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### DAFTAR ISI

THE EFFECT OF MAULI BANANA STEM EXTRACT GEL ( <i>Musa acuminata</i> ) APPLICATION IN CONCENTRATION OF 37.5% ON EPITHELIAL THICKNESS (Research report)	
<i>Monica Thiodora Limay, Maharani Laillyza Apriasari, Irham Taufiqurrahman</i>	1-8
MUSCULOSKELETAL DISORDERS (MSDs) RISK ANALYSIS IN WEST SUMATERA DENTISTS AND DENTAL STUDENTS (Research report)	
<i>Abu Bakar, Valendriyani N, Elyza DG, Nadhifa S, Chusnul</i>	9-13
THE EFFECT OF GIANT SNAKEHEAD ( <i>Channa micropeltes</i> ) EXTRACT ON THE NUMBER OF MACROPHAGE IN DIABETES MELITUS WOUND HEALING (Research report)	
<i>Khairunnida, Amy Nindia Carabelly, Maharani Laillyza Apriasari</i>	14-20
EXFOLIATIVE CHEILITIS (Case report)	
<i>I Wayan Arya K.Firdaus, Maharani Laillyza Apriasari</i>	21-24
MAJOR CONTRIBUTORY FACTORS IN THE INITIATION OF ROOT RESORPTION (Article review)	
<i>Syamsiah Syam, Aries Chandra T, Muhammad Ruslin</i>	25-30
PRIMARY MANAGEMENT OF BURNING MOUTH SYNDROME FOR GENERAL DENTIST (Article review)	
<i>Rinda Prawidiastuti, NurIsmah Gitasari, Yunita Fatmala, Wahyu Hidayat, Indah Suasani Wahyuni</i>	31-36
COMPRESSIVE STRENGTH OF TYPE III GYPSUM MIXED WITH WATER OF DIFFERENT WATER HARDNESS LEVEL (Research report)	
<i>Dewi Puspitasari, Suci Fikriyati, Debby Saputera</i>	37-40

THE DIFFERENCE OF SATISFACTION LEVEL IN JAMINAN KESEHATAN NASIONAL (JKN) AND GENERAL PATIENTS TOWARDS QUALITY OF DENTAL HEALTH SERVICES AT DENTAL CLINIC IN WEST KALIMANTAN (Research report)	
<i>Erdianto SetyaWardhana, Sandy Christiono</i>	41-45
FLAVONOID LEVEL ANALYSIS OF BINJAI LEAF EXTRACT ( <i>Mangifera caesia</i> ) IN ETHANOL, METHANOL, AND N-HEXANE SOLVENTS (Research report)	
<i>Didy Adham, Irham Taufiqurrahman, Zairin Noor Helmi</i>	46-49
FLAVONOID LEVEL TEST ON ETHANOL EXTRACT OF BINJAI LEAF ( <i>Mangifera Caesia</i> ) (Research report)	
<i>Ana Azizah Ansari, Irham Taufiqurrahman, Nurdiana Dewi</i>	50-54
ANTIOXIDANT ACTIVITY OF BINJAI LEAVES ( <i>Mangifera caesia</i> ) ETHANOL EXTRACTS (Research report)	
<i>Anita Diana Putri, Irham Taufiqurrahman, Nurdiana Dewi</i>	55-59
TOTAL FLAVONOID CONTENT ANALYSIS OF RAMANIA LEAVES' EXTRACT USING ETHANOL, METHANOL AND N-HEXANE AS SOLVENTS (Research report)	
<i>Risa Hermalinda, Irham Taufiqurrahman, Zairin Noor Helmi</i>	60-63
THE VARIANCE OF RADIOPAQUE IMAGES COMMONLY FOUND IN PERIAPICAL LESION USING PERIAPICAL RADIOGRAPHIC TECHNIQUE (Article review)	
<i>Indra G, Ria F, Lusi E, Azhari</i>	64-69
ATROPHIC GLOSSITIS AS A CLINICAL SIGN FOR ANEMIA IN THE ELDERLY (Case report)	
<i>Rahmatia Djou, Indah Suasani Wahyuni</i>	70-76
THE EFFICACY OF GREEN TEA ( <i>Camellia sinensis</i> ) LEAVES EXTRACT AS CORROSION INHIBITOR FOR ORTHODONTICS STAINLESS-STEEL WIRE (Research report)	
<i>Neny Roeswahjuni, Delvi Fitriani, Annisa Dwi Wardanianti</i>	77-82

THE EFFECT OF BIOLOGICAL MEMBRANES TO FIBROBLAST PROLIFERATION: PLATELET-RICH FIBRIN RELEASATE VS AMNIOTIC (Research report)	
<i>Rezmelia Sari, Sri Pramestri Lastianny, Alya Nur Fadhilah</i>	83-86
CORRELATIVE STUDY BETWEEN MALOCCLUSION SEVERITY LEVEL AND ORAL HYGIENE STATUS AMONG STUDENTS AT SMP LPP WAKAF FOUNDATION UNIVERSITAS MUSLIM INDONESIA IN 2017 (Research report)	
<i>Rafika COUNSUL, Yustisia Puspitasari, Sarahfin Aslan</i>	87-93
RADIOGRAPHIC FEATURES OF INTERNAL STRUCTURE IN JAW LESIONS (Review article)	
<i>Norlaila Sarifah, Ria N. Firman, Farina Pramanik, Lusi Epsilawati</i>	94-100
MANAGEMENT OF VARICELLA ZOSTER IN ADULT PATIENT (Case report)	
<i>Maharani Laillyza Apriasari, Selviana Rizky Pramitha</i>	101-105
DIFFERENCE IN SETTING TIME AND WORKING TIME INTERVAL OF CONVENTIONAL ZINC PHOSPHATE CEMENT AND NANOPARTICLE ZINC PHOSPHATE CEMENT (Research report)	
<i>Chandra Sari Kurniawati, Delvi Fitriani, Sheila Ananda Putri</i>	106-109

**DENTINO**  
**JURNAL KEDOKTERAN GIGI**  
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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.<sup>1</sup>

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.<sup>2</sup>

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.<sup>6</sup>

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.<sup>3</sup>

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.<sup>4</sup> Flavonoid has osteoblast stimulating effects which express anti-osteoporosis properties.<sup>5,6,7</sup> 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.<sup>8</sup>

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.<sup>9</sup> 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.<sup>10</sup>

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).<sup>11</sup>

Polar solvents can extract quaternary 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, saponins, and flavonoids from plants.<sup>9</sup>

## 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 Mandiingin 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:

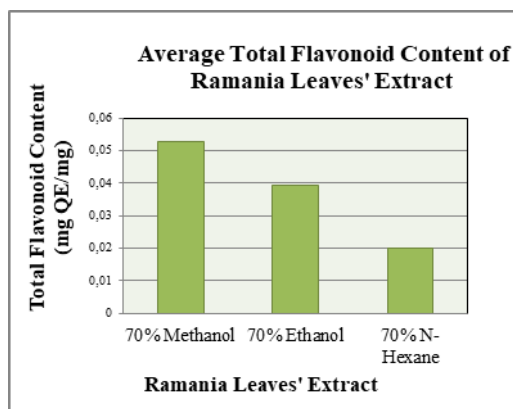
$$y = ax + b$$

$y$  : 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 ( $\alpha=0,05$ ) 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

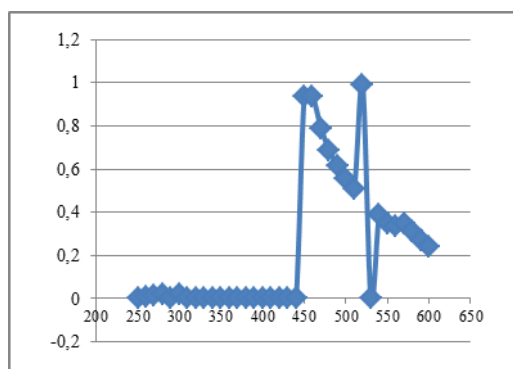
The results of total flavonoid contents in Ramania leaves' extract using ethanol and methanol solvents in preliminary study of drug preparations for wound healing process were shown in figure 1.



**Figure 1.** Average total flavonoid content of Rhamnus leaves extract

The chart demonstrates the differences of total flavonoid content in Rhamnus 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.** Rhamnus leaves' extract using methanol as solvent presenting in chart

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

## DISCUSSION

The charts showed that there were differences of total flavonoid content in Rhamnus 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.<sup>26</sup> 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.<sup>13</sup>

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.<sup>9</sup>

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

The flavonoid in Rhamnus leaves' extract has antioxidant and anti-inflammatory properties. As proposed by Indriasari I (2012), it has anti-inflammatory properties by inhibiting TNF- $\alpha$ .<sup>14</sup> In conclusion, this study reported that there were differences of total flavonoid content in Rhamnus leaves' extract using ethanol, methanol, and n-hexane as solvents.

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