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TOTAL FLAVONOID CONTENT ANALYSIS OF RAMANIA LEAVES' EXTRACT USING ETHANOL, METHANOL AND N-HEXANE AS SOLVENTS
(Research report)

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ABSTRACT
Background*: Wound healing process consists of three phases: inflammation, proliferation, maturation and remodelling. Secondary metabolites are chemical compounds that have potential to be explored and developed in search of raw materials for drugs to assist wound healing process; one of them is flavonoid. Flavonoid is classified as natural phenolic compound that has antioxidant properties coexisted with its antimicrobial and anti-inflammatory effect. Flavonoid is usually available in the form of glycosides and soluble in polar solvents, such as methanol and ethanol. The bark and leaves of Ramania, a native plant of Kalimantan, contains flavonoid compound with non-optimal utilization of its potential.* ***Purpose****: To determine the differences in total flavonoid content of Ramania leaves' extract obtained using ethanol and methanol solvents.* ***Methods****: This study is a true experimental study using post-test only with control group design; Samples collection were comprised of 27 specimens, categorized into three groups: two treatment groups using 70% ethanol solvent, one group using 70% methanol solvents, and one control group using 70% n-hexane solvent.* ***Results****: There were significant differences between ethanol group and methanol group result ($p<0.05$); ethanol group and n-hexane group result ($p<0.05$); and methanol group and n-hexane group result ($p<0.05$).* ***Conclusion****: This study concluded that there were significant differences in the total flavonoid content of Ramania leaves' extract using ethanol and methanol as its solvents.*

Keywords*: ethanol solvent, methanol solvent, ramania leaves, total flavonoid,*

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INTRODUCTION

Tooth extraction is a common daily practice conducted in dental workplace. It is an invasive approach that will trigger post-extraction regeneration known as wound healing process to restore the balance of impaired tissues and their compartments. Wound healing is a very dynamic process that includes haemostasis, inflammatory phase, proliferative phase, maturation and remodelling.[1]

Medicinal plants are plants with assisting properties for wound healing process, such as helping to reverse pain in inflammatory phase, supporting host's immune response against microbial invasions, and repairing damaged organs. The utilization of local medicinal plants is an alternative to conventional choice of drugs, with vast potentials to be explored using advanced technology in concern of evolving diseases.[2]

Secondary metabolites are chemical compounds with bioactive properties which mainly demonstrate protective function in medicinal plants. This compound has countless potential to be explored and developed as raw

materials for alternative choice of drugs, yet its exact application for medication is very limited. So far, Ramania is only perceived as a plant used for basic needs where the woods are cut and used as agricultural tools, while its young leaves are cooked and its fruits are to be eaten, either directly from the trees or as ingredient for various processed foods such as Indonesian salad, pickles, extracts, or lime substitute.[6]

Novalianti (2006) had performed phytochemical test on Ramania's bark which revealed the presence of phenolic and flavonoid compounds. Another study by Arwita D (2013) on Ramania's leaves obtained from Sumatera, Java, Ambon, and Kalimantan demonstrated flavonoid, saponin and triterpenoid as the most common secondary metabolites found in the leaves.[3]

Flavonoid is a natural phenolic compound with antioxidant properties; it has glucose side chains or preserved in a free form called aglycone. The antioxidant activity works by releasing hydrogen atom as glucoside.[4] Flavonoid has osteoblast stimulating effects which express anti-osteoporosis properties.[5,6,7] It also plays as a key supporting role in wound healing; the astringent exhibits not only high antioxidant activity but also antimicrobial properties, aiding in wound contraction, and increasing epithelialisation rate.[8]

Flavonoid is a polar compound because it has a number of non-substituted hydroxyl groups. Polar solvents, such as ethanol, methanol, ethyl acetate or their mixtures, can be used to extract flavonoid from plants' tissues.[9] A substance is soluble in a certain solvent if they have similar polarity values: polar substance is soluble in polar solvents and insoluble in non-polar solvents.[10]

The most common technique used to isolate active antioxidant substances from plants is solvent extraction, a method to separate plants' compounds using a solvent. Solvent is chosen based on its capability to dissolve maximum active substances and obtain final extracts (the end-result containing chemical compounds).[11]

Polar solvents can extract quartenary alkaloids, phenolic compounds, carotenoids, tannins, sugar, amino acids, and glycosides. Flavonoids are commonly found in glycosides form which is more soluble in polar solvents. The choice of solvent will affect the kind of herbal drug preparations produced. Methanol and ethanol are universal solvents with extracting ability for polar and non-polar analytes, and also isolating alkaloids, steroids, saponnins, and flavonoids from plants.[9]

METHODS

This is a true experimental study using post-test only control group design. Samples were collected using simple random sampling, categorized into three groups. According to Federer formula, the minimal repetition for each group is 9 times, thus the total samples needed were 27 specimens. Samples in this study were Ramania leaves taken from Mandiangin village, Karang Intan sub-district, Banjar Martapura district, South Kalimantan.

Accumulated data was then measured using Spectrophotometer UV-Vis to assess the total flavonoid content in ethanol, methanol, and n-hexane extracts. The equation used in this measurement is depicted below:

$$y = ax + b$$

y : absorbance value
x : flavonoid content
a, b : constants

Data was analyzed using Shapiro-Wilk normality test and Levene's variance homogeneity test. The data was then evaluated using One way ANOVA test with 95% confidence interval (α=0,05) to assess significant difference between each group. To detect which group expressed significant difference, the data was further evaluated using Post Hoc LSD (Least significant Different) test.

RESULTS

The results of total flavonoid contents in Ramania leaves' extract using ethanol and methanol solvents in preliminary study of drug preparations for wound healing process were shown in figure 1.

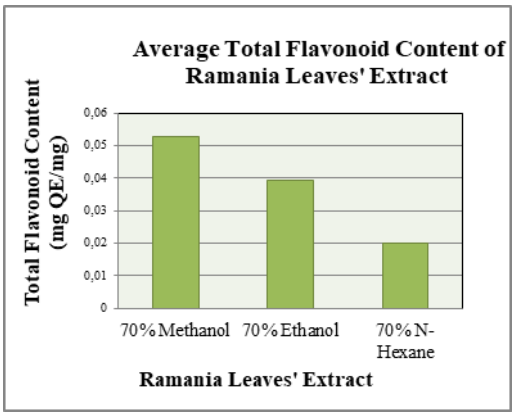

Figure 1. Average total flavonoid content of Ramania leaves extract

The chart demonstrates the differences of total flavonoid content in Ramania leaves' extract using ethanol, methanol, and n-hexane as solvents. The highest flavonoid content was found in group using methanol solvent (0,0526mgQE/mg), followed by ethanol group (0,0392 mgQE/mg) and n-hexane control group (0,0200 mgQE/mg) with the lowest total value.

Based on this results, methanol group has the highest flavonoid content, thus further study was performed. The result is illustrated in figure 2.

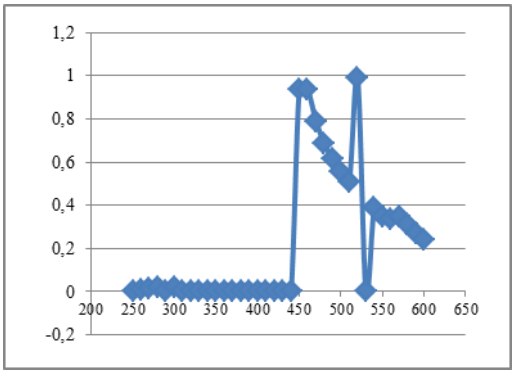

Figure 2. Ramania leaves' extract using methanol as solvent presenting in chart

Using spectrophotometer UV-Vis at 250–600 nm wavelengths, the flavonoid content in Ramania leaves' extract expresses the characteristic of anthocyanidin, with maximum absorbance values at 270-280 nm (tape II) and 465-550 nm (tape I).[12]

DISCUSSION

The charts showed that there were differences of total flavonoid content in Ramania leaves' extract using ethanol, methanol, and n-hexane as its solvents.

According to Markom M (2007), each extraction solvent has different polarity where ethanol contained 5.2; methanol 5.6; and n-hexane 0.1 polarity value.[26] The study result is also in accordance with Markom's which mentioned that each solvent produced different flavonoid contents due to different polarity values. Nuraida L (2008) implied that solvents induced simplicia pores dilation and diffused simplicia particles out of the surface.[13]

Methanol group has the highest total flavonoid content, followed by ethanol and n-hexane group. This result is in accordance with Romandanu (2014) study, which explained the polarity values of each solvent, from highest to lowest were methanol, ethanol, and n-hexane.[9]

This study's results revealed that Ramania leaves' extract contained flavonoid. Using spectrophotometer UV-Vis, the flavonoid type was analyzed and classified as anthocyanidin.[12]Arja F S (2013) mentioned that anthocyanidin is an anthocyanin aglycone which is formed after anthocyanin was hydrolysed by acids.[14]

The flavonoid in Ramania leaves' extract has antioxidant and anti-inflammatory properties. As proposed by Indriasari I (2012), it has anti-inflammatory properties by inhibiting TNF-α.[14] In conclusion, this study reported that there were differences of total flavonoid content in Ramania leaves' extract using ethanol, methanol, and n-hexane as solvents.

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