

## COMBINATION OF GOTU KOLA (*Centella asiatica* (L.)) ETHYL ACETATE EXTRACT AND VIRGIN COCONUT OIL (VCO) AS BURN HEALING

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### ABSTRAK

Luka bakar diakibatkan oleh paparan sumber panas seperti api, bahan kimia, atau radiasi dan dapat menyebabkan hilangnya atau kerusakan jaringan. Penyembuhan luka bakar secara tradisional sering menggunakan obat herbal seperti pegagan dan kelapa, biasanya ekstrak minyak. Penelitian ini bertujuan untuk mengidentifikasi efek penyembuhan luka bakar dengan pemberian kombinasi ekstrak etil asetat herba pegagan dan minyak kelapa murni (VCO) pada mencit. Mencit dibagi menjadi 7 kelompok yaitu kontrol negatif (K(-)) yang diberikan tween 20; kontrol positif (K(+)): luka bakar obat oles yang beredar di pasaran, P1: ekstrak Ethylacetate 10% pada tween 20; P2: VCO; P3: ekstrak Ethyl acetate 10% dan VCO 50% pada tween 20; P4: Ethyl acetate 5% ekstrak dalam VCO P5 : ekstrak etil asetat 10% dalam VCO dan diberi perlakuan selama 21 hari berturut-turut dengan frekuensi dua kali sehari. Hasil penelitian menunjukkan bahwa kombinasi ekstrak etil asetat 10% dari herba pegagan dalam VCO menunjukkan aktivitas penyembuhan tertinggi untuk luka bakar, sebanding dengan obat oles kontrol positif (K(+)) yang beredar di pasaran. Semakin tinggi konsentrasi ekstrak dan VCO, semakin baik aktivitas penyembuhan luka bakar.

**Kata kunci:** Ekstrak etil asetat, pegagan, minyak kelapa murni, penyembuhan luka bakar

### ABSTRACT

Burns result from exposure to heat sources like fire, chemicals, or radiation and can lead to tissue loss or damage. Traditional healing of burns often involves using herbal remedies like gotu kola and coconut, typically oil extracts. This study aims to identify the healing effects of burns by administering a combination of ethyl acetate extract of gotu kola herb and virgin coconut oil (VCO) to mice. The mice were divided into 7 groups, namely negative control (K(-)) was given tween 20; positive control (K(+)): burn topical medicine on the market, P1: 10% Ethylacetate extract in tween 20; P2: VCO; P3: Ethyl acetate extract 10% and VCO 50% in tween 20; P4: 5% Ethyl acetate extract in VCO; P5: extract 10% Ethyl acetate in VCO and treated for 21 consecutive days with a frequency of twice a day. The results showed that the combination of 10% ethyl acetate extract of gotu kola herb in VCO exhibited the highest healing activity for burns, comparable to the positive control (K(+)) topical

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medicine on the market. The higher the extract concentration and VCO, the better the healing activity of burns.

**Keywords:** Ethyl acetate extract, gotu kola, virgin coconut oil, burn healing

## INTRODUCTION

*Centella asiatica* or gotu kola is a plant found throughout Indonesia. One of the functions of gotu kola is to revitalize cells that accelerate the healing of burns. Gotu kola contains saponins, including *asiaticoside*, *asiatic acid*, and *madecassoside* stimulate the production of collagen I, vitamin B, suspected triterpenoid glycoside *asiaticoside* which has antileprosy and wound healing activity<sup>1</sup>. Some studies describe that gotu kola affects good wound healing. The effect is to increase collagen secretion, stimulate fibroblast proliferation, and increase angiogenesis<sup>2,3</sup>.

The use of gotu kola as a burning medicine can be used alone or combined with other ingredients that also have a healing effect on burns. The use of a combination can increase the healing effects of burns, as shown in Mayefis's research (2019), the combination of gotu kola (*Centella asiatica* (L.) Urban) and aloe vera (*Aloe vera*) herbal extracts influence the healing of burns in mice<sup>4</sup>. While Wijaya's research (2013) showed that burn test results from aloe vera extract with VCO can heal burns<sup>5</sup>.

Traditionally the use of herbal extracts in topical treatment is to soak herbs into coconut oil then the results of the bath are applied to the affected part of the body. VCO or known as virgin coconut oil, is a modification of the coconut oil production process, which is processed at low temperatures or without heating so that the critical content in the oil can still be maintained<sup>6</sup>. VCO when used topically, has a function as a skin protector, prevents infection, protects the skin from free radicals, and moisturizes the skin. VCO contains phytosterols that can provide anti-inflammatory effects. VCO contains medium-chain fatty acids, especially lauric acid, which is easily absorbed, has the potential to accelerate cell metabolism, moisturize wounds, and has anti-inflammatory activity<sup>5</sup>. Based on this, this study aims to determine the activity of using ethylacetate extract of gotu kola and VCO respectively, and their combination against burns in vivo using mouse test animals (*Mus musculus*).

## METHODS

### Tools and materials

The tools used in this study were a set of glassware such as drip pipettes, micropipettes, beakers, Erlenmeyer, etc., rotary vacuum evaporator (Buchi®), analytical balances (Precisa®), aluminum foil, water bath, beaker (Pyrex®), porcelain dishes, 20 mm diameter metal coin, bunsen and spiritus,

calipers, shavers, equipment for minor surgery, equipment for make histological and staining preparations, scissors, gloves, blenders, microscopes and cages of mice.

The ingredients in this study were gotu kola herb (*Centella asiatica* (L.) Urban) obtained from Kendari city, has been determined at the Laboratorium Pendidikan Biologi FKIP UHO based on Letter No. 27/BIO/PB/VII/2022, VCO from brands on the market, mice (*Mus musculus*), aquadest, ethyl acetate (Merck®), alcohol swab, positive control (burn healing gel that available in the market), sterile gauze, ketamine (KTM-100®), xylazine (Xyla®), tween 20 (Brataco®), reagents for phytochemicals screening.

### Extraction

Simplisia powder from gotu kola herb as much as 1 kg macerated using ethyl acetate as much as 5 liters for three days with maceration every day using the same solvent. Macerat is collected and then evaporated using a *rotary evaporator* at 60°C until a thick extract is obtained. The remaining solvent is removed by heating using a *water bath* with a temperature of 60°C until a viscous extract is obtained<sup>7</sup>.

### Phytochemical screening

Phytochemical screening of secondary metabolites of alkaloids, phenols, flavonoids, tannins, saponins, and steroids/terpenoids was carried out qualitatively using the Farnsworth method (using reagents such as Dragendorff for alkaloid test, Lieberman-Burchard for steroid/terpenoids etc.)<sup>8</sup>.

### Animal preparation

Male mice (*Mus musculus*) aged 10 weeks with a body weight of 25-30 g as much as 28 acclimatized for a week by loading mice in cages filled with husks and fed and drinking moderately. Then animals were randomly grouped into 7 groups, where each group consisted of 4 mice<sup>9</sup>, namely negative control (K(-)) was given tween 20; positive control (K(+)): burn topical medicine on the market, P1: 10% Ethylacetate extract in tween 20; P2: VCO; P3: Ethyl acetate extract 10% and VCO 50% in tween 20; P4: 5% Ethyl acetate extract in VCO; P5: extract 10% Ethyl acetate in VCO. During the acclimatization process, general condition observation and weight weighing are carried out.

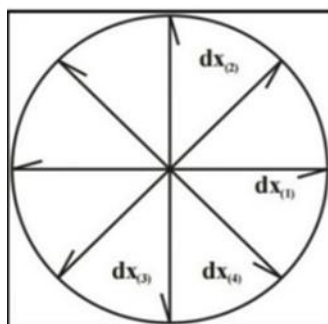
### Test of burn healing activity

Before the creation of burns on mice is carried out, the area to be wound is freed first from the hair using a sterile razor. After that, anesthesia is carried out using *ketamine IM* (dose: 50 mg/kg) and

*xylazine IM (dose 10 mg/kg)*<sup>10</sup>. The burn was made using a metal coin with 20 mm in diameter that heated in a bunsen fire for 1 minute and then affixed for 5 seconds to the back of the mouse. After the burn is made, then burn treatment is carried out by applying positive control and samples according to the predetermined treatment group 2 times a day. Test sampling is carried out for 21 days.

### Measurement of burn diameter and observation of burn condition

The percentage of burn healing is measured by the reduced diameter of the burn in mice. Measurement of burn diameter is carried out using a caliper tool with the Morton method, which measures four wound diameters permanently limited to the 21st day, then calculated the average value of the diameter of each measurement. Treatment of burns in test animals is carried out every day while measuring and taking data on the diameter of wounds in test animals is carried out every 3 days.



**Figure 1.** Four-way wound/burn diameter measurement<sup>11</sup>

The calculation of the percentage of burn healing is carried out with the following formula<sup>12</sup>:

$$\% \text{ burn healing} = \frac{L1}{Ln} \times 100\%$$

Legend: L1 = Area of zero day burns; Ln = Area of day n burns.

Where:

$$L = \pi r^2 \text{ or } \frac{1}{4} \pi d^2$$

Legend: d = average diameter of burns

In addition, burns on mice are visually observed by looking at the condition of the burn during the testing period, which is an inflammatory condition until the wound dries and closes.

### Preparation of skin histology

The preparation of skin histology is carried out by the paraffin embedding method<sup>13</sup> with the following stages: a. Harvesting of mouse organs; b. Fixation; c. *Washing*; d. Dehydration; e.

*Clearing*; f. Infiltration; g. *Embedding*; h. *Cutting*; i. *Attaching*; j. *Deparaffinization*; k. *Dealcoholization*; l. *Coloring*; m. *Mounting*; n. *Labeling*. The finished preparation is labeled and observed the histological structure of the tissues in the skin organs and closes the epidermal layer is.

### Histological observations of fibroblast cells

This observation parameter is to count the number of fibroblast cells with hematoxylin-eosin staining observed using a light microscope (Olympus BX 53 Microscope®) with a magnification of 100 times in 3 fields of each preparation which is then averaged<sup>14,15</sup>, which is connected to a camera (*Optic Lab Microscope Camera*).

### Data analysis

The data analysis method used in this study is the *one-way* Analysis of Variance (ANOVA) method using the *Statistical Product and Service Solution* (SPSS®) application version 26 with a significant level of  $\alpha = 0.05$ .






## RESULTS AND DISCUSSION

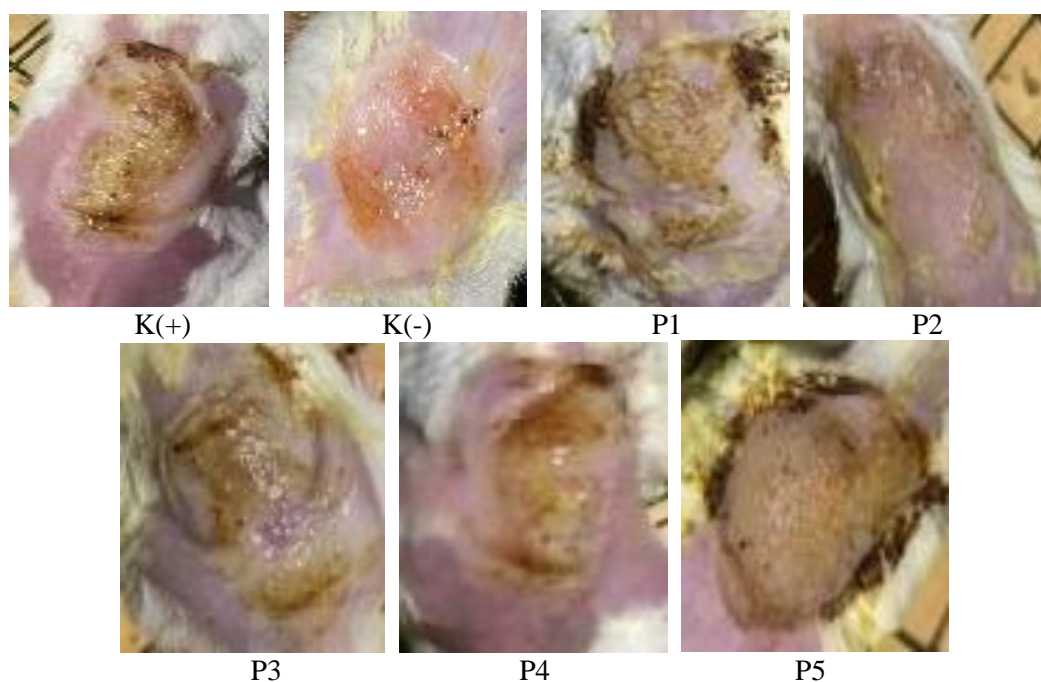
Gotu kola herb is extracted using ethyl acetate solvent and obtained a yield of 10.72%. Ethyl acetate solvent is semipolar so that it is expected to attract many compounds with a wide polarity range, both polar and nonpolar<sup>16</sup>. The phytochemical screening presented in table 1 showed that gotu kola contains all classes of secondary metabolites tested, namely alkaloids, flavonoids, tannins/polyphenols, saponins, and terpenoids.

Alkaloids can function as effective astringents and antimicrobials to help the process of reepithelialization of injured tissue, where the increased weight of dry granulation tissue and the production of hydroxyproline enzymes are caused by the high maturity of collagen tissue in the wound area. The content of alkaloids also plays a role in the process of strengthening collagen fibrils formed by preventing cell damage through DNA synthesis so that the growth of new tissue in wounds becomes faster. Flavonoids can accelerate the wound healing process by increasing the rate of wound contraction, decreasing the epithelialization period, increasing collagen deposition, and forming granulation tissue. Saponins are useful for affecting collagen (the early stage of tissue repair) by inhibiting excessive wound tissue production, sapogenin compounds also help stimulate the formation of new epithelial cells and support the reepithelialization process. Tannins can accelerate the formation of new tissue while protecting it from infection or as an antiseptic. Tannin compounds are astringents that work locally by precipitating blood proteins so that bleeding can be stopped<sup>11,17</sup>.

Triterpenoids have pharmacological effects on wound healing, namely as anti-inflammatory, and antibacterial, and encourage angiogenesis and synthesis of type 1 collagen<sup>17</sup>.

**Tabel 1.** Phytochemical screening of Gotu kola herb (*Centella asiatica* (L.) Urban)

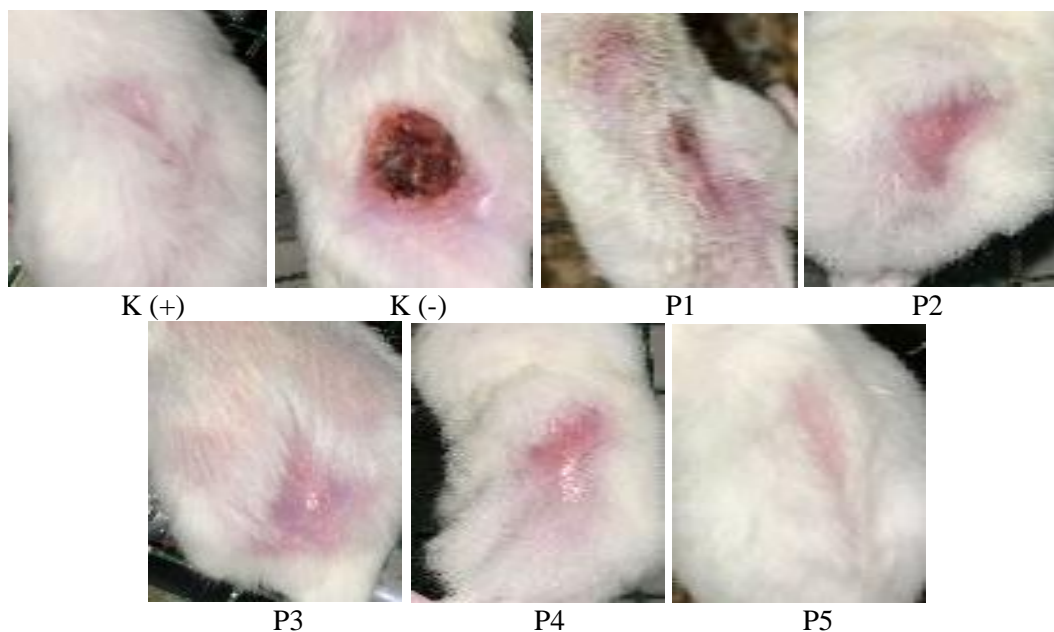
No.	Test	Reagen	Result	
1	Alkaloid	Dragendorrf	+ (brown-orange deposits form)	
2	Flavonoid	Mg + HCl	+ (orange color formed)	
3	Tannin and polyphenol	FeCl <sub>3</sub>	+ (purple-black color formed)	
4	Saponin	Hot water	+ (foam formed)	
5	Terpenoid	Liebermann-Burchard (acetic anhydrous + sulfuric acid)	+ (purple color formed)	



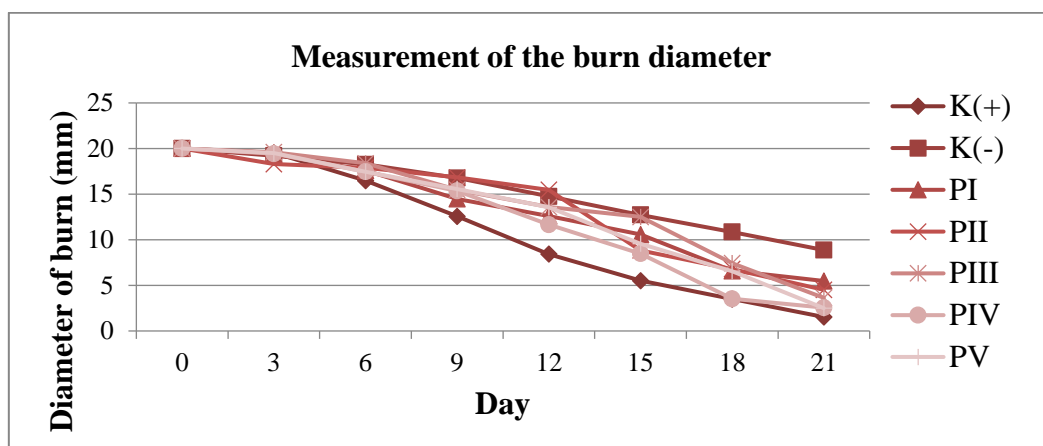
**Figure 2.** The condition of burns on the zero day. K(+) = positive control; K(-) = negative control (tween 20); P1 = ethyl acetate extract of gotu kola herb 10% in tween 20; P2 = VCO; P3 = Ethyl acetate extract of gotu kola herb concentration 10% and VCO 50% in tween



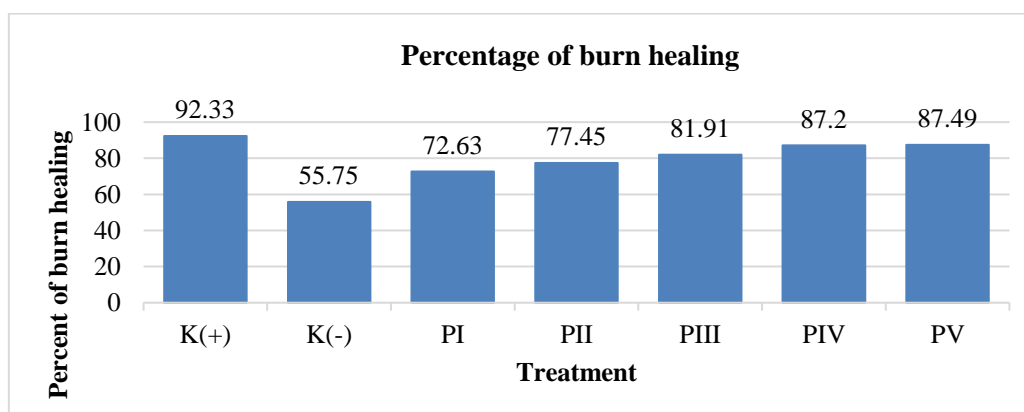
20; P4 = 5% ethyl acetate extract of gotu kola herb in VCO; P5 = ethyl acetate extract of gotu kola herb 10% in VCO.



**Figure 3.** The condition of burns on the 21<sup>st</sup> day



**Figure 4.** Graphic of measurement of the burn diameter



**Figure 5.** Graphic of the percentage of burn healing on the 21<sup>st</sup> day

The results of observations and data analysis showed that ethyl acetate extract, gotu kola herb, and VCO influenced the healing process of burns in mice. This influence is because there are compounds contained in the ethyl acetate extract of gotu kola herbs, namely alkaloids, flavonoids, tannins, triterpenoids, and saponins. While in VCO besides there are various types of fatty acids, especially medium chain fatty acids (MCT) are also rich in phenolic compounds and phytosterols<sup>5,18-20</sup>. Phytosterols can provide effects as anti-inflammatory and medium-chain fatty acids, especially lauric acid, are easily absorbed, have the potential to accelerate cell metabolism, and have anti-inflammatory activity<sup>5</sup>.

**Tabel 2.** LSD Test Analysis Results of burn healing on day 21

Group	K(+)	K(-)	P1	P2	P3	P4	P5
K(+)		0,000*	0,000 *	0,000*	0,000*	0,001*	0,682
K(-)	0,000 *		0,000 *	0,000*	0,000*	0,000*	0,000*
P1	0,000*	0,000*		0,000*	0,000*	0,000*	0,000*
P2	0,000 *	0,000 *	0,000 *		0,002*	0,000*	0,000*
P3	0,000 *	0,000*	0,000*	0,002*		0,000*	0,000*
P4	0,001*	0,000 *	0,000*	0,001*	0,000 *		0,002*
P5	0,682	0,000 *	0,000*	0,000 *	0,000*	0,002*	

Legend: \* = significant

The percentage of burn healing data was then analyzed by normality test and homogeneity test showed normal and homogeneous distributed burn healing percentage data. The next data analysis was carried out by the *ANOVA one-way* test with a value of ( $p < 0.05$ ) which is 0.000 which means that the percentage data on burn healing on the 21<sup>st</sup> day is significantly different. Thus, there are differences in healing abilities between the treatments given. To find out which treatment group gave a significant difference, statistical analysis continued with the LSD (*Least Significant Different*) test. The LSD test will show the significant difference in the percentage of burn healing data between one group and another. LSD test results can be seen in table 2.

Based on the results of statistical tests that all groups differ significantly except in P5 and K (+) which illustrates that P5 has a burn healing effect equivalent to K (+). P1, P2, P3, and P4 have significantly different values from K (-) and K (+). This shows that all treatments have wound healing activity but P1, P2, P3, and P4 activities are still below K (+) and P5. These results also show that the use of a combination of extract and VCO provides a better healing effect compared to single-use and the higher the concentration of extract and VCO given, the better the healing of burns. The use of a



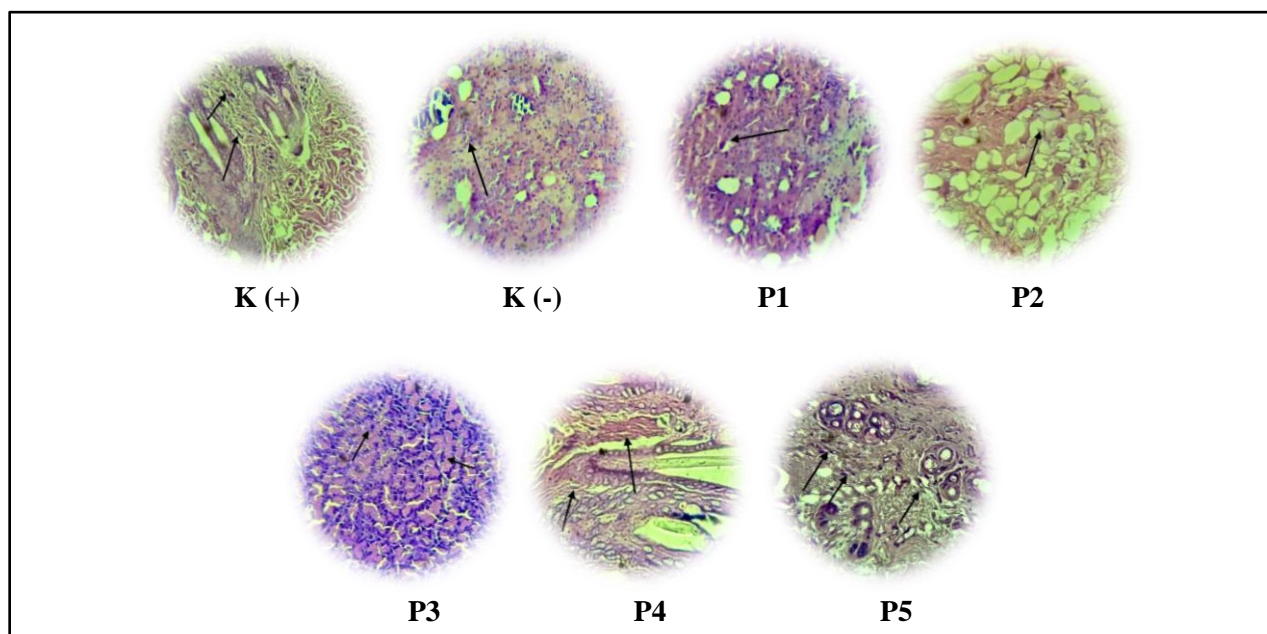
combination of 10% ethylacetate extract in VCO (P5) gives an effect equivalent to K (+), this can be interpreted that P5 can be used as an alternative to replace burn treatment on the market.

**Table 3.** Number of fibroblast cells in mouse burns on day 21

No.	Group	Number of fibroblast cells
1	K(+)	30
2	K (-)	8
3	P1	16
4	P2	20
5	P3	23
6	P4	25
7	P5	28

Based on the results of histological observations as shown in Fig.2 showed that fibroblast cells are flattened. Fibroblast cells play a role in the wound-healing process. Bainbridge rearch (2013) the wound-healing process will be disrupted if fibroblasts are not formed. The difference in the number of fibroblast cells formed due to the healing of burns is influenced by the chemical compounds contained in the test preparations given. It can be seen that the same results are shown by the presence of fibroblast cells where the positive control that has the largest percent healing has a large number of fibroblasts followed by P5 which means that it is in line with the percentage of burn healing.

According to Febram et al (2010), after injury, new fibroblast cells migrate to the wound area. The number of fibroblast cells will continue to increase The results of this study were conducted in line with Wijaya's research (2013) which described that mice given *Aloe vera* extract had an increase in the number of fibroblast cells from day 3 to day 10. The other research describes the number of fibroblast cells continuing to increase from day 2-3. During the formation of granulation tissue in a dermal wound, platelets, monocytes, and other cellular blood constituents release various peptide growth factors to stimulate fibroblasts to migrate into the wound site and proliferate, to reconstitute the various connective tissue component. Although the wound healing process is very complex and depends on the subtle interaction of many factors, normal wound healing can generally be broken down into four steps, including hemostasis (minutes to hours after injury), inflammation (days 1-3), proliferation and repair (days 4-21), and lastly, wound remodeling (days 21-365).



**Figure 6.** Histology of skin fibroblast cells in mice (*Mus musculus*) on the 21<sup>st</sup> day. The arrow (→) indicates one of the fibroblast cells.

## CONCLUSION

The combination of ethyl acetate extract of gotu kola herb (*Centella asiatica* (L.) Urban) and VCO can enhance the healing effect of burns and the combination with a 10% ethylacetate extract concentration in VCO provides effectiveness equivalent to burn preparations on the market. The combination of ethylacetate extract, gotu kola herb, and VCO has great potential to be developed as a preparation that will be distributed on the market but clinical trials are still needed

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