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Fri, Sep 23, 2022, 12:59 AM

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Fri, Sep 23, 2022, 7:57 PM

# <u>Effects of Stelechocarpus Burahol</u>, <u>BLUME</u>, leaf ethanol extract, <u>ointment on</u> burns healing

#### **ABSTRACT**

Stelechocarpus Burahol [Blume] Hook.f & Thomson, is a plant native to Indonesia that has antibacterial, antioxidant, antifungal and antiseptic properties. This study aims to determine the activity of a 70 % ethanol extract of Stelechocarpus Burahol, leaf, which is formulated as an ointment. Testing is done by modelling burns on rats with four parameters, namely the number of macrophages, the density of fibroblasts, the rate of re-epithelialisation and the measurement of the reduction in the burn area. The animals used for this study are 30 rats divided into five groups, namely the 3.25 % Stelechocarpus Burahol , leaf extract ointment group, the 6.5 % Stelechocarpus Burahol Jeaf extract ointment group, the 13 % Stelechocarpus Burahol value of the stele leaf extract ointment group, a negative control group (Vaseline flavum) and a positive control group (silver sulfadiazine [SSD]). Observations were on days 3, 7 and 14 histologically. Histological observations show a significant decrease in the number of macrophages, an increase fibroblasts density and re-epithelialisation compared to the negative control group, and at a concentration of 13 %, the ointment comparable results were comparable to silver sulfadiazine . It can be concluded that Stelechocarpus Burahol, leaf ointment extract can accelerate the healing of burn wounds, with the best results at a concentration of 13 %\_.

**Key words:** Stelechocarpus Burahol, Macrophages, Fibroblast, Re-epithelialisation

#### INTRODUCTION

Indonesia is a country with rich biological resources. Indonesia's biodiverse forests are a national asset that provides human beings with priceless benefits. One of these benefits is the use of the <u>Stelechocarpus Burahol</u>, plant as medicine. *Kepel* (*Stelechocarpus Burahol* [Blume] Hook.f. & Th) is a native Indonesian plant; it is the symbol of the Special Region of Yogyakarta and can be found in palaces in the region. A burn wound is a form of tissue damage or tissue loss caused by exposure to heat sources, i.e., fire, hot water, chemical substances, electricity or radiation. The severity of the wound is determined by two factors: The first is the width of the surface area exposed, and the second is the depth of the burn, which is categorised as a first-degree burn, second-degree burn or third-degree burn. A third-degree burn is a full-depth burn involving the epidermis, dermis and appendix parts of the skin. The healing process of a burn is very complex; thus, stabilising the general condition, providing

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healing care and offering prevention and treatment for the complications of a burn are considered costly, especially because the complications could lead to morbidity and mortality. There is a need to resolve these problems effectively, safely and reasonably affordably. One solution to these issues is the use of traditional medicine.

Based on Sunarni et al, study, 3 Stelechocarpus Burahol Jeaves have antioxidant properties. Another study also found that the Jeaves' juice at a concentration of 60 % show healing activity in open wounds on rats, with 59.84 % healing. 4 The antioxidant and antibacterial activity of Stelechocarpus Burahol, leaves, as well as its juice's role in the healing process of open wounds, suggest the possibility that 70 % ethanol extract of Stelechocarpus Burahol, leaves could boost the healing process of burn wounds. This study was, aimed at evaluation of, the potentials, of Stelechocarpus Burahol, leaves in healing burn wounds.

The study was conducted using rats that that were induced with third-degree burns. They were observed by measuring four parameters, namely the number of macrophages, the density of fibroblasts, the speed of re-epithelialisation and the decrease in the burn wound's surface area. The measurement was done by using the Image Raster 3.0 application. This parameter measurement supports the data for evaluation of the potential of Stelechocarpus Burahol leaves as burn wound medication.

# MATERIAL AND METHODS

# Chemicals

The following chemicals were used in this study: HgCl<sub>2</sub> (Merck), KI (Merck), CHCl<sub>3</sub> (Merck), FeCl<sub>3</sub> (Merck), metal Mg (Merck), methanol (Merck), xylol (Merck), paracetamol (Indofarma), ketamin HCl injections (Guardian Pharmatama Indonesia), Vaseline flavum (Pharma Laboratoria Bandung Indonesia) and silver sulfadiazine

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(SSD)/Burnazin® (Darya varia laboratoria Tbk). The following equipment was used: a microscope (Leica, Germany), a rotary vacuum evaporator (Eyela), a microtom (Thermo, USA), an analytical scale (Ohaus) and an oven (Memmert). All chemicals used in this study were of analytical grade.

#### Plant, Materials

The plant, material used was the leaf part of the <u>Stelechocarpus Burahol</u>, which was obtained from the Indonesian Research Institute for Spices and Medicines (BALITTRO). The <u>Stelechocarpus Burahol</u>, leaves was deposited, in the 'Herbarium Bogoriense', Botanical Field, Biology Research Centre, Indonesian Institute of Sciences (LIPI), with register no. 1592 / IPH.1.01 / If.07 / VI / 2017.

## Preparation of the Extract

The <u>Stelechocarpus Burahol</u> Jeaves (7 kg) were washed with running water and dried in the sun. The sample was grinded and sieved with a 40-mesh sieve. Then, 1.2 kg of the sample was, extracted with 8 L of 70 % ethanol via maceration. The maceration process was repeated twice for residue for the same duration (48 hours). The macerated <u>filtrate</u>, was then evaporated in a vacuum rotary evaporator and 40 °C water-bath until it attained the form of a thick extract. This extract was labelled *Kepel* leaves' ethanol extract (*KLEE*).

# **Preparation of the Test Animals**

Thirty Sprague Dawley male, rats weighing 150–200 g were used. They were acclimatised and given food and drink daily. The Health Research Ethics Commission of the University of Muhammadiyah Prof. Dr. Hamka approved the research procedure, with ethical approval letter number 02/17.10/017.

### **Determination of the Extract's Characteristics**

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The organoleptic observations of the KLEE included that of its shape, colour, odour and taste. Determination of loss on drying was done by using gravimetric analysis, where 2 g of thick extract was weighed in a calibration and dried at 105 °C in an oven for 30 minutes until it attained a constant weight.<sup>5</sup> Preliminary phytochemical screening was then conducted on the KLEE by testing several secondary metabolites. Following this, the extract was tested for its alkaloid content using the three reagents of Dragendorff, Mayer and Bouchardat, a flavonoid test (Shinoda and ammonia test), a tannin test (a test with gelatine and FeCl<sub>3</sub>), a saponin test (a foam test) and a steroid and terpenoid test (Liebermann Burchard test).<sup>6</sup>

#### Preparation of the Stelechocarpus Burahol Leaves' Ethanol Extract Ointment

The KLEE ointments were created with concentrations of 3.25 %, 6.5 % and 13 % (w/w) by weighing 0.325 g, 0.65 g and 1.3 g of KLEE, then adding Vaseline flavum until the ointment reached 10 g and crushing all ingredients until they were homogeneous.

# Generating Third-Degree Burns, and Treating the Test Animals

The rats were anaesthetised by using ketamine HCl injection at a dose of 40.08 mg/kg BW intramuscularly. A special metal plate 1.5 cm × 1.5 cm in diameter was then heated until it reached 100 °C and pasted for 30 seconds on the back part of the rat, which had already been shaved. After the wound had been generated, the rat was given analgetic medication orally (paracetamol tablet 51.37 mg/kg BW, a single dose). There were five test groups, namely the three KLEE groups with KLEE concentrations of 3.25 %, 6.5 % and 13%, the positive control (Burnazin®) group and the negative control group. Burnazin® was used as the positive control because it is the drug of choice in the topical treatment of burns and contains an active substance, namely SSD. Vaseline flavum (negative control) was then spread evenly over the

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wound surface twice daily (morning and afternoon) for each treatment for 14 days.<sup>7</sup> The dosing and treatment of the tested animals is presented, in Table 2.

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### **Histology Sample Preparations**

Skin tissue samples were taken from the biopsy of the burn wound and the subcutaneous fat tissue. The sample was taken on the third, seventh and 14<sup>th</sup> days after giving the test sample. Before the samples were taken, the test animals were anaesthetised using a ketamine injection. The specimens were then fixated by using a Buffer Neutral Formalin 10 % solution

#### **Histopathology Sample Preparations**

The tissue was fixated by using a Buffer Neutral Formalin (BNF) 10 % solution and left at room temperature for 24 hours. The tissue was then cut into pieces and placed in a specimen container made from plastic. Subsequently, it went through a dehydration process done with a graded alcohol concentration of 70 %, 80 % and 90 % for 2 hours each. Later, the clearing process was conducted using xylol to eliminate alcohol traces. After this, the moulding process was done by using paraffin blocks, and the moulds were stored in the fridge. These paraffin blocks were then sliced thinly, around 6–8 µm, by using a microtom (Thermo, USA). Afterwards, these pieces were floated on 60 °C warm water (a water-bath) to stretch the tissue and avoid creasing. These specimens were then lifted and placed on object glass to do the haematoxylin and eosin (HE) staining and were later observed under a microscope (Leica, Germany).8

# Data Analysis

Data were analysed statistically using One-way Analysis of Variance (ANOVA), followed by the Tukey–Kramer Post Hoc test for multiple comparisons. p < 0.05 was considered to represent statistical significance.

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# **RESULT AND DISCUSSION**

Stelechocarpus Burahol ethanol leaf extract was obtained using the maceration method, with 70 % ethanol as the solvent. The macerated residue was then evaporated by using a vacuum rotary evaporator at 50 °C until a thick extract was obtained. These 70 % KLEE ointments' characteristics were that they were semi-solid, and they had a unique smell, bitter taste and blackish-green colour. The phytochemical screening results showed that the extract contained flavonoids, saponin and tannin. This extract yielded 11.25 %, and the loss on drying was 8.92 %. Organoleptic and homogeneity observations of the 70 % KLEE ointment showed homogenous consistency, with the colour of the ointment darkening as the extract's concentration increased.

The parameters observed from the histology samples were the number of macrophages, the density of fibroblasts and the thickness of re-epithelialisation, which were obtained by observing 10 field views. The width of the burn wound was measured by processing the image using the *Macbiophotonic Image J* programme.

The burn wound model was made by inducing third-degree burns on the rats, which damaged the tissue until the dermis. The prepared samples used were 70 % KLEE ointments at concentrations of 3.25 %, 6.5 % and 13 %; Silver sulfadizine, for the positive control group; Vaseline flavum for the negative control group. Silver sulfadiazine, was chosen for the positive control group because it is the drug of choice, for topical burn wound treatment due to it containing SSD as its active agent. SSD inhibits bacterial DNA replication and damages the bacterial cell wall. The silver content in SSD also has antibacterial functions that help cleanse the wound, thus preventing tissue regeneration from being compromised. 9,10,11 The sample preparation in the form of ointment with a Vaseline flavum base had hydrocarbon characteristics, making it difficult to dissolve in water, thus prolonging contact between the medical ingredients and the skin. 12

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A burn wound is a form of tissue damage or loss caused by exposure to heat sources, i.e., fire, hot water, chemical substances, electricity or radiation.<sup>1</sup> Generally, the healing process is divided into three phases. The early phase of inflammation begins immediately after the injury occurs and involves the elimination of dead tissue to prevent infection. The second phase is the proliferation phase during which the balance between scar tissue formation and tissue regeneration occurs. The third phase is the maturation phase, which is aimed at maximising the structural strength and integrity of the wound.<sup>13</sup> The healing process of a burn wound has similarities with other wound healing processes, yet the durations of each of its phases differ.<sup>14</sup>

The macrophage cell calculation process was done by taking an image with a light microscope and then observing it and counting the macrophages using the *Image Raster 3.0* application. The number of macrophages found on the third-, seventh- and  $14^{th}$ -day observations showed significant differences in every group (p < 0.05). On the third day of observation, the number of macrophage cells in the 13 % concentration group was higher than that in the 6.5 %, 3.25 % and negative control groups (Table 3). This is because the macrophages became the predominant cell on the third day after the wound occurred. A macrophage is an effective cell in the phagocytosis process, as it phagocytoses pathogens, foreign bodies and other unnecessary cells. Macrophages in the tissue originate from monocyte cells in the blood that migrate to connective tissue. In the case of inflammation, the number of monocytes that migrate to the connective tissue will increase; thus, the macrophages are activated.  $^{15,16}$ 

On the seventh and 14<sup>th</sup> days of observation, the number of macrophages in the 13 % concentration group was compafrable, to that of the positive control group and the 6.5 % concentration group, yet it differed significantly from that of the negative control group and the 3.25 % concentration group (Table 3). This result shows that the

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inflammation process in the negative control group was still ongoing. The high number of macrophages in the negative control group indicated prolonged inflammation due to the growth of more microorganisms in the burn wound. The absence of active ingredients in the negative control group might have been the reason for the presence of microbes and the amount of tissue damage that the macrophage needed to phagocytose in the wound area. Thus, the wound healing process in the negative control group was prolonged and led to the proliferation phase being delayed. In the 13 concentration group, as well as in the other concentration groups, the number of macrophages was lower, indicating the end of the inflammation phase and the beginning of the proliferation phase.

During the proliferation phase, the macrophage is also needed to produce growth factors such as the fibroblastic growth factor and the transforming growth factor-Beta (TGF- $\beta$ ). Macrophages also activate fibroblasts and increase their migration, which plays a role in the tissue formation process and produces collagens. 18,27

The administration of the KLEE ointment can speed up the inflammation phase of burn wounds. This effect is\_related to the presence of secondary metabolite compounds in the <u>Stelechocarpus Burahol</u> leaf extract, such as flavonoids, saponin and tannin, that help the healing process by functioning as antioxidant and antimicrobial agents that affect wound healing. Tannin and saponin also have antiseptic properties. For example, saponin can trigger the vascular endothelial growth factor (VEGF) and increase the number of macrophages that migrate toward the wound area, thus increasing the production of cytokines, which activate fibroblasts in the wound tissue. 20,25

The third-day observation showed that the mean densities of fibroblasts in all of the concentration groups were significantly different from those of the negative control

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group. Figure 1 shows that the fibroblast densities in all of the concentration groups were still low since the fibroblast was yet to play a role in the inflammation phase. Fibroblasts start to play a part in both the proliferation and maturation phases. The seventh-day observation showed that the mean density in the 13 % concentration group was not significantly different from that of the positive control and 6.5 % concentration groups. A significant difference was only found between the negative control and 3.25 % concentration groups (Table 4). This is because the increased number of fibroblast cells triggers an increase in the number of collagen fibres, which speeds up the process of wound healing. The 14th-day observation showed that the mean density of fibroblasts in the 13 % concentration group was not significantly different from that of the positive control and 6.5 % concentration groups. This shows that the proliferation of fibroblasts determines the result of wound healing. Fibroblasts produce an extracellular matrix, which is replaced by collagen. Fibroblasts disappear immediately as the collagen matrix fills the wound cavity, and the formation of neovascular decreases through the apoptosis process.

Re-epithelialisation thickness was another wound-healing parameter that did not show any significant results during the third day of observation. On the seventh and 14<sup>th</sup> days of observation, however, the 13 % concentration group had a value equivalent to that of the positive control group: The mean value of re-epithelialisation thickness was 13.65µm ± 0.77 (Table 5). This could be interpreted as wound proliferation started on the fourth day and continued until the 14<sup>th</sup> day, when epithelial cell proliferation closed the wound that was affected by the epithelial cells' mitosis activity around the wound's edges. Subsequently, the mature epithelial cells moved from the wound's edges to the dermis, migrating to and attaching at the centre part of the wound.

In Figure 2, it can be seen that the positive control group and 13 % Stelechocarpus Burahol extract, ointment concentration group had thicker epithelial formation compared to the other test groups. During the proliferation phase, the thickness of the epithelial layer continues to increase until the wound area closes completely. The epithelium layered in the epidermis is composed of many layers of cells called keratinocytes. These cells are constantly renewed through the mitosis of cells in the basal layer, which are gradually shifted to the epithelial surface.<sup>22</sup>

The observation of the wound surface area was done using the Macbiophotonic Image J programme. Based on the microscopic observation from the first day until the 14th day, there was a decrease in the wound surface area. On the first day, the wound appeared pale white and was still wet. On the third day, the wounds in all test groups appeared large and swollen, which indicated that the inflammation process was still in progress. This is because the inflammation phase's role is to prevent the entry of bacteria, eliminate dirt particles from the wound tissue and prepare the wound for the advanced healing process.<sup>13</sup> On the seventh day, the wound appeared reddish-brown in the positive control group, while in the 6.5 % and 13 % concentration groups, the wounds showed the formation of scabs and shrinkage. On the 14th day, the wound had dried out, and the scabs started to fall off. The shedding of scabs indicates the growth of new cells, which help to speed up the process and attach the wound's edges. 23,24 The wound constriction percentage in the 13 % concentration group was 92.32 %, and in the positive control group, it was 95.31 %, on the 14th day of observation (Table 6). This proves that the 13 % KLEE ointment had the fastest rate of healing burn wounds, with a percentage that was proportional to that of the positive control group (Silver sulfadiazine).

CONCLUSION

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The 70% <u>Stelechocarpus Burahol</u> etnanol leaf extract, ointment with 13 % concentration shows burn wound healing acceleration activity, a decreased number of macrophages, wound surface area and an increase fibroblast density and reepithelialisation thickness.

CONFLICT OF INTEREST

No conflict of interest.

#### **ACKNOWLEDGEMENT**

Our gratitude is conveyed to the Research Institute and the Faculty of Pharmacy of the Prof. Dr. Hamka University that funded this research (internal grant with contract number 280/F.03.07/2017). We are also thankful to the Pathology Anatomy Laboratory of the Faculty of Medicine of Indonesia University for its provision of laboratory facilities.

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Table 1. Composition of the <u>Stelechocarpus Burahol</u> Leaves' Ethanol Extract Ointment

Concentration of the KLEE ointment	KLEE weight (g)	Vaseline flavum (g)
3.25 % concentration	0.325	9.675
6.5 % concentration	0.650	9.350
13 % concentration	1.300	8.700

Table 2. Dosing and Treatment of the Tested Animals

Days		Test animal	groups		
	3.25 % KLEE oint.	6.5 % KLEE oint.	13 % KLEE oint.	Negative control	Positive control
1	Shearing rate	S			
2	Rats were bu	urned with a spe	cial metal plate	at 100 °C for	5 seconds.
3–16	Rats were given 3.25 % KLEE ointment.	Rats were given 6.5 % KLEE ointment.	Rats were given 13 % KLEE ointment.	Rats were given Vaseline flavum.	Rats were given Burnazin <sup>®</sup> .
3,7,14		e was taken on o istological obse	•	en and 14 afte	er burns were

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Table 3. Effect of Stelechocarpus Burahol ethanol extract ointment on Macrophages density

Groups	The third day (cells±SD)	The seventh day (cells±SD)	The 14 <sup>th</sup> day (cells±SD)
Positive control	139.3±8.944	104.725±5.998	94.975±6.602
Negative control	109.875±7.221	119.95±7.083	112.3±4.231
3.25 % KLEE oint.	116.421±5.549 <sup>b</sup>	114.900±5.780 <sup>b</sup>	106.37±3.398 <sup>b</sup>
6.5 % KLEE oint.	124.175±7.322 <sup>b</sup>	109,925±4.332 <sup>b</sup>	99.875±4.678 <sup>b</sup>
13 % KLEE oint.	134.3±6.710 <sup>a,b</sup>	107.025±4.0343 <sup>a,b</sup>	97.025±3.927 <sup>a,b</sup>

Note: a not significantly different from the positive controls (p > 0.05)

Table 4. Effect of Stelechocarpus Burahol ethanol extract ointment on Fibroblast Density

Groups	The third day (cells±SD)	The seventh day (cells±SD)	The 14 <sup>th</sup> day (cells±SD)
Positive control	58.65±4.577 <sup>a</sup>	73.15±4.577 <sup>a</sup>	34.87±3.4674 <sup>a</sup>
Negative control	29.5±2.8191	49.55±3.1030	50.9±4.9139
3.25 % KLEE oint	45.95±3.2602 <sup>a</sup>	63.2±4.1688ª	43±3.9603ª
6.5 % KLEE oint	49.75±4.2534 <sup>a</sup>	67.35±4.3922 <sup>a</sup>	38.95±3.5388ª
13 % KLEE oint	51.2±3.6685 <sup>a</sup>	70.18±4.1372 <sup>a,b</sup>	37.02±3.0842 <sup>a,b</sup>

Note: a significantly different from the negative controls (p < 0.05)

Table 5. Effect of <u>Stelechocarpus Burahol</u> ethanol extract ointment Reepithelialisation Thickness

Groups	The third day (cells±SD)	The seventh day (cells±SD)	The 14 <sup>th</sup> day (cells±SD)	
Positive control	15.86±0.63	21.67±0.67	30.67±0.90	

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<sup>&</sup>lt;sup>b</sup> significantly different from the negative controls (p < 0.05)

b not significantly different from the positive controls (p > 0.05)

Negative control	7.72±0.55	10.84±0.60	25.06±0.94
3.25 % KLEE oint.	10.78±0.58 <sup>b</sup>	15.79±0.69 <sup>b</sup>	26.10±0.74 <sup>b</sup>
6.5 % KLEE oint.	12.72±0.59 <sup>b</sup>	18.22±0.65 <sup>b</sup>	28.95±0.73 <sup>b</sup>
13 % KLEE oint.	13.65±0.77 <sup>b</sup>	21.66±0.73 <sup>a</sup>	55±0.90 <sup>a,b</sup>

Note:  $^{a}$  = not significantly different from the positive controls (p > 0.05)

Table 6. Effect of Stelechocarpus Burahol, ethanol extract on percentages of Burn Healing,

Days to	Negative control	Positive control	3.25 % KLEE oint.	6.5 % KLEE oint.	13 % KLEE oint.
1	1.22±0.45	1.23±0.07	1.55±0.31	1.60±0.33	1.83±0.35
2	1.35±0.55	3.43±0.74 <sup>*</sup>	2.32±0.69	3.57±1.02	3.18±0.55 <sup>*</sup>
3	1.84±1.00	7.08±2.16 <sup>*</sup>	3.62±1.46	50±1.60	6.52±2.93 <sup>*</sup>
4	3.55±2.23	10.87±2.24 <sup>*</sup>	7.40±4.07	8.50±1.77	10.11±3.12 <sup>*</sup>
5	6.44±3.20	16.72±2.80 <sup>*</sup>	11.16±6.24	12.44±3.86	15.50±5.93 <sup>*</sup>
6	8.84±4.04	22.98±3.04 <sup>*</sup>	13.52±6.41	15.89±3.58	20.33±6.02 <sup>*</sup>
7	11.02±3.30	26.92±2.42 <sup>*</sup>	17.50±5.50	21.09±5.81 <sup>*</sup>	27.13±3.54 <sup>*</sup>
8	13.06±3.40	35.84±8.54 <sup>*</sup>	21.92±7.91	24.71±4.92 <sup>*</sup>	31.03±3.10 <sup>*</sup>
9	15.81±2.65	46.77±10.71 <sup>*</sup>	26.27±7.36	29.75±5.80 <sup>*</sup>	37.93±3.71 <sup>*</sup>
10	18.25±2.54	57.46±7.06 <sup>*</sup>	32.54±7.94 <sup>*</sup>	40.33±3.58 <sup>*</sup>	48.94±6.57 <sup>*</sup>
11	20.04±2.20	67.41±6.85 <sup>*</sup>	39.72±4.09 <sup>*</sup>	46.64±6.48 <sup>*</sup>	57.04±3.3 <sup>*</sup>
12	22.67±1.85	84.00±5.70 <sup>*</sup>	44.25±2.74 <sup>*</sup>	51.70±5.47 <sup>*</sup>	76.55±6.56 <sup>*</sup>
13	25.09±1.51	92.25±3.00 <sup>*</sup>	48±54±1.44*	56.30±4.12 <sup>*</sup>	84.34±4.67 <sup>*</sup>
14	26.72±1.77	95.31±2.72 <sup>*</sup>	51.64±2.49 <sup>*</sup>	61.70±4.34 <sup>*</sup>	92.32±2.58 <sup>*</sup>

Note:  $^*$  = significantly different from the negative controls (p < 0.05)

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 $<sup>^{\</sup>rm b}$  = significantly different from the negative controls (p < 0.05)

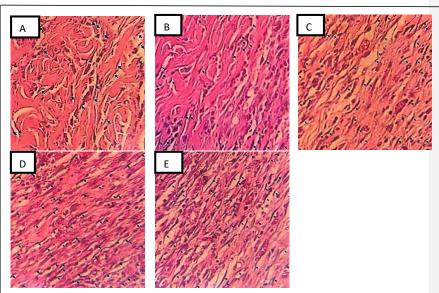


Figure 1. The histological picture on day 7 at 400x magnification under a light microscope (*Olympus*). Arrows indicate fibroblasts: A) negative control group; (B) 3.25 % KLEE ointment; (C) 6.5 % KLEE ointment; (D) 13 % KLEE ointment; E) positive control group. KLEE: *Kepel* leaves' ethanol extract

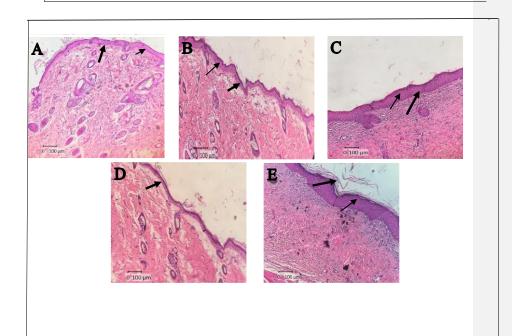


Figure 2. The histological picture on day 14 at 100x magnification, observed under a light microscope (*Olympus*). The arrows show re-epithelialisation: (A) 3.25 % KLEE ointment; (B) 6.5 % KLEE ointment; (C) 13 % KLEE ointment; (D) negative control group; E) positive control group. KLEE: *Kepel* leaves' ethanol extract



# **REVIEW FORM**

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# A. MANUSCRIPT

Journal	Tropical Journal of Natural Product Research
Manuscript Number	TJNPR AUG 115 AR
Type of paper	
Title of paper	POTENCY OF KEPEL LEAF ETHANOL EXTRACT (Stelechocarpus burahol [BLUME]) FOR BURNS (SEETHE MODIFIED TOPIC)
Name of Authors	

# B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Abstract	Rewrite the abstract to effect the corrections as indicated
Introduction	Ok. Effect the corrections as indicated
Methodology	The methods chosen are suitable and adequate for the study. Effect the corrections as indicated
Results	Ok. Effect the indicated corrections
Discussion	Ok. Effect the indicated corrections
Conclusion	The conclusion is supported by the results. Effect the correction as indicated
References	ok
Figures, Tables	ok

# C. REVIEWER'S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.	
NIL NIL	

# D. REVIEWER'S RECOMMENDATION

Please mark with "X" one of the options.

You state the article should:

Publish as it is	
Accept with minor revisions (editor will check), specific comments to the editor below	
Accept with moderate revisions as recommended by reviewer	X
Accept with major corrections (the article should be thoroughly changed)	
Full article	X
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Reject for reasons noted by the reviewer (please be specific)	

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