POTENTIAL OF *PLETEKAN* LEAF EXTRACT AS A NATURAL ANTISEPTIC FOR BURN HEALING

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ABSTRACT

Background: Pletekan leaf (*Ruellia tuberosa* L.) is a wild plant rich in flavonoids, steroids, triterpenoids, and alkaloids. These bioactive compounds function as bactericidal agents, which can inhibit bacterial growth and have great potential for wound healing. This study aimed to determine the natural antiseptic effect of pletekan leaf extract on burn healing.
Subjects and Method: This study was a randomized controlled trial conducted at the Biology Laboratory, Universitas Negeri Semarang, Central Java. A sample of 30 male white rats (*Rattus norvegicus* strain Wistar) was selected for this study, and divided into two groups: (1) The control group consisting of 15 white rats, which did not receive ethanol extract of pletekan leaf; and (2) The experimental group consisting of 15 white rats, which received ethanol extract of pletekan leaf. The dependent variable was burn healing. The independent variable was ethanol extract of pletekan leaf with 25% concentration.
Results: The mean healing area in white rats receiving ethanol extract of pletekan leaf was larger than those not receiving ethanol extract, and it was statistically significant ($p<0.050$).
Conclusion: The ethanol extract of pletekan leaf is effective for burn healing in white rats.

Keywords: ethanol extract, pletekan leaves, burn healing, white rat

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BACKGROUND

COVID-19 is a global pandemic, and it is not reassuring for the community. Transmission of this disease can be prevented by maintaining cleanliness, which can use antiseptics. Antiseptics are substances that can inhibit the growth and development of microorganisms without having to kill these microorganisms in living tissue. Antiseptic is a chemical substance that can destroy microorganisms or inhibit their work to prevent the occurrence of an infection. Antiseptics are used on living things, while disinfectants are used on inanimate objects. Antiseptics have a more general performance, while antibiotics are specific in their performance on certain microorganisms (Annisa and Chandra, 2019).

Pletekan leaf is a plant that contains flavonoids, steroids, triterpenoids and alkaloids. Vitalia et al. (2016); Susana et al. (2016); Rahmi et al. (2014) stated that *pletekan* leaves contain flavonoid compounds, sapo-

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nins, and alkaloids. Based on the phytochemical screening of *pletekan* leaves (Ruellia tuberosa L.) conducted by Rahmi et al. (2014), *pletekan* leaf water extract contains flavonoids, alkaloids, polyphenols, tannins, quinones, monoterpenoids, sesquiterpenoids. In addition, *pletekan* leaves contain hexadecanamide compounds, anti-inflammatory, anti-nociceptive, neuroprotective, anticonvulsant, and antifungal properties (Ida et al., 2016).

Flavonoids are active compounds in *pletekan* leaves as antibacterial (Suteja et al., 2016). Antibacterial compounds are active compounds that inhibit or kill a microorganism (Pambudi et al., 2014). Saponins can be used for the wound healing process because saponins function as antioxidants, anti-inflammatory, antibacterial and antifungal substances (Novitasari, 2014). Tannins are substances that play a role in vasoconstriction. Vasoconstriction is important in hemostasis. Tannins also function as astringents that can cause shrinkage of skin pores, harden the skin, stop exudate from closing wounds and prevent bleeding that usually occurs in wounds (Yenti et al., 2011).

Ethanol 96% in this study was used as a solvent because it is a polar solvent that is expected to extract alkaloids, phenolic compounds, and flavonoids, has a low boiling point and tends to be safe (Munawarah and Handayani, 2010).

A break in the continuity of the tissue or a breakdown in the integrity of the skin is called a wound. Burns are a type of damage to the skin that occurs when different layers of skin cells are damaged by hot liquids (boiling), hot solids (contact burns), or flames (fire burns). Burns can damage the skin, which protects from dirt and infection. Burned body surface areas can be life-threatening due to blood vessel damage, electrolyte imbalance and body temperature, respiratory and nerve function disorders (Azizah, 2017). Based on this background, this study aims to determine the natural antiseptic effect of *pletekan* leaf extract on healing burns.

SUBJECTS AND METHOD

1. Study Design

This was a Randomized controlled trial conducted at the Biology Laboratory Universitas Negeri Semarang, Central Java.

2. Population and Sample

A sample of 30 male white rats (Rattus norvegicus strain Wistar) was selected for this study, and divided into two groups: (1) The control group consisting of 15 white rats, which did not receive ethanol extract of pletekan leaf; and (2) The experimental group consisting of 15 white rats, which received ethanol extract of pletekan leaf.

3. Study Variables

The dependent variable was burn healing. The independent variable was ethanol extract of pletekan leaf with 25% concentration.

4. Operational Definition of Variables

a. Burn healing

Definition: evaluation of observations made within 21 days to assess wound diameter reduction

Measurement scale: Ratio

b. Pletekan leaf ethanol extract

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Definition: ethanol extract of pletekan leaves were diluted to obtain extract concentrations of 15%, 20%, and 25%
Measurement scale: ratio

5. Study Instruments

The ingredients used in the study were *pletekan* leaf extract, 96% ethanol, aquadest, magnesium (Mg), concentrated hydrochloric acid (HCl), ointment containing neomycin, sulfuric and biosulfate acid (bioplacenton), ether and male white rat (Rattus norvegicus strain wistar).

The tools used in the research were Bunsen, water bath, measuring cup, horn spoon, 40 mesh sieve, filter paper, syringe, dropper, iron measuring 1.5 x 1.5cm^2.

6. Data Analysis

The data from the test results of the concentration of pletekan leaf extract on (area of the burn) were then tested for normality and homogeneity. If the data is normally distributed and homogeneous, then it is tested with one-way ANOVA. If the data are not normally distributed and homogeneous. The bivariate data were analyzed by Mann-Whitney.

RESULTS

Observations of differences in healing rates began to appear when the characteristics of the wound proliferative phase, namely day 7, were then carried out at intervals of 3 days to determine the physical changes that occurred in each group. Table 1 shows that the percentage of burn healing rate in the 15% concentration group experienced an average healing rate of 83.16%, the 20% concentration group experienced an average healing rate of 82.48%, the 25% concentration group experienced an average healing rate of 84.71 %, positive controls experienced an average cure rate of 79%.

Meanwhile, the negative control group experienced an average healing rate of 70%, which was indicated by no more redness in the wound and shrinking of the wound.

The number of research subjects were 15 control groups and 15 intervention groups.

Table 1. Number of samples of mice in two groups

Subjects	Intervention		Control	
	N	%	N	%
White rats	15	50	15	50

Table 2. The use of pletekan leaf extract with a concentration of 15%, a concentration of 20%, and a concentration of 25% on the diameter of burn healing before intervention

Ethanol Extract of Pletekan Leaf	Mean	SD	p
15% concentration	0.38	0.03	0.001
20% concentration	0.39	0.05	0.001
25% concentration	0.36	0.12	0.001

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Table 3. The use of pletekan leaf extract with a concentration of 15%, a concentration of 20%, and a concentration of 25% on the healing of burns after the intervention

Ethanol Extract of Pletekan Leaf	Mean	SD	p
15% concentration	0.25	0.01	0.001
20% concentration	0.20	0.02	0.001
25% concentration	0.23	0.05	0.001

Table 4. Average percentage of burn healing rate

Rats	Positive Control	Negative Control	15% concentration	20% concentration	25% concentration
1	80.00%	64.00%	83.46%	84.44%	76.71%
2	80.00%	64.00%	84.17%	84.44%	82.57%
3	70.00%	72.26%	84.44%	78.84%	88.00%
4	84.17%	70.84%	81.86%	81.86%	88.53%
5	82.84%	77.00%	81.86%	82.84%	87.73%
Average	79.00%	70.00%	83.16%	82.48%	84.71%

DISCUSSION

Pletekan leaf extraction was carried out using the maceration method, a cold extraction method that aims to avoid damage to the thermolabile compounds in pletekan leaves. Stirring during maceration puts the surface of the simplicia powder in maximum contact with the solvent so that the active substance can be dissolved maximally. Ethanol has a high level of polarity in dissolving the antiseptic compounds present in the pletekan leaf powder. The yield of maceration of pletekan leaves using 96% ethanol solvent was 8.46%, with a thick extract of 84.57 grams.

At the phytochemical screening stage, qualitative tests were carried out for the presence of flavonoids, tannins, and saponins because these compounds functioned as antiseptics and affected wound healing. The test results for the ethanol extract of pletekan leaves (*Ruellia Tuberosa L.*) were positive for flavonoid compounds, saponins, and tannins. The identification of flavonoid compounds formed a reddish color that indicated a positive presence of flavonoids. Concentrated magnesium and HCl reduce the benzopyran core in the flavonoid structure so that red color is formed. The results of the saponin test were that large foam was formed, and after the addition of HCl, the foam remained stable, the identification of saponin compounds showed positive results. Saponins are easily soluble in water and have the characteristic of foaming when shaken. Saponins have a group.

Polar and non-polar are active on the surface to reduce surface tension like soap so that when shaken with water, saponins can form micelles. In the micelle structure, the polar groups face outward while the non-polar groups face inward. This state is what looks like foam. The qualitative test results for tannins showed a black color, and a precipitate occurred, indicating that the ethanol extract of the pletekan leaves was positive for tannins.

To determine the activity of ethanol extract of pletekan leaves on healing

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burns and the effect of the concentration of ethanolic extract of pletekan leaves on the healing of burns, a test was conducted on test animals, namely white rats of the Wistar strain. The rats were anesthetized first using ether so that when the burns were made, the rats did not feel excessive pain that caused stress or even death. Ether belongs to the standard class of inhalation anesthetics, which is good and can affect some living nerves but does not poison the test animal's body if it is not used excessively and is volatile (Heny et al., 2018). Burns that had been made were then treated according to the treatment group, namely the positive control group with bioplacenton ointment, the negative control group with 0.9% NaCl, and the 15%, 20%, and 25% concentrations. This study uses the ointment

bioplacenton as a positive control because the ointment contains 10% placenta extract, which functions to trigger the formation of new tissue so that it can be used for wound healing, and contains 0.5% neomycin sulfate, which functions to prevent or treat infection with gram-negative bacteria in the wound area. The treatment group was dropped evenly three times a day for 21 days. The results showed that the ethanol extract of pletekan leaves affected healing burns in rats. This burn healing effect is due to antiseptic compounds in the ethanol extract of pletekan leaves, namely flavonoid compounds, saponins, and tannins (Ida et al., 2016).

Flavonoids are polyphenolic compounds with various effects, including antioxidant, antitumor, anti-inflammatory, antibacterial, and antiviral effects. Tannins are astringent which can affect wound healing by several cellular mechanisms, one of which is increasing the re-formation of the dermis tissue in the wound tissue. Saponins help increase the number of macrophages migrating to the wound area, thereby increasing the production of Growth Factors that can increase new blood vessels.

On days 1-3 after the wound formation, the first stage of wound healing occurs, namely the inflammatory phase. The inflammatory phase aims to prevent colonization and infection by removing dead tissue and hemostasis (Kartika, 2019). Inflammation is characterized by the presence of rubor (redness), tumor (swelling), calor (heat), and dolor (taste) pain/pain) (Ghofroh, 2017).

Mice in all test groups experienced the inflammatory phase on day 1. The faster the inflammatory process occurs, the faster the subsequent wound healing process. The next stage of wound healing is the proliferative phase. The formation of a scab indicates that the wound healing process has entered an early proliferative phase. The proliferative phase occurs from day 3 to day 21. In the proliferative phase, the wound is filled with inflammatory cells, fibroblasts, collagen fibers, forming a reddish tissue with an uneven surface called granulation tissue (Ghofroh, 2017). Observations were made from the first day, and the wound diameter was measured after entering the proliferation phase, and there was a change in wound size. The negative control group recovered at day 24, with the first day appearing pale red, on day 14 scabs or crusts that dry on the wound are very little removed, and on day 24, the wound healed with no skin tissue

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marked colored Red. The positive control group on day seven has been seen.

The scab came off because the wound area after the measurement was reduced, and on day 21, there were no wounds. The concentration group was 15%, from day 7, the scabs had started to come off, and on day 18, there were no wounds with no wounds and scabs. The wounds healed markedly by no red skin tissue. The 20% concentration group was different from the 15% concentration group. From day 7, the scabs had started to come off, and on day 18, there were no wounds, no wounds, and scabs. The wounds healed with no red skin tissue. The 25% concentration group from the 7th day had many scabs coming off, and on the 17th day, the wound healed with no wounds and scabs, the wound healed markedly by the absence of red skin tissue. The 25% concentration group healed faster than the positive control so that the 25% extract concentration had a more significant and good effect on wound healing, followed by the 15% and 20% groups.

The data from the measurement of the diameter of the burn wound was converted in the form of a percentage to see the significance of the effect of the ethanol extract of pletekan leaves on the healing of burns. Based on table 1, the results of the average percentage of burn healing showed that the 25% concentration group showed a higher percentage of wound healing than the . group others, namely 84.71%. The positive control group showed 79% results, the 15% concentration group was 83.16%, and the 20% concentration group was 82.48%.

Based on the research that has been done, it can be concluded that the ethanol extract of pletekan leaves (Ruellia Tuberosa L.) has acted as a burn wound healing in white rats (Wistar). The ethanol extract of pletekan leaves contains antiseptic compounds that play an essential role in wound healing.

AUTHOR CONTRIBUTION

Yayuk Mundriyastutik as the lead researcher determined the formulation in the preparation of the extract concentration, Indah puspitasari helped make wounds on white rats. Muhammad Purnomo helped in smearing the extract and contributed to the drug as a control. Iffana Dani Maulida did the evaluation.

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CONFLICT OF INTEREST

There was no conflict of interest.

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