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Comparison of Burn Wound Healing Ability between Ethyl Acetate Extract Gel of Betel Nut (*Areca catechu*) and Snake Fruit Seed (*Salacca zalacca*) in Rats

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Abstract

The burn is the most common type of trauma that is found over the world. Betel nut and snake fruit belong to the family of Arecaceae have various phytochemicals that have antioxidant activity to accelerate the wound healing process. This study was aimed to explore the wound healing effect of ethyl acetate extract from betel nut and snake fruit. This study used 20 rats that divided into four groups include Standard (Bioplaceton©), Control, Gel of Snake fruit seed, and Gel of Betel nut. The gel was formulated from the ethyl acetate extract of snake fruit seed and betel nu by the maceration process. A steel plate conducted the burn under ketamine anesthesia. The parameters that were evaluated were wound contraction and epithelialization period. The result of this study showed that the wound contraction showed a significant difference up to 4 days of observation (P-value < 0.05). While, the group of standard, gel of snake fruit seed, and gel of betel nut have a significant difference in epithelialization period against the control group (P-Value < 0.05). Overall, both of the gel extracts have potential acceleration burn wound healing. However, the gel of betel nut is more effective than the gel of snake fruit seed.

Keywords: Betel nut; Snake fruit; Ethyl Acetate; Gel.

1. Introduction

The burn is the most common type of trauma that is found over the world. According to the WHO data, there are about 265.000 deaths due to burn wounds. The burn was found in low and middle countries.

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The annual incidence of the burn wound is estimated at around 187 per 100,000 population in the Eastern Mediterranean and 243 per 100,000 people in South-East Asia. The type of burn was various that may be due to flame burns, scalds, chemical burns, electric burns, and radiation burns [1]. Meanwhile, there has no recent surveillance data for burn wounds in Indonesia. The surveillance data of the health ministry of Indonesia in 2007 that known as Basic Health Research, showed that the prevalence of the burn wound is 2.2 percent. However, several studies were conducted locally. Primadina (2018) reported that there was 35 patients suffer from burn in the plastic surgery department of Syarif Ambami Rato Ebhu Government Hospital during 2015-2016, and these patients were most common aged between 41-60 years old [2]. On the other hand, Suzan and Andayani (2017) reported that the prolonged dehydration that due to the burn wound could lead to various morbidities like acute kidney injury, septic shock, reduction of liver function, or amputation [3]. Based on the fact above, it is needed to found more effective therapy to lower the effect of the burn wound and other complications due to the delayed recovery of the burn wound. Various natural sources have phytochemicals content that can accelerate wound recovery. The fruits that come from the family of *Arecaceae* like snake fruit and betel nut has several types of phytochemicals. The β-Sitosteryl-3β-glucopyranoside-6'-O-fatty acid Esters that can be found in the seeds of snake fruit and alkaloid, saponin, phenol, glucose, triterpenoid, and glycoside that can be found in the betel nut. These phytochemicals have antioxidant activity that can lead to accelerating the wound healing process [4,5]. Finally, this study was aimed to explore the wound healing effect from ethyl acetate extract of the betel nut and snake fruit seed among the rats as the animal trial.

2. Methods

This study was an experimental study that used *a pre-post-test control group design.* This study was conducted at the Riwandi Pet Shop and Animal House. The number of sample in this study was determined using G*Power 3.1. Based on the result of G*Power 3.1, the number of samples was at least eight rats for four groups. However, this study was used 20 rats that were divided into four groups. Betel Nut and Snake fruit that was used in this study were gotten from a traditional market in the Medan, North Sumatera. The betel nut and snake fruit seed were dried and meshed into dry simplicia. The dry Simplicia was macerated using ethyl acetate as the solvent for five days, the ratio of the dry simplicia ad solvent was 1:7.5.

Table 1: Formulation of the Gels

Materials	**Gel Base**	**Gel of 2% *Areca catechu***	**Gel of *2% Salacca zalacca***
Concentrated form of *Areca catechu* extract	-	2g	-
Concentrated form of *Salacca zalacca* extract	-	-	2 g
Carboxyl-methyl-Cellulose Sodium (Na-CMC)	5g	5g	5g
Glycerin	10g	10g	10g
Propylene-glycol	5g	5g	5g
Aquadest	100g	100g	100g

After five days, it was filtered, and the residue was re-macerated in the same way as before, while the filtrate was collected to evaporate by rotary evaporator—the result of evaporation known as concentrated form extract [6,7]. Both of ethyl acetate extract was screened the phytochemical content by Widowati and his colleagues

(2018). The phytochemicals were Alkaloid, Steroid/Triterpenoid, Saponin, Flavonoid, Tannin, and Glycoside [8]. The concentrated form of either betel nut or snake fruit seed extract was mix with several materials to form a gel. The material was shown in the following table. The rats that were used in this study were prepared a week before the intervention for acclimatization. All rats were burned by place a steel plate sized 2 x 2 cm at 90^{o}C for 10 seconds at the dorsal of each rat. To reduce the pain of the injury, the rats were injected 50 mg/kg BW IM of Ketamine. Then the rats were divided into four groups of intervention. The following table showed the intervention of each group.

Table 2: The Groups of Intervention

Groups	Intervention
Control	Apply the gel base every day
Standard	Apply the Gel bioplacenton® every day
Gel of 2% Arecha catechu	Apply the gel of 2% Arecha catechu every day
Gel of 2% Salacca zalacca	Apply the gel of 2% Salacca zalacca every day

These was observed for 21 days. The diameter of wound was measured every 3 days and the observation was ended when the scar of the wound was exfoliated. The diameter of the wound was calculated using the following formulation to determine the wound contraction. The time that was needed by the scar to exfoliate was known as the epithelialization period.

$$[(a-b)/b] \times 100\% \qquad (1)$$

a = Initial wound diameter

b = Specific day wound diameter

The descriptive statistic analyzed phytochemical contents, wound contraction, and epithelialization period. The analysis was followed by the Kruskal-Wallis and Mann-Whitney Test as a non-parametric statistic for wound contraction and epithelialization period. These analyses were purposed to compare the wound contraction and epithelialization period between each group of rats.

3. Result and Discussion

Table 3: Phytochemical Screening of Both of Ethyl Acetate Extracts

Phytochemicals	Betel Nut	Snake Fruit Seed
Alkaloid	+	+
Steroid/Triterpenoid	+	-
Saponin	-	-
Flavonoid	+	+
Tannin	+	+
Glycoside	+	+

The following table showed the result of phytochemical screening of both ethyl acetate extract. Based on table

3 above, both of ethyl acetate extract contain alkaloid, flavonoid, tannin, and Glycoside. However, ethyl acetate extract of betel nut content Steroid/Triterpenoid that was not found in the ethyl acetate extract of snake fruit seed. Meanwhile, the other study had been explored the phytochemicals of aqueous extract of betel nut that was alkaloid, saponin, phenol, glucose, triterpenoid, and glycoside. This difference may be due to the difference of the solvent that used to the extraction process [4]. Two parameters measured evaluation of the burn wound healing ability between both of the ethyl acetate include wound contraction and epithelialization period. The following table showed the comparison of wound contraction in the groups of the samples.

Table 4: Comparison of the Wound Contraction

Groups	**Wound Contraction (%)***						
	Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**	**Day 12**	**Day 14**
Control	0 (0)[a]	0 (0)[a]	0 (0)[a]	0 (0)[a]	0 (0)[a]	8.34 (5.55)[a]	16.67 (11.11)[a]
Standard	0 (5.56)[a]	5.56 (5.56)[b]	5.56 (5.55)[b]	5.56 (5.55)[b]	5.56 (11.11)[b]	25 (11.11)[b]	36.11 (22.22)[b]
Gel of *Arecha catechu*	2.78 (5.56)[a]	5.56 (11.11)[b]	5.56 (11.11)[b]	5.56 (11.11)[b]	5.56 (11.11)[b]	36.11 (11.11)[c]	55.56 (11.11)[c]
Gel of *Salacca zalacca*	2.78 (5.56)[a]	5.56 (5.56)[b]	5.56 (0)[b]	5.56 (0)[b]	5.56 (0)[b]	27.78 (16.66)[b]	61.11 (38.89)[c]
P-Value**	**0.392**	**0.025**	**0.025**	**0.025**	**0.025**	**0.005**	**0.009**

*Data is expressed as Median (Range). The small letter in the same column is significant at P-Value < 0.05. **The P-Value that was obtained by the kruskall-wallis test.

Based on table 4 above, the significant difference of wound contraction was observed after 12-14 days of observation between standard, the gel of betel nut (*Arecha catechu*), and snake fruit seed (*Salacca zalacca*). However, a significant difference had been observed between control and other groups after four days of observation. At the end of observation (14^{th} day), the group of intervention that was received gel of snake fruit seed (*Salacca zalacca*), followed by the group of intervention that was received gel of betel nut (*Arecha catechu*), standard, and control.

Table 5: Comparison of Epithelialization Period

Groups	**Epithelialization Period (Days)***	**P-Value****
Control	0 (0)[a]	0.012
Standard	14 (2)[b]	
Gel of *Arecha catechu*	12 (2)[b]	
Gel of *Salacca zalacca*	12 (2)[b]	

*Data is expressed as Median (Range). **P-Value was obtained by the Kruskal-Wallis test.

Based on table 5 above, there was a significant difference in the epithelialization period that was observed between the control group and other groups. The group of intervention that was received gel of betel nut (*Arecha catechu*) and snake fruit seed (*Salacca zalacca*) had the fastest epithelialization period. The previous study about the topical drug of the betel nuts showed that the ointment of betel nut had wound healing activity. Based on the Verma and his colleagues (2012) study, the tendency of 13th day wound contraction observation and epithelialization period among the rats that received ointment of 2% betel nut extract were around 87.23% and 15.67 days, respectively. Various factors affected both of the results; one of these factors is the solvent that was used in the study[9]. However, the Handayani (2016) showed a similar result with the recent study. Handayani (2016) reported that in the 14th day of observation, the average of wound contraction was 84.33% among the rats that received ointment of 20% betel nut [10]. Other studies also explore the oral effect of ethanol extract from betel nut. Bharat and his colleagues (2014) reported that ethanol extract of betel nut in the low and high dosage improved the wound contraction. The wound contraction showed a significant difference against the control group since the 13th day. The wound contraction in the 13th day between the lower and higher dosage was 72.34% and 77.08%, respectively. Meanwhile, the average epithelialization period for lower and higher dosage was 16.33 days and 17.67 days, respectively [6]. On the other hand, the study that explores the effect of snake fruit seed is not available yet. However, in the in vitro study that explores the therapeutic effect of snake fruit that may be facilitated, the wound healing effect has been done. These studies reported that the snake fruit peels contained caffeic acid, chlorogenic acid, ferulic acid, protocatechuic acid, rutin, and bound ligand. These phytochemicals that are found in the snake fruit peels have anti-aging and antioxidants effects. Although, there is a limit number of studies that explore the therapeutic effect of the seed [11,12]. The comparison of gel of betel nut and snake fruit seed showed that the gel of betel nut had better-wound healing activity than the gel of snake fruit. It may be due to the presence of a triterpenoid that is not found in the snake fruit seed. Triterpenoid has antioxidant activity that can neutralize the ROS and improve the microenvironment of the wound that accelerates the wound healing process [13].

4. Conclusion

Overall the ethyl acetate extract of betel nut and snake fruit seed has the wound healing activity. However, the betel nut has a more effective healing effect than the snake fruit seed.

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