



BURNS HEALING RATES IN RATS MEDICATED WITH LEAF EXTRACT OF TEKELAN (*CHROMOLAENA ODORATA* L.) OINTMENT

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ABSTRACT

Tekelan (*Chromolaena odorata* L.) is a type of plant that is known to contain antioxidants, anti-inflammatory, and antimicrobial properties that allow this plant to be used as a wound healing medicine, including burns. This study aims to determine the healing effect of ethanol fraction of tekelan leaf extract on wistar rats (*Rattus norvegicus*). By using completely randomized design five different ointments were prepared and given to five groups of experimental animals consisted of three rats each. To the each group, the treatments given are as follows: silver sulfadiazine cream (+ control), vaseline flavum (–control), tekelan extract of 5%, 10% and 15% respectively (as the test groups). Burn wound is inflicted by placing a metal rod (12 mm diameter)

heated to 100 °C in boiling water on the back skin of rats that had been previously shaved. Treatment was given once daily for 21 days. Observation of wound healing, based on percent closure wound area, was carried out on day 0 (immediately after the injury), day 7, 14 and 21. The results showed that all treatments could completely heal burns in test animals within 21 days. The only difference was seen in the weekly progress rate of wound closure, where only the tekelan extract with the highest concentration (15%) nearly matched the standard drug.

KEYWORDS: burns, wound, tekelan, *Chromolaena odorata*, tekelan extract ointment.

INTRODUCTION

Burn is an injury to the skin or other tissues due to external heat sources such as hot liquids (scalds), hot solids (contact burns), or flames (flame burns). Burn injuries accounting for estimated 180,000 deaths annually, mainly in the low-middle income countries.^[1,2] In Pakistan, for instance, the mortality rate was high in flame burns followed by scalds. Common cause of death in patient with flame burns was sepsis and irreversible shock.^[3]

To prevent burns from causing further damage to tissues and organs, due to infection, for example, burns need to be treated immediately. The treatment most commonly applied to burns, especially minor wounds, is to give topical medication, cream or ointments containing antibiotic applied to the skin. One of antibiotics cream that is world-widely used is *silver sulfadiazine cream*.^[4]

Considering adverse effect and high cost of the synthetics drugs, especially in the context of low-income countries, the plant-originated medicine still deserve to be considered as the alternative. One of plant species that is well known for its efficacy in burns healing is *Aloe vera*, *Artemisia sieberi*, and tekelan (*Chromolaena odorata*).^[5,6,7]

With regard to the tekelan plant (*Chromolaena odorata*), the wound healing activities of the herb might be due to the anti-oxidant, anti-inflammatory and antibacterial properties of the bioactive content of this plant. The bioactive which likely allow tekelan plant to have pharmacological and therapeutic activities mentioned above are flavanones, flavonols, flavones, quercetin, terpenes and terpenoids, essential oils, alkaloids, saponin, tannin, phenolic acid.^[8]

In Indonesia, there have been several studies on the pharmacological effects of tekelan leaf extract, but until now the results of this research have not been consistent regarding the methods and solvents of extraction, concentration, and the most effective formulas for the treatment of burns.^[9,10]

In an effort to find the most appropriate type of solvent, formulation, and concentration of tekelan leaf extract for use in the treatment of wounds, we tested in vivo the ethanol fraction of tekelan leaves macerated with n-hexane.

RESEARCH METHOD

Plant materials and extraction

Plant leaves sample of tekelan (*Chromolaena odorata L.*) were collected from suburb of Bandar Lampung, Lampung Province, Indonesia. Taxonomic determination of the plant were done by a botanist at the Center for Research and Development of Medicinal Plants and Traditional Medicine (*Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional-B₂P₂TOOT*) Tawangmangu, Karanganyar, Central Java Province, Indonesia.

The fresh leaves of the plants after being washed and rinsed with tap water were chopped into small pieces and then sun-dried under a black cloth cover in order to prevent sample from exposed to UV rays. The dried chopped leaves (400 g) are macerated using n-hexane (pro analysis from Merck). The filtrate were collected by filtration after 24 hours. The solid residues were then re-macerated using the same solvent every 24 hours until the extract is completely clear. The obtained filtrate was extracted with chloroform (pro analysis from Merck), and the filtrate was filtered. The residue was Soxhlet extracted with 70% ethanol. Lastly, the extract collected was concentrated using a rotary evaporator until a viscous extract of tekelan leaves are obtained.

Ointment preparation

There were three ointments of ethanol extract of tekelan leaves that were prepared in this study namely T₁, T₂, and T₃ containing of 5%, 10% and 15% extract respectively. The ointments were made by adding and mixing Vaseline Flavum (from Brataco) into the extract according to the mass proportion of the two components to fulfill the desired concentration up to a total mass of 100g.

Animals and Ethics

The experimental animals used in this study were male Wistar rats (*Rattus norvegicus*) with the age range of 5-6 months and weighing of \pm 200g. All test rats, obtained from Veterinary Centre Lampung, were kept for one week to adjust laboratory environment, given pellets and water ad libitum. All protocol for keeping and treating the test animals in this study has received approval from Ethical Commission of Medical and Health Research of the Faculty of Medicine, University of Lampung, Indonesia (Reg. No. 1785/UN26.18/PP.05.02.00/2020).

Research design

The experimental rats (n=15) were completely randomized into five groups (3 animals each) namely *Silver sulfadiazine* (positive control), Vaseline flavum (negative control), 5% extract, 10% extract and 15% extract.

Burns infliction and Treatment

Burn wounds were inflicted by placing metal rode (12 mm diameter) heated at 100°C in boiling water on the back skin of the rats that have been shaved previously. Before burn infliction the rats were anesthetized locally with lidoacain given subcutaneously. Immediately after wounding, the injured skin treated topically with the appropriate ointment. Treatments were given once daily for 21 days. Burns healing progress was assessed on day 0 (baseline), day 7, 14 and 21. The burns healing progress was indicated by percent closure of the wounds surface area calculated using equation formula bellows.

$$\% \text{ closure} = \frac{\text{wound area on day}_n - \text{wound area on day}_{n+1}}{\text{wound area on day}_n} \times 100$$

Phytochemicals identification

To ensure that the tekelan leaf extracts do contain bioactive compounds that have burns healing properties such as alkaloid, flavonoid, saponins, tannin, terpenoid and steroids, the qualitative phytochemical screening tests were applied. The presence of the alkaloids was determined using Wagner, Hager and Mayer's test. Tannin, saponins, steroid, and flavonoids were tested using Lead acetate and FeCl₃ test, Foam and Haemolysis tests, Salkowski test, Lead acetate test.^[11]

Data analysis

Data obtained were analyzed qualitative-descriptively and presented as mean followed by standard deviation (SD).

RESULTS

The effect of applying topical ointment on the wound surface area of experimental animals according to treatment days is presented in Table 1. Qualitatively, this data suggests that the ethanol fraction of tekelan (*Chromolaena odorata L.*) leaf extract gives a healing effect on burns that is similar to standard drugs, the silver sulfadiazine cream.

Table 1: Burns surface area in each group of rats by day of treatment.

Days after wounding	Burns surface area (mm ²)				
	(+) Control	(-) Control	5% Extract	10% Extract	15% Extract
0	113.04 ± 1.23	112.8 ± 2.12	113.4 ± 0.74	113.2 ± 4.33	112.8 ± 0.66
7	68.75 ± 0.62	76.85 ± 1.88	68.50 ± 0.66	84.91 ± 3.46	76.10 ± 0.66
14	6.56 ± 1.72	37.15 ± 0.66	16.16 ± 1.16	31.79 ± 2.25	13.25 ± 0.25
21	0.00	0.00	0.00	1.22 ± 1.2	0.00

*All values are presented as mean ± SD

The burns healing pattern in mice after given ointment topically for 21 days is pictured in Figure 1. The curve in the figure shows that the tekelan leaf extract ointment was effective in healing burns in mice within three weeks (21 days). The analysis on the progress rates of wound weekly healing resulted in the data as presented in Figure 2. Based on the data in Figure 2 it can be stated that the wound healing rate by the ethanol extract ointment of tekelan leaves, at a concentration of 15%, is similar to the rate of healing by standard drugs.

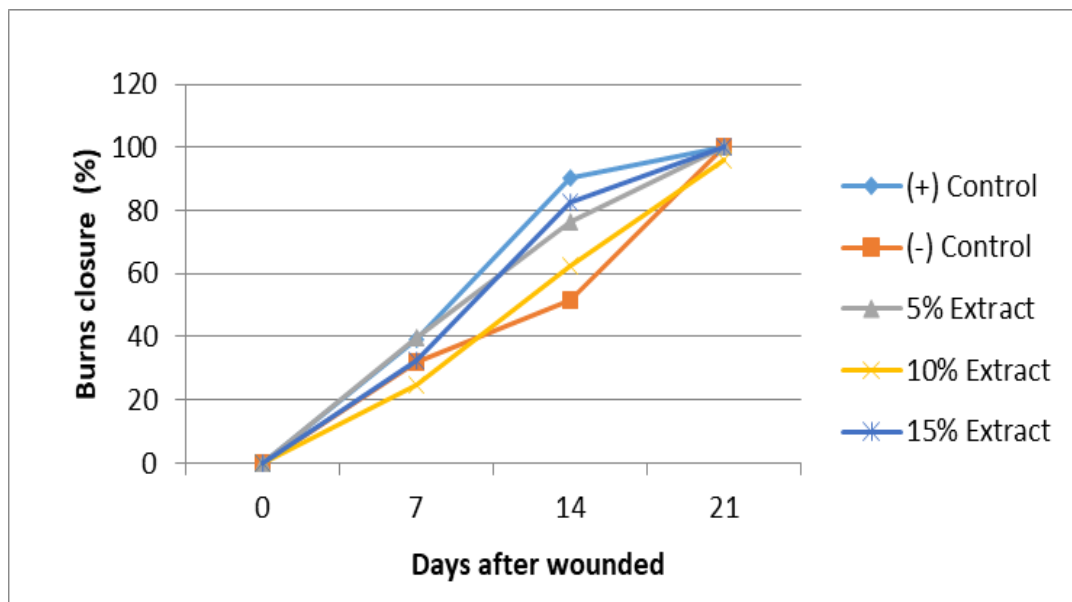


Figure 1: Burns healing pattern in mice after given ointment topically for 21 days.

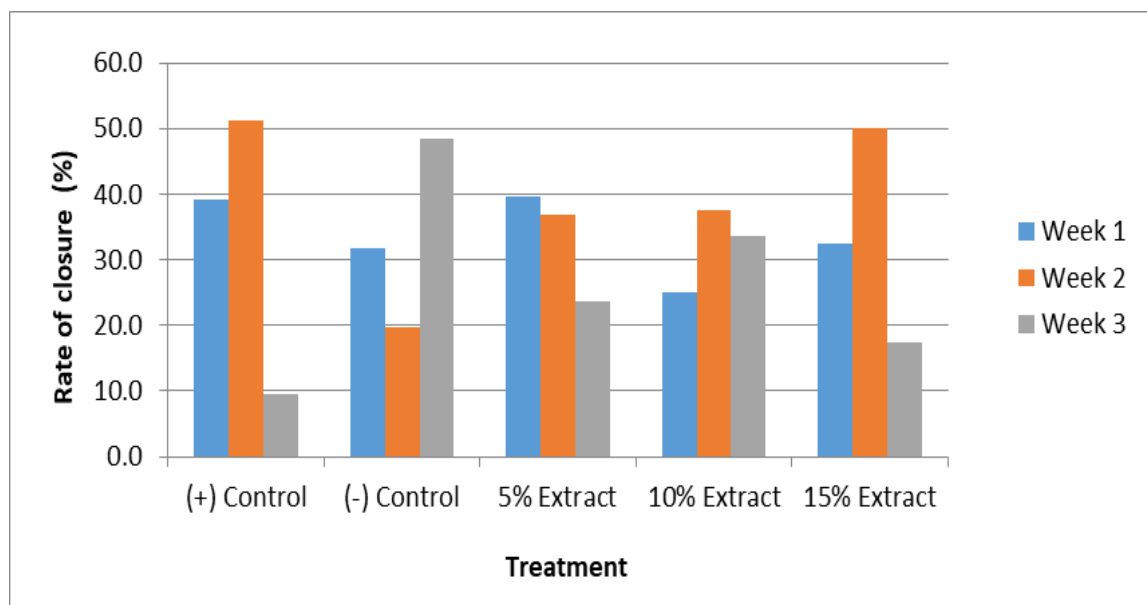


Figure 2: The comparison of weekly wound closure rates between rats treated with tekelan leaf extract ointment and standard medicine.

The results of the bioactive screening contained in the ethanol fraction of the tekelan leaf extract presented in Table 2. Except for steroids, all bioactive substances expectedly have burn wounds healing were positively detected in tekelan leaf extracts.

Table 2: Phytochemicals identified in ethanol fraction of tekelan leaf extracts.

Chemicals	Identification
Flavonoid	+
Alkaloid	+
Tannin	+
Terpenoid	+
Steroid	-
Saponin	+

DISCUSSION

All data presented in Table 1, Figure 1 and Figure 2 indicate that the ethanol fraction of the tekelan plant leaf extract has a healing effect on burns in rats. However, the question is, why did the burns of mice given only ointment base ingredient (vaseline flavum) also heal at the same time as the other groups of rats. The most likely explanation is because the ointment base functioned as moisturizer and softener mace skin and provide barrier for microbes to infect the wounds.^[12]

Burns healing properties of the ethanol leaf extract of *Chromolaena odorata* is very likely related to the high anti-oxidant activities and antibacterial properties of the phytochemicals

contained in this plant.^[13] The compounds that have antioxidant and antibacterial properties such as flavonoids, alkaloids, terpenoids, saponins and tannin were successfully detected in the ethanol extract of tekelan leaves applied this study.

Similar properties of plants containing alkaloids, tannins, terpenoids, and flavonoids with standard drugs, silver sulfadiazine cream, in healing burns are also found in the *Bixa orellana* plant. Such bioactive substances could increase the formation of fibroblast, proliferation of endothelial cells and keratinocytes, stimulate keratinocytes migration, and increase the regulation of protein production of extracellular matrix on keratinocytes.^[14]

Flavonoids are antioxidant that is inhibiting lipid peroxidation so that it can prevent or slow down the necrosis help revitalize the damaged blood vessels (*angiogenesis*).^[15] Anti-bacteria properties of the substances prevent synthesis of nucleic acid, slow down the function of cytoplasmic membrane, inhibit energy metabolism, inhibiting attachment and formation of bio-film.^[16]

Tannins and terpenoids are astringent that can minimize the skin pores, stop bleeding, blocking infection, forming protective layer on open tissues that makes wound safe from infection, and increase epithelialization.^[17,18]

CONCLUSION

Ethanol fraction of plant leaf extract of tekelan (*Chromolaena odorata* L.) revealed to show burns healing properties similar to that of standard medicine, the silver sulfadiazine cream. It suggests therefore that tekelan leaf extract worth recommended as burns topical ointment.

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Competing interests

Authors declare no competing interest.

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