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Studying the Formulation of Shallot (*Allium Ascalonicum L.*) Ethanol Extract Gel as Treatment of Excision Wounds in Rats

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Abstract

Onion bulbs (*Allium ascalonicum L.*) contain alkaloid compounds and saponins which can accelerate the wound healing process. Therefore, a study was conducted on the wound healing effect of excision of ethanol extract of onion bulbs (*Allium ascalonicum L.*) in the form of a gel against mice (Rattus novergicus). The purpose of this research was to study the stability of the preparation as well as the effectiveness and optimum concentration in excision wound healing from the ethanolic extract of shallot (Allium ascalonicum L.) in gel dosage form. The concentration variants used were 5%, 10%, and 20%. The method used was sample extraction by maceration and wound gel was made in 3 concentrations, namely 5%, 10%, and 20% then a stability test was carried out on the preparation by cycling test method at 4ºC and 40ºC for 6 cycles. Besides that, gel base was also used as a negative control and octadine® gel as a positive control, then the back skin of a rat (Rattus novergicus) was injured using a surgical knife. The results of the observation of the stability of the wound gel preparation were stable both at 4ºC and at 40ºC. Reduction of the length of the wound at the beginning of the treatment until the wound was completely closed showed that the ethanol extract gel preparation of shallot bulbs (*Allium ascalonicum L.*) could reduce and heal cuts in rats with a concentration of 20% which showed the most effective wound healing effect.

Keywords: Excision wound, Allium ascalonicum L., Gel

1. Introduction

Traditional medicine can be interpreted as ingredients or ingredients derived from plants that have been used therapeutically for generations, and in a way that refers to experience and genetic skills based on experience, traditional medicine for treatment or care, applies to the community for health services. Apply with an offset. Traditional medicines can be extracted in dry, concentrated or liquid dosage forms by extracting simplicia from the influence of direct sunlight in the right way (Wardani, et al., 2011; Sukmawati, et al., 2019).

Shallot (*Allium ascalonicum L.*) is one of the herbal plants that is widely used in traditional medicine. Derived from Syria in the Middle East, this plant is found in many tropical and subtropical regions, so it can be cultivated very well in Indonesia. Indonesian people not only use shallots as medicine, but also routinely use them as a spice in the kitchen. I use it for that purpose. Shallots are considered as a therapeutic agent for various ailments and are widely used as wound healing agents (Wongmekiat, et al., 2008).

As the outermost organ of the body, the skin acts as a protective layer for the body which is vulnerable to injury (Rashed, et al., 2003). With various forms of wound healing disorders, researchers are trying to find ingredients or formulations that can help speed up the process.

Many studies have been conducted on the benefits and formulations of traditional medicinal plants as alternative medicine. A study by Saenthaweesuk et al., (2015) Topical petroleum gel formulations made with ethanol extract of shallot bulbs at a concentration of 10% and 20% gave the best results with extracts at a concentration of 10% (78.61 ± 1.20%). % wound contraction, compared to 20% concentration of 78.55 ± 1.93%). Teo, et. al (2021) administration of shallot bulb extract at a concentration of 15% had a healing effect on burns on the 8th day with an average diameter of 0.81, while administration with doses of 10% and 5% had a healing effect on burns on the 3rd day 8 with an average diameter of 0.99.

Given this, red onion is a plant that is commonly grown by people, especially in the West Java region. We will often encounter onion bulb cultivation. One of them is in the Bayongbong Garut area west Java. It makes researchers

2. Research Methods

2.1. Tools and materials

The tools used in this study were glassware commonly used in laboratories, calipers (Pyrex), rat cages for each rat and drinking bottles for rats, flat-bottomed cups (Pyrex), corked bottles (Iwaki), crucibles and pliers. , desiccator (Iwaki), electric oven (Hammer), water bath (Yenaco) mortar and tamfer (Pyrex), analytical balance (Saka), pH meter (Hanna), rotary evaporator (RV10 IKA).

The materials used in this study were shallot bulbs (*Allium ascalonicum L.*), male white rats (Rattus norvegicus), aquabidest. The chemicals used are Bouchardat reagent solution, Mayer reagent solution, Dragendorff reagent solution, Liebermann-Burchard reagent solution, Molish reagent solution, chloroform, hydroxy propyl methyl cellulose (HPMC) 2%, propylene glycol, methyl paraben, propyl paraben, octadine. gel, povidone iodine, 70% alcohol, 96% alcohol.

2.2. Research Path

Interested in conducting research on the formulation of gel preparations from the ethanol extract of shallot bulbs on excision wound healing by observing changes in wound length and wound healing time.

1. Material Preparation and Extraction
   The sample used in this study was shallot bulbs (*Allium ascalonicum L.*) obtained from the Bayongbong area, Garut, West Java. The shallot bulbs obtained were then cleaned to remove adhering dirt, then chopped and dried. Furthermore, dry sorting is then carried out in a blender to become powder. Simplisia was extracted by maceration method using 96% ethanol solvent. Maceration was carried out for 3 days with solvent changes every 24 hours. Then the macerate was filtered and concentrated using a rotary evaporator until a thick extract was produced.
2. Determination of Drying Shrinkage
   The weighing bottle was heated at 105°C for 30 minutes. This is done until a constant weight of the weighing bottle is obtained or the difference in results between the 2 weighings does not exceed 0.005 g. Weigh 1,000 grams of the test material and put it in the weighing bottle. Then it was dried at 105°C for 5 hours and weighed again. The drying process is continued and dried again for 1 hour until the difference between successive weighings is not more than 0.25%.
3. Determination of Water Content
   Determination of water content using the gravimetric method, namely by heating an empty cup in an oven at 105°C for 30 minutes, cooling it in a desiccator for 15 minutes, then weighing it (W0). Then a 2 gram sample of simplicia powder or extract is put into a cup whose weight is known, weighed (W1), then dried in an oven at 105°C for 3 hours, cooled in a desiccator for 15 minutes, then the cup and its contents are weighed and dried again. for 1 hour, and cooled in a desiccator, weighed again (W2)
4. Phytochemical Screening
   a. Alkaloids
      As much as 2 ml of sample solution is put into a test tube. Then drip using 2N HCL, then divided into 3 test tubes. Each tube is added reagent. If it is positive for alkaloids, a brown precipitate will form on the addition of Wagner's reagent, a white precipitate will form on the addition of Mayer's reagent and an orange precipitate is formed on the addition of Dragendroff's reagent.
   b. Flavonoids
      As much as 10 g of powder or simplicia extract is added to 100 ml of hot water, boiled for 5 minutes and filtered hot. Into 5 ml of the filtrate, magnesium powder, 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol were added, shaken vigorously and allowed to separate. If there are flavonoids it is indicated by the appearance of red, yellow or orange on the amyl alcohol layer (Harahap, et al., 2019).
   c. Saponins
      As much as 0.5 g of simplicia powder or extract is put into a test tube, added to 10 ml of hot water, cooled and then shaken for 10 seconds. If foam is formed 1 to 10 cm high which is stable not less than 10 minutes and does not disappear with the addition of 2 N hydrochloric acid indicating the presence ofsaponins (Batubara, et al., 2020).
   d. Tannins
      As much as 0.5 g of simplicia powder or extract, is extracted with 10 ml of distilled water and then filtered, the filtrate is diluted with water until it is colorless. Take 2 ml of the solution and add 1-2 drops of 1% iron (III) chloride reagent. If it happens blue or green-black color indicates the presence of tannins (Aruan, et al., 2019).
   e. Steroids/Triterpenoids
      As much as 1 g of simplicia powder or extract is macerated with 20 ml of n-hexane for 2 hours, filtered, the filtrate is evaporated in an evaporating cup and to the remainder added 2 drops of Liebermann-Burchard. If

a purple or red color is formed, it turns purple or blue-green, indicating the presence of steroids/triterpenoids (Aruan, et al., 2019).

5. Formula Design

Table 1: Formula Design of Shallot Ethanol Extract Gel

Material	Function	Control (-)	Formula I	Formula II	Formula III
Onion Bulb Ethanol Extract %	active ingredient	-	5	10	20
HPMC (%)	Gelling Agent	2	2	2	2
Propylene glycol (%)	Humectants	15	15	15	15
Methyl Paraben (%)	Preservative	0.12	0.12	0.12	0.12
Propyl paraben (%)	Preservative	0.05	0.05	0.05	0.05
Distilled water ad (%)	Solvent	100	100	100	100

6. Preparation of Shallot Bulb Ethanol Extract Gel
   Distilled water as much as 20 times the weight of HPMC was heated to a boil, then removed and the HPMC developed in it for approximately 15 minutes, after swelling then added methyl paraben and propyl paraben previously dissolved in propylene glycol little by little while stirring until homogeneous, then added with distilled water up to 100 g.

7. Evaluation of Preparations

a. Organoleptic Testing
   Examination of the physical stability of the preparation includes shape, color and odor which are observed visually. Preparation stated stable if the shape, color and smell do not change visually during storage (Read, et al., 2003).

b. Homogeneity Testing
   A certain amount of preparation is smeared on a piece of glass, then the surface of the glass that has been smeared is pressed against another piece of glass, the preparation must show a homogeneous arrangement and no coarse grains are visible

c. Spreadability Testing
   A total of 1 gram of the preparation is placed on the watch glass and then covered with another watch glass and a weight of 200 grams is used on it and the diameter is measured after 1 minute.

d. pH testing
   Determination of the pH of the preparation was carried out using a Hanna pH meter. The pH meter was first calibrated using a standard neutral pH buffer solution (pH 7.0) and an acidic pH buffer solution (pH 4.0) and then dried with tissue paper. Measurements are made by dipping the pH meter into the gel preparation to be tested, until the tool shows a constant number. The number shown by the pH meter is the pH value of the preparation

e. Viscosity Testing
   Determination of the viscosity of gel preparations using a Brookfield viscometer. The gel preparation was put into the beaker glass until it reached a volume of 100 ml, then the spindle was lowered until it was immersed in the preparation. Then the tool is turned on by pressing the ON button. The spindle speed is set, then the scale is read (dial reading) where the moving red needle has stabilized. The viscosity value in centipoise (cps) is obtained from the multiplication of the reading scale with the correction factor (f) specifically for each spindle speed (Read, et al., 2003).

7. Testing of Gel on the Healing Effect of Excision Wounds
   a. Making an Excision Wound
      Making excision wounds in rats is carried out according to the method of Al-Bayaty & Abdulla (2012), which is as follows:
      1) Rats shaved their hair in the upper back area.
      2) When a wound is to be made, the rat is anesthetized first using chloroform.
      3) The upper back and surrounding areas are cleaned with providedone iodine then 70% alcohol.
      4) After that, a wound with an area of ±150 mm2 and a depth of 2 mm is made lengthwise, by tearing the skin using a scalpel to the subcutis, that is, to the dermis and connective tissue.
      5) The wound is then given preparations according to the test group, the preparation test is carried out after the wound is done.

8. Wound Healing Effect Test
   The wound healing effect test was carried out on 25 rats which were divided into 5 groups. Group I (positive control) was given otadine gel, group II (negative control) was given a gel base, group III was given a gel with a 5% onion bulb ethanol extract concentration, group IV was given a gel with a 10% onion bulb ethanol extract concentration, group V given a gel with a concentration of 20% onion bulb ethanol extract. Giving the gel is done topically by applying it on the part Wounds in treated rats used sterile cotton buds every day, from the 1st

day until the wound healed after being injured once a day in the afternoon. To prevent infection in the wound, it is carried out by maintaining the cleanliness of the cage both before and during treatment. The wound will also be cleaned once a day using 0.9% NaCl physiological solution. Observations of the wound were carried out visually every day starting from the beginning of treatment until the day the wound healed by measuring the reduction in wound length and calculating the percentage reduction in wound length.

9. Data Analysis
   The research data were analyzed using the SPSS (Statistic Product and Service Solution) 18 program. The data were analyzed using the Kolmogorov Smirnov method to determine its homogeneity and normality. Then the analysis uses the Kruskal-Wallis method to determine the average difference between groups. If there is a difference, it is continued by using the Mann Whitney test to see the real difference between groups treatment.

3. Results And Discussion

3.1. Extraction Results

Based on the results of the extraction of shallot bulb powder by maceration method using 96% ethanol solvent, the percent yield was 35.8% with an extract weight of 179.23 grams.

Determination of drying shrinkage is carried out to find out the compounds lost during the heating process. The missing compounds are water and volatile compounds such as essential oils. In determining the drying shrinkage, the results were 7.70% for powders and 8.10% for extracts. This indicates that the powder and extract from shallot bulbs have met the requirements for drying milk, namely <10%.

Determination of water content is carried out to determine the remaining water contained in simplicia. In determining the water content, the results were 8.20% for powders, and 8.00% for extracts. Phytochemical screening was carried out to determine secondary metabolites contained in simplicia and shallot bulb extract.

Table 2: Results of Phytochemical Screening of Simplicia and Shallot Extract

Skrining	Precipitate	Color/Reagent	Simplicia	Extract
Alkaloid	Mayer Bouchardat dragendroff	White precipitate Brown precipitate Orange	+	+
Flavonoid	Mg+HCl pekat	Red	+	+
Saponin	Air panas/dikocok	Foam	-	-
Tanin	FeCl3 1%	Blackish green	-	-
Steroid/triterpenoid	Lieberman-Buchard	Green color	+	+

Description:
(+) positive: contains a group of compounds
(-) negative: does not contain a class of compounds

3.2. Evaluation of Ethanol Extract Gel Preparations

Shallot Bulbs (*Allium ascalonicum L.*) Gel preparations with various concentrations of shallot bulb ethanol extract 5%, 10%, and 20%. Has the following characteristics:

Table 3: Evaluation of Gel Preparations

Parameter	Control (-)	Formula I	Form II	Formula III
Organoleptic	Semisolid Typical smell Clear White	Semisolid Typical smell Chocolate	Semisolid Typical smell Chocolate	Semisolid Typical smell Chocolate
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneos
Spread power	5.4±0.11	5.3±0.15	5.5±0.11	5.5±0.20
pH	5.4±0.05	5.5±0.15	4.8±0.20	4.9±0.17
Viscosity	2591	2723	3149	3421

Observation of the organoleptic test of shallot bulb ethanol extract gel consisted of odor and color. The resulting gel form complies with the gel form criteria, namely semisolid, the resulting shape and consistency did not change during observation and storage. Based on the color of the gel preparation produced by the shallot bulb ethanol extract gel which was brown in color, the color produced by the gel preparation did not change during observation. The odor produced is in the form of a weak characteristic odor of onion bulbs, where the odor is produced from the preparation gel formula I was stronger than gel formula II and III, the strength of the odor produced decreased during storage time. This is influenced by air so that the oxidation reaction occurs from the extract

The homogeneity of gel preparations is characterized by the absence of coarse grains and no clumping (Chizmadia & Brearley, 2008). Based on this, it shows that the mixing of the gel base with the ethanol extract of shallot bulbs is quite good so that the use can be evenly distributed.

Observation of the spreadability of the gel preparation was carried out to determine the amount of spreading force needed by the gel to spread on the skin or to determine the spreadability of the gel preparation when applied to the skin. A spread of 5-7 cm indicates a semisolid preparation consistency that is comfortable to use. The results in the initial test of the gel preparation obtained a spreadability value of 5.4 cm in the control (-), 5.3 cm in formula I, 5.5 cm in formula II, and 5.5 cm on the formula III. From the results of these observations the gel preparation can be said to be stable because it is still in the range of spreadability parameters.

pH monitoring aims to determine the acidity of a preparation, especially topical preparations. Ideally, topical preparations have the same pH value as the skin so that irritation does not occur on the skin surface. The results of observations of the pH of the gel preparation in the control (-) were 5.5, formula I 5.4, formula II 4.8, and in formula III 4.9, from the observations it can be said that the pH of the gel preparation stable because it is in the pH range the treatment takes place normally, that is indicated by changes in the reduction in the length of the wound that is getting smaller and the percentage of reduction in the length of the wound. increase. The results of monitoring injured mice showed changes in behavior such as calm and sleepy behavior, hair skin physiology, namely 4.5-6.5. The difference in pH value in each formula is caused by the addition of onion bulb ethanol extract which is acidic, causing the gel preparations in each formula to approach acidic pH but still in the physiological range of the skin.

Viscosity is carried out to determine the magnitude of a viscosity of the preparation, where the viscosity value represents the amount of resistance of a liquid to flow. The higher the viscosity value, the greater the resistance to flow. The results of the viscosity of the gel preparations in the control (-) were 2591cps, formula I 2723cps, formula II 3149cps, and formula III 3421cps. The good viscosity value of the gel preparation is in the range of 2000-4000cps. From the initial observations it can be said that the gel preparation is stable because it is in the range of the viscosity parameter.

3.3. Testing of Gel Preparations on Excision Wound Healing

Evaluation of wound healing effectiveness carried out on rats, where the process of falling out, decreased appetite and alcohol consumption increases.' due to rat stress due to wound pain. behind them. This is especially important in the first day gradually recovering from the treatment and returning to normal after the third day.

The results of the study and the average decrease in wound length were found to decrease wound length increments used to decrease wound closure until wound heals. Comparing the three methods of gel preparation of onion bulb ethanol extract can heal wounds for 16 days for onion bulb ethanol extract gel preparation with a concentration of 20%. The results showed that the ethanol extract gel of shallot bulbs could accelerate wound healing compared to the main gel. Based on the percentage change and reduction in the length of the lesion, it was seen that the increase in the percentage of the lesion increased to 100% from day 15 to the positive control used octadine® gel with the active ingredients octenidine hydrochloride 0.15% and allantoin 0.20% which can prevent thrush, followed by ethanol extract of shallot bulb gel total 20%, 10% and 5%.

The results of the percentage reduction in wound length obtained the effectiveness of good wound healing starting from octadine® gel as a positive control, then for test preparations 20% shallot bulb ethanol extract gel; 10% and 5%, and followed by a gel base. The graph of the percentage reduction in the length of the incision can be seen in Figure 1.

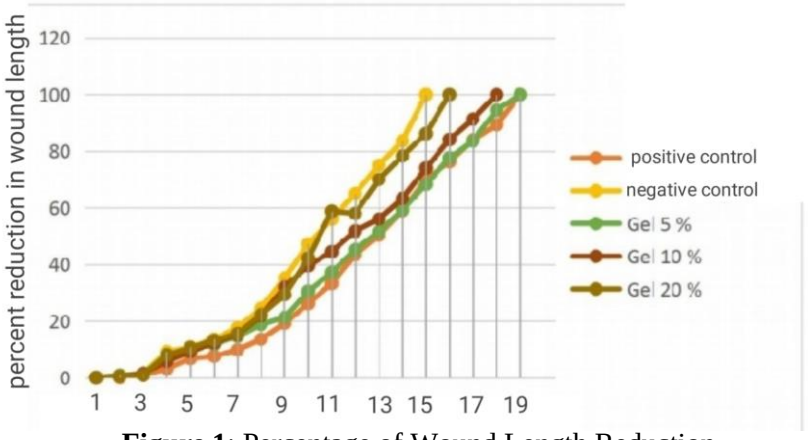

Figure 1: Percentage of Wound Length Reduction

The difference in treatment and release test was due to the 20% onion bulb ethanol extract gel preparation being suitable for treatment as a comparator drug, while the 10% shallot bulb ethanol extract gel preparation the dose is not maximal for healing cuts and in gel preparations of 5% shallot bulb ethanol extract, the healing of cuts is longer.

Compounds suspected of having wound healing activity in the extract Shallot bulb ethanol, namely saponins and flavonoids. Flavonoids work in the inflammatory phase by inhibiting the formation of prostaglandins and other inflammatory mediators, namely leukotrienes. The decrease in the production of prostaglandins and leukotrienes as inflammatory mediators will accelerate the inflammatory process to the next process, namely proliferation and maturation processes which result in faster wound healing.

while saponins work in the proliferative phase by increasing the proliferation of monocytes which can affect the number of macrophages. Increasing the number of macrophages around the wound area can affect many things, such as increasing the secretion of growth factors which play a role in the proliferative phase and increasing the number of fibroblasts migrating to the wound area accompanied by increasing the amount of collagen synthesized so that the proliferation process can be accelerated.

The length of the wound (mm2) in each experimental animal in each treatment was evaluated by the Kruskal-Wallis method, then followed by the Mann-Whitney test to see the actual difference between each treatment to see the treatment with the same or different effect. and the smallest effect on the largest one another. This test was carried out on all treatments from day first treatment until wound healing. Statistical test results can be seen in Appendix 17. Based on the statistical results of the Shapiro-Wilk normality test, the normality data test results showed that the data obtained was not normally distributed because the sig value was less than ($p < 0.05$), meaning that there was a significant difference between treatments on day 2. -1, 2, 3, 5, 10, 11, and 14. Continuing the homogeneity test using the Levene method shows the data obtained on days 5, 8, 9, and 10 has a significant value of less than 0.05, meaning the data obtained not homogeneously distributed. Because the normality and homogeneity tests were not fulfilled, ANOVA testing could not be carried out. So, for further analysis using Kruskalwallis and Man Whitney follow-up test. The results of the Kruskal-Wallis test obtained Asymp sig <α data, meaning that from day 4 to day 18 there was a significant difference in the activity of excision wound treatment during treatment. The Kurskal-Wallis test was followed by the Mann Whitney test to see differences between treatment groups.

The results of the Mann Whitney test in the positive control and negative control groups on day 1 to day 3 and day 19 showed no significant difference ($sig > \alpha$), whereas on day 4 to day 18 there is a significant difference compared to the negative control ($sig < \alpha$). For the negative control group with the 5% gel test preparation there was no significant difference on the 1st, 2nd, 3rd, 8th, 9th, 12th to 17th day, and 3rd day 19 there was no significant difference ($sig > \alpha$), whereas on the 4th, 5th, 6th, 7th and 18th days there were significant differences ($sig < \alpha$).

For the negative control group with 10% gel preparation there was no significant difference on day 1 to day 3, day 15, and day 19 ($sig > \alpha$), whereas on day 4 to day the 14th and 16th to 18th day there was a significant difference ($sig < \alpha$).

For the negative control group with 20% gel preparation on day 1 to day 3, and day 19 there was no significant difference ($sig > \alpha$), while on day 4 to day 18 there was a difference significantly compared to the negative control ($sig < \alpha$).

For the positive control group with negative control on day 1 to day 3, and day 19 there was no significant difference ($sig > \alpha$), while on day 4 to day 18 there was a significant difference compared positive control ($sig < \alpha$).

For the positive control group with 5% gel preparation on day 1 up to the 6th day, and the 19th day there was no significant difference ($sig > \alpha$), while on the 7th to 18th day there was a significant difference compared to the positive control ($sig < \alpha$).

For the positive control group with 10% gel preparations on the 1st, 2nd, 6th, 7th, 9th, 10th and 3rd day 19 there was no significant difference ($sig > \alpha$), while on the 3rd, 4th, 5th, 8th, 10th to 17th days there was a significant difference compared to the positive control ($sig < \alpha$).

For the positive control group with 20% gel preparations on day 1, up to 9, day 16 to 19 there was no significant difference ($sig > \alpha$), while on day 10, up to the 15th there is a significant difference compared to the positive control ($sig < \alpha$).

4. Conclusion

Based on the research that has been done it can be concluded that:

1. The ethanol extract of onion bulbs can be formulated in a stable gel dosage form.
2. Onion bulb ethanol extract gel preparation has a healing effect on wounds because the compounds contained in shallot bulb ethanol extract such as alkaloids, flavonoids, and steroids/tritepenoids can affect the healing process in wound. On the 15th day a concentration of 20% can affect wound healing 86.2%, a concentration of 10% can affect wound healing 74%, and a concentration of 5% can affect wound healing 68.3%.
3. The best concentration of shallot bulb ethanol extract gel preparations begins with gel preparations with a concentration of 20% (can affect wound healing on the 16th day), 10% concentration (can affect wound healing on the 17th day), and 5% concentration (may affect wound healing on day 19).

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