

Formulation And Activity Test Of Acne Spot Gel Binahong Leaf Extract (*Anredera Cordifolia* (Ten.) Against *Propionibacterium Acnes*

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Abstract

Acne is the most common skin disease and is characterized by small spots such as blackheads to large spots filled with pus on the pilosebaceous (hair follicles, hair base and sebaceous glands). Acne is a health problem that is quite serious and disturbing for some people. One of the plants that is efficacious as an acne medicine is Binahong leaves (*Anredera cordifolia* (Ten.)). Binahong leaf extract (*Anredera cordifolia* (Ten.)) contains flavonoid and saponin compounds which are formulated as active substances in this acne spot gel preparation. This research the aim was to determine the physical properties of the preparation and the antibacterial activity of acne spot gel from Binahong leaf extract (*Anredera cordifolia* (Ten.)) against *Propionibacterium acnes* bacteria using the disc diffusion method. This research used an experimental method using binahong leaf extract with concentrations of 30%, 35% and 40%. Then a physical stability test was carried out with test parameters including organoleptic, homogeneity, pH, spreadability, stickiness, irritation and hedonic. The results of the research based on the physical stability test showed that organoleptically, homogeneity, pH, spreadability, stickiness, irritation and hedonic in all 4 formulas met the requirements. Based on the acne spot gel antibacterial test against *Propionibacterium acnes*, the results showed that the concentration of binahong leaf extract had an effect on inhibiting the growth of *Propionibacterium acnes* bacteria with an inhibitory power in formula 1 14.4mm, formula 2 14.4mm, and 16.8mm for formula 3 with a strong inhibition zone category. Then, for the statistical analysis results, a value of $p < 0.05$ was obtained, namely 0.001, which indicates that the results are significant to see whether there is an effect of concentration on the Acne spot gel (*Anredera cordifolia* (Ten.)) preparation and the comparison on the *Propionibacterium acnes*.

Keywords: Acne spot; binahong leaves; *propionibacterium acnes*

1. Introduction

Acne (*Acne vulgaris*) is a common condition in both women and men and is often present during puberty or adolescence. The development from adolescence to early adulthood is a transition period with various problems such as hormonal, physical, psychological and social changes. These changes create health problems, including acne [1]. Acne usually occurs on the skin of the face, neck, chest and back.

Untreated acne can result in scarring that is difficult to treat in the long term and has negative physical and psychosocial effects [2]. The cause of acne is still unknown because acne is a multifactorial inflammatory disease and its pathogenesis is still evolving [3]. Acne is caused by four pathogenic factors namely increased sebum production, hyperkeratinization, *Propionibacterium acnes* bacteria and inflammatory response.

There are various types of acne medications on the market. There are creams, gels, masks, tablets, and more. The application of acne creams sometimes leaves unpleasant marks on the skin. Gel preparations are used because they are one of the dosage forms that are easy to apply, easy to dry, give a cool feeling, easy to clean and oil-free. The application of gel preparations on acne is very easy to do by applying it to the skin of the face with acne then left to dry and rinsed using clean water.

One plant that is empirically effective in treating acne is binahong leaves (*Anredera cordifolia* (Ten.)). This plant grows in the shade and produces vines. [2] states that binahong is empirically able to

treat various diseases. Binahong contains alkaloid, saponin, flavonoid and polyphenol compounds. Saponin compounds have antimicrobial properties.

Based on the background that has been described, the author is interested in testing the activity of binahong leaf extract (*Anredera cordifolia* (Ten.) in the form of acne spot gel preparations against *Propionibacterium acnes*.

2. Methodology

This research was conducted in September to October 2023 and was carried out at the Pharmacy Laboratory, Faculty of Health Sciences, University of Muhammadiyah Palopo.

Research Variables

Independent variable: variation in the concentration of binahong leaf extract as the active substance in the formulation of acne spot gel preparation which is monitored from the amount of % (30%, 35%, 40%) of binahong leaf extract in the acne spot gel preparation.

Dependent variable: characteristics of the acne spot formulation from ethanol extract as anti-acne which includes organoleptic test, homogeneity, PH, spreadability, adhesiveness, antibacterial activity test of the most stable preparation, irritation test, and hedonic test.

Tools and materials

The tools used were analytical scales, digital scales, blenders, stirring rods, beaker glass, drop pipettes, porcelain cups, erlenmeyer, ruler, paper discs, ose needles, parchment paper, sterile tweezers, gel pots, mortar and pestle, oven, hot plate, autoclave, parchment paper, pH universal, petri dish, watch glass, aluminum foil, plastic wrab, spritus, glass jar, vortex mixer, magnetic stirrer, wooden rattles, test tube, horn spoon, spatula, viscometer, deck glass, spreader, and spoit. While the materials used were Binahong Leaf Extract (*Anredera cordifolia* (Ten.), distilled water, 70% ethanol, propylene glycol, methylparaben, carbomer, TEA, NA (Nutrient Agar) media, *Propionibacterium acnes* bacterial isolate, and 0.9% NaCl.

Sample Processing of Binahong Leaf (*Anredera cordifolia* (Ten.)

The simplisia used is binahong leaf (*Anredera cordifolia* (Ten.). The criteria for binahong leaf taken are fresh and the leaves used are young leaves, the selection of young leaves makes it easier for the leaf crushing process because it is easier to crush than older leaves, then picking and then washing with water to separate dirt from the leaves. Then cut into small pieces, then dried the binahong leaf sample by aerating. After drying, then the sample is mashed using a blender. After that, the smooth binahong leaf sample is ready to be extracted.

Preparation of Binahong Leaf Extract (*Anredera cordifolia* (Ten.)

Preparation of the extract begins with working on binahong leaf powder (*Anredera cordifolia* (Ten.) as much as 500grams put into a maceration vessel and added 70% ethanol until submerged and then stirred until homogeneous, closed immediately then stored in a room that is protected from sunlight for 3 days. During soaking every day stirring is done for 15 minutes [4]. Then filtered using a thin white cloth to separate the filtrate and residue. The filtrate obtained still contains a lot of solvent so it must be concentrated and dried using a fan, the result of this concentration is called a thick extract.

Preparation of Acne Spot

Binahong leaf extract gel is made using carbomer 940 base with various extract concentrations of 30%, 35% and 40%. Put Carbomer 940 into the mortar then add the heated distilled water, then stir until a gel base is formed. TEA is added to the base and mixed until homogeneous. Dissolve methylparaben and propylene glycol in a porcelain cup. Add the remaining distilled water to the gel base then stir until homogeneous, and add binahong leaf extract according to the reference formula.

Preparation of Positive Control and Negative Control

The preparation of the control solution is by using a marketed acne spot (brand X), while the negative control uses the preparation base.

Evaluation of Acne spot Preparation

Organooleptic Test

Organooleptic testing is done visually and seen directly in shape, color, and scent.

pH test

pH test is carried out using a universal pH device that is inserted into the sample. The pH value of the preparation that meets the skin pH criteria and does not irritate the skin is around 4,5 to 6,5.

Homogeneity Test

The homogeneity test is carried out by applying the gel to two pieces of glass or other suitable transparent material. The gel needs to be homogeneous and without coarse grains. If there are no coarse grains then the test preparation is declared homogeneous.

Spreadability Test

Stick the millimeter paper on the petri dish, then 0,5 gram of gel placed on a petri dish. The initial measurement of the diameter of the gel distribution was carried out using a petri dish without a weight, after which a load of 50 grams was added every 1 minute until the total weight reached 200 grams. Then note the diameter of the gel spread. The spreadability of a good preparation is 5-7 cm or 5,54-6,08 cm (based on SNI standards). Preparations that are difficult to spread or spread too much will reduce the level of comfort of use and efficiency of use of the preparation, as a result preparations that are too dilute can cause their adhesive power to decrease and therefore the contact time of the active substance with the application site can also be reduced [5].

Adhesion Test

The adhesion test was carried out by placing 0,5 gram of gel on a glass object then covered with another glass object, and given a load of 1 kg for 3 minutes. Determination of adhesion strength is the time required for the two glass objects to separate. The adhesion requirement is more than 1 second [5].

Viscosity Test

Viscosity measurements are carried out by placing the sample in a viscometer until the spindle is submerged and then measuring the viscosity using a Brookfield viscometer using spindle no. 64 with a speed of 50 rpm [6].

Irritation Test

A total of 14 people expressed personal responses to the binahong leaf acne spot gel, had no previous skin problems, and were willing to participate in the study. The irritation test was conducted by applying the preparation on the forearm with a diameter of 2 cm, left exposed for 2 hours and

observed what happened. A positive irritation reaction is characterized by redness, itching, or swelling on the skin of the treated panelist's forearm [5].

Hedonic Test

A total of 14 people expressed personal responses to the binahong leaf acne spot gel, had no previous skin problems, and were willing to participate in the study. To measure liking or disliking of binahong leaf acne spot gel, a questionnaire in the form of a googleform was used to represent feelings of dislike, dislike, like and really like. Observed in the binahong leaf acne spot gel were shape, color and scent.

Tool Sterilization

All tools to be used were sterilized first. Tools are sterilized in an oven at 170 ° C during $\pm$ 2 hours, use needles and tweezers are burned by burning over direct heat and the media is sterilized in an autoclave at 121 ° C for 15 minutes.

Bacteria Rejuvenation

Propionibacterium acnes was taken from 1 sterile loop needle and then inserted into the slanted Nutrient Agar (NA) medium by scraping. Next, it was incubated in an incubator at 37°C during 24 hours.

Zone of Inhibition Measurement

Category of inhibition zone for *Propionibacterium acnes* in the literature (Mila., 2019), which states that weak inhibition occurs if the diameter of the antibacterial inhibition zone is >5 mm, strong inhibition if the diameter of the antibacterial inhibition zone ranges from 10-20 mm, moderate resistance if 5- 10 mm and the barrier is very strong if the diameter of the antibacterial barrier zone is >20-30 mm.

3. Result and Discussion

3.1. Result

Table 1. Results of Extract Yield Determination of *Anredera cordifolia* (Ten.)

No.	Extraction Method	Weight of simplicity used	Weight of extract obtained	Yield	Evidence
1.	Maceration	500 gr	169,8 gr	33,96%	Fulfil

Table 2. Results of Determination of Drying Loss and Water Content

No.	Simplicia standard parameters	Check up result (%)	Requirement (%)	Evidence
1.	Drying Shrinkage	7%	<10%	Fulfil
2.	Water Content	4%	<10%	Fulfil

Table 3. Organoleptic Test Results of Spray Deodorant

Formulation	Testing	Length of Observation (Week)			
		1	2	3	4
F0	Shape	Gel	Gel	Gel	Gel
	Color	Brown	Brown	Brown	Brown
	Scent	Greentea	Greentea	Greentea	Greentea
F1	Shape	Gel	Gel	Gel	Gel
	Color	Brown	Brown	Brown	Brown
	Scent	Greentea	Greentea	Greentea	Greentea

F2	Shape	Gel	Gel	Gel	Gel
	Color	Brown	Brown	Brown	Brown
	Scent	Typical Binahong	Typical Binahong	Typical Binahong	Typical Binahong
F3	Shape	Gel	Gel	Gel	Gel
	Color	Brown	Brown	Brown	Brown
	Scent	Typical Binahong	Typical Binahong	Typical Binahong	Typical Binahong

Description: F0 = Acne spot gel base with 1.5% carbopol concentration

F1 = Acne spot gel with an extract concentration of 30% carbopol 1%

F2 = Acne spot gel with an extract concentration of 35% carbopol 1%

F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%

Table 4. Homogeneity Test Results

Formulation	Length of Observation (Week)				Range
	1	2	3	4	
F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	A preparation that has good homogeneity must show a homogeneous composition and no visible coarse grains (Robert et al., 2023)
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous	
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	

Description: F0 = Acne spot gel base with 1.5% carbopol concentration

F1 = Acne spot gel with an extract concentration of 30% carbopol 1%

F2 = Acne spot gel with an extract concentration of 35% carbopol 1%

F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%

Table 5. pH Test Results

Length of Observation (Week)	Formulation				Range
	F0	F1	F2	F3	
1	5	5	5	5	pH requirement for a good gel preparation is that it matches the pH of the skin, namely between 4.5-6.5 (Wulandari et al., 2019).
2	5	5	5	5	
3	5	5	5	5	
4	5	5	5	5	
Average	5	5	5	5	

Description: F0 = Acne spot gel base with 1.5% carbopol concentration

F1 = Acne spot gel with an extract concentration of 30% carbopol 1%

F2 = Acne spot gel with an extract concentration of 35% carbopol 1%

F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%

Table 6. Viscosity Test ResultS

Length of Observation (Week)	Formulation				Range
	50 RPM				
	F0	F1	F2	F3	
1	9932 cP	2772 cP	2916 cP	3480 cP	The requirement for the viscosity of gel preparations is 500-10,000 cP (Rahmatullah et al., 2020).
2	9744 cP	2604 cP	2580 cP	2904 cP	
3	9636 cP	2424 cP	2436 cP	2628 cP	
4	9552 cP	2232 cP	2256 cP	1992 cP	
Average	9716 cP	2508 cP	2547 cP	2751 cP	

Description: F0 = Acne spot gel base with 1.5% carbopol concentration
F1 = Acne spot gel with an extract concentration of 30% carbopol 1%
F2 = Acne spot gel with an extract concentration of 35% carbopol 1%
F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%
RPM = Revolution per minute
cP = Centipoise

Table 7. Spreadability Test Results

Load	Length of Observation (Week)	Formulation				Range
		Second				
		F0	F1	F2	F3	
1kg	1	5	5	4	5	The adhesion requirement is more than 1 second [5].
	2	4	4	2	4	
	3	3	2	2	4	
	4	3	2	2	4	
	Average	3,75	3,25	2,25	4,25	

Description: F0 = Acne spot gel base with 1.5% carbopol concentration
F1 = Acne spot gel with an extract concentration of 30% carbopol 1%
F2 = Acne spot gel with an extract concentration of 35% carbopol 1%
F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%

Table 8. Adhesion Test Results

Load	Length of Observation (Week)	Formulation				Range
		Second				
		F0	F1	F2	F3	
1kg	1	5	5	4	5	The adhesion requirement is more than 1 second [5].
	2	4	4	2	4	
	3	3	2	2	4	
	4	3	2	2	4	
	Average	3,75	3,25	2,25	4,25	

Description: F0 = Acne spot gel base with 1.5% carbopol concentration
F1 = Acne spot gel with an extract concentration of 30% carbopol 1%
F2 = Acne spot gel with an extract concentration of 35% carbopol 1%
F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%

Table 9. Irritation Test Results

Formula		Sukarelawan													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Reddish skin	F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Itchy skin	F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Swollen skin	F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Description: + = Irritation occurs

- = No irritation occurs

Table 10. Hedonic Test Results (Favorability)

Formula	Indikator	SS	S	KS	TS
F0	Warna	4	10	-	-
	Aroma	2	12	-	-
	Bentuk	3	11	-	-
F1	Warna	2	9	3	-
	Aroma	1	12	1	-
	Bentuk	3	11	-	-
F2	Warna	3	9	2	-
	Aroma	1	11	2	-
	Bentuk	4	10	-	-
F3	Warna	3	6	5	-
	Aroma	-	9	5	-
	Bentuk	2	10	2	-

Description: SS = Really like

S = Like

KS = Less like

TS = Don't like

Table 11. Antibacterial Activity Test Results of Acne Spot Binahong Leaf (*Anredera cordifolia* (Ten.)

Formulation	Replication			Average	Evidence
	1	2	3		
F0	0 mm	0 mm	0 mm	0 mm	Does not provide activity
F1	13,75 mm	13 mm	16,5 mm	14,4 mm	Strong
F2	12,75 mm	11,5 mm	19 mm	14,4 mm	Strong

F3	15,25 mm	11 mm	24,25 mm	16,8 mm	Strong
K+	12,5 mm	12,25 mm	13,25 mm	12,6 mm	Strong

Description: F0 = Acne spot gel base with 1.5% carbopol concentration
F1 = Acne spot gel with an extract concentration of 30% carbopol 1%
F2 = Acne spot gel with an extract concentration of 35% carbopol 1%
F3 = Acne spot gel with an extract concentration of 40% carbopol 1.5%
K+ = Acne spot gel brand X on the market

Table 12. ANOVA Test Results

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between groups	536.792	4	143.198	10.231	0,001
Within groups	131.167	10	13.117		
Total	667.958	14			

3.2. Discussion

The initial stage of this research was collecting raw materials. The raw material used is binahong leaves (*Anredera cordifolia* (Ten.) which have been taken from a garden located in the Palopo City area with the criteria being green, young and fresh, with collection time in the morning. Next, simplicia is made. First -Firstly, the binahong leaves that have been taken are sorted wet to separate foreign materials and other impurities from the simplicia material. Next, they are washed using running water, then drained, after that the chopping process is carried out and then the drying process is carried out by letting it air for 5 days. Then Dry sorting is carried out to separate dirt that is still attached to the simplicia [7]. Next, the simplicia is powdered using a blender to make pollination easier.

Drying loss is a non-specific parameter which aims to provide a maximum limit (range) regarding the amount of compounds lost in the drying process. The drying shrinkage parameter is basically a measurement of the remaining substance after drying at a temperature of 105°C to constant weight, which is expressed as a percent value [8]. Water content is used to determine the water residue after the drying process. This determination was carried out using the gravimetric method. The results obtained can be seen in table 2.

In determining the drying loss parameters of simplicia binahong leaf extract, a drying loss value of 7% was obtained. This is in accordance with the requirements for drying losses of less than 10%, because drying losses also represent the evaporated water content. The results of determining the water content are 4%, this is in accordance with the requirements for water content, namely no more than 10% (Depkes RI., 2017).

The yield determination was carried out to determine the comparison between the weight of the extract obtained and the weight after the extraction process. The results obtained from the yield of binahong leaf extract (*Anredera cordifolia* (Ten.) using the maceration method were 33.96%. This is in accordance with the requirements for the yield of thick extract, namely not less than 10% [9]. According to [3] that a high yield value indicates a large number of bioactive components contained in it, so that if the yield number is higher, the number of active compounds contained in the sample will also increase. As has been

reported by [5] that the high number of active compounds contained found in a sample is indicated by the high amount of yield obtained from binahong leaves (*Anredera cordifolia* (Ten.).

As for the results of the preparation testing carried out in organoleptic testing, observation results were obtained during 4 weeks of storage which showed that the acne spot gel without or with the addition of 30%, 35% and 40% binahong leaf extract did not experience changes in shape, color and odor. This is in accordance with the requirements which prove that the acne spot gel preparation in the formula is organoleptically stable within a storage period of 4 weeks.

Homogeneity testing is a test of the mixing of ingredients in the acne spot gel preparation which shows a homogeneous composition [10]. Tests were carried out on gel base and gel with concentrations of 30%, 35% and 40%. All acne spot gel formulas show a homogeneous composition which is characterized by the absence of coarse granules. This is in accordance with the homogeneity requirements which proves that the acne spot gel preparation in each formula is homogeneously stable within a storage period of 4 weeks.

pH testing is carried out to see the acidity level of the preparation so that the acne spot gel preparation does not cause irritation to the skin. The suitability of the skin's pH to the pH of the topical preparation influences the skin's acceptance of the preparation. The results of the pH test on each formula in this study showed that there was no change each week, namely week 1 was 5 as well as weeks 2, 3 and 4. This is in accordance with the pH requirements for the skin, namely 4.5- 6.5.

Viscosity testing is carried out to determine the viscosity of the acne spot gel. The results of the viscosity test before and after storage for 4 weeks decreased every week. In F1 with 1% carbopol basis, there was a decrease in the 1st week to 2772 cP, 2604 cP in the 2nd week, 2424 cP in the 3rd week and 2232 cP in the 4th week. Meanwhile, in F2 with 1% carbopol basis, there was a decrease in the 1st week to 2916 cP, 2580 cP in the 2nd week, 2436 cP in the 3rd week and 2256 cP in the 4th week. Then in F3 with 1.5% carbopol basis there was a decrease in the 1st week to 3480 cP, 2904 in the 2nd week, 2628 cP in the 3rd week and 1992 cP in the 4th week. The results for F0 on a 1.5% carbopol basis also showed a decrease in the 1st week of 9932 cP, the 2nd week 9744 cP, the 3rd week 9696, and 9554 cP in the 4th week. The viscosity of the gel changes due to the length of storage and also changes in temperature. The results of this viscosity test are in accordance with the requirements for gel viscosity preparations, namely 500-10,000 cP.

Testing the spreadability of the preparation is carried out to determine the amount of force required for the gel to spread on the skin or to determine the ability to spread the gel preparation when applied to the skin. The results of the spreading power test showed that there was a decrease in the spreading power in F1, F2 and F3, while there was no change in F0. In F0, the spreadability results in the 1st week were 4.5cm as well as in the 2nd, 3rd and 4th weeks. Meanwhile, in F1 the results were 4.9 cm in the 1st week, 4.6 cm in the 2nd week, 3rd week 4 cm, and 4th week 4cm. For F2, the results were 4.6 cm in the 1st week, 4.6 cm in the 2nd week, 4.6cm in the 3rd week, and 4.2 cm in the 4th week. As for F3, there

was also a decrease, namely in the 1st week the results were 5.2 cm, in the 2nd week 5.1 cm, in the 3rd week 5.1 cm, and 5.1 cm in the 4th week. Results from F0, F1 and F2 decreased, this was due to oxidation factors during storage of the preparation. Meanwhile, F3 can be said to be stable because it is still within the spreading power range, this is in accordance with the spreading power requirements, namely 5-7 cm [5].

Gel adhesion testing is carried out to determine the bond between the gel and the skin. The higher the adhesion of the gel indicates the stronger the bond between the gel and the skin, thus allowing higher drug absorption by the skin [11]. In this adhesion test, the results obtained from F0 in the 1st week were 5 seconds, 4 seconds in the 2nd week, 3 seconds in the 3rd week, and 3 seconds in the 4th week. Meanwhile, in the 1st week of F1 the results were 5 seconds, in the 2nd week 4 seconds, in the 3rd week 2 seconds, and 2 seconds in the 4th week. Then the results of F3 in the 1st week were 5 seconds, 4 seconds in the 2nd week, 4 seconds in the 3rd week, and 4 seconds in the 4th week. In this test, each formula has an adhesive power that tends to decrease over time. According to [3], the adhesive power of the acne spot gel preparation decreases due to oxidation of the preparation components, resulting in a decrease in adhesion. This change is still within the range of adhesion parameters so that all preparations can be said to be stable. This is in accordance with the adhesion requirement, namely more than 1 second [5].

Irritation testing is carried out to determine the irritating effect of acne preparations spot gel of binahong leaves (*Anredera cordifolia* (Ten.) after being used on the skin, so that the safety level of the preparation can be known. This irritation test aims to prevent side effects on the skin. The results obtained for acne preparations Binahong leaf extract spot gel with concentrations of 30%, 35% and 40% did not show any side effects in the form of skin redness, itching and swelling caused by the preparation. From the results of the irri

tation test it can be concluded that the acne spot gel preparation made safe to use on skin.

Hedonic testing was carried out to measure the respondent's level of preference for the preparation. The results of the hedonic test on the acne spot gel preparation of binahong leaf extract (*Anredera cordifolia* (Ten.) include the parameters of shape, color and aroma. There are 4 determination scales, namely very like, like, dislike and dislike. The hedonic test involved 14 respondents using the Slovin formula using an error of 20%. This is because the population used is small. In the hedonic test respondents preferred colors in F0 compared to F1, F2 and F3. For aroma respondents preferred F1 and F0 whereas in terms of form, respondents prefer F1 and F0.

In this study, the antibacterial activity of the acne spot gel formula of binahong leaf extract (*Anredera cordifolia* (Ten.) was tested using the disc diffusion method. In this study, the acne spot gel formula brand X was used as a positive control, while the acne spot gel formula without extract was used as a negative control to clarify whether the basic ingredients of the formula do not have antibacterial activity, but if the antibacterial activity comes from binahong leaf extract which has been proven to be able to inhibit bacterial growth.

The results of the average diameter of the inhibition zone for the acne spot gel

preparation in table 4.9 which uses binahong (*Anredera cordifolia* (Ten.)) leaf extract against *P. acnes* bacteria are as follows, the negative control (F0) without the extract does not cause obstacles with values an average of 0 mm is categorized as a weak barrier, F1 has an average zone diameter resistance of 14.4 mm which is classified as a strong obstacle, F2 has an average resistance zone diameter of 14.4 mm which is also classified as a strong obstacle, F3 has an average resistance zone diameter of 16.8 mm which is categorized as a very strong obstacle. strong, and the positive control (acne spot gel These results are in accordance with the category of inhibition zone for *P. Acnes* bacteria in the literature, which states that weak inhibition occurs if the diameter of the antibacterial inhibition zone is >5 mm, strong inhibition if the diameter of the antibacterial inhibition zone ranges from 10-20 mm, moderate inhibition if 5-10 mm and very strong resistance if the diameter of the antibacterial resistance zone is $>20-30$ mm.

Based on the results of the antibacterial activity test in the table above, it can be seen that the acne spot gel preparations F1, F2, and F3 all have antibacterial activity, while F0 (without extract) which is the negative control in this study does not provide inhibition zone activity. This indicates that the basic ingredients of acne spot gel do not have antibacterial activity.

In this research, the data analysis used was a One Way Anova variant test. The One Way Anova test aims to find out whether there is a difference in the average of the test samples. Before carrying out calculations using One Way Anova, one of the mandatory requirements needs to be fulfilled, namely a normality test on the existing data, followed by a homogeneity test. The data normality test is carried out with the aim of measuring whether the data used has a normal distribution or not. Meanwhile, the data homogeneity test aims to test the similarity (homogeneity) of several samples, namely whether the variations in samples taken from the same population are uniform or not.

The antibacterial activity test using the positive control acne spot gel brand X with antibacterial claims showed an average inhibition zone of 12.2mm. Furthermore, it was proven by the results of statistical analysis using SPSS that a significant value for the normality test was $p=0.463$, so it could be said that it was normally distributed because the sig value was ($p>0.05$). The negative control used in this study was acne spot gel F0 (without extract). The inhibition zone in brand X acnes spot gel is in the strong category. This is in accordance with the mechanism of action of *Centella asiatica* as an active ingredient which functions as an antibacterial by inhibiting the work of bacteria in cell formation and even growth [12]. So it can inhibit bacterial growth and produce an inhibitory zone in the bacterial growth medium. The use of F0 (without extract) as a negative control is to clarify whether the basic ingredients of the formula do not have antibacterial activity, but rather if the antibacterial activity comes from binahong leaf extract which has been proven to be able to inhibit bacterial growth. The results of the antibacterial activity test showed that the negative control (F0) did not produce an inhibition zone with an average obstacle diameter of 0 mm. Therefore, no statistical analysis results using SPSS were found.

Of the three acne spot gel formulas, binahong leaf extract is included in the strong

antibacterial category. It can be seen that F3 with an extract concentration of 40% produces the largest inhibition zone, this can be seen from the average inhibition zone formed, namely 16.8mm. Previously, normality was tested first to ensure the data was normally distributed and the data had to be homogeneous. The data normality test shows that the significant value obtained was $p=0.391$ for F1 with an extract concentration of 30%, $p=0.298$ for F2 with an extract concentration of 35%, and $p=0.610$ for F3 with a concentration of 40% which shows $p > 0.05$ which means the data is normally distributed.

In testing the homogeneity of the data, a significant value of $p=0.015$ was obtained, so it can be said that the data was not normally distributed because the sig value was ($p > 0.05$). Therefore, an alternative test for the average concentration was carried out, namely by carrying out a correction test using Brown-Forsythe.

After carrying out a correction test using Brown-Forsythe, a significant value of $p=0.684$ was obtained, so the data can be declared homogeneous. The results of the normality test are normally distributed and the results of the homogeneity test show that they are homogeneous, so statistical tests can then be carried out using the one-way ANOVA test.

Analysis using One Way Anova shows a significant value of $p=0.001$, where at a value of $p < 0.05$ then H_0 is accepted, so it can be concluded that the hypothesis is proven correct that there is a difference in the effectiveness of the antibacterial power of binahong leaf extract (*Anredera cordifolia* (Ten.) One test function Way Anova is to differentiate the average between concentrations from an experiment that has samples from more than 2 groups.

Based on the results of post hoc tests, which shows that $p < 0.05$ means the data is significantly different or significantly different from other concentrations marked with an asterisk (*). The post hoc test showed that the diameter of the inhibition zone for *P. acnes* bacteria for KN had a significant difference compared to KP and all formulas. Meanwhile, F1, F2, F3 and KP have significant differences compared to KN. It can be seen that the higher the concentration of the extract used, the larger the barrier zone formed.

From the results of the research carried out, it was found that the best preparation from the physical stability test and antibacterial activity was the preparation in formula 3 with an extract concentration of 40%. These results indicate that binahong leaf extract is able to inhibit the growth of *P. acnes* bacteria, which is one of the causes of acne. Binahong leaf extract contains secondary metabolite compounds, namely saponins and flavonoids. This compound contains active compounds that can be used as antibacterials [13]. The mechanism of action of saponins is by increasing the permeability of cell membranes, so that hemolysis occurs in cells. If saponin interacts with bacterial cells, the bacteria will burst or lyse. Meanwhile, the mechanism of action of flavonoids is as an antibacterial, namely by inhibiting cell membrane function and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form complex compounds with extracellular proteins that can damage bacterial cell membranes, followed by the release of intracellular compounds from the bacteria [14].

4. Conclusion

Based on this study, the acne spot gel preparation of binahong leaf extract (*Anredera cordifolia* (Ten.) which has good physical quality test results is in formula 3 with 40% extract concentration. Antibacterial activity of acne spot gel preparations of binahong (*Anredera cordifolia* (Ten.) that is most effective against the growth of *Propionibacterium acnes* is formula 3 with 40% extract concentration.

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