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Research Article

Ointment Formulation of Tapak Dara (*Catharanthus roseus* (L.) G. Don) Flower Ethanol Extract and its Activity in Burn-Healing

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Abstract

Treatment done on burn wounds is intended to provide local therapy to heal as quickly as possible. The content of secondary metabolites in the tapak dara (*Catharanthus roseus* (L.) G. Don) flower can help the healing process of burns, namely alkaloids, saponins, tannins, and flavonoids. Alkaloids act as antibacterial; saponins can trigger collagen formation; tannins as astringents that cause shrinkage of skin pores and stop minor bleeding in wounds; and flavonoids have anti-inflammatory effects. This study aimed to formulate an ointment of *C. roseus* flower ethanol extract and determine its physical characteristics such as organoleptic test, homogeneity, pH value, dispersion, and stability test of the preparation and examine the activity as a burn healer in white male rats. The research data were analyzed statistically using the ANOVA method, followed by the LSD test (least significant difference) to see how the ointment-containing extract reduced the diameter and percentage of the burn wounds. The results show that all ethanol extracts of *C. roseus* flower ointments met the requirements for its physical characteristic tests. It offers a good activity as a burn healer in white male rats. The most effective concentration is an ointment containing 15% of ethanol extract from *C. roseus* flower (F3 group), which shows a significant difference ($p \leq 0.05$) from the blank and the other group formula in burn wound healing.

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INTRODUCTION

Burns are skin tissue damage or loss due to contact with heat sources such as fire, hot water, chemicals, electricity, and radiation. The extent of tissue damage during burns impairs angiogenesis, collagen reorganization, and granulation tissue formation and induces free radical-mediated damage resulting in delayed tissue repair[1]. Burn recovery requires lengthy hospitalizations, expensive drugs, and prolonged rehabilitation periods[2,3]. Inappropriate burn care will lead to complications, infection, and bleeding. Burns that are not treated promptly will be inhabited by pathogenic bacteria that rapidly undergo exudation with the absorption of large amounts of water, protein, and electrolytes and often require skin grafting from other parts of the body to produce permanent wound closure[4,5].

The problem of burns is still a global problem that needs to be resolved[6]. Indonesia's potential, rich in medicinal plants, is expected to be able to answer these problems[7]. One of the medicinal plants commonly used empirically as a healer for wounds and burns is tapak dara (*Catharanthus roseus* (L.) G. Don). *Catharanthus roseus* is a plant from the Apocynaceae family originating from Central America and is generally grown as an ornamental plant. *Catharanthus roseus* is a type of herbal plant that can grow up to 1 meter, including perennial plants, or can live for approximately two years. The leaves are green, oval in shape, and the flowers have five trumpet-shaped crowns, the color of the flowers is white, pink, or white with a red

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spot in the middle[8]. The components of the active compounds found in *C. roseus* are phenolic acids, flavonoids, alkaloids, saponins, terpenoids, and tannins[9,10]. The purpose of this study was to determine the activity of the ethanol extract of the *C. roseus* flower, which had been formulated in the form of an ointment as a burning medicine, and to find out the best extract concentration capable of healing burns compared to the positive control.

MATERIALS AND METHODS

Materials

The tools used were digital scales, rotary evaporator, measuring cups, and pH meter (Hanna Instruments). The test animals used in this study were male white rats weighing 200 - 300 g. The sample and materials used in this research were *C. roseus* flower, adeps lanae, vaseline album, nipagin, vanilla oil, 70% ethanol, aquadest, lidocaine, and betadine ointment.

Methods

Plant collection and determination

Catharanthus roseus flowers were picked purposively from Medan Helvetia, Deli Serdang Regency, North Sumatra, then dried and processed into fine simplicia powder. Plant determination was carried out at the Herbarium Medanense, Center for Biology Research, Universitas Sumatera Utara, Medan, North Sumatra. The determination result stated that the plant used as the sample was the flower of *C. roseus*, with certificate number 5961/MEDA/2021.

Extraction

The fine simplicia powder was put into a maceration vessel, then immersed in 70% ethanol solvent. This immersion was carried out for five days. Stirring was done so that the solvent was immersed in all the simplicia powder. After five days, the soaking results were then filtered using filter paper. The filtrate was taken and accommodated. The residue was macerated again for two days, then collected and concentrated with a rotary evaporator at 45°C to obtain a thick extract[11].

Ointments formulation

Ointment formulations are presented in **Table I**. There were four formulas (0, 1, 2, and 3), with the difference being the concentration of *C. roseus* extract used. All ingredients were weighed according to the calculation. Nipagin was put into the mortar, French vanilla oil was added, then ground until homogeneous. Then, the vaseline album was added and ground homogeneously. Adeps lanae was added and grounded until homogeneous. The extract of *C. roseus* flower was added little by little and ground until homogeneous, then the remaining part of the vaseline album was grounded until homogeneous. The base formulation of the ointment without extract was made as a blank[12].

Table I. Ointment formulation

Ingredients	F0 (blank)	F1 (5%)	F2 (10%)	F3 (15%)
Catharanthus roseus flower extract (g)	-	5	10	15
Adeps lanae (g)	15	15	15	15
Nipagin (g)	0.05	0.05	0.05	0.05
Vanilla oil (mL)	0.05	0.05	0.05	0.05
Vaseline album ad (g)	100	100	100	100

Evaluation of ointment

Stability test: The stability test was performed using the cycling test method. The ointment preparation was stored at a temperature of ±4°C for 24 hours, then transferred to a temperature of ±40°C for 24 hours (1-cycle). Tests were carried out in the 1st to 6th cycle by observing organoleptic, homogeneity, pH, and dispersion[13].

Organoleptic test: The organoleptic examination of the ointment was observed visually, including the physical form, color, and odor. Organoleptic analysis was performed every cycle on the cycling test (6 cycles)[14].

Homogeneity test: The homogeneity test was carried out by weighing 1 g of the ointment preparation and then smeared on a slide and tightly closed with another slide; then, the homogeneity of the ointment was observed. Homogeneous ointments

were characterized by the absence of lumps and granules, an even structure, and a uniform color. Homogeneity analysis was performed every cycle on the cycling test (6 cycles)[15].

pH test: pH value was measured using a pH meter. First, the instrument was calibrated using the acid and aqueous buffer, then the electrode was rinsed with distilled water and wiped with a tissue. About 1 g of the ointment was dissolved in 100 mL of distilled water. Then the electrode was dipped into the solution until the pH meter showed a constant pH value. Analysis of the pH of the preparation was carried out before the preparation was tested for stability and every 1 cycle of the cycling test stability test[16].

Spreadability test: As much as 0.5 g of the ointment was placed on the center of the petri dish. Another choice of petri dish was placed on top of the gel and left for one minute. As much as 50 g and 100 g of load were put in; then, the diameter constant was measured. The analysis of the dispersion of the preparation was carried out before the stability test and after the 6 cycle cycling test stability test[17].

Burns on rats

This research has obtained research ethics approval from the Animal Research Ethics Committee, Universitas Sumatera Utara, with certificate number 0504/KEPH-FMIPA/2021. Method of applying burns was performed with 15 male white rats that were adapted for seven days. On the first day of the study, they were divided into five groups, each consisting of three rats. Each rat was marked or labeled on its tail using a waterproof marker according to its group. The hair in the area to be injured was shaved first and disinfected with 70% ethanol. Then, the mice were anesthetized using lidocaine. Administration of burns on the backs of rats was done using an iron coin plate heated on a blue fire for 3 minutes and then affixed to the back of the rat for 5 seconds until a second-degree burn was formed[18,19].

Calculation of burn diameter

Burns formed were measured using a caliper, then the diameter of the burn was calculated by **Equation 1**. After the burn diameter was obtained, the percentage of burn healing was calculated using **Equation 2**.

$$d = \frac{d_1+d_2+d_3+d_4}{4} \quad [1]$$

d: wound diameter

d_1, d_2, d_3, and d_4: wound diameter measured from various directions

$$P_x = \frac{d_1-d_x}{d_1} \times 100\% \quad [2]$$

P_x: percentage of healing day x

d_1: first-day wound diameter

d_x: wound diameter on day x

Data analysis

Burn healing time data were analyzed statistically using the ANOVA (One-way Analysis of Variant) method, followed by the LSD (least significant difference) test with a 95% confidence level.

RESULTS AND DISCUSSION

Extraction

The extraction method used is maceration, a simplicia extraction process with organic solvents carried out several times by stirring at room temperature[11]. A total of 200 g of *C. roseus* flower simplicia powder was soaked in 2,000 mL of 70% ethanol. The filtrate was then concentrated with a rotary evaporator to obtain a thick extract of 68.67 g. The yield obtained is 34.33%.

Evaluation of ointment

Evaluation of the ointment includes stability testing using the cycling test method (6 cycles) by observing organoleptic, homogeneity, pH, and dispersion[13]. Organoleptic stability testing includes the ointment's shape, color, and odor. The ointment was observed during the cycling test. The ointment has a semi-solid form which is characteristic of the ointment

itself. In the ointment base without extract, it has a yellowish-white color which, when combined with the extract of *C. roseus* flower, becomes brown. The higher the concentration level, the darker the brown color. The aroma of ointment smells of French vanilla due to adding fragrance to the modified formula to avoid the preparation from a rancid odor caused by adding extract. The organoleptic properties of all ointment formulas did not change after the cycling test treatment, which showed the stable properties of the preparation[14,15].

The homogeneity test results of the ointment during the cycling test obtained from the control and the formula using extracts with three different concentrations showed that all ointment preparations were homogeneous. The homogeneity of the ointment preparation is seen from the absence of substances that have not been mixed, so there is no homogeneity difference during the cycling test stability test[16]. The pH stability test was carried out on each ointment formula during the cycling test, in which the average pH obtained was 5.8-6.1. The pH test aims to determine the safety of the ointment preparation so as not to irritate the skin. The pH of the preparation is good according to the pH of the skin, which is 4.5-6.5. If the pH of the preparation is too acidic, it will irritate the skin, and if the pH of the preparation is too alkaline, it causes dry skin[17].

The dispersion results measurement obtained after the cycling test decreased compared to before the test. Although the dispersion results decreased after the test, each preparation was still within the range of good ointment spreadability between 5-7 cm. The dispersion test was carried out to ensure satisfactory drug administration. The wider the preparation is spread, the greater the diffusion coefficient, which results in increased drug diffusion[20].

Visual observation of burns and wound healing

Observations of burns were analyzed until 21 days on days 1, 7, 14, and 21 to see the physical changes that occurred in the treatment area[21,22]. The results of visual observations of burns in test animals can be seen in **Table II**.

Table II. Visual observation of burns

Formula	Rat	Description	Observation results (days)			
			1	7	14	21
F0 (blank)	1	Color	RB	DB	R	R
		Scab formed	-	-	✓	✓
		New skin formation	-	-	-	✓
	2	Color	RB	DB	C	R
		Scab formed	-	✓	✓	✓
		New skin formation	-	-	✓	✓
	3	Color	RB	DB	C	R
		Scab formed	-	-	✓	✓
		New skin formation	-	-	-	✓
F1 (5%)	1	Color	RB	DB	R	R
		Scab formed	-	-	✓	✓
		New skin formation	-	-	✓	✓
	2	Color	RB	DB	C	R
		Scab formed	-	-	✓	✓
		New skin formation	-	-	✓	✓
	3	Color	RB	DB	R	R
		Scab formed	-	-	✓	✓
		New skin formation	-	-	✓	✓
F2 (10%)	1	Color	RB	C	R	P
		Scab formed	-	-	✓	✓
		New skin formation	-	✓	✓	✓
	2	Color	RB	DB	R	P
		Scab formed	-	✓	✓	✓
		New skin formation	-	-	✓	✓
	3	Color	RB	DB	R	R
		Scab formed	-	✓	✓	✓
		New skin formation	-	-	✓	✓
F3 (15%)	1	Color	RB	C	P	W
		Scab formed	-	✓	✓	✓
		New skin formation	-	✓	✓	✓
	2	Color	RB	DB	R	W
		Scab formed	-	✓	✓	✓
		New skin formation	-	✓	✓	✓
	3	Color	RB	DB	R	P
		Scab formed	-	✓	✓	✓
		New skin formation	-	-	✓	✓

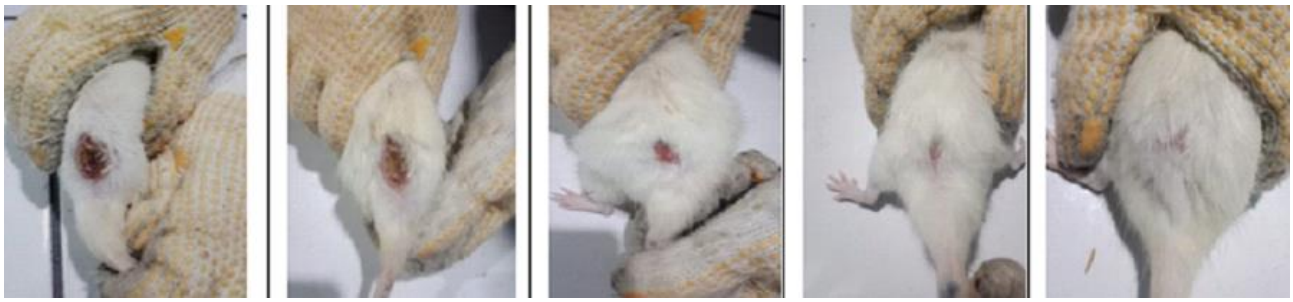
Positive control (C+)	1	Color	RB	C	P	W
		Scab formed	-	✓	✓	✓
		New skin formation	-	✓	✓	✓
	2	Color	RB	C	P	P
		Scab formed	-	✓	✓	✓
		New skin formation	-	✓	✓	✓
	3	Color	RB	C	P	W
		Scab formed	-	✓	✓	✓
		New skin formation	-	✓	✓	✓

Note: Brownish red (RB); Dark brown (DB); Chocolate (C); Red (R); Pink(P); White (W); Occur (✓); Not occur (-)

Measurement data on the average diameter of burns and the percentage of healing in the negative control, positive control, and test group with a concentration of 5%, 10%, and 15% on day 1 to 21 are presented visually in **Figures 1** and **2**, with calculations can be seen in **Tables III** and **IV**.

F0 **F1** **F2** **F3** **C+**

Figure 1. Day 1 observations.



F0 **F1** **F2** **F3** **C+**

Figure 2. Day 21 observations.

Table III. The average diameter of burns

Group	Average ± SD burn diameter (days)			
	1	7	14	21
F0	20.23 ± 0.15	19.03 ± 0.15[b]	18.10 ± 0.10[b]	14.60 ± 0.40[b]
F1	20.20 ± 0.10	18.13 ± 0.15[ab]	17.33 ± 0.15[ab]	9.86 ± 0.20[ab]
F2	20.23 ± 0.15	17.83 ± 0.20 [ab]	16.93 ± 0.15[ab]	7.20 ± 0.36[ab]
F3	20.30 ± 0.10	17.43 ± 0.15[a]	9.86 ± 0.30[a]	3.03 ± 0.68[a]
C+	20.26 ± 0.20	17.36 ± 0.20[a]	9.40 ± 0.55[a]	2.56 ± 0.76[a]

Note: [a]: Significantly different from negative control; [b]: Significantly different from positive control

Table IV. Burn wound healing percentage

Group	Average ± SD percentage of burn healing (%)		
	After 7 days	After 14 days	After 21 days
F0	5.93 ± 0.04[b]	10.54 ± 0.68[b]	27.83 ± 2.22[b]
F1	10.23 ± 0.33[ab]	14.19 ± 1.08[ab]	51.15 ± 0.86[ab]
F2	11.86 ± 0.84[ab]	16.31 ± 0.46[ab]	64.42 ± 1.67[ab]
F3	14.12 ± 1.17[a]	51.39 ± 1.67[a]	85.05 ± 3.43[a]
C+	14.31 ± 0.48[a]	53.60 ± 3.12[a]	87.32 ± 3.61[a]

Note: [a]: Significantly different from negative control; [b]: Significantly different from positive control

In this study, an ointment base was used as a negative control. This was done to ensure that the extract of *C. roseus* flower gave the effect of healing burns. Betadine ointment was used as a positive control because it is a pharmaceutical preparation in the form of an ointment that can also be used as a burn healer, readily available in the market. In comparison, the secondary metabolite compounds in the *C. roseus* flower that can help the healing process of burns are flavonoids, alkaloids, saponins, and tannins[23,24].

Flavonoids work as antibacterial by forming complex compounds against extracellular, which disrupt cell integrity. In addition, flavonoids also have anti-inflammatory effects that function as anti-inflammatory and can prevent stiffness and pain[25]. Alkaloids also have the ability as antibacterial; the mechanism is thought to be a way of disrupting the peptidoglycan component in bacterial cells so that the cell wall layer is not formed and causes the death of the cell[26]. Saponins can trigger the formation of collagen where the more collagen there is, the faster it will attract fibroblasts to the edges of the wound so that the fibroblasts will experience a phenotypic change to become myofibroblasts which accelerates the wound contraction process so that the size of the wound quickly decreases[27]. Tannins also play an essential role in the healing process of burns, which are helpful as astringents that cause shrinkage of skin pores, stop minor bleeding so that they can cover wounds, and prevent bleeding that usually occurs in wounds[28].

Figure 3 shows the healing of burns in test animals that occurred for 21 days. The group that was given a formula containing active compounds showed a better healing process than the wounds that were given blanks. The best activity is shown by Formula 3, whose results are close to the positive control.

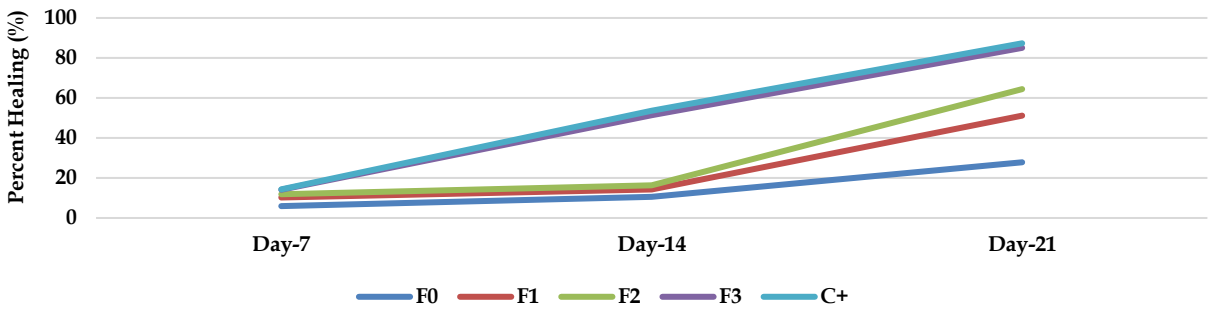


Figure 3. Healing wound percentage.

CONCLUSION

All the formulas for the ethanol extract of *C. roseus* ointment met the requirements for the evaluation of stability, organoleptic, homogeneity, pH, spreadability, and the best activity is shown by Formula 3 with 15% extract content.

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AUTHORS' CONTRIBUTION

L determined, designed the study, supervised, analyzed data, and partook in writing the original draft of the article. **TNKS** supervised the laboratory, managed the work, and partook in writing the original draft of the article. **RS** conducted the search, interpreted data, and partook in writing the original draft of the article. **IH** and **BI** individually revised the final article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

DATA AVAILABILITY

The data that support this study are available from the corresponding author **L**, upon reasonable request.

CONFLICT OF INTEREST

All of the authors have no conflict of interest to declare.

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