The Effect of Pagoda Flower Extract (*Clerodendrum Paniculatum L*) on Collagenization and Histopathological Features of Skin Tissue in the Healing Process of Dermapen Wounds in Male Wistar White Rats

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ABSTRACT

This study was conducted to test the effect of pagoda flower extract (Clerodendrum Paniculatum L) on collagenization and histopathological features of skin tissue in the healing process of wounds from dermapen in male white Wistar rats. The treatment was given in the form of 10%, 15%, and 20% cream on the healing process of wounds from dermapen. Phytochemical tests found that pagoda flowers positively contained active compounds such as alkaloids, flavonoids, saponins, and tannins. From the observations of wound healing, the control group had 71.5% of wounds closed. In treatment group 1, the percentage of wound healing was 100% on the 13th day, and in treatment groups 2 and 3 on the 12th day. In histopathological observations, the collagen density of the wound was already tight in treatment groups 2 and 3, with 15% and 20% pagoda flower cream extracts. So, pagoda flower extract is effective in healing wounds from dermapen. Kolmogorov-Smirnov test normality test The results of wound healing data in each group are normally distributed with a significance value of 0.200> 0.05 in all test groups.

Keywords: Collagenization, Pagoda flower, Dermapen Skin

INTRODUCTION

The skin protects the inside of the body from external physical disturbances, chemical disturbances, heat disturbances, and infectious disturbances, especially from fungi and bacteria. The skin is the body's outermost layer covering the muscles, organs, and internal organs. The skin comprises three main layers: the epidermis, the dermis, and the hypodermis.

All three are present in human skin and perform different roles. One form of skin damage is a wound. A skin wound is a pathological condition caused by disease, injury, or physiochemical damage. During daily activities in nature, humans often experience injuries. Injuries can occur due to accidents, accidents, or deliberate attacks. Basic aid is the first action that must be taken after someone is injured to reduce pain and prevent bleeding. These actions include stopping bleeding, cleaning the wound, covering the wound with sterile gauze covered with cotton and bandaged, and then covering the wound with medicine. In addition, treatment can be done using traditional medicine available in the environment. Dermapen is a motorized microneedle therapeutic system tool that can be adjusted to skin problems. Dermapen, an ergonomic device, can adjust the needle length from 0.25 to 2 mm using disposable needles.

LITERATURE REVIEW

The skin is the outer layer that covers the body of a vertebrate. The skin consists of the epidermis, dermis, and hypodermis. The skin functions as an excretory organ because sweat glands (sudoriferous glands) are in the dermis layer (Aziz *et al.*, 2018). The skin is a barrier that protects internal tissues from trauma, ultraviolet (UV) radiation, extreme temperatures, toxins, and bacteria. Other important skin functions include sensory perception, immune system surveillance, temperature regulation, and fluid loss control. The skin is also essential for us to understand our environment and communicate through skin receptors (Picture of the Skin, 2021). Because the skin is the largest body organ, it is susceptible to trauma and injury (Graduation, 2015).

Many things can cause wounds, but mechanical trauma is the most common. Mechanical trauma can occur due to blunt or sharp objects. Wounds can also be divided into two types: open and closed. The replacement and restoration of damaged tissue function is known as wound healing. The healing process for each wound varies depending on the location, severity, and extent. The wound healing process consists of three stages (Fadhilah, H. 2022), inflammation, proliferation, and remodeling (Ermi Girsang, 2021). The body responds and triggers wound healing if damaged or missing tissue is damaged (Pradipta, 2010). Wounds are categorized into acute and chronic wounds. Acute wounds are caused by traumatic physical/chemical damage or surgical procedures, while non-healing wounds are caused by diseases such as infections, diabetes, vascular disease, and cancer.

Dermapen is a motorized microneedle therapeutic system that can be adjusted to skin problems. It is important to use a derma pen with sterile needles separately. One dermapen can treat narrow areas such as the nose, lips, and eyes without damaging adjacent skin (Purbosetyo, 2020). In the research, dermapen is often used to create wounds on the skin of mice, which are then studied for the healing process by plant extraction. The healing process will be observed for collagenization of the skin. Collagen is needed to repair and rebuild connective tissue to support the development of strong muscles, skin, and joints (Ivanalee et al., 2018). Chlorogenic acid (CA) is a phenolic compound known for its antioxidant properties against oxidative stress (Ermi Girsang et al., 2021).

In traditional medicine, pagoda flowers (Clerodendrum paniculatum L.) often relieve various inflammatory conditions, such as joint pain and sore throat. The benefits of this flower are that it can also be used for wounds on the skin, such as wounds due to bruises, boils, scabs, and other external wounds that can be treated using pagoda flowers. The antioxidant content in this flower can help the body produce skin collagen tissue faster in the wound-healing process. Antioxidants are compounds that can prevent and repair damage to cells in the body (Regitha Claudia, 2024).

METHODS

Laboratory experimental research examines the effectiveness of administering pagoda flower extract cream in skin collagenization of male rats (Rattus norvegicus) given dermapen treatment and observing the histopathology of rat skin tissue. The research was conducted at the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara in May - July 2024. Ethical Clearance will be submitted to the Health Research Ethics Commission (KPEK) Universitas Prima Indonesia. The study sample used male Wistar rats (Rattus norvegicus) weighing 160-250 grams. The research sample of 24 mice was divided into 4 groups (Kendall *et al.*, 2018) (Control group (K), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3). The dermapen was applied to the skin of white rats according to the desired area, which is 2x2cm. The wound-healing process was observed daily for 14 days. After 14 days, all rats were euthanized by inhalation of excess technical chloroform (Calsum, 2018). In preparing pagoda flower extract cream, dried pagoda flowers are extracted using 96% ethanol, filtered, and the filtrate is collected; the residue is then macerated. The ethanol content is evaporated using a rotary evaporator to obtain a thick extract. The evaporation results were then thickened using a water bath. The resulting thick

extract is used for making creams. Ingredients in making creams include Pagoda flower extract in preparations (10 g, 15 g, and 20 g), cetyl alcohol, glycerin, triethanolamine, stearic acid, methyl paraben, propyl paraben, distilled water. The group of rats that have passed the acclimation period and have been given dermapen action will then be given the treatment of pagoda flower extract for 14 days, where group K was not given pagoda flower extract cream, P1 with pagoda flower extract cream with a concentration of 10%, P2 with a concentration of 15%, and P3 with a concentration of 20%. On the 15th day, the white mice were euthanized through decapitation and previously anesthetized, and their skin tissue was taken to make histopathological preparations. The observations were made with 40x and 100x magnification at 5 fields of view by anatomical pathologists with repeated readings—microscopic observation by looking at the state of the epidermis and the structure of collagen density. Then, histopathological scoring was carried out to determine the density of collagen tissue distribution (Rizka, A., 2013). Then, the wound healing process and histopathology of the mouse skin tissue were observed, which will be presented descriptively with Kolmogorov-Smirnov test data normality analysis, continued by testing the significance between trial groups using the one-way variance analysis technique or One Way ANOVA at a 95% confidence level ($p < 0.05$) and further testing was carried out using the Post Hoc Test with the LSD technique (Ghozali, 2018).

RESULTS

This study used 24 male rats (Rattus norvegicus) of the Wistar strain that had received dermapen treatment as samples once. However, the rat's hair was shaved first. After being shaved, the rats were made unconscious using a combination of ketamine (80 ml/kg BW) and xylazine (5 ml/kg BW) so that the rats did not feel pain and avoided excessive movement by the rats that arose due to the derma pen treatment. Furthermore, dermapen treatment was given to the skin of white rats according to the desired area, which was 2x2cm. The derma pen treatment adjusted the needle depth from 0.25 - 2 mm. This study has a needle depth of 2 mm and a wound width of 2 cm. Then, the rats were treated according to each group's treatment.

After the wound was given, the mice on H0 got the same wound width, which was 2 cm. Then, after observations were made after the treatment, those who experienced perfect wound closure were in treatment groups 1, 2, and 3, which were 0 cm. On the 14th day, only the control group did not experience perfect wound closure with a wound width of 0.57 cm. So,

if the data is presented in the percentage of wound healing, then the difference in the average percentage of healing from the control and treatment groups is seen. The average percentage of wound healing from dermapen on the last day of the control group was 71.5%. Treatment group 1 got a wound healing percentage of 100% on the 13th day, while treatment groups 2 and 3 on the 12th day.

The pagoda flower plant (Clerodendrum paniculatum L.) is considered a medicinal plant because of its many properties, which are then made into an extract cream for healing wounds. The phytochemical tests carried out revealed that it appears to contain secondary metabolite compounds, namely flavonoids, saponins, tannins, and alkaloids.

Reporting Research Results

The following is the conceptual framework in this study:

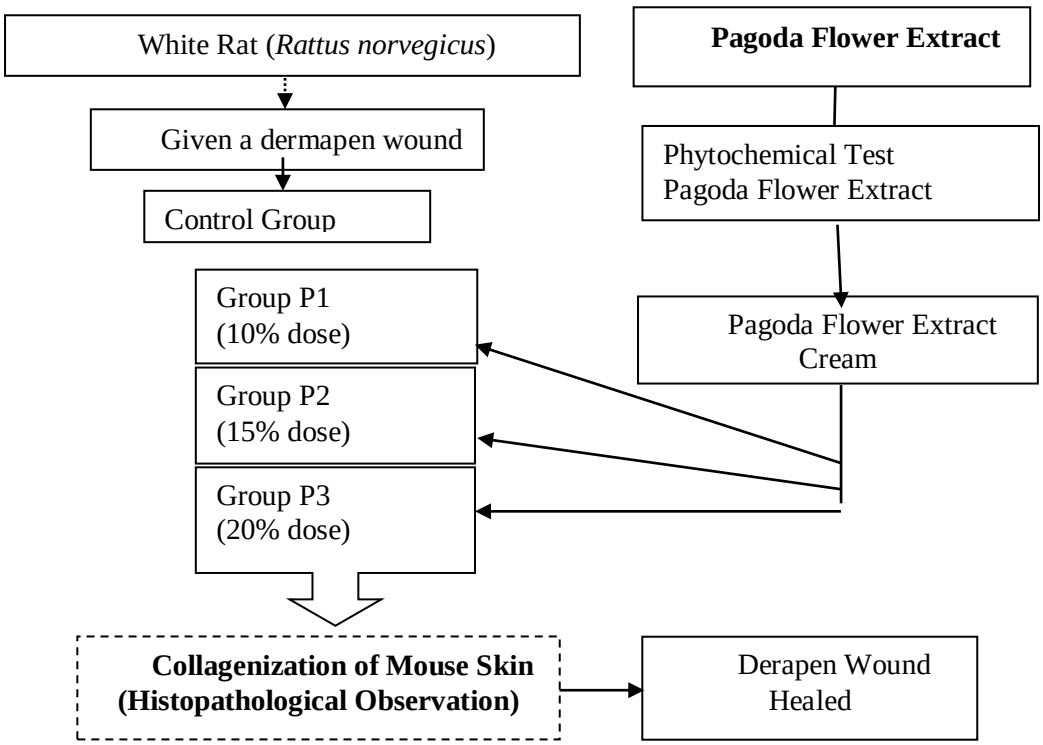


Figure 1. Conceptual Framework

This study hypothesizes that administering pagoda flower extract (Clerodendrumpaniculatum) affects collagenization and histopathological features of skin tissue in the healing process of wounds from derma pen in male Wistar strain white rats.

The pagoda flower plant (Clerodendrum paniculatum L.) is medicinal because of its many properties. The results of the phytochemical test of pagoda flower extract can be seen in the following table.

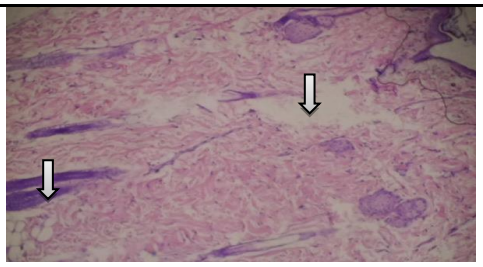
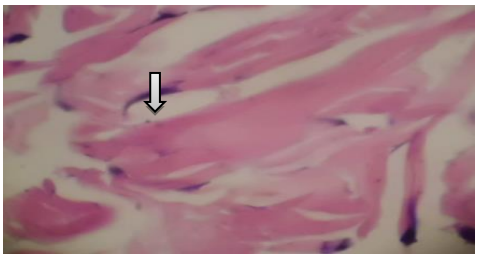
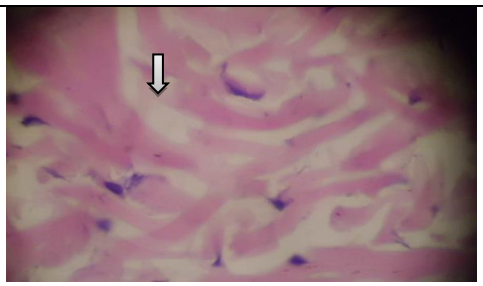
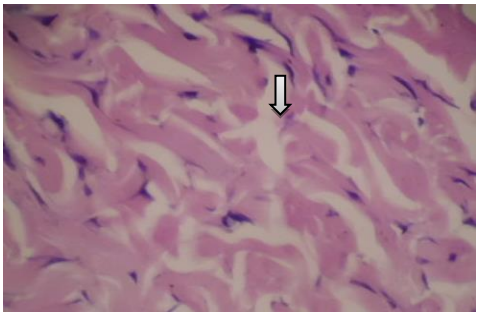
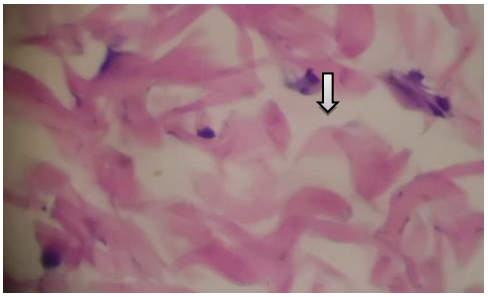
Table 1. Phytochemical Screening Results of Pagoda Flower Extract

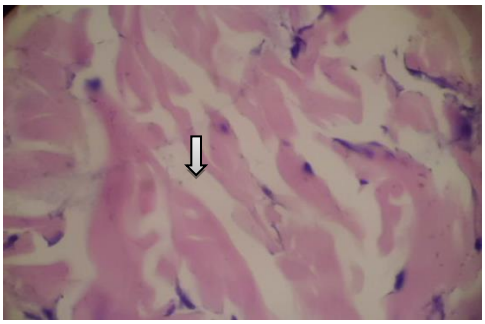
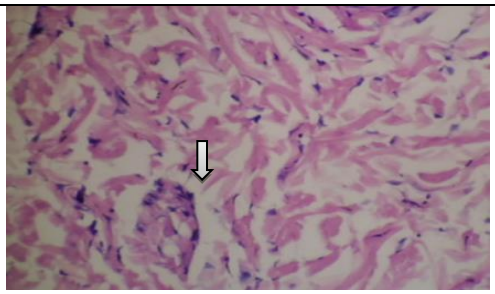
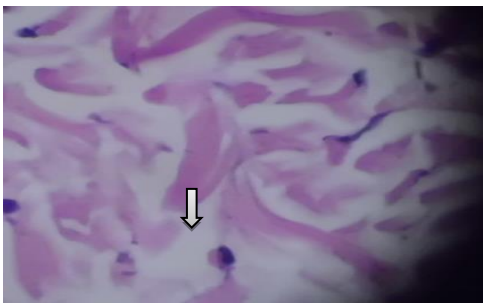
Secondary Metabolites	Testing	Color	Results
Flavonoid	Wilstater	Red	+
Saponins	Forth	Blue and foamy	+
Tannin	FeCl3	Blackish green	+
Alkaloid	Wagner	Yellow	+
Triterpenoid	Lieberman – Burchard	Green	-

A red extract is formed when testing flavonoids, which contain flavonoids. There is foam in the extract, which means it contains saponins. In the results of the tannin test, a blackish-blue liquid appears, which means it contains tannins. The alkaloid test results are yellow, meaning it contains alkaloids. In the steroid/triterpenoid test, the color that comes out is green, which means it contains negative triterpenoids. The study results prove that the extract pagoda flower (Clerodendrum paniculatum L.) positively contains active compounds such as alkaloids, flavonoids, saponins, and tannins. So, pagoda flower extract has good antioxidant benefits for the body.

After wound healing, histopathological observation of mouse skin tissue was carried out. This observation aimed to see the structure and morphology of cells, especially collagen cells, in each dermapen wound specimen in the treatment group with base cream (0) and pagoda flower extract cream with concentrations of 10%, 15%, and 20%. The application of the cream was carried out twice a day, namely in the morning and evening.

Table 2. Histopathological Image of Mouse Skin Tissue

No	Kel	**Histopathological Image of Skin Tissue**
1	K (0%)	 
2	P1 (10%)	 
3	P 2 (15%)	

		
4	P 3 (20%)	
		

The results of this study show that administering pagoda flower extract cream(*Clerodendrum paniculatum L.*)can affect the density of skin collagen in rats undergoing dermapen wound treatment on male white rats (*Rattus norvegicus*) Wistar strain. This is proven by the difference in collagen density between treatment groups.

Next, to analyze the data, the normality test in this study used the Kolmogorov-Smirnov test with the following results:

Table 3. Normality Test Results

Group	df	Sig
Control	14	.200
P1	14	.200
P2	14	.200
P3	14	.200

The results of the test obtained a significance result of 0.200 in all groups that had been measured in 14 days of treatment with p> 0.05, which is 0.200 so that the data has been normally distributed. Furthermore, homogeneity testing was carried out using the Levene test with a significance level of 5%, the following table:

Table 4. Homogeneity Test

Levene static	**df1**	**df2**	**Sig**
1,595	3	52	.202

The probability value of significance obtained is greater than 0.05, which is 0.202, so it can be concluded that the control group, treatment group 1, treatment group 2, and treatment group 3 come from a population that has the same variance. Furthermore, testing the significant effectiveness between the trial groups with one way anova.

Table 5. One Way Anova Test Results

	Amount	df	Mean square	F	Sig
Inter Group	2,894	3	.965	2,081	.000
In Group	24.104	52	.464		
Total	26,997	55			

The results of the One-Way ANOVA test in the table above show that the significance value produced is 0.000 or <0.05. Based on these data, there is a significant difference between the control and treatment groups. Then, the post-hoc LSD further test was conducted to analyze the differences in the average levels of total cholesterol between groups with the following results:

Table 6. Results of LSD Post-Hoc Test

Group		Mean difference	Sig
Control	Treatment 1	.33500	.000
	Treatment 2	.53214*	.000
	Treatment 3	.57643*	.000
P1	Control	-.33500	.000
	Treatment 2	.19714	.000
	Treatment 3	.24143	.000
P2	Control	-.53214*	.000
	Treatment 1	-.19714	.000
	Treatment 3	.04429	.864
P3	Control	-.57643*	.000
	Treatment 1	-.24143	.000
	Treatment 2	-.04429	.864

A Post Hoc LSD test was used to determine whether the group had a significant difference in wound healing compared to other groups. The results of the analysis showed that there was a significant difference between the control group and treatment groups 1 ($p = 0.000$), 2 ($p = 0.000$), and 3 ($p = 0.000$). While treatment group 2 and treatment group 3 did not have a significant difference ($p = 0.864$).

DISCUSSION

Obesity occurs when you consume more calories than you burn. One form of skin damage is a wound. A skin wound is a pathological condition caused by disease, injury, or physiochemical damage. Wounds are divided into acute wounds and chronic wounds based on their healing duration. Acute wounds are caused by trauma or surgical wounds, and they heal within 8–12 weeks, depending on the size and depth of the wound (Farahani, 2011). Wounds usually undergo inflammatory, proliferative, and remodeling phases to repair tissue damage. Collagen, an important part of the extracellular matrix, is critical in regulating the

wound healing phase, either in its native form, fibrillar conformation, or soluble form in the wound environment (Mathew-Steiner, 2021).

This study used test animals such as male white rats (Rattus norvegicus) and Wistar strains weighing 160-250 gr. The test animals were divided into 4 groups: the control group, which was only given base cream, treatment group 1, which was given pagoda flower extract cream (ClerodendrumPaniculatum) with different concentrations, namely 10%, 15%, and 20%. The study was conducted by collecting data related to the observation of the treatment procedure. First, the rats were given preconditioning treatment in the form of wounds from dermapen. The procedure began by eliminating the consciousness of the test animals using a combination of ketamine (80 ml/kg BW) and xylazine (5 ml/kg BW) so that mice do not feel pain and avoid excessive movements made by mice which arise as a result of the derma pen action.

The average percentage of wound healing results from dermapen on the last day of the control group was 71.5%. Treatment group 1 obtained a wound healing percentage of 100% on the 13th day, while treatment groups 2 and 3 on the 12th day. The researcher can conclude that the control group did not experience total healing, and treatment groups 2 and 3 required the fastest time to experience total healing compared to treatment 1 because the wound closed perfectly before the 14th day.

Observations were continued on the histopathological picture of the skin tissue to see the collagenization of wound healing in the control group (K), which was only smeared with distilled water, resulting in minimal collagen growth. However, there were still several points of needle puncture wounds that had not entirely closed in the mouse skin tissue, so it received a score of 1 (less than 10%). Meanwhile, treatment group 1 was given pagoda flower extract cream.(Clerodendrum paniculatum L.)A concentration of 10% shows collagen growth, and the fibers look scattered even though the collagen fibers are not yet dense and thick, score 2, namely (10-50%). Treatment groups 2 and 3 were given pagoda flower extract cream(Clerodendrum paniculatum L.)with concentrations of 15% and 20%, and there was an increase in dense collagen fibers so that it appeared to dominate the results and a score of 4 was given. Collagen fibers in the wound area are very dense (90-100%). So, it can be concluded that the density of collagen formed in the histopathology of the skin of white rats (Rattus norvegicus) of the Wistar strain that was given dermapen treatment cannot be separated from the phytochemical compound content of pagoda flower extract.(Clerodendrum paniculatum L.).

CONCLUSION

1. In this study, it was found that pagoda flower extract (*Clerodendrum paniculatum L.*) affects the collagenization of the skin of mice treated with derma pen and the histopathological picture of skin tissue in the healing process of wounds from derma pen in male white Wistar rats.
2. The content of secondary metabolites in pagoda flower extract (*Clerodendrum paniculatum L.*) through phytochemical tests shows pagoda flowers. Positively contains active compounds such as alkaloids, flavonoids, saponins, and tannins. So, pagoda flower extract has antioxidant, anti-inflammatory, and anti-aging benefits.
3. The average percentage of wound healing results from dermapen on the last day of observation (day 14) was the Control group with 71.5%. Treatment group 1 obtained a wound healing percentage of 100% on day 13, while treatment groups 2 and 3 on day 12, so the administration of extract creamPagoda flower extract (*Clerodendrum paniculatum L.*) is effective in healing wounds and the fastest wound healing at doses of 15% and 20%.
4. In the histopathological picture of the skin tissue of mice, treatment groups 2 and 3 were given pagoda flower extract cream.(*Clerodendrum paniculatum L.*) With concentrations of 15% and 20%, dense collagen fibers increased, appearing to dominate the results, and a score of 4 was given. Collagen fibers in the wound area are very dense (90-100%).

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