used Quasi experimental with pre-experimental design with quantitative testing. The total samples were 32 male marmots, which divided into 2 treatment groups consisted of ramania bark extract group and ramania leaf extract group. Total levels of flavonoids were calculated using a UV-Vis Spectrophotometer. **Results:** The mean value of total flavonoid in ramania bark extract was 11.14 µg/mg and the leaf extract was 17.15 µg/mg. The Independent T-test showed that there was significant difference between the treatment groups ($p < 0.05$). **Conclusion:** It can be concluded that the total flavonoid content found in ramania (*Bouea macrophylla* Griffith) leaf extract is higher than the bark extract.

Keywords: Flavonoid, ramania leaf, ramania stem bark, maceration

Correspondence: Sofyan Erwandi, Dentistry Faculty Lambung Mangkurat University, Veteran St 128B, Banjarmasin, South Borneo, email: erwandisofyan636@gmail.com

INTRODUCTION

Wound is defined as a disorder of the functional anatomical structure of the body. It is usually caused by mechanical trauma such as sharp or blunt objects, changes in temperature, chemical substances, explosions, electric shock and animal bites. In the medical field, efforts to accelerate wound healing effectively, safely and practically are things that get a lot of attention. Wounds that do not heal for a long time, with various reasons, are common problems. This incidence is one of the major causes of disease which increases the mortality rate, causes psychological disorders,

increases the treatment and budgets maintenance and loss of productivity.¹

The purposes of wound healing treatment are to stop bleeding and cleanse foreign objects, dead cells and bacteria from the wound so that the healing process can be achieved. One of the factors that affect the wound healing process is the type of drugs used for the treatment.² Appropriate treatment and wound care management is a way to optimize wound healing conditions and reduce the risk of infection. Therefore, they can prevent the occurrence of chronic wounds.³

Drug interactions is a situation where a substance affects the activity of a drug that increases or decreases its effect, or produces

undesirable new effects.⁴ It is known that modern chemical drugs have many negative effects, either directly or indirectly. Moreover, drug prices continue to rise which reduce the selling rate of the drug. The conditions of Indonesian who still live in rural areas and the occurrence of economic crisis create expectation in the use of traditional medicine as an alternative treatment with affordable prices. Traditional medicines, mainly from herbs, have been used for a long time and it is also used for researches in wound healing field. Some doctors recommend using medicinal plants as a healing product for wounds.⁶

In Indonesia, there are more than 20,000 types of medicinal plants known, but only about 1,000 new plant species are recorded and only about 300 are recorded as traditional medicine. One alternatives to meet the basic needs of the community in the medical field is to use traditional medicine.⁷ There are many medicinal plants that can be used to accelerate wound healing, one of which is Ramania (*Bouea Macrophylla* Griffith). Previous research by Novalianti (2006) and Dina Harliany, et al (2016) has done phytochemical test on bark and leaf of ramania which stated that bark and ramania leaf contain flavonoid compound.⁸ The study from Landy et al (2013) showed that the juice of ramania fruit has antioxidant activity because the extract contains phenol component which detected through phytochemical test.

Flavonoid is a polyphenol compound that has a function as an antibacterial compound by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes. In addition, flavonoid is also a phenol compound that can function as protein coagulator.⁷ Flavonoids function to maintain normal growth, infection and damage. Flavonoids have been introduced as anti-carcinogenic or anti-cancer, anti-allergic, tumor growth inhibition, antibacterial agent and often used as traditional medicine. Flavonoids are also known as antimicrobials and antioxidants. The application on the skin may inhibit bleeding.⁹ This study aims to analyze the differences in total flavonoid in ramania (*Bouea Macrophylla* Griffith) bark and leaf extract.

MATERIALS AND METHOD

This research began with the making of research permit and ethical clearance issued by Faculty of Dentistry Lambung Mangkurat University No.013/KEPKG-FKG ULM/EC/VIII/2017. The research method was quasi experimental with pre-experimental design

involving quantitative testing with total sample of 32. This study was conducted to determine the comparison of total flavonoids in ramania bark ethanol extract with maceration method of extraction.

Sampling technique for the ramania bark and leaf used simple random sampling with 2 treatment groups and 16 repetitions for each group, consisted of P1: bark extraction with ethanol solvent 95% and P2: leaf extraction with ethanol solvent 95%.

Making of Simplicia Powder

First, bark and leaf were cleansed and washed with running water to remove the dirt. The cleansed bark was then mashed and dried by winding them up without direct exposure to sunlight for 3x24 hours. The dried bark and leaf were then blended until smooth enough to form powder. The simplicia powder then sieved with mesh number 40 and dried again using an oven with a temperature of 60°C. The simplicia powder was stored for 4 hours in a clean, dry container and avoided from sunlight during further extraction process.

The Extraction Process

Dry simplicia was weighed with analytic scale and obtained 50 mg weight of each simplicia stem bark and ramania leaf and then separated into two glass containers with ramania bark and ramania leaf containers. The two maceration glass containers containing stem bark and ramania leaf were filled with 95% ethanol solvent. Maceration was carried out for three days and was protected from sunlight. The stirring was done several times a day so the solvent penetrated to all surface of the simplicia powder. After 3 days, the solvent were filtered and the filtrate was evaporated using rotary evaporator at a temperature of 50°C to obtain viscous concentrate of ramania bark and leaf extract. The filtrate was poured in a vapor plate, then evaporated in fume hood. The viscous concentrate of ramania bark and ramania leaf ethanol extract was obtained. Re-maceration was done for the dregs, until clear filtrate was obtained.

Determining the Maximum Wavelength

Determining the maximum wavelength was done by weighing 2 mg quercetin and then diluted with 95% ethanol till 100 ml. A total of 0.5 ml of solution was taken and then reacted with 2 ml of aquades and 0.15 ml of NaNO₂ 5% and after which were left it for 6 min. After that, 0.15 ml AlCl₃ 10% solution was added and left it for 6 minutes. The solution was reacted with 2 ml of NaOH 4% and diluted till the total volume of 5 ml

reached and left for 15 min, then the solution absorbance was measured using a UV-Vis spectrophotometer at wavelengths between 250-600 nm.

The preparation of Default Curve

The standard quercetin was made by preparing 5 pieces of 10 ml measuring flask. Each flask were then poured a standard solution of 0.01 g, 0.02 g, 0.03 g, 0.04 g, and 0.05 g. After that the solution was added with aquades up to 10 ml. The absorbance of each solution was measured at the maximum wavelength as the previous procedure. Then a standard curve was made between the absorbance (A) and quercetin (Q) concentration.

The Content test for Total Flavonoid

The samples were weighed to 2 mg then dissolved with ethanol solvent with concentration of 95% to 100 ml to obtained 20 ppm concentration. A total of 0.5 ml of each extract solution was reacted with 2 ml of aquades and 0.15 ml of NaNO₂ 5% then left it for 6 min, after which 0.15 ml of AlCl₃ 10% was added in the solution and left it for 6 min. Then the solution was reacted with 2 ml of 4% NaOH and diluted to a total volume of 5 ml and left for 15 minutes. The absorbance of the extract solution was measured with the maximum wavelength of quercetin solution using a UV-Vis spectrophotometer. The total flavonoid content was determined based on the calculation result of the regression equation of quercetin calibration curve. Total flavonoid was stated as the total of equivalent quercetin per mg of extract (mgQE / mg).^o

RESULT

The result showed that the maximum standard wavelength of quercetin flavonoids was 415 nm. Based on the calculation of absorbance of standard solution of quercetin at various concentration then a standard curve of quercetin can be created. Linear regression equation can be obtained with $y = 0.0081x + (-0.0648)$, where x is total flavonoid and y is absorbance (A). The equation is used for the quantitative analysis on measuring the content of flavonoid compounds in the bark and leaf of ramania extracts to obtain the total flavonoids content. The standard curve of quercetin standard solution can be seen in Figure 5.1..

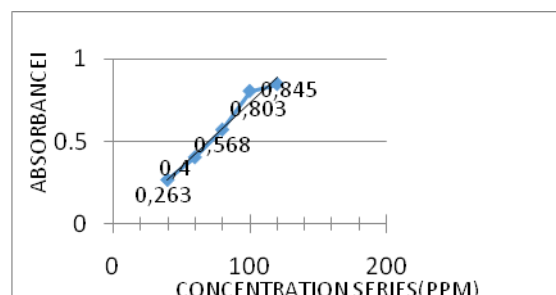


Figure 5.1 Absorbance Value of Quercetin Standard

The result of absorbance value of flavonoid content in ramania leaf extract was calibrated with linear regression equation. The value of flavonoids content of each sample was obtained and averaged and the obtained value of total content of flavonoids can be seen in the picture below 5.2.

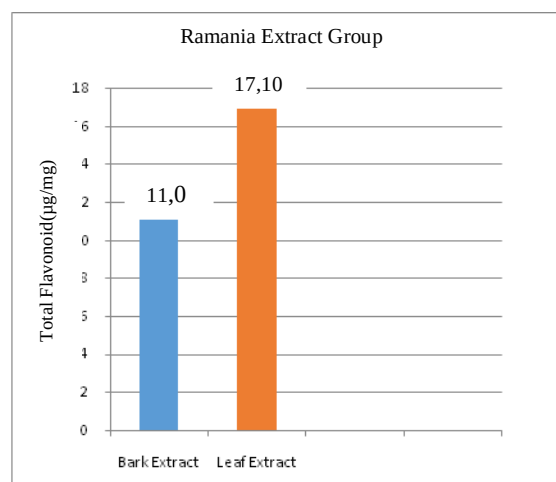


Figure 5.2 Average value of Total Flavonoid

Figure 5.2 shows the average of total flavonoid in each group. The content's total flavonoid of ramania leaf maceration extract was higher than ramania bark maceration extract.

Table 5.1 Result of *Shapiro-wilk* normality test

Treatment	Bark Extract Significance(p)	Leaf Extract Significance (p)
Ethanol Maceration 95%	0,102	0,056

* Normally Distributed ($P > 0,05$)

Based on Table 5.1, it can be seen that the significance value of both groups of bark extract

and leaf extract were $p > 0,05$. This result means that the data was distributed normally, so parametric test of *Independent T-test* can be performed.

Table 5.2 Result of *Independent T-test*

Treatment	Bark Extract Mean $\pm$ SD	Leaf Extract Mean $\pm$ SD	Significance
Ethanol Maceration 95%	11,08 $\pm$ 1,00	17,10 $\pm$ 0,43	(p) 0,000

Table 5.2 above shows that the significance value of bark extract and leaf extract were $p < 0,05$. The results of the *Independent T-test* showed that there was a significant difference from the total content of flavonoids between ramania bark extract and ramania leaf extract.

DISCUSSION

Quercetin is a flavonol glycoside belongs to the flavonoid derivative. This compound is a phenol compound. It is used as a standard solution for the determination of flavonoid levels because quercetin is a flavonoid derivative from flavonol group that has ketone groups on the C-4 atoms and also hydroxyl groups on C-3 and C-5 that are next to it. Figure 5.1 is a quercetin calibration curve that helps to determine the levels of flavonoid compounds in the sample through the linear regression equation of the quercetin calibration curve. Figure 5.1 shows the higher the concentration (ppm), the higher the absorbance capacity of the quercetin solution.^{10,11}

The result of this study indicates that the total content of flavonoids in ramania leaf is higher than in ramania bark. Flavonoids are polar compounds that are easily soluble in polar solvents such as ethanol, methanol, butanol and acetone. Flavonoids are included in the largest group of phenol compounds that have very active properties to slow the growth of viruses, bacteria, and fungi. Chemical compounds of flavonoids are generally known as antioxidants and have been utilized as one component of basic materials in the manufacture of medicines. Flavonoids are also water-soluble compounds which can be extracted with ethanol and remain present in the solvent after fractionation with non-polar solvent. Flavonoids are phenol compounds that can change color when added with bases or ammonia so they can be easily detected in the form of solution. Flavonoids contain a

conjugated aromatic group that shows a strong absorbance in spectrophotometry. The total flavonoid in Figure 5.2 shows ramania leaf extract with maceration method using ethanol solvent have higher total flavonoids than bark extract. Commonly used solvents for extraction of active compounds are water, ethanol, ether, methanol, chloroform and acetone. This indicates that the 95% ethanol solvent used is suitable for leaves. It is according to the results of the Harliany D et al (2016) study which states that 95% or higher concentration of ethanol solvents are more likely to attract the flavonoid compounds.^{12,7,13,14}

The significant difference in Table 5.2 proves that the part of plant taken for extraction affects the yield of the total flavonoids. This is in accordance with the fact that there are 3 basic parameters affecting the extraction result, which are part of the plant, the solvent used for the extraction and the method used in extraction procedure. The extraction method used is maceration extraction method by immersing simplicia in a certain solvent with occasional shaking and stirring at room temperature. This process is done several days until the ratio of simplicia and solvent determined has been obtained. The material used is called simplicia, which is a dry material that undergone the mashing or smoothing process but has not experienced the processing of compounds separation in the material.¹³

Handayani (2013) study on flavonoid content of fire-tree (*Avicennia marina* (Forks.) Vierh.) bark and spruce as an active antioxidant compound showed the same result as this study, where higher amount of flavonoid content is found in leaf than the bark. The flavonoids contain antioxidants that can accelerate the process of wound healing by neutralizing or suppressing the negative effects of free radicals. The research of Princess et al (2017) on the influence of tapak dara (*Catharanthus Roseus*) extract on the amount of fibroblasts in wound healing process in the oral mucosa shows the effect of the extract in the amount of fibroblasts during wistar rat (*Rattus norvegicus*) wound healing process. The wound healing process becomes faster because it was given leaf extract topically. Tapak dara leaf contains alkaloid, polyphenols and derivatives, flavonoids, tannins and steroids as well.^{15,16}

Based on the result of this research, it can be concluded that the total of flavonoid content in ramania leaf extract is higher than bark extract.

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