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The Efficacy of Watermelon (*Citrullus lanatus*) Rind Extract in Healing Burns in Balb/C Male Mice

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ABSTRACT: *Burns are skin tissue damage that occurs as a result of direct contact with a heat source. One of the natural treatments for burns is watermelon (Citrullus lanatus) rind. Watermelon rind contains citrulline, an alkaloid that plays a role in wound healing. This study aims to explore the potential of watermelon rind to become extract gel as a burn remover in male Balb/C strain mice. This research method is a true experiment that begins with the preparation of watermelon rind extract by maceration with ethanol. Furthermore, the polyphenol, flavonoid, saponin, and alkaloid content of the extract was identified through the phytochemical screening. The next stage was the preparation of a gel with three variations of extract concentrations, namely 25%, 50%, and 75% w/v. The gels were characterized by organoleptic, pH, and spreadability. The final stage tested the gel's activity as a burn wound healing in male Balb/C strain mice. The results showed that the extract contained saponins, polyphenols, flavonoids, and alkaloids. The spreadability test results of the gel showed that all gels met the requirements. All gels had activity against burns in male Balb/C strain mice. Thus, watermelon rind extract can be used as a medicine for burns.*

Keywords: *healing burns; male Balb/C strain mice; watermelon*

1. Introduction

Burns are events that cause skin tissue damage due to direct contact with heat sources, such as fire, hot water, chemicals, electricity, and radiation. Burns not only damage the skin, but can also affect the body's system [1]. The principle burn healing treatment is to restore the tissue function and shape to normal with minimal side effects [2]. Another reference mentioned that the principle of burn wound healing treatment is to prevent secondary infections, stimulate the formation of collagen tissue, and develop the remnants of epithelial cells to close the opening in the burn wound [1].

Healing burns in general can be done using various forms of medicine. One of them is by using synthetic drugs. Synthetic drugs used are drugs that have antibacterial, anti-inflammatory, and analgesic uses [3]. Some other drugs that can be used for healing burns are bioplacenton, silver sulfadiazine, and bacitracin [4]. The use of synthetic drugs has drawbacks, such as side effects due to interactions with other drugs, allergies, and some synthetic drugs are not recommended for use by pregnant and lactating women [3]. Currently, people prefer to use products from natural medicines. The use of natural medicine is currently more desirable because it has lower side effects compared to synthetic drugs [1]. One of the natural treatments for burns is watermelon rind. Watermelon rind contains various active compounds including alkaloids, phenols, saponins, and terpenoids [5]. Watermelon (*Citrullus lanatus*) rind also contains citrulline, which is a class of alkaloids that play a role in wound healing because of its effects on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodeling [2]. The combination of watermelon rind extract and mangosteen peel extract has been shown to reduce burn diameter [2].

So far, people only consume watermelon (*Citrullus lanatus*) for the red or yellow flesh, while the white layer is less attractive for public consumption. However, not many people know that the inner white skin of a watermelon, which is considered trash, actually contains a lot of nutrients. By exploiting and exploring the potential of watermelon rind into pharmaceutical preparations in gel form and using it as a burn remover, waste disposal of watermelon rinds can be reduced, and this waste can have a higher value, especially in the field of pharmaceutical technology.

2. Materials and method

2.1. Chemical

The equipment used in this research belongs to the pharmaceutical technology laboratory at the Jember Pharmacy Academy, and includes Ohaus CP 214 analytical balance, Ardir food dehydrator ARD-PM77, a members uf55 laboratory oven 53 L, a flannel cloth, 40W 220V soldering iron, and laboratory glass hand tools. The materials used in this study were watermelon purchased from a watermelon farmer in Jember, etanol 96% (food grade), aquadest, magnesium tape (Merk), hydrochloric acid 37% (Merck), iron (III) chloride hexahydrate (Merck), sulfuric acid 98% (technical grade), Mayer's reagent (Smart Lab), CMC-Na (food grade), polysorbate 80 (Smart Lab), glycerin 99% (rofa brand), methylparaben (pharmaceutical grade), propylparaben (pharmaceutical grade), disodium EDTA (Merck), ketamine HCl injection (Bernofarm), and Bioplacenton gel (Kalbe Farma). The animals used in this study were male BALB/C strain mice purchased from animal breeders in Jember.

2.2. Methods research

2.2.1. Watermelon (Citrullus lanatus) rind extraction

As much as 1.5 kg of watermelon rind was cleaned of dirt and washed with water. Watermelon rind was cut into small pieces and then dried by aerating with the help of a Ardir food dehydrator ARD-PM77. The dried watermelon rind

was then ground into a powder (simplicia). Simplicia was sieved with a B30 sieve, and 621.4 g of watermelon rind powder (*Citrullus lanatus*) was extracted using the maceration method with 3 liters of 97% ethanol for 2 days. The mixture was stirred every 2 hours. After 2 days, the extract was filtered through a flannel cloth to obtain filtrate and residue. The residue was re-macerated with 2 liters of 96% ethanol for 2 days, and the extract was filtered again to produce another set of filtrate and residue. All the filtrates were combined and evaporated in a Memmert uf55 laboratory oven 53 L at 70ºC to obtain a thick watermelon rind extract.

2.2.2. Phytochemical screening test

The phytochemical screening test of watermelon rind extract aims to identify the chemical compounds in the extract. The screening test was conducted to qualitatively identify the content of saponins, polyphenols, flavonoids, and alkaloids.

a. Identification of Saponins

A total of 0.05 grams of concentrated liquid extract of watermelon rind (*Citrullus lanatus*) was added with 5 ml of distilled water and heated for 2 minutes. Then, the watermelon rind extract (*Citrullus lanatus*) was filtered and the filtrate was shaken. The presence of saponins was indicated by the emergence of foam for approximately 2 minutes [1].

b. Identification of Flavonoid

A total of 0.05 grams of thick extract of watermelon rind (*Citrullus lanatus*) was mixed with 3 ml of 70% ethanol, then shaken, heated, shaken again, and filtered. The filtrate obtained was then added with Mg metal and 2 drops of concentrated HCl. The formation of a red color on the ethanol layer indicates the presence of flavonoids [1].

c. Identification of Polyphenol

A total of 0.05 grams of thick extract of watermelon rind (*Citrullus lanatus*) was mixed with 5 ml of 70% ethanol then shaken, and 0.1% iron (III) chloride solution was added. A positive result gives rise to a strong green, red, purple, blue, or black color [6].

d. Identification of Alkaloids

The test was carried out by taking 2 mL of watermelon rind (*Citrullus lanatus*) which had been extracted with water and ethanol as solvents into 2 different test tubes [7]. After that, one of the test tubes contains the extract plus 10 drops of 2N sulfuric acid and 6 drops of Mayer's reagent. If each solution forms a white precipitate, the positive sample contains alkaloids [8].

2.2.3. Preparation of watermelon (Citrullus lanatus) peel extract gel

Watermelon peel extract gel (*Citrullus lanatus*) was made using CMC NA, polysorbate, glycerin, nipagin, nipasol, EDTA, and aquadest as the gel base. Then, the gel base that had been made was added with ethanol extract of watermelon rind (*Citrullus lanatus*) with concentrations of 25%, 50%, and 75% to obtain 30 grams of watermelon rind extract gel (*Citrullus lanatus*) [9].

2.2.4. Physical properties test of watermelon (Citrullus lanatus) peel extract gel

a. Organoleptic Test

Observations of the preparations included the color, aroma, and texture of each gel preparation formulation which were observed 3 times by the respondents.

b. pH Test

The pH test was carried out using a universal pH paper dipped in diluted gel sample. The color changes that occured matched the universal pH standards.

c. Spreadability Test

This test was carried out by weighing 0.5 grams of gel and then placing it in a round glass, Another glass was placed above it, and left for 1 minute. After that, 150 grams of the load was added, allowing it to stand for 1 minute and the constant diameter was measured.

2.2.5. Burn test on balb/C male mice

The initial step before testing the burn activity of watermelon peel extract gel in male Balb/C strain mice was obtaining an ethical clearance permit from the Faculty of Dentistry, University of Jember. As a result, the Research Ethics Committee of the Faculty of Dentistry, University of Jember stated that the above protocol complied with the ethical principles so that it could be implemented, according to letter No. No.1790/UN25.8/KEPK/DL/2022.

The mice were acclimatized for 1 week before the study. Then, the experimental mice were shaved in the upper back area one the day before the burn wound. Next, the mouse was anesthetized using ketamine 0.12 mL IM and a special circular metal plate with a diameter of 2 cm x 2 cm was heated to 100 °C and placed on the upper back of the mouse for 30 seconds to induce burns. The burns were considered circular [10].

Mice that had burns were given treatment by applying chemical/synthetic and natural preparations. Each group consisted of 5 male balb/c strain mice and received the following treatment:

1) Treatment of group 1: Burns were treated with Bioplacenton (positive control)
2) Treatment of group 2: Burns were smeared with gel base (negative control)
3) Treatment of group 3: Burns were treated with 25% watermelon rind extract gel (*Citrullus lanatus*)
4) Treatment of group 4: Burns were treated with 50% watermelon rind extract gel (*Citrullus lanatus*)
5) Treatment of group 5: Burns were treated with 75% watermelon rind extract gel (*Citrullus lanatus*).

Experimental animals that suffered burns were treated by administering gel base (negative control), Bioplacenton (positive control), and fruit peel extract gel with concentrations of 25%, 50%, and 75% once a day for 14 days. The measurement of wound area was carried out after the inflammatory phase which was named on the fourth day, using graph paper. The measurement results of the burn area were analyzed for the normality of the data obtained using the Kolmogorov-Smirnov method. If the data were normal, the analysis was continued with the One Way ANOVA and post hoc methods to determine differences in burn activity at various gel concentrations.

3. Result and discussion

3.1. Phytochemical screening test

In the test for the content of saponins, flavonoids, polyphenols, and alkaloids of watermelon rind extract, foam formed for 2 minutes successively, a green color faded to red in the ethanol layer, a red color in the ethanol layer, and a white precipitate formed. Thus, watermelon rind extract contains saponins, flavonoids, polyphenols, and alkaloids as shown in Table 1. Alkaloid compounds in watermelon rind play a role in wound healing due to their effects on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodeling [2].

3.2. Physical Properties Test of Watermelon (Citrullus lanatus) Peel Extract Gel

The physical properties of the gel were evaluated through several tests, including organoleptic test, pH test, and spreadability test. The results of the evaluation of the physical properties of the gel are described in Table 2. The results of organoleptic observations showed that the watermelon rind gel had a characteristic odor, with the extract having moss green and a thick and semi-solid texture. This shows that there is no change in shape, smell, and color across the three concentrations. The higher the addition of extract to the formula, the darker the resulting gel [5].

Examination of pH is a physicochemical parameter that must be carried out for topical

Table 1. Phytochemical screening test of watermelon (*Citrullus lanatus*) peel extract

No.	Test	Result	Conclusion
1.	Identification of saponins	Presence of foam for ± 2 minutes	Positive
2.	Identification of Flavonoid	The presence of a green color fades to red in the ethanol layer	Positive
3.	Identification of Polyphenol	The presence of a red color in the ethanol layer	Positive
4.	Identification of Alkaloids	The presence of a white precipitate in the test tube	Positive

Table 2. Physical properties test of watermelon (*Citrullus lanatus*) peel extract gel

Parameters	Results		
	F1 (25%)	F2 (50%)	F3 (75%)
Organoleptic	Moss green, characteristic odor of watermelon rind extract (*Citrullus lanatus*), thick, semi-solid	Moss green, characteristic odor of watermelon rind extract (*Citrullus lanatus*), thick, semi-solid	Moss green, characteristic odor of watermelon rind extract (*Citrullus lanatus*), thick, semi-solid
pH	7.00	7.00	7.00
Spreadability	5.74	5.71	5.09

preparations, because pH is related to the effectiveness and stability of the active substance and preparation, as well as the skin comfort when applied. Based on the observed results of the pH test, it can be seen that the three concentrations of watermelon rind extract gel have a neutral pH of 7.00. The pH of the gel does not meet the requirements for a topical preparation, which are 4.5 - 6.5 [11]. However, pH 7.00 is neutral so it does not damage the skin's surface. Overly acidic conditions may cause skin irritation, while highly alkaline conditions may result in skin dryness [12].

The spreadability test of the gel preparation was intended to determine the ability to spread a gel when applied to the skin. Based on the measurement results, the diameter of the dispersive power of the watermelon rind extract gel from formulation 1, formulation 2 and formulation 3 had good dispersibility, namely 5.74 cm; 5.71 cm; and 5.09 cm. The three formulations met the predetermined dispersion requirements of 5.00 – 7.00 cm. Based on the spreadability test method, the constant diameter measurement is formed when 0.5 g of gel is given a load of 150 g for 1 minute. The decrease in dispersal value of the three formulas was due to the increased concentration of watermelon rind extract, resulting in thicker gels and reduced spreadability.

3.3. Burn test on balb/C male mice

The burns observed in this study were second-degree burns which were marked by a reddish color in male mice [13]. Second-degree burns involve the entire epidermis and part of the dermis causing bulla, reddish color, slight edema, and severe pain. If handled properly, second-degree burns can heal within 7 to 20 days, leaving scar tissue [14].

The method for creating the mice burns begins with anesthetization using an injection of 0.03 ml of ketamine at a concentration of 50 mg/kg BW. This is followed by shaving the mice using a razor on the back of the mice which aims to make it easier when making burns on their backs. Then, the male mice were burned on the upper thighs using 0.5 cm diameter solder, down to the dermis and the connective tissue underneath causing blisters and skin to peel off in certain areas and forming second-degree burns which are characterized by a reddish color in male mice [11]. The injured mice were treated with ointment once a day every morning according to their respective

groups and observations of burn wound healing were carried out for 14 days.

Table 3 shows the Observational data on the decrease in the average area of second-degree burns. The measurement of the wound area was carried out after the inflammatory phase, which was named after the fourth day using graph paper.

Based on Table 3, there was a decrease in the size of the burns on the backs of the mice. Based on these data, it can be seen that the decrease occurred from day 0, which is the day of induction, to day 2. This was part of the inflammatory phase that occurs in the area around the burn. In this phase, mast cells release histamine which increases the permeability of blood capillaries and causing swelling. The data from the measurement of the area of the burn wound was then converted into a percentage which can be seen in Figure 1.

The data on the extent of burns and the percentage of burn healing were statistically analyzed using the Kolmogorov-Smirnov normality test with an error rate in decision-making set at $\alpha = 5\% = 0.05$. The selection criteria used sig.α or p-value, where if sig.α <α, the H0 was rejected [15]. The results showed that all burn wound areas and burn healing percentages for all groups were not normally distributed (p<0.05), so the analysis was carried out using non-parametric analysis. Non-parametric test results showed that all treatments on days 2, 5, 8, 12, and 14 showed no significant difference (p<0.05). Thus, the watermelon rind extract gel has the potential to relieve burns and has an activity similar to a burn reliever drug that is already on the market (Bioplacenton).

Table 3. Average burn area on observation day

Group	Average burn area (mm^2)				
	Day 2	Day 5	Day 8	Day 12	Day 14
Bioplacenton	12	6,33	5,5	3,5	0,5
Concentration of 25%	13	8	5,17	3,67	1,5
Concentration of 50%	16,5	11,67	6,83	4,83	3
Concentration of 75%	13	11,67	8,67	3,67	1
Gel Basis	21,33	10,167	7,83	6	3,33

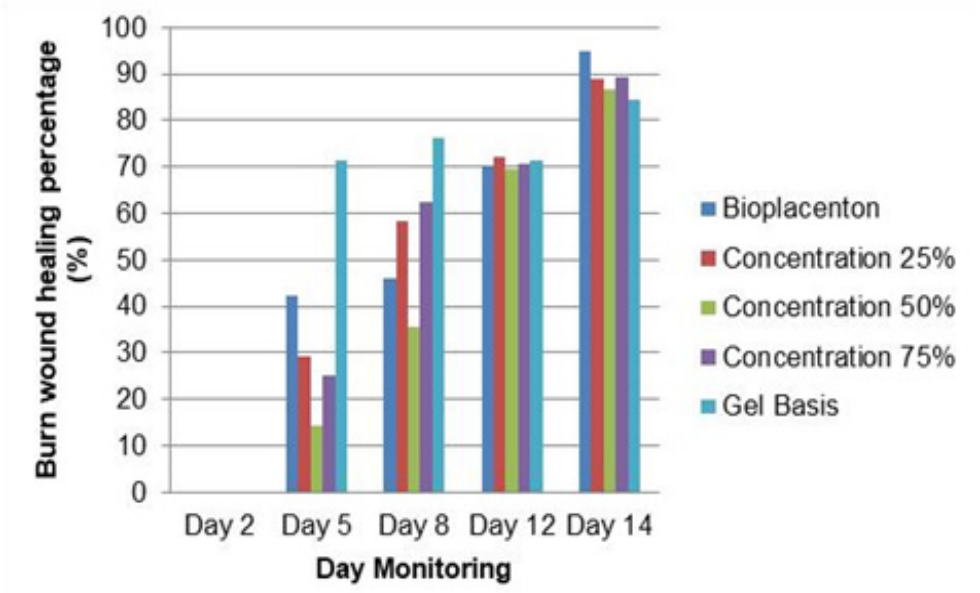

Figure 1. Graph of average burn healing percentage

4. Conclusion

Watermelon peel extract gel (*Citrullus lanatus*) has activity in healing second-degree burns. The effective concentration in burn healing is a concentration of 25% with the smallest wound diameter, the highest healing percentage and faster scab formation and release time.

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