



# Wound healing activity of *Dillenia ochreatea* leaves ethanol extract in Wistar rats

[Actividad de cicatrización de heridas del extracto de etanol de hojas de *Dillenia ochreatea* en ratas Wistar]

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## Abstract

**Context:** *Dillenia ochreatea* is a medicinal plant used traditionally for scabies treatment. *D. ochreatea* leaves contain secondary metabolites of triterpenoids, phenolics, and steroids that have the potential to accelerate the wound healing process.

**Aims:** To determine the effect of the ethanolic extract of *D. ochreatea* leaves on the speed of healing of burns and incisions.

**Methods:** The test animals were divided into six groups: a positive control group (1% silver sulfadiazine ointment for burns and 10% povidone-iodine ointment for wound incision), a negative control group, and four treatment groups with an ointment concentration of 2.5, 5, 7.5, and 10%. Burns were made using a heated iron plate, and incision wounds were made using a sterile scalpel blade with a length of 3 cm. Parameters observed included burn area, incision length, percent recovery, and histopathology.

**Results:** It was observed that the 10% concentration of *D. ochreatea* leaf ethanol extract showed the best results in healing burns and incision wounds, with 100% healing within 12 days for burns and five days for incisions. The treatment group had a significantly different wound healing activity ( $p < 0.05$ ) compared with the negative control and was not significantly different ( $p > 0.05$ ) from the positive control.

**Conclusions:** *D. ochreatea* leaf ethanol extract (10%) could accelerate the healing of burns and incisions.

**Keywords:** burn wound; *Dillenia ochreatea*; incision wound; skin histopathology.

## Resumen

**Contexto:** *Dillenia ochreatea* es una planta medicinal utilizada tradicionalmente para el tratamiento de la sarna. Las hojas de *D. ochreatea* contienen metabolitos secundarios de triterpenoides, fenoles y esteroides que tienen el potencial de acelerar el proceso de cicatrización de heridas.

**Objetivos:** Determinar el efecto del extracto etanólico de hojas de *D. ochreatea* sobre la velocidad de cicatrización de quemaduras e incisiones.

**Métodos:** Los animales de prueba se dividieron en seis grupos: un grupo de control positivo (ungüento de sulfadiazina de plata al 1% para quemaduras y ungüento de povidona yodada al 10% para incisión de heridas), un grupo de control negativo y cuatro grupos de tratamiento con una concentración de pomada de 2,5; 5; 7,5; y 10%. Las quemaduras se realizaron con una placa de hierro caliente y las incisiones se realizaron con una hoja de bisturí estéril de 3 cm de longitud. Los parámetros observados incluyeron el área quemada, la longitud de la incisión, el porcentaje de recuperación y la histopatología.

**Resultados:** Se observó que la concentración al 10% de extracto etanólico de hoja de *D. ochreatea* mostró los mejores resultados en la cicatrización de heridas por quemaduras e incisiones, con un 100% de cicatrización en 12 días para quemaduras y cinco días para incisiones. El grupo de tratamiento tuvo una actividad de cicatrización de heridas significativamente diferente ( $p < 0,05$ ) en comparación con el control negativo y no fue significativamente diferente ( $p > 0,05$ ) del control positivo.

**Conclusiones:** El extracto etanólico de hoja de *D. ochreatea* (10%) podría acelerar la cicatrización de quemaduras e incisiones.

**Palabras Clave:** *Dillenia ochreatea*; herida de incisión; herida por quemadura; histopatología de la piel.

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## INTRODUCTION

Wounds damage the protective function of the skin, accompanied by loss of continuity of epithelial tissue, with or without injury to other tissues. Treatment of burns includes preventing infection and providing an opportunity for the remnants of epithelial cells to proliferate and cover the wound surface. Wounds have a high risk of disease, so preoperative antiseptic techniques are needed to reduce infection in the wound area (Dumville et al., 2015). Many antimicrobial ointments have been sold to reduce wound infection; however, these topical antimicrobial agents have some side effects and are only partially effective in healing the wound (Somboonwong et al., 2012). Therefore, new drugs are needed to heal wounds.

The use of traditional medicinal plants for skin infections has long been prevalent in human society, and one of such plants is *Dillenia ochreatea* (Miq.) Teijsm. & Binn. ex Martelli (family *Dilleniaceae*). In Indonesia, this plant is known by the local names simpur, simpor, and semprawang. In Malaysia, this plant is known as simoh; in Myanmar, Mai-masam; in Thailand, san-masan; in Philippines, Kad-mon; and in Brunei, simpor (Heyne, 1987). This plant has been used empirically, especially by the Musi tribe, Banyuasin, South Sumatra, to treat skin infections (Muharni et al., 2017). Information on the chemical content and biological activity of the *D. ochreatea* plant is still very limited. Ethanolic extract of *D. ochreatea* leaves contained triterpenoid, steroid, and phenolic compounds and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Muharni et al., 2017). Antibacterial activity plays a role in wound healing, suppressing bacterial growth (Ming et al., 2021).

Other species of *Dillenia*, such as *D. indika*, reported already as traditionally were used for treatment against cough and dyspnea, containing major chemical compounds betulin (pentacyclic triterpenoid) and betulinic acid. These compounds show a broad spectrum of pharmacological activities such as anti-HIV, anti-inflammatory, anti-cancer, anti-malarial, and wound healing. There are many *Dillenia* species, but only a few are proven scientifically (Barua et al., 2018). Phytochemical studies on *Dillenia* species have shown triterpenoids as the major compound mainly of the lupane and oleanane types (Macahig et al., 2011). *Dillenia ochreatea* is a well-known medicinal plant for scabies therapeutic (Yustian et al., 2012). The use of *D. ochreatea* leaf as traditional medicine for scabies therapeutic used as approach for determining the pharmacological activity of *D. ochreatea* in burn wounds and incision wound healing. *Dillenia* (Dille-

niaceae genus), especially *Dillenia ochreatea* warrant further studies on their therapeutic potential as an alternative to new drug candidates for the treatment of various diseases (Saiful and Armania, 2014).

## MATERIAL AND METHODS

### Chemicals and reagents

Other materials were ethanol absolute (Merck®), sodium carboxymethylcellulose (Na-CMC) (Brataco®), NaOH (Merck®), lanolin (Dexa Medica®), silver sulfadiazine (Dexa Medica®), povidone-iodine (Dexa Medica®), vaseline (Sigma Aldrich).

### Plant material

*D. ochreatea* leaves (2 kg) were collected from Musi Banyuasin district in South Sumatera Province, Indonesia (3°34'1.434"S 104°77'18.19"E), (3°13'07.3"S 104°38'43.8"E) in October 2019. The sample was identified as *Dillenia ochreatea* (Miq.) Teijsm. & Binn. ex Martelli at Herbarium Bogoriense as Research Center for Biology, Indonesian Institute of Science Bogor, registered B-82/IV/D1.01/i/2021.

### Extraction

*D. ochreatea* leaves were dried at room temperature and ground into a powder. The *D. ochreatea* leaves powder (500 g) was macerated using ethanol for 3 × 24 hours and then filtration. The maceration was carried out with three repetitions (Pratiwi et al., 2021). The pooled filtrated were dried under reduced pressure using a rotary evaporator (Buchi®) at a temperature of 70°C to give the ethanol extract at a constant weight of 51.4 g (10.28% yield of fresh plant).

### Preparation of *D. ochreatea* leaf extract ointment

The preparation of *D. ochreatea* leaf extract ointment using the method described by Kiran et al. (2017). The standard formula of the ointment base used was a fatty base containing 15 g of lanolin and 85 g of vaseline to prepare a 100 g product, which was heated over boiling water. The mixture was stirred at a constant speed (1000 rpm) until it became homogeneous and formed an ointment base. The ointment was used as a vehicle for the preparation of each extract for topical application. The extract for topical application was used in experiments with concentrations of 2.5, 5, 7.5, and 10% (w/w). The crude extract 0.5, 1.0, 1.5, and 2 g, respectively, added of 19.5, 19, 18.5, and 18 g of vehicle to make up to 20 g of the crude extract ointment so the final concentrations of 2.5, 5, 7.5, and 10% (w/w) (Table 1).

**Table 1.** Ointment composition *D. ochreatea* leaves extract.

Ointment concentration	<i>D. ochreatea</i> leaves extract (g)	Ointment base (g)	Ointment weight (g)
Extract of <i>D. ochreatea</i> 2.5%	0.5	19.5	20
Extract of <i>D. ochreatea</i> 5%	1	19	20
Extract of <i>D. ochreatea</i> 7.5%	1.5	18.5	20
Extract of <i>D. ochreatea</i> 10%	2	18	20

### Experimental animals

The animal test used in the experiment were Male albino rats (Wistar strain, aged 2-3 months, weighing 180-250 g, 30 males) obtained from the Animal Laboratory Center at Palembang South Sumatera, Indonesia. Animal experiments in this study were carried out following the Animals in Research: Reporting In Vivo Experiments Guidelines and the Guide for the Care and Use of Laboratory Pharmacology, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya. The ethics committee approved the experimental protocol of the Ahmad Dahlan University No. 022109040.

### Effect of *D. ochreatea* extracts on the healing of burn wounds

A total of 60 male Wistar rats weighing 150–200 g obtained from the Laboratory Animal Center, Sriwijaya University, south Sumatera, Indonesia, were used in this study. The test animals were acclimatized to the laboratory environment for 7 days under adequate lighting (12 h of light) and a room temperature of 22°C. During the acclimatization process, the test animals were provided standard food and drink. The animals were randomly divided into incision and burn wound groups, with each group having 30 animals. Each group was further divided into six subgroups composed of five animals per subgroup: 1) negative group (untreated group); 2) positive group (1% silver sulfadiazine for burn wounds and 10% povidone-iodine for incision wounds); 3) 2.5% ethanol extract group; 4) 5% ethanol extract group; 5) 7.5% ethanol extract group; and 6) 10% ethanol extract group.

The effect of *D. ochreatea* extract on the healing of burn wounds was investigated using the method described by Akhoondinasab et al. (2014). The animals were anesthetized by intraperitoneal injection of 1% lidocaine during wound induction. The back of the animal under the ear was shaved and plucked using a Veet and razor, marked in a rectangular shape measuring 3 × 2 cm. Sterilization was done by applying 70% alcohol to the cleaned part. Next, burn wounds were created by placing a hot plate (3.0 × 2.0 cm) at 75°C in the prepared area for 10 seconds. The

test substance (ointments) was applied to the burned area twice a day for 15 days after burning. The burn area was measured immediately after the burn and on days 1 through 14, after the burn at interval 2. Negative (untreated) and positive controls (treated with 1% silver sulfadiazine) were used for comparison. Evaluated parameters included burn wound area and percent recovery in test animals. The burn area was measured using the ImageJ application, which can use to measure the surface area.

### Effect of *D. ochreatea* extracts on the healing of incision wounds

The effect of *D. ochreatea* extract on the healing of incision wounds was investigated using the model of Kokane et al. (2009). The animals were anesthetized using 2 mL of 2% lidocaine during the induction of the wound. The right side of the back of each animal was shaved, and then a midline incision was made (3 cm) through the skin with a sharp scalpel no. 11. After creating the wound, the test substance was topically applied to the affected area twice daily for five days. Negative controls (untreated) and positive controls (treated with 10% povidone-iodine ointment) were used for comparison. The first observation of healing of the incision wound was evaluated 24 hours after the incision was made. The healing of the incision wound was considered at 1-day intervals from the first day to the eighth day. The measurement of wound length was carried out at 1-day intervals. Observations were made visually by paying attention to changes in wound length, percentage of wound healed, and wound recovered days.

### Histopathology

On day 14, after burns and incision wounds were made, the animals were sacrificed by intraperitoneal injection of sodium pentobarbital (100 mg/kg BW). The skin specimens (1.5 × 1.5 cm) were collected from each animal for histological examination. The tissue was fixed in a 10% buffer neutral formalin solution, and then the sections were stained with hematoxylin and eosin. The histology slides were then scanned using Olyvia software at 40× and 4× magnifications. The epithelialization and collagen thickness were measured starting from the edge of the wound bed

down to the lower dermis. Measurements were made on the left, middle, and right sides, and then the average value was taken and compared with the control group (Kiran et al., 2017).

### Statistical analysis

The data were analyzed using data processing software SPSS version 26 and presented as the mean  $\pm$  standard deviation (SD). The data were tested for normality using the Shapiro-Wilk. If the resulting data were normally distributed ( $p > 0.05$ ), it could be continued to test the data using the one-way ANOVA test analysis, so differences between experimental groups could be compared and considered significant when  $p < 0.05$ . If the data were not normally distributed, then the data could be analyzed using the Mann-Whitney test.

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## RESULTS

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### Effect of *D. ochreatea* extracts on the healing of burn wounds

Wound healing activities of the ethanolic extract of *D. ochreatea* leaves were tested in the incision and burn wound models using the albino rat *Rattus norvegicus* (Wistar strain). The animals were randomly divided into incision and burn wound groups, with each group having 24 animals. Each group was further divided into six subgroups composed of four animals per subgroup: negative group (untreated group), positive group (1% silver sulfadiazine for burn wounds and 10% povidone-iodine for incision wounds), 2.5% ethanol extract group, 5% ethanol extract group, 7.5% ethanol extract group, and 10% ethanol extract group. Parameters observed for burn wound healing activity consist of the burn area, percent recovery, burn healing time, and skin histopathology (Rahman et al., 2019). The results of the average burn area are presented in Table 2; percent recovery is shown in Table 3 and burn wound healing time in Table 4.

Based on Table 2, the average area of burns in each treatment group decreased. Effect of *D. ochreatea* extract at all concentrations showed a higher average burn wound healing value than the negative control and positive control groups. The positive control group showed complete healing on day 14 (burn area 0 cm<sup>2</sup>), while the treatment group at all concentrations experienced complete healing on day 12, and the negative control group had not fully healed until day 14 (burn area 0.24  $\pm$  0.22 cm<sup>2</sup>). Based on the area of wound healing, the percentage of recovery was calculated, as shown in Table 3. The increase the value of

the percentage of healing was in line with the decrease in the burn area in all groups. The percentage of burn healing increase varied in each group. All treatment groups achieved 100% cure on the 12<sup>th</sup> day, while the positive control showed on the 14<sup>th</sup> day. The negative control until the 14<sup>th</sup> day only had a healing percentage of 96.03  $\pm$  3.64%.

Table 4 shows that the positive control group gave an average percent value of wound healing of 7.1% per day, while the treatment group that was given *D. ochreatea* leaf extract at all concentrations gave a higher average value of recovery (8.3%) than the positive control and negative control (6.8%). The percentage average burn healing activity of the positive control group per day was lower than the treatment group. Burn healing time of positive control was longer than all treatment groups and all treatment groups had the same total healing time. The data indicated extract at concentration 7.5% more effective in wound burn healing compared to other concentration.

### Incision wound healing activities

Wound healing parameters were determined based on the length of the wound, percent recovery, wound healing time, and skin histopathology. The measurement of the decrease in the incision length was carried out at 1-day intervals. The results of the average burn area are presented in Table 5; percent recovery is shown in Table 6, and incision wound healing time in Table 7.

Table 5 shows that each group's average length of incisions decreased. The positive control group had a wound length of 0 cm on the fourth day, while the negative control group had a wound length of 0 cm on day 6 and all treatment groups on day 5. Table 6 shows that each group offers a wound healing process characterized by increased percent recovery. The percent recovery in the positive control group on day 4 reached 100%. The treatment group at all test concentrations had 100% recovery on the 5<sup>th</sup> day. The negative control group on day 5 had a percent recovery of 90.83  $\pm$  8.33.

Table 7 shows the wound healing time in each treatment group. The positive control group had a healing time of 4 days, the negative control group had a healing time of 6.6 days, and the treatment group had a healing time of 5 days. All treatment groups have a wound healing of incisions activity was lower than positive control but higher compared to the negative control. The healing time of the treatment group was not significantly different ( $p > 0.05$ ) from the positive control group.

**Table 2.** Effect of ethanolic extract on the area of the burn wound.

Group	Burn area (cm <sup>2</sup> )							
	Day							
	0	2	4	6	8	10	12	14
Positive	5.93 ± 0.07	5.77 ± 0.08	5.72 ± 0.05	5.15 ± 0.39	4.00 ± 0.40	1.25 ± 1.50 <sup>a</sup>	0.15 ± 0.29 <sup>a</sup>	0 ± 0
Negative	5.97 ± 0.14	5.93 ± 0.15	5.92 ± 0.15	5.06 ± 0.12	4.46 ± 0.50	1.79 ± 0.72 <sup>b</sup>	0.86 ± 0.57	0.24 ± 0.22
Extract of <i>D. ochreatea</i> 2.5%	5.74 ± 0.15	5.69 ± 0.11	4.79 ± 0.09	3.32 ± 0.13	2.21 ± 0.47	0.65 ± 0.20 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 5%	5.25 ± 0.46	5.20 ± 0.44	3.56 ± 0.10	2.48 ± 0.13	1.15 ± 0.58	0.40 ± 0.35 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 7.5%	5.98 ± 0.06	5.90 ± 0.01	5.87 ± 0.05	4.16 ± 0.67	3.43 ± 0.96	0.81 ± 0.35 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 10%	5.61 ± 0.13	5.56 ± 0.12	4.86 ± 0.10	2.74 ± 0.29	1.77 ± 0.50	0.44 ± 0.52 <sup>a</sup>	0 ± 0	0 ± 0

Data are expressed as mean ± SD (n = 4); Different letters indicated the significantly different (p<0.05): compare to the negative group, <sup>a</sup> compare to the positive group.

**Table 3.** Effect of ethanolic extract on recovery of the burn wound.

Group	Recovery (%)							
	Day							
	0	2	4	6	8	10	12	14
Positive	0 ± 0	2.78 ± 0.70	3.63 ± 0.55	13.22 ± 5.99	32.56 ± 6.21	79.00 ± 24.99 <sup>a</sup>	97.56 ± 4.87 <sup>a</sup>	100 ± 0 <sup>a</sup>
Negative	0 ± 0	0.68 ± 0.46	0.87 ± 0.58	15.25 ± 0.48	25.36 ± 7.02	70.09 ± 11.81 <sup>b</sup>	85.54 ± 9.39 <sup>b</sup>	96.03 ± 3.64 <sup>b</sup>
Extract of <i>D. ochreatea</i> 2.5%	0 ± 0	0.92 ± 0.76	16.59 ± 3.82	42.20 ± 3.77	61.40 ± 9.26	88.63 ± 3.79 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>
Extract of <i>D. ochreatea</i> 5%	0 ± 0	1.05 ± 0.76	32.05 ± 3.91	52.49 ± 5.44	77.46 ± 12.42	91.96 ± 7.04 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>
Extract of <i>D. ochreatea</i> 7.5%	0 ± 0	1.37 ± 0.82	1.80 ± 0.29	29.97 ± 10.81	41.83 ± 15.95	88.26 ± 7.83 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>
Extract of <i>D. ochreatea</i> 10%	0 ± 0	0.85 ± 0.49	13.28 ± 2.15	50.99 ± 6.16	68.31 ± 9.57	92.02 ± 9.33 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>

Data are expressed as mean ± SD (n = 4); Different letters indicated as significantly different (p<0.05): <sup>a</sup>compare to the negative group, <sup>b</sup> compare to the positive group.

**Table 4.** Burn wound healing time.

Group	Recovery day-14 (%)	Average burn healing every day (%)	Time burn wound healing (day)
Positive	100	7.1	14
Negative	93.03	6.8	14.57
Extract of <i>D. ochreatea</i> 2.5%	100	8.3	12
Extract of <i>D. ochreatea</i> 5%	100	8.3	12
Extract of <i>D. ochreatea</i> 7.5%	100	8.3	12
Extract of <i>D. ochreatea</i> 10 %	100	8.3	12

**Table 5.** Effect of ethanolic extract on length of the incision.

Group	Day							
	0	1	2	3	4	5	6	7
Positive	3 ± 0	2.82 ± 0.09	1.97 ± 0.12	1.05 ± 0.31 <sup>a</sup>	0 ± 0	0 ± 0 <sup>a</sup>	0 ± 0	0 ± 0
Negative	3 ± 0	2.87 ± 0.05	2.72 ± 0.09	1.32 ± 0.26 <sup>b</sup>	0.82 ± 0.29 <sup>b</sup>	0.27 ± 0.25 <sup>b</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 2.5%	3 ± 0	2.86 ± 0.05	2.66 ± 0.15	1.10 ± 0.10 <sup>a</sup>	0.56 ± 0.20 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 5%	3 ± 0	2.80 ± 0.10	2.56 ± 0.15	0.91 ± 0.15 <sup>a</sup>	0.56 ± 0.15 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 7.5%	3 ± 0	2.90 ± 0.08	2.65 ± 0.17	1.20 ± 0.21 <sup>a</sup>	0.17 ± 0.35 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0	0 ± 0
Extract of <i>D. ochreatea</i> 10%	3 ± 0	2.77 ± 0.09	2.52 ± 0.15	1.02 ± 0.18 <sup>a</sup>	0.12 ± 0.25 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0	0 ± 0

Data are expressed as mean ± SD (n = 4). Different letters indicated the significantly different (p<0.05): <sup>a</sup>compare to the negative group, <sup>b</sup> compare to the positive group.

**Table 6.** Effect of ethanolic extract on percent recovery of incision wound.

Group	% Recovery							
	Day							
	0	1	2	3	4	5	6	7
Positive	0 ± 0	5.83 ± 3.19	34.16 ± 4.19	65 ± 10.36	100 ± 0 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0	100 ± 0
Negative	0 ± 0	4.16 ± 1.67	9.16 ± 3.19	55.83 ± 8.76	72.50 ± 9.95 <sup>b</sup>	90.83 ± 8.33 <sup>b</sup>	100 ± 0	100 ± 0
Extract of <i>D. ochreatea</i> 2.5 %	0 ± 0	4.44 ± 1.92	11.11 ± 5.09	63.33 ± 3.33	81.11 ± 6.93 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0	100 ± 0
Extract of <i>D. ochreatea</i> 5 %	0 ± 0	6.67 ± 3.33	14.44 ± 5.09	57.77 ± 5.09	85.57 ± 0.17 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0	100 ± 0
Extract of <i>D. ochreatea</i> 7.5 %	0 ± 0	3.33 ± 2.72	11.67 ± 5.77	60.11 ± 7.20	94.16 ± 11.66 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0	100 ± 0
Extract of <i>D. ochreatea</i> 10 %	0 ± 0	7.51 ± 3.19	15.83 ± 5.01	65.83 ± 6.30	95.83 ± 8.33 <sup>a</sup>	100 ± 0 <sup>a</sup>	100 ± 0	100 ± 0

Data are expressed as mean ± SD (n = 4). Different letters indicated the significantly different (p<0.05): <sup>a</sup>compared to the negative group, <sup>b</sup> compare to the positive group.

**Table 7.** Incision wound healing time.

Group	Recovery incision wound day 5 (%)	Average incision wound healing every day (%)	Time of incision wound healing (day)
Positive	100	25	4
Negative	93.03	15.1	6.60
Extract of <i>D. ochreatea</i> 2.5%	100	20	5
Extract of <i>D. ochreatea</i> 5%	100	20	5
Extract of <i>D. ochreatea</i> 7.5%	100	20	5
Extract of <i>D. ochreatea</i> 10%	100	20	5

**Table 8.** The average thickness of the epithelium and collagen.

No.	Group	Average epithelial thickness (µm)	Average collagen thickness (µm)
1	Burn wound	145.91 ± 1.15 <sup>a</sup>	1220.56 ± 5.22 <sup>a</sup>
2	Incision wound	71.68 ± 2.75	816.71 ± 8.16 <sup>a</sup>
3	Normal	65.02 ± 1.14	2067.99 ± 7.22

Data are expressed as mean ± SD (n = 4); <sup>a</sup>significantly different (p<0.05) compared to the normal group.

## Histopathological analysis

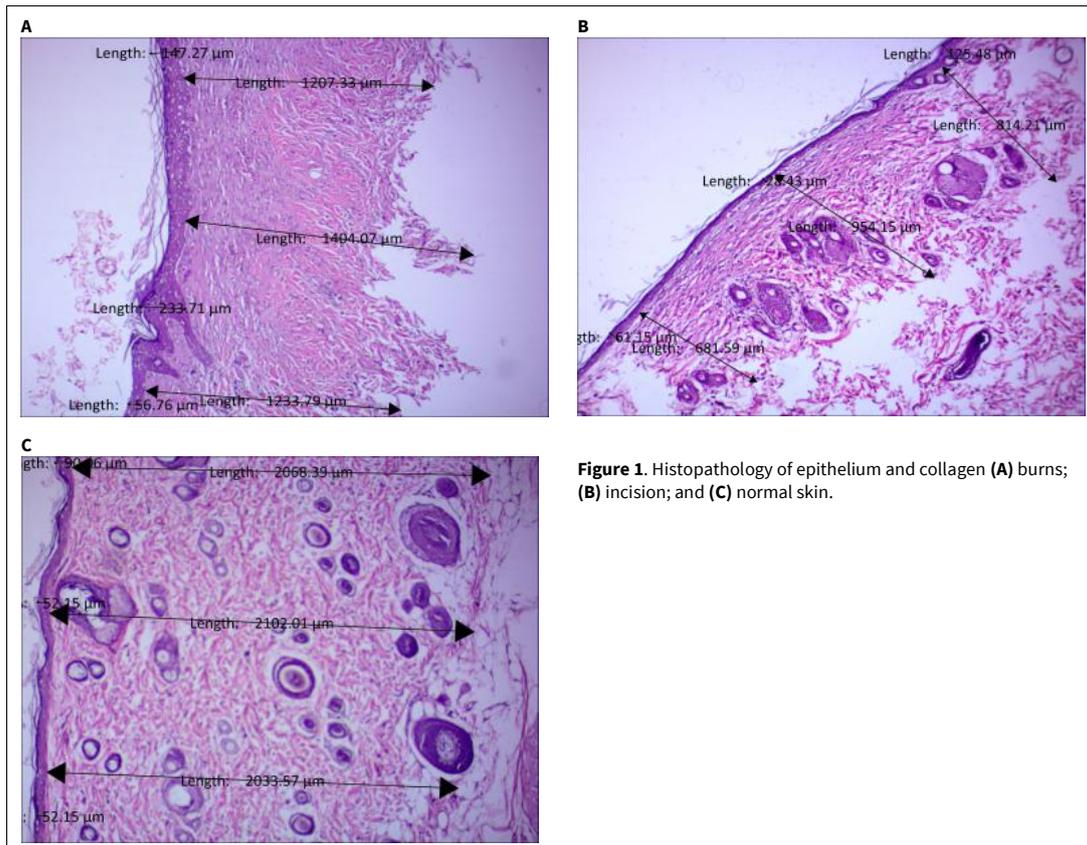
Histopathological observation of rat skin was performed to see changes in cell structure in organs with epithelialization and collagenization parameters. Observations were made by comparing the histopathological features of the burn group and the incision wound group with the normal group. Samples were selected from the group with the highest extract ointment concentration. Table 8 and Fig. 1 showed that the burn group had a more increased epithelial thickness than the incision group. The treatment group showed a significant difference (p<0.05) from the normal group.

## DISCUSSION

Effect of *D. ochreatea* ethanolic extract on the healing of burns showed a higher average burn wound healing value than the positive control group. These results indicated that all treatment groups had better burn healing activity than the positive control because

the crude extract contains a complex mixture of medicinal plant metabolites (Tiwari et al., 2011). It indicates that the ethanolic extract of *D. ochreatea* leaves contains bioactive compounds as antibacterial and anti-inflammatory and stimulates epithelial formation. Acts synergistically to accelerate wound healing also reported the percentage of burn wound healing of ethanolic extract of *Scutellaria barbata* leaves with 20% dose, higher compared to povidone-iodine standard, which is 100% wound healing was observed in 14.4 ± 0.89 days, while povidone iodine ointment treated group 13.67 ± 0.38 days (Kiran et al., 2017).

All treatment groups experienced a decrease in the burn area and an increase in percent recovery, which was significantly different (p<0.05) from the negative control. The treatment group had a burn healing activity that was not significantly different (p>0.05) from the positive control. The ethanolic extract of *D. ochreatea* leaves contains bioactive compounds that can potentially increase the speed of burn healing



**Figure 1.** Histopathology of epithelium and collagen (A) burns; (B) incision; and (C) normal skin.

(Muharni et al., 2017). The higher the test concentration, the higher the levels of bioactive compounds contained. The average percent recovery from day 1 to day 5 for each group was <20%. At this time, the wound enters the inflammatory phase so that the wound healing process in forming new cells has not been maximized. In the inflammatory phase, hemostasis, loss of dead tissue, and prevention of colonization and infection by pathogenic microbial agents occur (Landén et al., 2016).

An increase in percent recovery characterized the process of wound healing. The use of povidone-iodine as a positive control in wound care reduced redness due to its antibacterial content. All treatment groups had a wound length of 0 cm on the fifth day. The healing of rat wounds was indicated by the closure of the cut and the formation of white skin. All treated groups showed a wound healing of incision activity was lower than the positive control but higher than the negative control. Statistical analysis showed that the treatment group had a significantly different wound healing activity ( $p < 0.05$ ) against the negative control and not significantly different ( $p > 0.05$ ) against the positive control.

The ethanol extract of *D. ochreate* leaves contains secondary metabolites triterpenoids, phenolics, and steroids (Muharni et al., 2017). The mechanism of

triterpenoids as antibacterial reacts with transmembrane proteins on the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in the destruction of the protein transmembrane so that bacterial growth is inhibited or dies (Chung, 2020).

Phenolic compounds also have an antibacterial activity that can play a role in wound healing (Sukmawan et al., 2021). The antibacterial mechanism of phenol compounds in killing microorganisms is by denaturing cell proteins. The hydrogen bonds between phenol and protein cause the protein structure to be damaged. The hydrogen bonds affect the cell wall's and cytoplasmic membrane's permeability (Madduluri et al., 2013). Steroid class compounds act as antibacterial agents by increasing the speed of wound healing. The mechanism of steroids as antibacterials is related to lipid membranes and sensitivity to steroid components that cause liposome leakage. Steroids can interact with cell phospholipid membranes, which are permeable to lipophilic compounds, causing decreased membrane integrity and cell membrane morphology to change, which in turn causes cells to become brittle and lysed (Makarewicz et al., 2021). In addition to antibacterial activity, steroidal compounds are potent anti-inflammatories by inhibiting phospholipase A2 so that arachidonic acid is not formed (Xie et al., 2015).

Histopathological observation of rat skin shows that the burn group had a higher epithelial thickness than the incision group. These results indicated that the active compounds in the ethanolic extract of *D. ochreatea* leaves could increase the epithelialization process, thus accelerating the wound healing process. Epithelial formation begins during the transitional phase, after the inflammatory phase, and before the proliferative phase (Ahmad et al., 2021). In this transitional phase, inflammatory mediators are still released on the burned skin, so epithelialization has not occurred correctly (Tiwari, 2012). Day 14 is part of the proliferative phase, where keratinocytes and fibroblasts are migrated, which accelerates wound closure. Fig. 1 shows the difference in thickness between the normal and treatment groups. Collagen thickness in the treatment group had a very significant difference ( $p < 0.05$ ) compared with the normal group, where the normal control had a greater collagen thickness than the test group. For observation, rat skin preparations were taken on day 14, when the proliferative phase started. In this phase, fibroblasts on the wound surface slowly produce new collagen fibers. The main process of fibroblast growth occurs on days 7 to 14 after treatment, and after that, it will continue to improve until the skin structure returns to normal (Gonzalez et al., 2016).

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## CONCLUSION

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*D. ochreatea* leaf ethanol extract ointment at a concentration of 10% demonstrated the best results in healing burns with 100% recovery on day 12 and incision wounds with 100% recovery on day 5. There was no significant difference ( $p > 0.05$ ) in the healing activity of burn and incision wounds compared to positive control.

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## CONFLICT OF INTEREST

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The authors declare no conflicts of interest.

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**AUTHOR CONTRIBUTION:**

Contribution	Muharni M	Annisa A	Fitrya F	Anas M
Concepts or ideas	x	x	x	x
Design	x	x	x	x
Definition of intellectual content	x	x	x	
Literature search	x	x		x
Experimental studies	x	x		x
Data acquisition	x	x	x	x
Data analysis	x	x	x	x
Statistical analysis	x		x	x
Manuscript preparation	x	x	x	x
Manuscript editing	x	x	x	x
Manuscript review	x	x	x	x

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