

Characterization Test of Binahong (*Anredera Cordifolia* (TEN.) STEENIS.) Leaves and Aloe Vera (Aloe Vera) Leaves Extracts using Infudation Method in Making Liquid for External Wound Healing

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Abstract

Binahong leaves and aloe vera leaves contain flavonoid compounds and saponin compounds that can help the wound healing process, therefore a liquid medicine for external wound healing is made from extracts of binahong leaves and aloe vera leaves. The process of making liquid medicine begins with the manufacture of binahong leaf extract using binahong leaves carried out by the infundation method. The infundation method was carried out through an extraction process using distilled water at a temperature of (80-90)°C for 15 minutes. The results of the hypothesis show that at a temperature of (80-90)°C for 15 minutes there are no variables that affect the manufacture of binahong leaf extract. The binahong leaf extract and aloe vera were mixed according to the specified formula. The formulation used was the ratio (binahong leaf extract: aloe vera leaf) = 1:1 as formula A; 1:2 as formula B; 2:1 as formula C; 1.5:1 as formula D and added alcohol in the ratio (alcohol: a mixture of binahong leaf extract and aloe vera leaf) 1:4 to prevent oxidation. The preparation obtained is liquid so that it is easier to apply. The test results showed that the external wound healing liquid formulation with a volume ratio of aloe vera leaf extract and binahong 2:1 (Formulation C = 1.8467%) showed a high flavonoid content compared to formulas A, B, or D. The chosen method is that every 1 L of external wound healing liquid medicine consists of 536 ml of binahong leaf extract, 268 ml of aloe vera leaf extract, 201 ml of 70% alcohol from the total volume.

Keywords: Binahong Leaf, Aloe Vera Leaf, Infundation Extraction, Flavonoid, Saponin

INTRODUCTION

Indonesia has a lot of potential biological natural resources as a source of food and medicine (Kinho et al., 2011). Traditional medicines derived from plants and pure natural ingredients have side effects, the level of danger and risk is much lower than chemical drugs (Muhlisah, 2005). Traditional medicine is an ingredient or ingredient in the form of plant material, animal material, mineral material, preparation of extracts (galenic), or a mixture of these materials which have been used for generations for treatment, and can be applied in accordance with the prevailing norms in society. External Medicine Liquid is a Traditional Medicine preparation in the form of oil,

solution, suspension, or emulsion, made of *Simplicia* and/or *Extract* and used as external medicine (BPOM, 2019). In plants, flavonoids function as growth regulators, photosynthetic regulation, antimicrobial and antiviral. Flavonoids can be used as a traditional medicine because flavonoids can work as respiratory inhibitors, inhibiting aldol reductase, monoamine oxidase, protein kinase, DNA polymerase, and lipoxygenase (Robinson, 1995). Flavonoids have antibacterial properties. The inhibition of the development of these bacteria will affect the development of tissue damage and the wound healing process can be accelerated (Umar et al., 2012). According to (Ristanti A. 2019), doing an extraction with 500 grams of material added 1 liter of distilled water heated for 5 minutes at a temperature of 90°C. The total flavonoid content was 1.962% from 500 grams of wet binahong leaves and 1.393% levels were obtained from 500 grams of dried binahong leaves. From research (Sari et al., 2018), it is known that the results of determining the levels of flavonoids in ethanol extract of aloe vera (*Aloe Vera*) using UV-VIS spectrophotometry are 0.1333%.

Saponins are surface-active compounds that can form foam when shaken in water. Saponins also have hemolytic properties and if injected directly into the bloodstream will be very toxic, but will not be harmful if taken by mouth, therefore saponins can be used as additives in non-alcoholic beverages/beverages (Evans W.C., 2002). The infundation method is a filtration process that is generally used to extract water-soluble substances at a temperature of 90°C for 15 minutes (Sujatmiko, 2014). One of the water-soluble substances is flavonoids. Flavonoids are compounds that can be dissolved in polar solvents such as ethanol, methanol, butanol, dimethylformamide, and water (Markham, 1988). The advantages of using the infusion method include: the unit of equipment used is simple, the operational cost is relatively low.

EXPERIMENTAL SECTION

The research method used is one-sided ANOVA. It will test the hypothesis about the average value of a normal population with the number = k of the same size. From each population, samples are taken independently with size = n and it is estimated that each population has the same variant. The Calculation hypothesis is carried out as follows:

$$c = \frac{T^2}{k \cdot n} \quad (1)$$

Sum of squares between samples

$$SSB = \frac{\sum_{i=1}^k T_i^2}{k} - c \quad (2)$$

$$SST = \sum_{i=1}^k \sum_{j=1}^n X_{ij}^2 - c \quad (3)$$

$$SSE = SST - SSB \quad (4)$$

Table 1. ANOVA

Source of Variation	Degrees of Freedom	Number of Squares	Squared mean value
Among Samples	(k-1)	SSB	$MSB = \frac{SSB}{(k-1)}$
Deviation	k(n-1)	SSE	$MSE = \frac{SSE}{k(n-1)}$
Total	k n - 1	SST	

F Stats

$$F = \frac{MSB}{MSE} \quad (5)$$

Hypothesis Testing

H₀ is rejected at the significance of if $F > F_{\alpha}$, H₀ is accepted if $F \leq F_{\alpha}$, F_{α} is obtained at the value of for F at degrees of freedom (k-1) and [k(n-1)] $F_{\alpha} = 5\%$ obtained in the table significant 0.05 and degrees of freedom for: Variable A = (k-1) and [k(n-1)].

Estimated average value of each population (μ):

$$\mu_1 = \frac{T_i}{n} \quad (6)$$

For = 5%

- i. H₀ has rejected, it means that there is a variable effect on the observation
- ii. H₀ has accepted means that there is no effect of the variable on the observation (Kasmiyatun, 2013).

Trial Steps

A. Making binahong leaf extract

1. Preparing Binahong Leaves 1830.5 gram
2. Then wash the prepared binahong leaves with clean water
3. After that cut into pieces about 1cm square
4. Then every weighing 6 grams of binahong leaves that have been cut into pieces.
5. Put 1 ml of aquadest in the vessel and extract it using the infusion method at 90°C for 15 minutes
6. Filtering the results of the extraction then obtained filtrate I (extract from the leaves of binahong)

7. Filter filtrate I with a vacuum filter to get filtrate II (extract from binahong leaves).

B. Making aloe vera leaf extract

1. Prepare 1000 gram of aloe vera leaves.
2. Peel and cut it.
3. Then smooth it using a mixer until it becomes a gel.
4. After that, filtering 1 obtained the extract.
5. Then the second filtering with a vacuum filter obtained aloe vera extract which will be used for formulation.

C. Manufacture of wound liquid preparations

1. Mix the extract from binahong leaves, aloe vera leaf extract, alcohol in a vessel according to the formulation made. The following is a liquid formulation of a combination of binahong leaf extract and aloe vera leaf:

Table 2. The formulation for making liquid preparations from binahong leaf extract and aloe vera leaf.

Material Name	Formula			
	A	B	C	D
Extract (ml) (Binahong : Aloe vera)	1:1	1:2	2:1	1,5:1
Alcohol (ml) (v/v total) (Ethanol 70%)	1:4	1:4	1:4	1:4

2. Stir the mixture until it is homogeneous
3. Then pack it in bottles.

D. Result Analysis

The liquid preparations made were analyzed physically, chemically and their effectiveness. Physically, the organoleptic test is carried out. Chemically, the pH test, flavonoid content test and saponin test were carried out.

1. Physical properties test (organoleptic test)

Organoleptic is assessing and observing the texture, color, shape, aroma, taste of a food, drink, or medicine (Nasiru, 2014).

2. Chemical properties testing

a. pH test

The pH test was carried out with digital pH. This pH measurement aims to determine whether the cream that has been made is acidic or alkaline. Skin preparations should have a pH that is approximately the same as the pH of the skin so it is not easy to irritate the skin, which is between 5-7 (Solanum, 2015).

b. Flavonoid Quantitative Analysis Test

1. Determination of Maximum Wavelength

A standard curve was made by connecting the concentration of a standard solution of quercetin with the absorption results obtained from measurements using a UV-VIS spectrophotometer at a wavelength of 437 nm.

2. Quercetin Standard Curve Creation

- Weighed 25 mg of standard quercetin and dissolved in 25 ml of 95% ethanol. 100 ppm quercetin mother liquor was prepared.
- Prepared standard solution of quercetin concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.
- From each standard solution of quercetin, 0.5 ml was taken and 0.1 ml of 10% AlCl₃ and 0.1 ml of 1 M potassium acetate were added.
- Standard incubated for 30 minutes at room temperature.
- Absorbance was measured by UV-Vis spectrophotometry at a maximum absorption wavelength of 437 nm.

3. Determination of Flavonoid Levels Binahong leaf extract and aloe vera leaf.

- Weighed 100 mg of extract, dissolved in 100 ml of 95% ethanol, made a concentration of 1000 ppm.
- From the standard solution, 0.5 ml was taken and then 0.1 ml of 10% AlCl₃ and 0.1 ml of 1M potassium acetate were added.
- Samples were incubated for 30 minutes at room temperature.
- Absorbance was measured by UV-VIS spectrophotometry.

4. Data Analysis

The data obtained were obtained from the absorbance of the quercetin comparison solution. A calibration curve was made and a linear regression equation was obtained. The concentration of the compound is calculated by entering into the linear regression equation $y = ax + b$ with $y =$ absorbance, $x =$ content in ppm (mg/L) (Ristanti A., 2019).

c. Saponin content test

- Preparation of Test Solution

The binahong leaf extract was weighed as much as 0.5 grams, then dissolved in 10 ml of 95% ethanol in a 100 ml volumetric flask until completely dissolved, then added with distilled water up to 100 ml.

- Saponin Compound Test Ten drops of the test solution were put into a test tube and 5 drops of hot water were added, shaken for 15 seconds until the foam was formed. Then added 1 drop of 2N iodide acid. If the foam does not disappear, it indicates the presence of saponins (Helmidanora et al., 2020).

RESULTS AND DISCUSSION

The process of making external wound liquid medicine from extracts of binahong leaves and aloe vera using 1830.5 gram of fresh binahong leaves resulted from the extraction of 494 ml. 800 g of fresh aloe vera leaves obtained a filtrate of 400 ml. Binahong leaf extract and aloe vera are then mixed according to the specified formula. The extract mixture obtained is still subject to oxidation, therefore alcohol is added. The results obtained comparison (alcohol: a mixture of binahong leaf extract and aloe vera leaf) 1:4 did not change color. This proves that the addition of alcohol helps slow down the oxidation process. This comparison is used for each formula used, by changing the ratio of the volume of the binahong leaf extract and aloe vera leaf extract. The formulation used is the ratio (binahong leaf extract: aloe vera leaf) = 1:1 as formula A; 1:2 as formula B; 2:1 as formula C; 1.5:1 as formula D. The preparation obtained is liquid so that it is easier to apply. You can use a spray or a bottle and use it like betadine in general on external wounds.

From hypothesis testing, $F = 0.611$. At = 0.05 (5%) with dk = 6; 7 so that $F_{0.05} = 3.87$ from the results of research conducted there are no variables that affect the manufacture of binahong leaf extract. $F < F_{\alpha} \rightarrow H_0$ is accepted, indicating that the extraction temperature does not affect the extraction of binahong leaves. The results obtained, the extraction of binahong leaves was carried out between temperatures (80 – 90)°C and 15 minutes. This is supported by the results of chemical tests which showed the presence of flavonoids and saponins with the highest flavonoid content in formula C and the study of external wound healing gels conducted by Aprilianingsih 2018 on mice with the same composition as having the most effective healing compared to other formulas and positive control in the form of betadine. From the three tests, it was found that formula C had the best results as a liquid medicine formula for external wound healing from binahong leaf extract and aloe vera leaf, namely Formula C with a composition of 536 mL Binahong leaf extract, 268 mL aloe vera leaf extract and 70% alcohol 201 mL with a total one formula 1005 ml.

Substances that play an active role in external wound healing liquid drugs are flavonoids and saponins. Wound healing liquid medicine that has been made, needs to be tested several times to prove its quality. The tests carried out include tests of chemical properties, tests of physical properties, and biological tests. The physical test consists of the organoleptic test. Chemical tests are in the form of pH tests, quantitative tests for flavonoid content, qualitative tests for saponin content while biological tests are in the form of microbial contamination based on the Regulation of the Agency for Drug and Food Control Number 32 of 2019.

Physical properties test

Organoleptic observations of liquid preparations on formulas A, B, C, D were carried out for 2 weeks. An organoleptic test is intended to see the physical appearance of preparation including shape, aroma, and color. The results of organoleptic observations of wound healing liquid drugs from binahong leaf extract and aloe vera leave-in formulas A, B, C, D are the same. The first is color, where changes in the color of the preparation can be affected by various treatments such as processing and cooking, where the presence of a colorant in the preparation is sensitive to heat and the observation is dark green blackish, the aroma of a product can be produced from the base material itself. namely the distinctive smell of binahong leaves and aloe vera with a texture that can be observed from a physical point of view and the form of a product in these formula preparations is liquid.

Chemical properties test

1. pH test

The pH test on the preparation plays an important role because it is related to the safety level of use on the skin, the pH of the liquid preparation is measured using a digital pH. The result of pH formula A 6.9; formula B 7.1; formulas C and D are the same, namely 7.0. Skin preparations should have a pH that is approximately the same as the skin's pH, so it is not easy to irritate the skin, which is between 5-7 (Solanum, 2015). In this study, the pH in formulas A, C, and D met the normal pH of the skin, namely 6.9 and 7.

2. Test the flavonoid content

Flavonoids from binahong leaf extract have pharmacological activities as an anti-inflammatory, analgesic, and antioxidant (Mardiana, 2013). Binahong contains flavonoids that can accelerate wound healing. The following is a picture and table of results from testing the flavonoid content in liquid medicine for external wound healing from binahong leaf extract and aloe vera leaf.

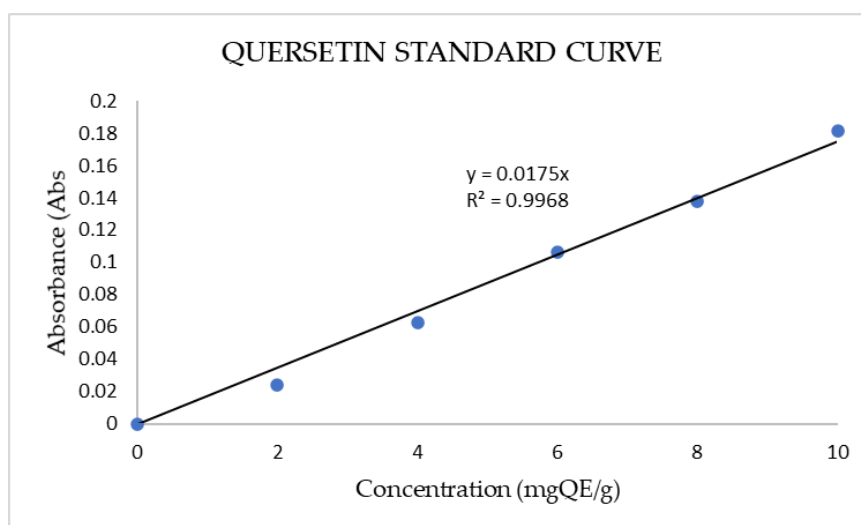


Figure 1. Graph of the quercetin standard curve

Table 3. Flavonoid Content Test Results

Extract Volume Comparison (Binahong: Aloe Vera)	Flavonoid Content (mgQE/g)	Flavonoid Content (%)
Formula A (1 : 1)	12,744	1,2744
Formula B (1 : 2)	8,821	0,8821
Formula C (2 : 1)	18,467	1,8467
Formula D (1,5 : 1)	14,472	1,4472

The largest flavonoid content in formula C where the composition of binahong leaf extract: aloe vera leaf (2:1) was found to be 1.8467% at 2000 grams of the wet weight of binahong leaves and 1000 grams of aloe vera leaf weight. In-gel preparations (Aprilianingsih, 2018), formula B heals wounds faster than other formulas. Formula Breached 100% healing percentage on day 7, while formula A, C, D the percentage of wound healing had not reached 100% with positive control using betadine producing 40% wound healing percentage and negative control without treatment in mice. This is because formula B contains more binahong extract. Binahong contains flavonoids that can accelerate wound healing. With the same comparison of the composition of binahong leaf extract and aloe vera leaf on wound healing in mice, namely formula C in this manufacture. So that it can be used as a position where the test results are in formula C as a wound liquid medicine that is effective in healing external wounds.

3. Saponin content test

Saponins are surface-active compounds that foam when shaken in water and at low concentrations often cause hemolysis of red blood cells. Some saponins work as anti-

microbial, saponins have the ability as a cleanser so it is effective for open wounds. Saponins are soluble in water and ethanol but insoluble in ether (Robinson, 1995). In testing the saponin content of the nutrition Laboratory at Airlangga University, it was found that formulas A, B, C, D showed positive saponin results so that the external wound liquid medicine contained saponins and the role of saponins was able to clean open wounds and effectively help the external wound healing process.

Biological Properties Test

Biological tests on the external wound healing liquid drug formula from binahong leaf extract and aloe vera leaf were tested for microbial contamination based on the Regulation of the Agency for Drug and Food Control Number 32 of 2019 at the Health Laboratory of the Sukoharjo Health Office.

Table 4. Formula test results based on the Regulation of the Agency for Drug and Food Control Number 32 of 2019 on microbial contamination

Category	Condition	Formula			
		A	B	C	D
Total Plate Number (ALT)	$\leq 2 \times 10^2$ colony/mL	3×10^1	0	1×10^1	0
Yeast Mold Number (AKK)	$\leq 2 \times 10$ colony/mL	0	0	0	0
<i>Staphylococcus aureus</i>	Negative /mL	Negative	Negative	Negative	Negative
<i>Pseudomonas aeruginosa</i>	Negative /mL	Negative	Negative	Negative	Negative

It can be seen from the test results that all formulas are included in the requirements for liquid medicine for wounds based on the Regulation of the Agency for Drug and Food Control Number 32 of 2019 on microbial contamination so that they are safe for consumption.

CONCLUSION

Obtaining external wound healing liquid medicine from binahong leaf extract and aloe vera leaf by extracting binahong leaves using the infusion method. The results obtained, the extraction of binahong leaves was carried out between temperatures (80 – 90) oC and 15 minutes. $F < F_{\alpha} \rightarrow H_0$ is accepted, indicating that the extraction temperature does not affect the extraction of binahong leaves. Then make 4 formulas, namely the ratio (binahong leaf extract: aloe vera leaf) = 1:1 as formula A; 1:2 as formula B; 2:1 as formula C; 1.5:1 as formula D. The four liquid drug formulas each contain

saponins and flavonoid levels in formula A of 1.2744%, 0.8821% of formula B, 1.8467% of formula C, 1.4472% formula D. So that formula C is most effective in healing external wounds with a composition of 536 mL Binahong leaf extract, 268 mL aloe vera leaf extract and 201 mL 70% alcohol with a total of 1005 ml formula. Judging from the results of microbial contamination, the Total Plate Number (ALT) of formula A was found to be 3×10^1 colonies/mL and formula C 1×10^1 colonies/mL, formulas B and D showed 0 colonies/mL. The Yeast Mold Number (AKK) formula A, B, C, D shows the number 0 colonies/mL. the four formulas on the contamination of *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed negative results. So that the four formulas meet the requirements of BPOM number 32 of 2019.

SUGGESTION

Further research is needed on formulation testing. It can be in terms of biological testing, testing of healing effects, and phytochemicals. Then in the organoleptic test, it is necessary to add respondents. Further research is needed for liquid preparations for external wounds of binahong leaf extract and aloe vera leaf with other bases.

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