

The Effectiveness of Ointment of Patah Tulang Stem's (*Euphorbia Tirucalli*) Ethanol Extract for Burn Wound Healing on White Rats (*Rattus Norvegicus*)

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Abstract. Plants of patah tulang (*Euphorbia tirucalli*) is one of the plants that has been widely known by the public for a long time and is used as a traditional medicine, one of them as a wound medicine. Plants of patah tulang contains saponins, tannins and flavonoids that may help in wound healing. This study aimed to determine the effect of ointment of patah tulang stem's ethanol extract with various concentration (5%,10%,20%) for burn wound healing on white rats. Long of burn wound healing was determined by the percentage of burn wound healing obtained from the average of burn wound diameter on white rats in 26 days was observed. The data of burn wound healing percentage was analyzed with Kruskal-Wallis Test. The results showed that ointment with 20% extract concentration had the same effect as positive control in burn wound healing on white rats ($p>0,05$).

1. Introduction

According to the World Health Organization (WHO) in 2012, burn injuries are included into the 15th leading cause of death in children and young adults aged 5-29 years. The mortality rate due to burns approximately 195,000 lives per year [1]. Burns can occur in everyday life, especially in households such as scalding water, exposure to fire, chemicals, electricity and radiation. Burns that are often found are shallow burns of degree II [2].

Indonesia has a diversity of plants that can be used as a traditional medicine. Traditional medicine for generations has been widely used by Indonesians as an alternative treatment option [3]. One of them is the plants of patah tulang.

Plants of patah tulang (*Euphorbia tirucalli*) are empirically known as wound medicine. An ethanol extract of patah tulang stems at 10% concentration can wound healing because it contains flavonoid compounds, saponins and tannins. The study of [4] also showed that in patah tulang stem's ethanol extract contained flavonoid compounds, saponins and tannins. Saponins contained in plants can stimulate the formation of collagen that plays a role in the wound healing process [5], while tannins and flavonoids have activity as antiseptic and antibacterial [6]

This research needs to be done to know the effect of ointment of patah tulang stem's ethanol extract to burn wound healing as proof that it can be used as other wound medicine besides medicine for cutting wound [7].



2. Methods

2.1. Materials

Patah tulang stem's, ethanol 95%, hydrochloric acid 5 M, magnesium powder, FeCl₃, aquades, vaselin album, ethyl chloride spray, white rats (*Rattus norvegicus*) of wistar strain(150-200gr), erlenmeyer, porcelain cup, stirring bar, measuring cylinder, analytical balance (Shimadzu), oven (Mettler), blender, knife, waterbath, evaporator (Ika RV-10 basic), mortar dan stamper, weighing paper, ointment cup 25 g, pH meter, zinc metal plate (2cm), white rats cage, steril gauze, shearers, ruler or steering wheel.

2.2. Licensing of ethical clearance

This research using test animals is required to permit ethical clearance to ensure that this test does not use methods that violate animal testing regulations. This permission is submitted to the ethics commission in Faculty of Medicine, University of Jenderal Soedirman.

2.3. Determination of plants

Plants was obtained in Mersi Village, East Purwokerto, Banyumas are determined to find out the true identity of the plant being tested. Determination was done at the Laboratory of Plant Taxonomy, Faculty of Biology, University of Jenderal Soedirman.

2.4. Extract preparation

Patah tulang stems are cut into pieces and dried to dry in a room that is not exposed to sunlight for 1-2 days. Next, a sample of 1075 g was put into the oven at 500°C for 4 days. The dried samples were then weighed again and blended. 760 grams of simplicia powder was soaked using 95% ethanol for 5 days with occasional stirring. The residue and filtrate are separated by using filter paper. Conducted remaseration by adding 625 ml ethanol to residue for 2 days with occasional stirring. The overall filtrate obtained was evaporated using an evaporator, then evaporated using a waterbath at a temperature of 600°C to obtain a concentrated extract.

2.5. Compounds identification

2.5.1. Flavonoid test (shinoda method). The extract was taken 2 g and added enough magnesium powder and 10 drops of 5 M hydrochloric acid. The presence of flavonoids is characterized by the formation of reddish black [6].

2.5.2. Tannin test. The extract was taken 2 g and added with 10 ml of hot water, then spilled iron (III) chloride. The presence of tannins in the sample is characterized by the appearance of a blackish-green color [6].

2.5.3. Saponin test. The extract was taken 2 g and added with 10 ml of aquades then shaken strongly for approximately 1 minute. Further stays for 10 minutes and observed the foam or foam formed. The presence of sapogenin compounds in the sample is characterized by the formation of a stable foam for 10 minutes with a height of 3 cm [6].

2.6. Ointment formulation and evaluation

Table 1. Formula of Ointment of Patah Tulang Stem's Ethanol Extract (Qomariah, 2014)

Material	Formulation			
	FI 5%	FII 10%	FIII 20%	NC (-)
Extract	1,5 g	3 g	6 g	-
Vaselin Album	28,5 g	27 g	24 g	30 g
Mf. Ungt	30 g	30 g	30 g	30 g

Note: X brand ointment as a positive control

Ointment formulation was done, then it was evaluated such as organoleptic, homogeneity, pH, disperse, and adhiveness test. Organoleptic test was conducted by observing ointments from its form, smell, and colour [8] And then, each ointment formula is applied to a transparent piece of glass to know the homogeneity of ointment. The ointment is said to be homogeneous if there are no coarse grain presence. [9].

pH test uses pH meter in order to know the acidity of ointment. Furthermore, disperse test was done by method of 0.5 g ointment is placed on a round glass (d: 15 cm), other glass is placed on it and left for 1 minute. The diameter of the ointment was measured, after which 100g of load was added and then measured a constant diameter. While for the adhesiveness test was done by method of 0.5 g ointment on top of object glass and other object glass placed on the ointment. Then pressed with a load of 1 kg for 5 minutes. Mounted object glass on the test equipment, load as much as 80 g then released and recorded the time until the second glass of this object is released [2].

2.7. Test animal

Male rats were used with age 2 months and BB \pm 150-200g. And then, methodology employed in the care, use, inflicting of wound, administration of treatment and evaluation of wound on white rats were done in strict compliance to the Code of Practice (ethical clearance).

2.8. Creation of burn wound

The zinc metal plate (2.5cm) is heated on a flame, the temperature is 1000oC. White rats were anesthetized using ethyl chloride spray, sprayed on the skin and hair on the backs of shaved white rats, and then plastered with hot zinc metal for 5 seconds (dermis along with underlying tissue under which blistering and peeling skin). The wound is considered a circle.

2.9. Evaluation of burn wound healing

25 male white rats were divided into 5 groups:

- | | | |
|---------|---|--|
| Group 1 | : | X brand ointment as a positive control (PC) |
| Group 2 | : | Ointment base as a negative control (NC) |
| Group 3 | : | Ointment with 5% extract concentration (FI) |
| Group 4 | : | Ointment with 10% extract concentration (FII) |
| Group 5 | : | Ointment with 20% extract concentration (FIII) |

Burn wounds were observed in the average diameter of burn wound healing with measurements using the sliding / shovel length to recover approximately for 2-3 weeks.

3. Data Analysis

The results of this study will be analyzed descriptively and statistically. Descriptive analysis was used on phytochemical screening and physical properties test, while percentage of burn wound healing was analyzed statistically using One Way Anova if it fulfilled normality and homogeneity of data requirement.

4. Results and discussion

4.1. Patah tulang stem's extraction

Extracts of patah tulang stem were obtained through maceration process using 95% ethanol solvent due to its capability to dissolve almost all substances including polar, semi-polar, and nonpolar substances [10]. The obtained dense extract was 33.41 g with 6.682% of rendemen percentage.

4.2. Compounds identification

Compounds identification was to determine the presence of saponin, tannin, and flavonoid compound in the extract of patah tulang stem. This was because those compounds were alleged to be effective in healing wound.

Table 2. Phytochemical Screening Result

Group of Compounds	Reactors	Results
Flavonoid	Mg + dense HCl	-
Tannin	FeCl ₃	+
Saponin	<i>aquades</i>	+

Note: (-) = undetected, (+) = detected

4.3. Ointment evaluation

4.3.1. Organoleptic tests. Organoleptic tests were conducted by observing ointments from its form, smell, and colour.

Table 3. Organoleptic Tests' Result

Formula tion	Form	Organoleptic Tests	
		Colour	Smell
FI	semi-solid	dark green	typical of plants
FII	semi-solid	black green	typical of plants
FIII	semi-solid	dark black	typical of plants
NC	semi-solid	white	no smell

Notes:

- FI : ointment with 5% extract concentration
- FII : ointment with 10% extract concentration
- FIII : ointment with 20% extract concentration
- NC : ointment base as a negative control

4.3.2. Homogeneity test. The research result showed that homogenous result was obtained from ethanol extract's ointment of patah tulang stem on 5, 10, 20% concentration and negative control. The ointment was characterized homogenous if no coarse grain presence [9].

4.3.3. pH test. The ointment's pH in this research (Table 5) has met the requirement, i.e. having pH corresponding to skin's physiological pH of about 4.5-6.5 [11]. Excessively low pH values can cause irritation, while too high pH values can cause scaly skin [2].

Table 4. Average Result of pH

Formulation	pH Average \pm SD
FI	5,5 \pm 0,0
FII	4,5 \pm 0,0
FIII	4,7 \pm 0,0
NC	5,0 \pm 0,0

Notes:

- FI : ointment with 5% extract concentration
- FII : ointment with 10% extract concentration
- FIII : ointment with 20% extract concentration
- NC : ointment base as a negative control

4.3.4. Disperse test. Disperse test was conducted to determine the ability of ointment's diffusion on the skin. The easier the ointment is flattened on the skin, the greater the absorption of its active substances. According to [12], a good dispersing power is 5.6-6.4 cm.

Table 5. Disperse Tests' Average Result

Formulation	Dispersing power's average (cm)± SD
FI	5,6 ± 0,0
FII	5,7 ± 0,0
FIII	5,7 ± 0,0
NC	5,6 ± 0,0

Notes:

- FI : ointment with 5% extract concentration
 FII : ointment with 10% extract concentration
 FIII : ointment with 20% extract concentration
 NC : ointment base as a negative control

4.3.5. Adhesiveness test. Adhesiveness test was to determine the ointment's ability to persist longer on the skin. A good adhesiveness is not less than 4 seconds [13]. Adhesiveness test's result showed that the adhesiveness was poor because it was less than 4 seconds. This was due to the used ointment's base (vaseline album/ white vaseline) whose adhesiveness was less than 4 seconds.

Table 6. Adhesiveness's Average Result

Formulation	Adhesiveness (seconds)
FI	3,6 ± 0,05
FII	3,4 ± 0,05
FIII	3,2 ± 0,05
NC	3,8 ± 0,05

Notes:

- FI : ointment with 5% extract concentration
 FII : ointment with 10% extract concentration
 FIII : ointment with 20% extract concentration
 NC : ointment base as a negative control

4.4. Evaluation of burn wound healing. Experimental animals were given II degree burn wound characterized by damage to epidermis and dermis, such as dry skin, hair follicle damage, dry glands, and sebaceous glands. Wound healings for II degree burn wound need 2-3 weeks [14]. The healing process consists of 3 phases, i.e. inflammation, proliferation, and matrix tissues remodeling [14]. The inflammation phase is characterized by swelling. Proliferation phase is characterized by exudate and fibroblast formation which looks like a scab on the wound. Moreover, remodeling phase is characterized by a small new tissues formation on the wound or a recovered wound [2]. The inflammation process occurs for about 3 days after the injury [15]. Without any inflammation, wound healing process will never occur.

In this research, it can be seen that the inflammation phase of each ointment was different. The inflammation phase of negative control began on day 1 to day 5. In the ointment from 5% concentration of patah tulang stem's ethanol extract, the inflammation phase began on day 1 to day 4. Meanwhile, in the ointment from 10 and 20% concentration of patah tulang stem's ethanol extract and positive control, the inflammation phase began on day 1 to day 3 (Table 7).

Table 7. Result of burn wound healing in 3 phases, i.e. inflammation, proliferation, and remodeling

Formulation	Inflammation	Proliferation	Remodelling
NC	day 1 - 5	day 5 - 10	day 11 - 26
PC	day 1 - 3	day 4 - 8	day 9 - 21
FI	day 1 - 4	day 4 - 9	day 10 - 24
FII	day 1 - 3	day 3 - 8	day 9 - 23
FIII	day 1 - 3	day 3 - 7	day 8 - 20

Notes:

- FI : ointment with 5% extract concentration
 FII : ointment with 10% extract concentration
 FIII : ointment with 20% extract concentration
 NC : ointment base as a negative control
 PC : X brand ointment available on the market

After inflammation phase, the next stage was proliferation phase. This proliferation phase characterized by fibroblasts formulation which looked like a scab. Proliferation phase of each ointment was different and it can be seen in Table 7.

The final stage of burn wound healing was the phase of remodeling matrix's tissues. This phase was the longest phase in burn wound healing process. A dynamic process of wound contraction and scarring occurred. During this phase, the newly formed tissues will be structured in such a way as the original ones [2]. The observation result showed that each formula had different healing time (Table 7 and 8).

Table 8. Healing's Diameter and Percentage

Formula	Healing's Diameter (d) and Percentage % of day-													
	20		21		22		23		24		25		26	
	d	%	d	%	d	%	d	%	d	%	d	%	d	%
NC	5,9	76,4	4,9	80,4	3,9	84,4	2,7	89,2	1,7	93,2	0,6	97,6	0	100
PC	0,4	98,4*	0	100	-	-	-	-	-	-	-	-	-	-
FI	4,5	82*	3,5	86	2,5	90	1,5	94	0,4	98,4	0	100	-	-
FII	2,7	89,2*	1,7	93,2	0,7	97,2	0	100	-	-	-	-	-	-
FIII	0	100*#	-	-	-	-	-	-	-	-	-	-	-	-

Notes:

- * : significantly different from KN
 # : not significantly different from KP
 FI : ointment with 5% extract concentration
 FII : ointment with 10% extract concentration
 FIII : ointment with 20% extract concentration
 NC : ointment base as a negative control
 PC : X brand ointment available on the market

The rats given formula III recovered 100% on day 20. In formula II, they recovered 100% on day 23, and in formula I, they recovered 100% on day 25. In the negative control, they recovered 100% on day 26 and in the positive control, they showed 100% recovery on day 21. An analysis was done to the data of recovery percentage on day 20 using Kruskal-Wallis Test because the result of normality test indicated that abnormal data distribution was found ($p < 0,05$). Based on the analysis result, it was discovered that there was a difference on burn wound healing in each group's treatments of the ointment from 5, 10, and 20% concentration, positive and negative control ($p < 0,05$) of patah tulang stem's ethanol extract. Moreover, an analysis using Mann-Whitney test was conducted to find out which groups were significantly different (the result can be seen in Table 8). Formula III had the same effect with positive control in healing burns on rats.

Based on those data, it can be inferred that the ointment from 20% concentration of patah tulang stem's ethanol extract gives more rapid effect in healing burns compared with other ointments. This is because patah tulang stem's ethanol extract has flavonoid, tannin, and saponin compound. Flavonoid inhibits bacterial growth by destroying the permeability of bacteria's cell wall, microsomes, and lysosomes as a result of an interaction between flavonoid and bacteria's DNA. Furthermore, it also inhibits the motility of bacteria. Tannin serves as an astringent which can shrink the skin pores, harden the skin, and stop exudate and mild bleeding so that it can cover the wound and prevent the bleeding.

Moreover, saponin contained in plants can stimulate collagen formation which functions to heal the wound [16].

5. Conclusion

The ointment from 20% concentration of patah tulang stem's ethanol extract has the same effect with positive control in burn wound healing process on white rats ($p > 0,05$).

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