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Effect of Ethanol Extract of Rosella Flower Petals (*Hibiscus sabdariffa L.*) on Healing Cut Wounds in White Rats (*Rattus norvegicus*)

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ABSTRACT

Background: Wounds are skin disorders in the form of damage to continuity due to a pathological process, if not treated properly it can cause serious complications such as failure to heal and chronic problems due to untreated wound infections, especially post-operative infections and scar tissue. The next problem is that excessive antibiotics can cause side effects and increase the risk of antimicrobial resistance and delay wound healing. So a new strategy is needed with herbal-based therapy that has the effect of treating infections and does not cause side effects in patients.
Subjects and Method: The study was conducted with a post test only randomized control group design to test macroscopic observations of the average size of the wound length, hyperemia and swelling in 30 white Wistar rats (*Rattus norvegicus*) after receiving ethanol extract of rosella petals (*Hibiscus sabdarifa L.*). The dependent variables of the study were wound closure time, hyperemia and wound swelling. The independent variable of the research was varying doses of ethanol extract of rosella flower petals (*Hibiscus sabdarifa L.*) 6%, 12% and 15%. The parameters assessed are the length of the wound and the macroscopic assessment of the wound. Data were analyzed using the Kruskal-Wallis test, $p<0.05$ was considered statistically significant.
Results: Administration of *Hibiscus sabdariffa L.* 6%, 12% and 15% ethanol extract gel on the 12th day resulted in a significant acceleration of the wound healing process (p=0.024). There was a significant difference between negative control (Mean=3.28 mm; SD=1.68 mm), Positive control (Mean= 2.81 mm; SD= 1.11 mm), 6% rosella flower petal extract gel (Mean=2.92 mm; SD=1.22 mm), 12% (Mean=1.290 mm; SD=1.09 mm), and 15% (Mean= 1.04 mm; SD= 0.95 mm) (p=0.024), in the macroscopic assessment there was no significant difference in the wound healing process on day 3, 6, 9, 12 and 15 except for signs of swelling on days 6 (p=0.038) and 9 (p=0.038) which had significant differences compared to controls.
Conclusion: Pemberian gel ekstrak etanol *Hibiscus sabdariffa L.* 15% memiliki waktu tercepat dalam penutupan dan penyembuhan luka (Mean=1.04 mm; SD= 0.95 mm) selama 12 hari (p= 0.024), waktu terlama dalam penutupan dan penyembuhan luka adalah kontrol negatif (gel plasebo) (Mean= 0.98 mm; SD=1.10 mm) yaitu selama 15 hari (p=0.158).

Keywords: ethanol extract gel, hyperemia, Roselle, wound healing

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BACKGROUND

A wound is a disruption of the integrity of the epithelial layer of the skin or mucosa due to mechanical trauma or external pressure (ElHawary et al., 2023). The wound healing process has several phases, namely hemostasis, inflammation and angiogenesis which respond to the extracellular matrix, collagen and other compounds (Rodrigues et al., 2019). The wound healing process begins after damage to the skin occurs which results in extravasation of blood from damaged blood vessels so that platelets contact fibrillar collagen, fibronectin and matrix proteins (Kirby et al., 2015). The appearance of collagen in this phase functions to induce platelets and result in the deposition of fibrin in the wound (Kirby et al., 2015). In the inflammatory phase, the process of immune cells occurs, encouraging pro-inflammatory cytokines to influence the migration of fibroblasts, epithelial and endothelial cells. Inflammatory phase fibroblasts contribute to collagen deposition, by degrading collagen triggering angiogenesis and reepithelialization (Xue and Jackson, 2015). Re-epithelialization occurs several hours after the wound, through specific cytokines, proliferation and migration from the wound border area to approach the edge and thicken with the aim of closing the wound (Gonzalez et al., 2016).

Wounds that occur less than 5 days later and continue with the hemostasis and inflammation phase are referred to as acute wounds. Physiologically, acute wounds have a wound healing time of around 0-21 days for the wound to close (Nasution and Muslimdjas, 2021). A wound is said to have failed to heal if the wound does not close after 12 weeks or is usually called a chronic wound (Lindholm and Searle, 2016). Some of the reasons why wounds fail to heal are due to untreated wound infections, especially post-operative infections and scarring (Worster et al., 2015). Excessive use of antibiotics causes side effects and risks antimicrobial resistance (Punjataewakupt et al., 2019). And according to research, it is said that the use of antibiotics in chronically infected wounds can affect the delay in wound healing (Caldwell, 2020). Infections in wounds are not easily treated with conventional medicine, apart from the emergence of side effects due to antibiotics, the high price means that patients do not seek treatment so that infections are difficult to control resulting in increased morbidity, so new strategies are needed to treat infected wounds. The pathophysiology of wound healing has extensive factors in biochemical and molecular mechanisms so that therapeutic strategies are quite challenging (Pang et al., 2017). Current therapy generally aims to accelerate wound healing, but infection control is often neglected. So it is hoped that there will be the development of natural or herbal therapies to increase skin repair in wound healing (Vilkickyte et al., 2022). These factors can be overcome with complementary therapies, one example of which comes from rosella flower petals (*Hibiscus sabdariffa L.*). The potential benefits and treatments vary, one of which is effective in treating inflammation,

oxidative stress, hypertension, hyperlipidemia, carcinogenicity, infections, diabetes, Alzheimer's, etc. (Vijayakumar et al., 2018).

The nutritious contents of rosella flower petals come from bioactive compounds in the phenol, tannin, flavonoid, alkaloid and anthocyanin groups which have antibacterial and antioxidant activity (Purbowati, 2015). The tannin content has antibacterial power by inhibiting the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells cannot form (Zhen et al., 2016). The polyphenol content works in killing microorganisms by denaturing cell proteins, and the anthocyanins in the extract act as antioxidants to ward off free radicals (Cruz et al., 2019). Based on the description above, the aim of the research is to examine in more depth the effectiveness of ethanol extract of rosella petals as a wound healing medicine.

SUBJECTS AND METHOD

1. Study Design

This was a randomized control trial.

2. Population and Sample

This research was conducted using a true experimental type of research with a post test only randomized control group design using male white Wistar rats treated with incisional wounds as research subjects. The selection of mice as experimental animals was based on the consideration that genetically, mice are similar to humans and have the ability to adapt to the laboratory environment. The inclusion criteria in this study were age 2.5 - 3 months, body weight 150 - 200 grams, male gender, healthy condition (active and not disabled). Sample size estimation uses the Federer formula. Each treatment group contained a minimum of 5 male mice. Researchers chose to use 6 male mice per group to maintain the mortality of experimental animals with a total of 5 treatment groups so that the total number of research samples was 30 mice. Samples were obtained using a simple random sampling method, while rosella flowers were obtained using a purposive sampling method, taken from Pancur Batu Village, Deli Serdang Regency, North Sumatra Province.

3. Study Variables

The dependent variable were duration of wound healing, macroscopic observation of hyperemia, granulation and wound closure. The independent variable was the administration of rosella flower petal extract gel.

4. Operational Definition of Variables

The ethanol extract dose of rosella flower petals is an extract obtained in dry conditions which is extracted using the maceration method using 96% ethanol solvent and filtered to obtain a filtrate. The filtrate obtained was then evaporated using a rotary evaporator to become a thick ethanol extract. The process of making and extracting simplicia is carried out in the integrated laboratory of the Faculty of Medicine, Methodist University of Indonesia. The extract is administered topically using a gel preparation at a concentration dose of 6%, 12%, and 15% on days 0 to 15. An assessment was carried out of the fastest duration for the wound to close in the wound healing process which was the result of an analysis of the average measurement of wound closure, assessment of hyperemia and macroscopic swelling on days 0, 3, 6, 9, 12 and 15.

5. Study Instruments

Wound healing examinations are carried out in an integrated laboratory at the Faculty of Medicine, Indonesian Methodist University, using the method of measuring the average wound closure and macroscopic assessment of the wound.

6. Data analysis

Wound length data are expressed as Mean, SD. Statistical analysis was performed

using statistical software. Data normality and homogeneity tests were carried out. If the data is not normally distributed and homogeneous then an ANOVA test is carried out, if the data is not normally distributed and homogeneous then the Kruskal-Wallis test is carried out. In this study, statistical test decisions were taken with a p value <0.05 which was considered significant. Macroscopic data assessing hyperemia, swelling and wound closure after receiving ethanol extract of rosella flower petals (*Hibiscus Sabdarifa L.*) were analyzed descriptively.

7. Research Ethics

The use and handling of experimental animals in research laboratories is carried out in accordance with the ethical rules for animal research regulated in the Declaration of Helsinki and obtained ethical clearance from the ethics committee of the Faculty of Medicine, UMI Medan No. 42/-KEPK-FKUMI/EC/2023.

RESULTS

1. Wound Length

The results of observations of the duration of the wound healing stages were measured after 15 days of administration of rosella flower petal extract (*Hibiscus sabdariffa L.*) and compared with the control group as shown in Table 1.

Table 1. Description of differences in wound length between study groups

	Day 3		Day 6		Day 9		Day 12		Day 15	
Group	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Placebo	15.33	2.56	12.31	1.73	6.68	1.83	3.28	1.68	0.98	1.10
Control +	14.84	1.96	15.40	2.46	7.79	2.15	2.81	1.11	0.82	1.55
Extract 6%	13.68	1.73	12.02	2.07	8.53	2.13	2.92	1.22	0.39	0.96
Extract 12%	17.36	1.68	13.36	1.44	6.33	1.66	1.29	1.09	0.00	0.00
Extract 15%	17.40	1.56	12.80	1.86	6.45	1.80	1.04	0.95	0.00	0.00
P value	0.021		0.077		0.23		0.024		0.158	

From Table 1 it is known that the fastest duration of wound healing was in the group given 15% rosella flower petal ethanol extract gel (Mean= 1.04 mm; SD= 0.95 mm), followed by the 12% rosella extract gel group (Mean= 1.29 mm; SD= 1.09 mm) the ability to accelerate wound healing was higher compared to the placebo control group (Mean= 3.28 mm; SD= 1.68 mm) and positive control (Mean= 2.81 mm; SD= 1.11 mm) using the Kruskal-Wallis test analysis p value= 0.024 which means there is a significant difference.

2. Macroscopic Wound Assessment

The healing process is observed through macroscopic assessment of the hyperemic process and swelling of the wound until it closes.

Table 2. The healing process is observed through macroscopic assessment of the hyperemic process and swelling of the wound until it closes

Group	Negative Control					Positive Control					Rosella flower petal extract gel 6%					Rosella flower petal extract gel 12%					Rosella flower petal extract gel 15%				
Repetition Day	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
1	A	A	A	A	C	A	A	A	A	C	A	A	A	A	C	A	AB	AB	A	C	A	A	A	A	C
2	A	AB	AB	A	C	A	A	A	A	C	A	A	A	A		A	A	A	A	C	A	A	A	C	C

Group	Negative Control					Positive Control					Rosella flower petal extract gel 6%					Rosella flower petal extract gel 12%					Rosella flower petal extract gel 15%				
Repetition Day	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
3	A	A B	A B	A B	A B	A	A	A	A	C	A	A	A	A	C	A	A B	A B	A	C	A	A B	A B	C	C
4	A	A B	A B	A B	A	A	A B	A B	A B	C	A	A	A		C	A	A	A	C	C	A	A	A	A	C
5	A	A B	A B	A	A	A	A	A	A	A	A	A	A	A	C	A	A	A	A	C	A	A B	A B	A	C
6	A	A B	A B	A	C	A	A	A	A		A	A	A	A	A	A	A	A	C	C	A	A	A	A	C

Note:

C : Wound Closure

A : Hyperemic

B : Swelling

Table 3. Differences in hyperemia, swelling and wound closure on examination days 3, 6, 9, 12 and 15

	Day 3	Day 6	Day 9	Day 12	Day 15
Hyperemic	1.000	1.000	1.000	0.326	0.138
Swelling	1.000	0.038*	0.038*	0.220	0.406
Wound Closure	1.000	1.000	1.000	0.153	0.160

* The results of the Kruskal Wallis Test are significantly different

Tables 2 and 3 show the results of descriptive analysis of hyperemia on days 3, 6, 9, 12, and 15. It was found that the negative controls were still hyperemic until day 15 with an insignificant p value. In the positive control, 6%, 12% and 15% rosella flower petal extract gels experienced hyperemia until the 12th day. So, it can be concluded that administering rosella extract gel at doses of 6%, 12% and 15% was able to eliminate hyperemia on the 12th day. The results of descriptive analysis of wound swelling showed that swelling occurred up to day 9 in 6%, 12% and 15% rosella extract gel. This means that 6%, 12% and 15% doses of rosella extract gel have the ability to inhibit significant swelling (p= 0.038) until the 9th day. The results of the descriptive analysis of wound closure showed that the wound healing effect was obtained with the assessment that the wound closed perfectly on the 15th day, which was obtained in the positive control, gel extract 6%, 12%, and 15%, while the negative control showed that the wound closed on the 15th day. that the administration of rosella extract gel was able to speed up wound closure compared to the negative control.

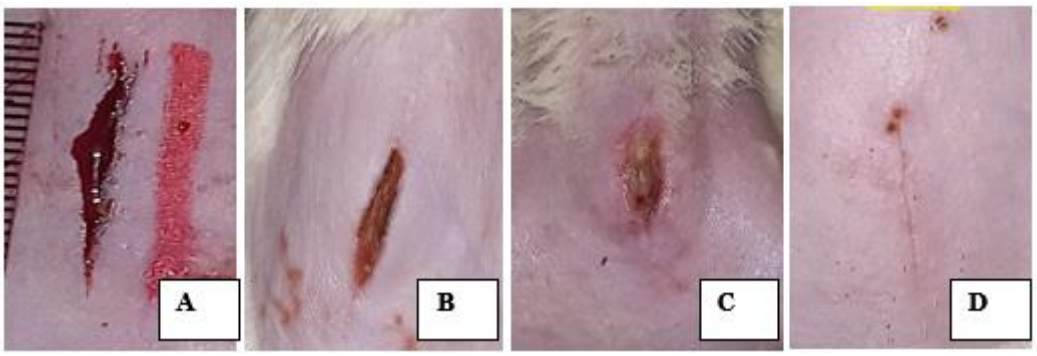

Figure 1. Description of the Incision Wound Process in Wistar Galus Rats

Note:

A : Initial Incision Wound

B : Hyperemic

C : Swelling

D : Wound Closure

DISCUSSION

Testing the effect of rosella flower petal extract (*Hibiscus sabdariffa L.*) on healing incisional wounds began with determining the identity of the extract with the aim of avoiding errors in administering the extract in the research. Then, selection and drying of samples of rosella flower petals was carried out with the aim of removing foreign substances and dirt left on the simplicia (Purbowati, 2015). In making simplicia, rosella flower petal extract uses the boiling method using hot water and stirring to ensure the balance of the extract concentration (Styawan et al., 2021). The examination of simplicia characteristics carried out on roselle flower petal extracts included macroscopic analysis, water content determination, water soluble extract determination, ethanol soluble extract determination, total ash content determination, and determination of acid insoluble ash content and total flavonoid content. It has been standardized to make ointment preparations with the active ingredient rosella flower petal extract in concentrations of 6%, 12% and 15% and has been standardized according to the Indonesian Herbal Pharmacopoeia (Siahaan et al., 2021).

This research was carried out using macroscopic observations which aimed to see the effectiveness of the test extract preparation given in a topical preparation on the process of signs of inflammation, namely signs of hyperemia and swelling in the wound and the wound closure process. Observations on the 3rd day showed that macroscopic results showed hyperemia and the length of the incision wounds were quite heavy in all test groups and no swelling was found in all test groups. Macroscopic observations on day 6 and day 9 showed signs of hyperemia in all groups and signs of swelling mostly occurred in the negative control group. Observations on the 12th day in the 15% rosella flower petal extract gel group showed that the wound had closed compared to the other test groups. And on observation on the 15th day, all wounds in the 6%, 12% and 15% rosella flower petal extract groups and the positive control group experienced wound closure compared to the negative control. The results of observations of the average length of wounds in all groups were analyzed using SPSS. The results of the Kruskal-Wallis non-parametric test on the 3rd day of observation (p=0.021) and the 12th day (p=0.024) concluded that there were significant differences in the test for each group, while on the 6th day of observation (p=0.077), 9 (p=0.232), and 15 (p= 0.158) which concluded that there were no significant differences in each group. And macroscopic observations carried out the non-parametric Kruskall-Wallis test showed that the results of observations on days 6 (p=0.038) and 9 (p=0.038) showed significant differences in signs of wound swelling, while observations on day 3 (p= 1.000), 12 (p=0.220) and 15 (p=0.406) and observation of signs of hyperemia and wound closure did not have significant differences in all treatment groups. From the data obtained, it is known that all groups experienced signs of hyperemia, however swelling occurred most in the negative control group. There are signs of hyperemia and swelling due to the inflammatory phase process due to bleeding during the incision treatment (Tottoli et al., 2020).

Physiologically, acute wounds have a healing time of around 0-21 days before they close (Nasution and Muslimdjas, 2021). In the wound healing process during the inflammatory phase, the wound will limit further damage, close the wound and remove cell debris and bacteria, and encourage cell migration so that the phase

has an important role in the wound healing process. In the re-epithelialization phase, the process of formation and healing of damaged tissue occurs, replaced by regeneration of parenchymal cells with fibroblast tissue (Coger et al., 2019). The process in the initial phase of wound inflammation is characterized by hyperemia or redness and swelling of the wound which is caused by increased blood flow in the arteries to the damaged or traumatized tissue so that plasma proteins and phagocytes reach the wound surface thereby preventing secondary infection in the wound (Tottoli et al., 2020). Blood that goes to tissue that has experienced wound trauma will experience vasodilation and increased vase permeability in the wound area due to platelet degranulation, hageman factor and the release of quinine and plasmin. This vasodilation causes swelling and pain in the initial phase of the wound (Singh et al., 2014). The process of reducing the length of the wound accompanied by reduced hyperemia or reddish color in the inflammatory phase of the incisional wound is a sign that the wound healing process is starting to occur. The presence of external factors such as wound treatment can speed up the wound healing process (Tottoli et al., 2020) . In the research given, rosella flower petal extract is known to contain various phytochemical contents with various benefits, for example bioactive compounds in the group of phenols, tannins, flavonoids, alkaloids and anthocyanins which generally have antibacterial and antioxidant activity. The flavonoids in rosella flower petal extract function in inhibiting neutrophil degranulation and have the potential to inhibit the release of histamine from mast cells so that edema or swelling can be avoided (Zhen et al., 2016). In addition, flavonoids have potential in the re-epithelialization process, are antimicrobial and astringent which have an effect on wound contractility and the rate of re-epithelialization (Shedoeva et al., 2019). Polyphenols in rosella flower petal extract are able to reduce redness or hyperemia by inhibiting the activation of Mitogen-activated Protein (MAP) kinase in mice thereby suppressing Nuclear Factor Kappa B (NF-kB) and the Cyclooxygenase-2 (COX-2) protein enzyme which is a mediator inflammation (Li et al., 2019). Apart from that, the polyphenol content works in killing microorganisms by denaturing proteins in cells, and the anthocyanin content in the extract acts as an antioxidant and can ward off free radicals (Tsai et al., 2002). The tannin content plays a role in accelerating the cicatricial process and wound contraction and inhibiting the formation of phagocytosis and macrophages, as well as triggering epithelialization due to the antimicrobial effect of tannin. Apart from that, tannins have a role in transcription and translation in Vascular Endothelial Growth Factor (VEGF) (Fajarningrum, 2022). Alkaloids play a role in the initial phase of wound closure by stimulating the production of fibroblasts in the wound (Fernandes et al., 2018). In the inflammatory phase, Reactive Oxygen Species (ROS) will always be produced and this will hamper the wound healing process (Yadav et al., 2018). One of the triggers for free radicals, Tumor Necrosis Factor alpha (TNF-α), through ferritin degradation, produces many hydroxyl radicals (-OH) in cell membrane peroxidation so that it can initiate cell damage (Siahaan et al., 2022). The antioxidant content has a role in repairing tissue damage and can accelerate the disappearance of hyperemia and swelling in wound healing (Yadav et al., 2018).

The phytochemical content in plants has an important effect in reducing free radicals. Non-enzymatic antioxidants and

enzymatic antioxidants in plants have a role in protecting against oxidative stress by reducing oxygen radicals that damage membranes and biological structures (Siahaan et al., 2020). The phytochemical content of rosella flower petals is able to reduce the inflammatory phase and produce a significant acceleration of wound repair as shown in the rosella flower petal extract gel group on days 3 and 12 where at a dose of 15% extract gel it has the fastest effect in healing wounds. The lowest average wound length was in the 15% rosella flower petal extract gel group, followed by the 12% rosella flower petal extract gel group. The speed of shortening the length of the wound was higher than the placebo group and the positive control group. When tissue is injured or damaged, phospholipase expression can appear on the cell membrane. The phosphorlipase enzyme converts phospholipids into arachidonic acid which, with the help of the cyclooxygenase and lipoxygenase enzymes, will be converted into pro-inflammatory mediators and prostaglandins (Lubis et al., 2020). The flavonoid content contained in the ethanol extract of rosella flower petals will inhibit the phosolipase enzyme so that the formation of arachidonic acid can be inhibited and the cyclooxygenase and lipoxygenase pathways are disrupted and inhibit the synthesis of pro-inflammatory mediators such as leukotrins and prostaglandins. Reduced synthesis of pro-inflammatory mediators and prostaglandins causes a decrease in the vasodilatory effect so that inflammatory cells are reduced and the hyperemic appearance of the wound also decreases (Ma and Khachemoune, 2023). The antioxidant function in anthocyanins plays a role in wound healing by breaking the chain of propagation of Reactive Oxygen Species (ROS) so that damage or uncontrolled cell growth that leads to cell death in body tissue can be inhibited (Pangaribuan, 2016).

In macroscopic assessment, swelling still occurred until the 9th day in the positive control, 6%, 12% and 15% extract gel, while the negative control occurred until the 12th day. The cause of swelling usually occurs due to hyperemia and comes from fluid and cells in the blood circulation to the interstitial tissue (Bowden et al., 2016). With the tannin compounds contained in rosella flower petal extract, it can inhibit the process of hypersecretion of mucosal fluid and reduce pro-inflammatory proteins. Apart from that, tannins also have an affinity for protein so they can reduce the swelling effect on wounds during the inflammatory phase (Mumpuni et al., 2021).

This research shows that 15% rosella flower petal extract gel has the potential to accelerate the wound healing process better based on macroscopic assessment, although not all days of treatment had statistically significant differences between groups. This is also supported by other research which reports that ethanol extract of rosella leaves (*Hibiscus sabdariffa L.*) is effective in healing wounds based on histological assessment of skin epithelial thickness (Rambe et al., 2022). Ethanol extract of rosella flower petals provides a new opportunity for further research into its potential as a wound healing agent.

AUTHOR CONTRIBUTION

Ade Dwi Pratiwi as a research member who carried out extraction, handling of experimental animals, induction and elisa examination. Jekson Martiar Siahaan as head researcher is responsible for selecting ideas, monitoring and evaluating research implementation, and data collection. Endy Juli Anto, Putri Chairani Eyanoer carried out

data analysis and assisted in data interpretation.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

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