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Effectiveness of Mango Gedong Gincu Peel Extract Gel (*Mangifera indica* L.) on Cut Wounds in Male Wistar White Rats (*Rattus norvegicus*)

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ABSTRACT

Background: A wound is an injury to a part of the body where the skin and the underlying tissue lose continuity. The wound healing is a complex process consisting inflammatory phase, proliferation phase, and maturation phase. Open wounds often occur in everyday life and if left behind and untreated can potentially lead to bacterial infection. Mango gedong gincu peel extract (*Mangifera indica* L.) contains flavonoids, tannins, phenols, triterpenoids, steroids, alkaloids, and saponins which have roles in wound healing.
Aims: To analyses the effectiveness of mango gedong gincu peel extract gel (*Mangifera indica* L.) against the healing of cut wounds in male wistar white rats (*Rattus norvegicus*).
Methods: The study was an experimental study with a post-test only control group design using 25 male wistar white rat as the subject and was carried out in the FK UGJ Research Laboratory. The study used five groups : negative control group, positive control group, and treatment group using mango gedong gincu peel extract gel (*Mangifera indica* L.) with concentrations of 5%, 10%, and 15%.
Results: The results of the study revealed that mango (*Mangifera indica* L.) gedong gincu peel is a source of various phytochemical such as flavonoids, tannins, phenols, triterpenoids, steroids, alkaloids, and saponins. This study showed that the 5% and 10% concentration treatment groups had the same average length measurement wound of 0.86 cm. The fastest observation of erythema was the 5% concentration group with a healing rate of 8 days, in the observation of edema the 10% concentration group was the fastest of 6 days, and the observation long of healing time was 5% and 10% concentration had the same rate of 11 days.
Conclusion: Mango gedong gincu peel extract gel (*Mangifera indica* L.) was effective against wound healing with optimum concentrations of 5% and 10% which gives the best healing effect against wounds in male wistar white rats (*Rattus norvegicus*).

Keywords: *Mangifera indica* L.*, Cut wounds, Rattus norvegicus, Wound healing*.

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1. Introduction

Wound is an injury to a part of the body where the skin and the underlying tissue lose continuity. Open wounds often occur in daily life and can lead to bacterial infections if not treated. Infected wounds heal slowly and often result in the formation of exudate and toxins, along with the death of regenerative cells. Therefore, stimulation is

also needed to heal the injured areas of the body, restore normal function, prevent infection, and alleviate discomfort and pain caused by the wounds (Rema, 2023).

Indonesia is a tropical country with a variety of plants and fruits. Indonesia is striving to increase fruit production, with the hope of meeting domestic needs and playing a role in exporting high-quality fresh fruits to other countries (Awaliyah F, 2018). One of the agricultural commodities that is very significant in Indonesia due to its high economic value and is a type of mango that is widely exported is the mango gedong gincu. The production center for mango gedong gincu in West Java Province includes Indramayu Regency, Majalengka Regency, and Cirebon Regency. In addition to being a production center, Cirebon Regency also serves as the marketing hub for mango gedong gincu from various other regencies. The marketing of mango gedong gincu is now starting to expand into international markets (Awaliyah F, 2018).

Mango can produce up to 60% of polluting by-products, including its peel. Mango contains metabolic compounds such as alkaloids, saponins, tannins, terpenoids, flavonoids, and mangiferin (Suhatri *et al.*, 2022). The most important components in mango are flavonoids and mangiferin, which have been proven to be involved in the wound healing process (Espinosa L *et al.*, 2022).

The administration of antibiotics is important for preventing wound infections. The antibiotics given can be either natural or synthetic compounds that function to inhibit and stop microbial infections. Improper administration of antibiotics can lead to adverse effects such as microbial resistance to antibiotics, increased drug side effects, and even death (Febryanto *A et al.*, 2023). The negative impact has led to a shift in perspective from the use of chemical substances to natural materials. Until now, many studies have been conducted to find natural extracts that stimulate and promote wound healing regeneration. The use of natural material extracts is generally a practice that has been carried out by traditional communities for generations. The extract of natural active ingredients is a wound healing agent that fights infection and accelerates wound healing (Risa AM *et al.*, 2018).

One of the plants used in traditional medicine is mango (*Mangifera indica* L.). However, the utilization of mangoes is still limited to fruit consumption alone, whereas mango peel is a potential source that has not been optimally utilized. Several studies indicate that mango peel is a potential source of antioxidants, anti-inflammatory agents, anti-diabetic properties, antibacterial effects, and anti-proliferative compounds. This is why mango peel could be used as a potential remedy for addressing various clinical issues in the future (Umamahesh K *et al.*, 2020).

In this study, the extract of mango gedong gincu peel will be formulated into a gel preparation. The formulation of gel has advantages such as not easily flowing on the skin's surface due to its high viscosity and adhesive properties, as well as being thixotropic, which allows it to spread easily when applied. It does not leave marks and only forms a thin layer like a film, is easy to rinse off with water, leaves a cooling sensation after application, absorbs better than cream, is very suitable for use on hairy areas, and is more cosmetically preferred. The gel easily melts and becomes smooth upon contact with the skin (Rosida *et al.*, 2018).

2. Methods

Study Design/Research Procedures

This research is an experimental study with a "post-test only control group design," using male wistar white rats (*Rattus norvegicus*) as the research subjects. This study uses 5 groups: 1 negative control group, 1 positive control group, and 3 treatment groups, which were conducted randomly using the simple random sampling method. This research was conducted at the Research Laboratory of the Faculty of Medicine at Swadaya Gunung Jati University in Cirebon over a period of 4 months, from March 2024 to July 2024. The sample size for each group is determined using the *Federer* formula, resulting in a sample size (n) of 5, and the number of groups in this study is 5, so this research uses 25 male wistar white rats with a weight of 180-250 grams and an age of 2-3 months.

a. Materials

The materials used in this research include mango gedong gincu peel, aquadest, ethyl chloride spray, neomycin sulfate and placenta extract, glycerin, Na-CMC, propylene glycol, methyl paraben, hair removal

cream, 70% ethanol, and phytochemical reagents (HCL2N, Ether, Hager, Mayer, Methanol, Bouchardat, Dragendorff, 1% $FeCl_3$, H_2SO_4, 10% NaOH).

b. Preparation of Rats

The research procedure began with the preparation of the subject of the study : 25 male wistar white rats (*Rattus norvegicus*) were divided into 5 groups of each group consisting of 5 rats. All research subjects were acclimatized for 7 days in a laboratory environment and were given standard feed for rats, namely pellets and aquadest, *ad libitum*. The test rats fur was trimmed with scissors, and then the fur was removed using hair removal cream on the back area where a cut would be made. Local anesthesia was then administered using ethyl chloride spray. Next, the wound is created by making a cut on the back of the rats with a diameter of approximately ±2 cm and a depth of about ±2 mm by lifting the skin on the back of the rats with forceps and then making a sterile cut with a scalpel.

c. Extraction Process

10 kilograms of mangoes were peeled, resulting in approximately 950 grams of peel. The obtained mango peel was then sorted and washed thoroughly. Next, it was sun-dried on aluminum foil for about 5 days, yielding 157 grams of dried mango peel. Finally, the dried mango peel was coarsely chopped and blended until smooth. Then it was filtered using a sieve to obtain 142 grams of mango peel simplicia. Next, the simplicia was macerated with 70% ethanol by placing it in a dark bottle. After that, let it sit for 3 x 24 hours with stirring once a day, then filter it using filter paper. The obtained macerate was then evaporated using a rotary vacuum evaporator at a temperature of 50°C and subsequently concentrated with a waterbath, resulting in a thick extract weighing 29 grams.

d. Gel Preparation

The gel preparation was made with a formulation according to **Table 1** as follows : aquadest was heated to a temperature of 70°C, then Na-CMC was added and stirred until homogeneous. Next, glycerin, propylene glycol, and methyl paraben were added and stirred again until homogeneous. After the gel base is formed, extract from the mango gedong gincu peel is added. The gel that has formed is then stored in a place that is not exposed to direct sunlight for one night.

Table 1. Formulation of Mango Gedong Gincu Peel Extract Gel

Materials	Concentrations						Benefits
	(%)	(g)	(%)	(g)	(%)	(g)	
Gedong gincu mango peel extract	5	1.5	10	3	15	4.5	Active substance
Na-CMC	1	0.3	1	0.3	1	0.3	Gelling agent
Glycerin	10	3	10	3	10	3	Humectant
Propylene glycol	15	4.5	15	4.5	15	4.5	Humectant
Methyl Paraben	0.5	0.15	0.5	0.15	0.5	0.15	Preservative
Aquadest ad	100	30	100	30	100	30	Solvent

e. Physical Stability Test Of Gel

The gel preparation underwent physical stability testing, which includes:

1). Organoleptic Test: The preparation is visually observed for changes in shape, the emergence of odor or lack thereof, occurrence of syneresis or not, and color changes.

2). Homogeneity Test: The preparation is placed between two glass slides and then examined for the presence of coarse particles or non-homogeneity under light. Homogeneity is indicated by the absence of coarse grains in the preparation.

3). pH Test: The pH test can be conducted using a pH meter. This test is performed to assess the acidity level of the gel formulation to ensure that it does not cause skin irritation. The formulation's pH should be between 4.5 and 6.5 to match the requirements for skin pH.

4). Spread Test: To make sure the gel is applied to the skin evenly, the spread test is carried out. The gel is weighed at 0.5 g, it is positioned in the middle of the circular scale. A weight and round glass, or other transparent material are placed on top of the gel so that the combined weight of the two is 150 g. After leaving it for a minute, the spread's diameter is measured. The gel should be evenly distributed between 5 and 7 cm.

f. Phytochemical Test

1) Phenol Test: A total of 0.5 g of extract was added to 2 mL of methanol, then filtered, and the filtrate is treated with 3 drops of H_2SO_4. The formation of a red color indicates a positive result for phenols.
2) Tannin Test: A total of 0.5 g of extract was added to 10 mL of hot water, then filtered, and treated with 1% $FeCl_3$. The formation of a dark green-black color indicates a positive result for tannins.
3) Flavonoid Test : A total of 0.5 g of extract was added to 2 mL of methanol, then filtered, and the filtrate is treated with 10% NaOH. The formation of a reddish-black, yellow, or orange color indicates a positive result for flavonoids.
4) Saponin Test: A total of 0.5 g of extract was placed in a test tube and then 10 mL of heated aquadest is added. The mixture is vigorously shaken for about 1 minute. It is then allowed to sit for 10 minutes, and the foams or bubbles that form indicate a positive result for saponins.
5) Alkaloid Test: A total of 0.5 g of extract was added to 1 mL of HCl2N and 9 mL of heated aquadest. Then it is filtered, and the filtrate is transferred into four test tubes, each with a volume of 2.5 ml. The four solutions were tested with Bouchardat, Dragendorff, Hager, and Mayer + Methanol + Bouchardat reagents. A positive result with the Bouchardat reagent is indicated by the formation of a brown-black precipitate, while the Dragendorff reagent produced a white precipitate, the Hager reagent resulted in a yellow precipitate, and the Mayer + Methanol + Bouchardat reagents yielded a brown-black precipitate.
6) Steroid and Triterpenoid Test: A total of 0.5 g of extract was placed into a test tube, then 2 mL of concentrated H_2SO_4 is added. The solution is gently shaken and left for a few minutes. The blue-green color indicates a positive result for the steroid test, while the reddish-brown to purple color indicates a positive result for the triterpenoid test.

g. Manage of Rats During Experiment

A total of 25 white rats are randomly divided into 5 groups. Then each group was given different treatments ; the negative control group was given a gel base, the positive control group was given neomycin sulfate and placenta extract, and the three treatment groups were given gel made from gedong gincu mango peel extract at concentrations of 5%, 10%, and 15%. Wound care and the administration of test materials were carried out twice a day, in the morning and evening, for 14 days. Then, for observation and measurement of the cut wound, it was done once a day in the evening.

Statistical Techniques

Data analysis using One Way Anova and Kruskal-Wallis tests, followed by Post-Hoc Duncan test, because the research is a type of comparative study that is unpaired with numerical and categorical scales. Therefore, for normally distributed or homogeneous data used the Parametric test (One Way ANOVA) and for non-normally distributed or heterogeneous data used the Non-Parametric test (Kruskal-Wallis). This is then followed by the Post-Hoc Duncan test to observe the differences in means between groups.

Ethical Clearance

This research has received ethical clearance from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine at Swadaya Gunung Jati University with the number 6/EC/FKUGJ/IV/2024.

3. Results

a. Results of Phytochemical Testing

Phytochemical testing is one method to identify the secondary metabolites contained in a substance. This phytochemical test is very useful for determining the group of compounds found in the mango (*Mangifera indica* L.) gedong gincu peel as a treatment for cut wounds. The metabolites contained in the mango (*Mangifera indica* L.) gedong gincu peel can be seen in **Table 2**.

Table 2. Results of Phytochemical Testing

No.	Phytochemical Compounds	Results	Conslusions
1.	Saponins	Foam forms that lasts for 10 minutes, with a height of approximately 2 cm	(+)
2.	Tannins	A black color is formed	(+)
3.	Flavonoids	A red color is formed	(+)
4.	Phenols	A red color is formed	(+)
5.	Alkaloids	Test tube 1: Bouchardat reagent forms a brown precipitate	(+)
		Test tube 2: Dragendorff reagent forms a white precipitate	(+)
		Test tube 3: Hager reagent shows a yellow precipitate	(+)
		Test tube 4: Mayer + methanol + Bouchardat reagent forms a black precipitate	(+)
6.	Triterpenoids	A reddish color is formed	(+)
7.	Steroids	A greenish color is formed	(+)

b. Results of the Measurement Length of Cut Wounds

Table 3. Average Length of Cut Wounds

Groups	Length of Cut Wounds (cm)					Average (cm)
	(1)	(2)	(3)	(4)	(5)	
C (-)	0.91	0.97	0.82	0.91	1.05	0.93
C (+)	0.73	0.82	0.62	0.80	0.84	0.76
P1 5%	0.76	0.90	0.59	0.82	0.80	0.77
P2 10%	0.51	0.80	0.58	0.94	1.03	0.77
P3 15%	0.81	0.87	1.47	0.66	0.84	0.93

From the average length of the cut wounds over 14 days in **Table 3**, it was found that in the control group (-), the average length of the wound was 0.93 cm, in the control group (+) the average length of the wound was 0.76 cm, in the 5% concentration group the average length of the wound was 0.77 cm, in the 10% concentration group the average length of the wound was 0.77 cm, and in the 15% concentration group the average length of the wound was 0.93 cm.

Saponins (+) → foam forms

Tannins (+) → A black color is formed

Flavonoids (+) → A red color is formed

Phenols (+) → A red color is formed

Alkaloids (+) → There are precipitates in each of the reagents

Triterpenoids (+) → A reddish color is formed

Steroids (+) → A greenish color is formed

Figure 1. Results of Phytochemical Testing

c. Results of the Morphological Observation of Cut Wounds

1). Erythema

Table 4. Average Healing of Erythema

Groups	Healing of Erythema (Days)					Average (Days)
	(1)	(2)	(3)	(4)	(5)	
C (-)	9	9	9	10	12	9.8
C (+)	10	12	8	11	10	10.2
P1 5%	8	9	5	9	10	8.2
P2 10%	4	12	6	12	11	9
P3 15%	9	11		7	10	9.25

From the observation results of erythema over 14 days in **Table 4**, it was found that the control group (-) disappeared on day 10, the control group (+) disappeared on day 10, the 5% concentration group disappeared on day 8, the 10% concentration group disappeared on day 9, and the 15% concentration group disappeared on day 9.

2). Edema

Table 5. Average Healing of Edema

Groups	Healing of Edema (Days)					Average (Days)
	(1)	(2)	(3)	(4)	(5)	
C (-)	6	8	6	7	11	7.6
C (+)	6	6	6	9	9	7.2
P1 5%	7	8	2	7	9	6.6
P2 10%	2	9	2	9	10	6.4
P3 15%	6	9		4	8	6.75

From the observation results of edema over 14 days in **Table 5**, it was found that for the control group (-) it disappeared on day 8, the control group (+) disappeared on day 7, the 5% concentration group disappeared on day 7, the 10% concentration group disappeared on day 6, and the 15% concentration group disappeared on day 7.

3). Long of Healing Time

Table 6. Average Long of Healing Time

Groups	Long of Healing Time (Days)					Average (Days)
	(1)	(2)	(3)	(4)	(5)	
C (-)	13	13	12	13	14	13
C (+)	11	14	10	13	12	12
P1 5%	11	13	7	13	12	11.2
P2 10%	6	14	9	14	13	11.2
P3 15%	12	13		9	13	11.75

From the observation results of the healing duration over 14 days in **Table 6**, it was found that for the control group (-) on day 13, the control group (+) on day 12, the 5% concentration group on day 11, the 10% concentration group on day 11, and the 15% concentration group on day 12.

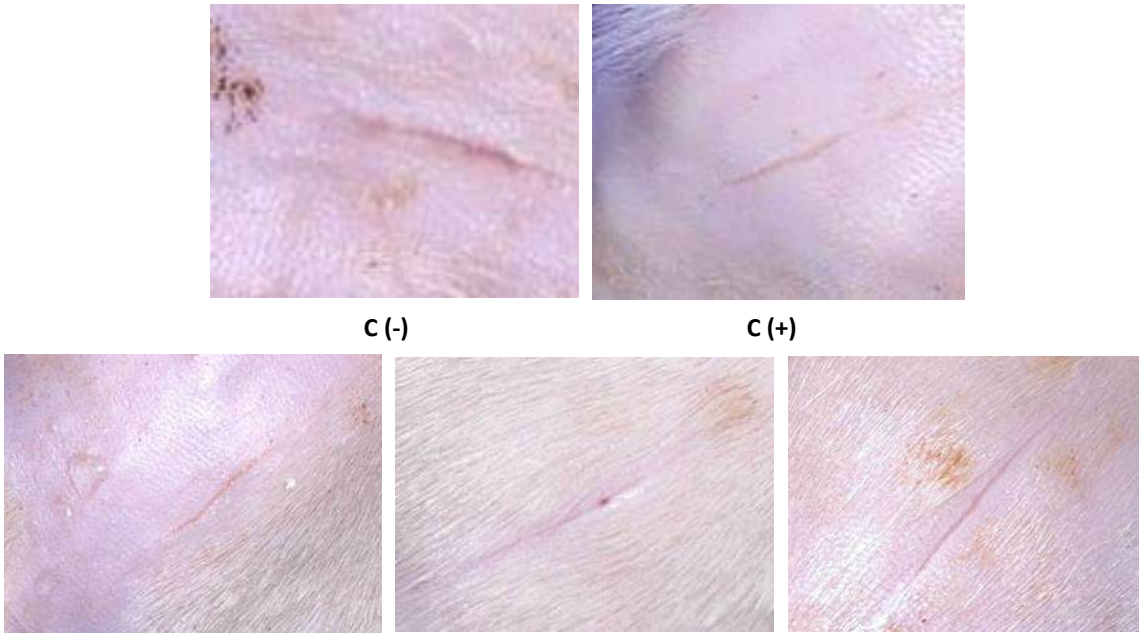

Figure 2. Cut Wounds in Day 14

4. Discussion

From the measurement results of wound length, it was found that the control group (+) showed the best healing of wound length, followed by the 5% and 10% concentration groups which had the same results, and then the 15% concentration group and control group (-) also had the same results. The wound healing process is influenced by several chemical compounds found in the extract of mango gedong gincu peel, such as triterpenoids and steroids that have the ability to promote fibroblasts which will synthesize collagen, saponins that act as antiseptics, stimulate the proliferation of epidermal cells, and affect the migration rate of keratinocytes to the wound area, thereby enhancing wound epithelialization, flavonoids that act as antioxidants by inactivating free radicals or enhancing antioxidant functions, and also tannins as antimicrobials and antioxidants to protect and prevent wound areas from damage caused by free radicals and inhibit the growth of pathogenic bacteria in the surrounding wound area that cause light bleeding to stop and wounds to close.

The research results by Risa AM *et al.,* (2018) indicate that erythema disappeared in the treatment group between days 4 and 6. In contrast, in this study, erythema disappeared between days 8 and 10. Furthermore, in that study, edema disappeared in the treatment group between days 7 and 10, while in this study, edema disappeared between days 6 and 8. Additionally, the duration of healing in that study was reported to be between days 7 and 11 for the treatment group, whereas in this study, the healing duration was between days 11 and 13.

In the 15% concentration group, there was one rat that experienced an infection, which caused the wound not to heal until the end of the research. This also affected the average length of the wound in that group resulting in it not being too high, and the morphology of the cut wound could not be assessed until the end of the research. In this study, several factors were identified that may influence the healing of incision wounds, including the varying processes of cell regeneration in the test animals. For example, some animals had rapid fur regrowth, while others did not grow fur until the end of the study. The wound closure processes also varied; in some test animals, the wounds closed tightly, while in others, a crust formed first, yet the width of the wound did not decrease. Additionally, the thickness of the skin layers in each test animal differed; some had thin subcutaneous depth, while others had thicker subcutaneous layers. There were also diseases affecting the test animals, which made their wounds difficult to heal. From these factors, it is possible that they could hinder and affect the healing process of cut wounds in rats. This is also supported by the research of Grada A *et al.,* (2018) titled "Research Techniques Made Simple: Animal Models of Wound Healing" which mentions that several factors can influence wound healing, including aging, infection, medication, nutrition, obesity, diabetes, venous insufficiency, and peripheral artery disease. Chronic wounds are wounds that do not successfully progress through the normal healing phases in a regular and timely manner. Chronic wounds in animals can arise from acute wounds induced by diabetes, mechanical pressure, ischemia, or reperfusion injury.

The limitations of this study are that the measurement and observation of cut wounds only extend to the proliferation phase, and research on the percentage of phytochemical compounds contained in mango gedong gincu peel has not yet been conducted. For further research, more time is needed regarding the wound healing process to reach the maturation phase and it is also necessary to assess the percentage of phytochemical compounds contained in mango gedong gincu peel.

5. Conclusion

From the research results, it can be concluded that the extract of mango (*Mangifera indica* L.) gedong gincu peel contains several bioactive compounds, including saponins, tannins, flavonoids, phenols, alkaloids, triterpenoids, and steroids, as indicated by phytochemical tests. The gel derived from this extract is effective for the healing of cut wounds; however, there is no significant difference observed among the treatment groups. The optimal concentrations for healing in male wistar white rats (*Rattus norvegicus*) are found to be 5% and 10%. It is recommended that further studies assess the effectiveness of this extract on human patients. In addition, future research should explore the effectiveness of mango (*Mangifera indica* L.) gedong gincu peel extract at varying doses and compare gel formulations with ointments or creams. Investigating the potential toxic effects of the extract is also necessary.

Conflict of Interest

There is no conflict of interest. Nothing to disclosure.

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