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Antibacterial Activity Test Of Miana (*Coleus Atropurpureus*) Leaf Extract As Acne Spot Treatment Against Acnes Propionibacterium

Yuyun Sukmawati[1] , Chitra Astari[2*] , Anugrah Umar[3]
[1]Pharmacy, University of Muhammadiyah Palopo, 91959, Indonesia
[2]Pharmacy, University of Muhammadiyah Palopo, 91959, Indonesia
[3]Pharmacy, University of Muhammadiyah Palopo, 91959, Indonesia

Corresponding Author Email : chitraastari@umpalopo.ac.id

Abstrak
Indonesia is a country that has a tropical climate so pathogenic bacteria easily breed in Indonesia. P. acnes is part of the normal flora of human skin, this bacteria is found in the sebaceous follicle area of the skin and can cause acne if it sticks. Acne vulgaris (acne) is a skin disease caused by bacteria that is very common among young people because it can damage their self-confidence. The results of the analysis showed that Miana (*Coleus atropurpureus*) leaves were effective against Propionibacterium acnes bacteria. Antibacterial activity testing was carried out using the agar diffusion method and using paper discs. Made in gel dosage form and subjected to several physical stability tests. From the results of the research carried out, the acne spot treatment preparations with the best physical stability, irritation and hedonic tests were found in F1 and F2 preparations with a concentration of F1 5% and F2 10%, as well as the best antibacterial activity found in F2 with an extract concentration. 10%. In testing the antibacterial activity to inhibit the antibacterial growth of miana (*Coleus atropurpureus*) leaf extract, each formula with an extract concentration of 5%: 27mm, 10%: 29mm, and 20%: 27mm was effective as an antibacterial to inhibit the growth of the Propionibacterium acnes bacteria that causes acne. the diameter of the inhibition zone is in the very strong category (>20 mm).

Keywords: antibacterial; miana leaves (*Coleus atropurpureus*); *Propionibacterium acnes.*

1. Introduction

Indonesia is a country with a tropical climate, so bacteria including pathogenic bacteria easily multiply in Indonesia. Diseases caused by bacteria are still a big problem because they cause various types of diseases such as skin infections, intestinal infections, gastrointestinal infections, and respiratory infections.

Acne vulgaris (acne) is a skin disease caused by bacteria that is very common among young people as it can damage their self-confidence. This skin condition is caused by chronic inflammation of the sebaceous glands. (Wibawa & Winaya, 2019). With the onset of puberty, the frequency of acne vulgaris gradually decreases. However, sometimes it persists into the 30s and beyond. In males, symptoms are more severe but generally resolve more quickly (Wasitaatmadja, 2010). Propionibacterium acnes (P. acnes) has chemokines that attract polymorphonuclear leukocytes to the lumen of comedones. When polymorphonuclear leukocytes engulf P. acnes and release hydrolytic enzymes, they damage the follicular wall and cause rupture, allowing the follicular contents (lipids and keratin) to penetrate the dermis, causing inflammation (Zaenglein, 2008). (Zaenglein, 2008).

Acne vulgaris affects almost everyone, especially young adults, with a prevalence of about 85%. The highest prevalence is 83-85% in females aged 14-17 years and 95-100% in males aged 16-19 years. According to surveys in Southeast Asia, 40-80% of people suffer from acne vulgaris. According to Indonesian Aesthetic Dermatology Research, the number

of cases in Indonesia was 60% in 2006 and 80% in 2007. In 2009, 90% of patients had acne vulgaris (Saragih et al., 2016).. It is said that 80% of adolescents have suffered from this disease and the clinical manifestations are blackheads, papules, pustules, nodules and scarring that damage their appearance. This disease can be influenced by various factors, namely changes in the pattern of keratinization, increased sebum production, formation of free fatty acid fractions, increased numbers of bacteria, increased androgen hormones and psychological factors. It can also be triggered by factors such as age, race, diet, and climate. (Wibawa & Winaya, 2019). Applying acne cream sometimes leaves marks that make the skin feel uncomfortable. Acne blemishes slowly disappear, but the process of covering up blemishes often causes the skin to become dry and flaky. This is the latest acne treatment offered by products in the acne blemish treatment category. Its function is to hide acne scars. Therefore it should be used when acne is normal i.e. not inflamed. Acne spot treatment is actually very gentle on the skin. Unlike using gel or cream type acne medication that causes the skin to peel and dry out. Currently, there are still many people who choose to use traditional cosmetics. Although it is a bit complicated to use, it is safer for skin health. There are many plants around us that can be used for health and beauty. One of the plants from the genus Coleus native to Indonesia contains medicinal compounds, namely miana (Coleus atropurpereus) which has properties to relieve pain and anti-inflammatory, antioxidant, antibacterial and accelerate wound healing. (Tari et al., 2013).

So far, miana leaves that are converted into certain antibacterials are still little utilized. Most people only use miana leaves as medicine and drink it. Therefore, based on the description above, a study was conducted to determine the antibacterial activity of miana leaf extract (Coleus atropurpureus) as a gel to treat acne blemishes, because this innovation is not yet on the market.

2. Methodology

2.1. Type of Research

This research was carried out using experimental methods in a pharmaceutical laboratory, at Palopo Muhammadiyah University.

2.2. Time and Place of Research

This research will be carried out in July – November 2023 andcarried out at the Pharmacy Laboratory, Faculty of Health Sciences, University Muhammadiyah Palopo.

2.3. Tools and Materials

a. Tools

The tools used in this research are analytical scales, blender, stirring rod, sieve, beaker, dropper, porcelain cup, vernier caliper, *paper disc*, ose needle, parchment paper, sterile tweezers, gel pot, mortar and pestle, oven, millimeter paper, universal pH, petri dish, watch glass, tampah, petri dish, *aluminum foil*, glass jar, funnel, *rotary evaporator*, *waterbath*.

b. Materials

The materials used in this study were miana leaf extract (*Coleus atropurpureus*), distilled water, 70% ethanol, propylenglycol, glycerin,

methylparaben, carbomer 940 and TEA (*Triethanolamine*), NA (*Nutrient Agar*) media, *Propionibacterium acnes* bacterial isolate.

2.4. Research Procedures

A brief description of the methods that will be used in this research is as follows:

2.4.1 Analytical Stage

a. Simplisia Preparation

Making miana leaf simplisia (*Coleus atropurpureus*) is by collecting brownish red miana leaves. The criteria for miana leaves taken are that they are fresh and the leaves used are young leaves, the selection of young leaves makes it easier for the leaf crushing process because they are easier to crush than older leaves, and are picked at 09.00-12.00 when the photosynthesis reaction is perfect. Then do wet sorting with the aim of cleaning plants from foreign objects. Next, wash the leaves with clean running water, drain and then weigh the wet weight of 3 kg. Next, chop the miana leaves with a size of 1-2 cm, then dry in the oven at 40-50 ℃ until the simplisia is dry and easy to break. (Lady Yunita Handoyo & Pranoto, 2020).

Next, dry sorting is carried out to separate foreign objects such as unwanted plant parts and other impurities that are still left in the dried simplisia. Weigh the dried simplisia and mix it into powder. Then the simplisia powder is sieved with a sieve to produce smaller simplisia powder particles. This is because the finer powder is easier to extract because the surface area of the Simplisia powder in contact with the liquid is wider. The finished simplisia is stored in a glass container. (Rohmawati, 2008).

b. Preparation Process of Miana Leaf Extract (*Coleus atropurpureus*)

A total of 300 g of miana leaf powder was extracted using the maceration method. Miana leaf powder was soaked in a closed container using 70% ethanol solvent. The ratio between simplisia powder and solvent was 1:10. Maceration was carried out at room temperature for three days with remaceration every 24 hours and periodic stirring. (Khasanah, 2016).

The results of maceration extraction in the form of maserat, filtered using filter paper to separate the maserat with the residue. The filtered macerate is then concentrated using a *rotary* evaporator evaporated on a 40 ℃ *waterbath* for 3 days to remove any remaining solvent that may still be present until a thick extract of miana leaves is obtained. (Khasanah, 2016).

2.4.2 Gel Preparation

The preparation of the gel base was carried out by dissolving Carbomer 940 in a mortar and adding heated distilled water, stirring to form a gel base. Furthermore, TEA (Triethanolamine) was mixed into the gel base little by little, stirring until homogeneous. Methyl paraben, glycerin, and propylenglycol were dissolved into a porcelain cup. The remaining distilled water was added to the base until a gel was formed. After the base is formed, it is weighed with the calculation (base ─ amount of extract). Miana leaf extract was dissolved first in distilled water and added little by little until homogeneous. The preparation that has been formed is put into a glass pot or plastic pot. The gel base to be

made in this study has different concentrations of miana leaf extract, namely 5%, 10% and 20%.

2.4.3 Evaluation of the preparation

Evaluation of gel preparations carried out on physical characteristics includes orgnoleptical test, homogeneity test, pH test, spreadability test and shelf time test.

a. Organoleptical Test

Organoleptical testing is done visually by observing changes that occur including color, odor, and consistency. (Ansel, 1989).

b. Homogeneity Test

The homogeneity test is carried out by applying the gel on two pieces of glass or other suitable transparent material. The gel should be homogeneous and there are no coarse grains. If there are no coarse grains then the test preparation is declared homogeneous (Nikam, 2017).

c. pH test

The pH test is carried out using a universal pH which is inserted into the sample. Then match it with the pH indicator. Observe the matching color on the pH indicator. The pH value of the preparation that meets the skin pH criteria and does not irritate the skin is 4.5-6.5. (Nikam, 2017).

d. Spreadability Test

Millimeter paper was attached to a Petri dish, then 0.5 g of gel was placed on the Petri dish. Initial measurement of the gel spreading diameter with a Petri dish without load, then added 50 grams of load every 1 minute until the total load weight reached 200 grams. Then record 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 too spread will reduce the level of comfort of use and the effectiveness of the use of the preparation, while preparations that are too dilute will cause their adhesion to decrease so that the contact time of the active substance with the application site is also reduced. (Yusuf et al., 2017).

e. Viscosity Test

The viscosity test uses a viscometer tool which is carried out by placing the preparation in a glass beaker, then installing the appropriate spindle and after that measuring at the appropriate rpm speed. Where the viscosity of good skin preparations ranges from 2000-50000 cPs (Hikmah, 2022).

f. Irritation Test

The irritation test was carried out by applying the preparation to the upper part of the hand arm, the upper part of the arm is a sensitive part like the face so it can be used for irritation testing. Then observe the symptoms that arise such as redness, itching and swelling of the skin. (Wahyuni et al., 2021).

g. Hedonic Test

The hedonic test was conducted to observe the organoleptic and level of liking of the gel preparation by distributing questionnaires using google form to several panelists. (Kusumawati & Bintang, 2023).

2.4.4 Antibacterial Activity Testing

a. Media Creation

Nutrient Agar (NA) and Inclined Agar Media For *Nutrient Agar* (NA) solution, as much as 2.8 g was dissolved with 100 mL of distilled water in an erlenmeyer, then homogenized with a magnetic stirrer, the NA solution was used for making inclined agar media. A total of 5 mL of NA solution was poured into a test tube and placed in an inclined position and allowed to solidify. Slanted agar media is used for rejuvenation of test bacteria (Aldo et al., 2020).

b. Tool sterilization

The tools to be used in the test were thoroughly washed and then dried. After that, they were wrapped in aluminum foil and then sterilized together with NA media using an autoclave at a pressure of 1 atmosphere with a temperature of 121 ℃ for 15 minutes. (Titaley, 2014).

c. Bacterial Rejuvenation

Propionibacterium acnes bacteria derived from pure culture, taken as much as 1 ose and then inoculated by scraping on a slanted Nutrient Agar (NA) medium and then incubated at 37 ℃ for 18-24 hours. (Dewi et al., 2019).

d. Preparation of Test Bacteria Suspension

Test bacteria that have been rejuvenated, taken one ose and then suspended in 10 mL of sterile 0.9% NaCl solution, after which it is homogenized. (Aldo et al., 2020).

e. Basic Media Preparation and *Nutrient Agar* (NA) Seeding

Media preparation was carried out by preparing ingredients for the medium, namely by weighing 5.6 grams of *Nutrient Agar* (NA) media then dissolved with 200 ml of distilled water. then stirred until homogeneous, after homogeneous sterilized by autoclaving at 121 ℃ for 15 minutes. Gel base (negative control), *acne spot treatment* (positive control) and acne spot treatment gel preparations with concentrations of 5%, 10%, and 20% were weighed as much as 0.1 gram each and then dripped into the wells. Then incubated at 37℃ for 24 hours. After incubating for 24 hours, observations were made.

f. Antibacterial activity test

A total of 15 mL of *Nutrient Agar* (NA) medium was put into a Petri dish and then allowed to solidify. After solidifying, 1 ose of bacteria was taken which had been measured based on the Mc.Farland standard of 108 col / mL, then scratched evenly on the surface of the medium, then inserted each *paper disc* which had been dripped with 20 µL of miana leaf extract aseptically using sterile tweezers and as a positive control (+) tetracycline paper dics were used and as a negative control (-) the distillation liquid used. *Paper discs* containing extracts were inserted into the surface of the medium with a distance of 2-3 cm from each other on the edge of the Petri dish. Then the *paper disc* was incubated at 37°C for 1x24 hours. Then the clear diameter formed was observed and measured the diameter of

the inhibition area with a caliper. This treatment was then repeated three times. The difference in bacterial sensitivity to antibacterials is influenced by the structure of the bacterial cell wall. Gram-positive bacteria tend to be more sensitive to antibacterials because the cell wall structure of gram-positive bacteria is simpler than the cell wall structure of gram-negative bacteria, making it easier for antibacterial compounds to enter gram-positive bacterial cells (Yunita et al., 2012). (Yunita et al., 2012).

Table 3.2 Categories of Zone of Inhibition of *P.acnes* Bacteria (Davis & Stout, 1971)

Inhibition zone diameter (mm)	Category
>20 mm	Very strong
10-20 mm	Strong
5-10 mm	Weak

3. Result and DiscussionResult and Discussion

3.1. Simplisia Moisture Content Test

The water content test is one of the parameters to see the quality of the miana leaf plant (*Coleus atropurpureus*). This aims to maintain the quality of the simplisia that will be used for the next process. The results of the water content test can be seen in the table below:

Table 4.1 Observation Results of Water Content Test of Miana Leaf Simplisia (*Coleus atropurpureus*)

Testing	Content (%)	Terms
Water Content	8,5%	≤ 10%

The results of the water content test of miana leaves (*Coleus atropurpureus*) amounted to 8.5%. This is in accordance with the requirements stated in the Indonesian Herbal Pharmacopoeia Second Edition 2017, which is no more than 10%. If the moisture content in simplisia is more than 10%, this will accelerate the growth of microorganisms so that decay occurs, because water is a good medium for the growth of microorganisms so that it can result in a decrease in the quality of simplisia.(Setyaningrum, 2012). Determination of water content is carried out to provide a minimum limit of water content that can still be tolerated in simplisia.

3.2 Simplisia Drying Shrinkage Test

The drying shrinkage test is a non-specific parameter that aims to provide a maximum limit (range) on the amount of compounds lost in the drying process. (Ministry of Health, 2014). The results of the drying shrinkage test can be seen in the table below:

Table 4.2 Observation Results of Drying Shrinkage Test of Miana Leaf Simplisia (*Coleus atropurpureus*)

Testing	Content (%)	Terms
Drying Shrinkage	5%	≤ 10%

The drying shrinkage value obtained from miana leaf extract (*Coleus atropurpureus*) is 5%. This shows the amount of water content and compounds lost during the drying process is 5%. A good requirement for drying shrinkage is less than 10% because drying shrinkage also represents the evaporated water content (Fadillah et al., 2015). (Fadillah et al., 2022)..

3.3 Extraction

3.3.1 Miana Leaf Extraction (*Coleus aropurpureus*)

The extraction process of miana leaves is carried out by maceration method. Miana leaf powder (*Coleus atropurpureus*) was soaked in a closed container using 70% ethanol solvent. Maceration was carried out at room temperature for three days and stirred periodically. (Khasanah, 2016). The results of maceration extraction in the form of maserat, filtered using filter paper to separate the maserat with the residue. The filtered macerate is then evaporated on a *waterbath* at 40 ℃ to remove any remaining solvent that may still be present until a thick extract of miana leaves is obtained. (Khasanah, 2016).

The reason for choosing 70% ethanol solvent in this study is because 70% ethanol is a polar solvent, can extract or separate various kinds of polar compounds from polar to non-polar ones. The higher the concentration of ethanol, the less polar the solvent is. (Setyaningrum, 2012).

3.3.2 Calculation of the Yield of Miana Leaf Extract (*Coleus atropurpureus*)

$$\% \text{ Yield} = \frac{\textit{Berat ekstrak kental } (g)}{\textit{Berat simplisia } (g)} \text{ x } 100\%$$

$$= \frac{75\ gram}{300\ gram} \text{ x } 100\%$$

$$= 25\%$$

The yield obtained from sample extraction is 25%. Therefore, the yield of the extract obtained is declared good, because the yield is not less than 10%. This result meets the standardization requirements of the Indonesian Herbal Pharmacopoeia (FHI). (Ministry of Health, 2014). The higher the yield value indicates that the extract produced is better used and is included in the standardized value. (Badaring et al., 2020).

3.4 Physical Evaluation of Preparations

a. Organoleptical Test

Table 4.3 Organolepstic Test Observation Results of Gel *Acne Spot Treatment* Miana Leaf Extract (*Coleus atropurpureus*)

Observation	Sunday	F1	F2	F3	F0
Color	I	Blackish Brown	Blackish Brown	Blackish Brown	Clear
	II	Blackish Brown	Blackish Brown	Blackish Brown	Clear
	III	Blackish Brown	Blackish Brown	Blackish Brown	Clear
	IV	Blackish Brown	Blackish Brown	Blackish Brown	Clear
Aroma	I	Green Tea	Green Tea	Green Tea	Green Tea
	II	Green Tea	Green Tea	Green Tea	Green Tea
	III	Green Tea	Green Tea	Green Tea	Green Tea
	IV	Green Tea	Green Tea	Green Tea	Green Tea
Consistency	I	Consistent	Consistent	Consistent	Consistent
	II	Consistent	Consistent	Consistent	Consistent
	III	Consistent	Consistent	Consistent	Consistent
	IV	Consistent	Consistent	Consistent	Consistent

Description: F1 = Formulation with 5% extract concentration

F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

Organoleptical test is done to see the physical appearance of a preparation visually by observing the color, smell and consistency of each formula. (Rijayanti, 2014). The purpose of the organoleptical test is to determine whether there are organoleptical changes in the *acne spot treatment* gel preparation of miana leaf extract (*Coleus atropurpureus*) during 4 weeks of storage at room temperature.

From the research conducted, it is known that the four formulas each have differences based on the physical evaluation of the preparation. This is because the type of *gelling agent* has a different consistency in each formula. The test results of each formula showed no change in color, odor and consistency in the *acne spot treatment* gel preparation of miana leaf extract (*Coleus atropurpureus*) for 4 weeks of storage at room temperature.

b. Homogeneity Test

Table 4.4 Observation Results of Homogeneity Test of *Acne Spot Treatment* Gel of Miana Leaf Extract (*Coleus atropurpureus*)

Formula	Sunday			
	I	II	III	IV
F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous

Description: F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

This homogeneity test was carried out with the aim of knowing the level of homogeneity of the *acne spot treatment gel of* miana leaf extract (*Coleus atropurpureus*) by looking at the uniformity of the particles in the preparation. Of the four formulations of *acne spot treatment* gel preparations, each has a homogeneous composition characterized by no part that is not well mixed during 4 weeks of storage. Thus, all gel preparations have good homogeneity and meet the requirements of Indonesian Pharmacopoeia Edition III, namely if the gel is applied to a piece of glass or other suitable transparent material, it must show a homogeneous composition that can be seen by the absence of coarse particles and spread evenly.

c. Viscosity Test

Table 4.5 Observation Results of Viscosity Test of *Acne Spot Treatment* Gel of Miana Leaf Extract (*Coleus atropupureus*)

Formula	Sunday		
	I	II	III
F1	4940 cps	3040 cps	2480 cps
F2	3440 cps	3340 cps	3160 cps
F3	1440 cps	1200 cps	940 cps

F0	18540 cps	19380 cps	19940 cps

Description: F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

The viscosity test is carried out to determine the viscosity of the preparation, where a viscosity value states the amount of resistance of a liquid to flow. Viscosity measurement of *acne spot treatment* gel was carried out using a *Brookfield Viscometer*. The results of measuring the viscosity of the miana leaf extract (*Coleus atropurpureus*) gel preparation showed that the viscosity of the preparation before and after storage showed a significant difference for all preparations of each formulation. For a good gel formula, the viscosity value is between 2,000-50,000 cps. (MOH, 2014).

From the observations, there was a decrease in viscosity value after storage in the second and third weeks. This means that the viscosity of the preparation was not stable during the 3 weeks of storage at room temperature. The change in viscosity of the gel preparation is due to the presence of air bubbles that are still trapped when making the gel (Pramesthi et al., 2020). (Pramesthi et al., 2020).

d. Spreadability Test

Table 4.6 Observation Results of Gel Spreadability Test *Acne Spot Treatment* Miana Leaf Extract (*Coleus atropurpureus*)

Formula	Spreadability			Average	Range
	1	2	3		
1	3,85	5,25	4,1	3,86	5-7 (Sayuti et al., 2015)
2	6,6	6,15	6,9	6,6	
3	5,35	2,2	3,4	5,36	
0	3,85	5,15	5,25	3,86	

Description: F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

The spreadability test was conducted to determine the ability of the miana leaf extract (*Coleus atropurpureus*) *acne spot treatment* gel to spread on the skin surface. Semi-solid preparations are able to spread easily at the site of administration, without any significant pressure. The easier it is applied to the skin, the greater the contact surface area of the efficacious substance with the skin and the more optimal the absorption of the drug.

The test results that have been carried out show that the increase in the spreadable area is accompanied by an increase in the load given, and the best results are found in formulas II and III because they have a spreadability value that is included in the scale of good spreadability. Semi-solid preparations that are comfortable to use have a spreadability of 5-7 cm. (Rohmani & Kuncoro, 2019). Based on the test results that have been obtained, it is known that formulas I and 0 have poor spreadability because they do not enter the range of good spreadability. The increase and decrease in spreadability is strongly influenced by the consistency of the gel, because this is related to the viscosity value of the preparation. If the

viscosity value of the preparation is high, the resulting spreadability is low, otherwise the lower the viscosity value of the preparation will produce a wide spreadability as well. This happens because the high viscosity causes the gel to be difficult to flow so that the resulting spreadable area is small (Saputra et al., 2019).

e. pH test

Table 4.7 Observation Results of pH Test of *Acne Spot Treatment* Gel Preparations of Miana Leaf Extract (*Coleus atropurpureus*)

Duration of Observation (Week 1)	Formula				Library
	0	1	2	3	
1	5	5	6	4	The pH requirement for a good gel preparation is in accordance with the pH of the skin, which is between 4.5-6.5 (Wulandari et al., 2019).
2	5	5	4	6	
3	5	5	6	5	
0	5	5	5	5	
Average	5	5	5	5	

Description: F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

The pH test was conducted to determine the acidity of the *acne spot treatment* gel preparation of miana leaf extract (*Coleus atropurpureus*). The pH test is considered very important, especially for preparations used on the skin because preparations that have a pH that is too acidic will cause skin irritation while preparations that are too alkaline will cause dry skin. (Rohmani & Kuncoro, 2019). The results of pH testing of the four formulations carried out for 4 weeks obtained stable pH preparation results, namely at pH 4-6, which means that it meets the natural pH requirements of the skin, namely 4.5-6.5. (Rohmani & Kuncoro, 2019)..

f. Irritation Test

Table 4.8 Observation Results of Irritation Test of *Acne Spot Treatment* Gel Preparations Miana Leaf Extract (*Coleus atropurureus*)

Respondents	**Skin Reaction**			
	F1	**F2**	**F3**	**F0**
1	-	-	- -	
2	-	-	- -	
3	-	-	- -	
4	-	-	- -	
5	-	-	- -	
6	-	-	- -	
7	-	-	- -	
8	-	-	- -	
9	-	-	- -	
10	-	-	- -	
11	-	-	- -	
12	-	-	- -	
13	-	-	- -	

14	-	-	- -
15	-	-	- -

Description: (-) = No irritation
(√) = There is irritation
F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

Skin irritation test is conducted to determine the side effects of using acne spot treatment gel on the skin. Based on the tests that have been carried out, formulas I, II, III and IV give negative results to all respondents. This is in accordance with the parameters of irritation reactions such as redness, itching and swelling of the skin. The test results indicate that the *acne spot treatment gel* preparation of miana leaf extract (*Coleus atropurpureus*) with a concentration of 5%, 10%, and 20% is safe to use on the skin and does not cause irritation.

g. Hedonic Test

Table 4.9 Hedonic Test Observation Results of *Acne Spot Treatment* Gel Preparations Miana Leaf Extract (*Coleus atropurureus*)

Formula	Parameters	Respondent assessment			
		Very Favorable	Like	Enough Likes	Dislikes
F1	Color	6	9	-	-
	Aroma	4	8	3	-
	Texture	7	8	-	-
F2	Color	3	12	-	-
	Aroma	5	9	1	-
	Texture	5	9	-	-
F3	Color	6	8	1	-
	Aroma	5	8	2	-
	Texture	7	8	-	-
F0	Color	7	7	1	-
	Aroma	4	9	2	-
	Texture	8	7	-	-

Description: F1 = Formulation with 5% extract concentration
F2 = Formulation with 10% extract concentration
F3 = Formulation with 20% extract concentration
F0 = Gel Base

Hedonic test results on *acne spot treatment* preparations of miana leaf extract (*Coleus atropurpureus*) which include color, aroma and texture. There are 4 determination scales, namely very like, quite like and dislike. This test was conducted to see in what formula the respondents' interest in the preparation of *acne spot treatment of* miana leaf extract (*Coleus atropurpureus*). The hedonic test involved 15 respondents using the slovin formula (20% error). This is because the population used is small (Hsia et al., 2015). (Hsia et al., 2015).. In the hedonic test, respondents preferred the color of F1, for aroma respondents preferred F2, while in texture respondents preferred F3.

3.5 Antibacterial Activity Test

Microbiological tests were conducted to determine the antibacterial activity of *acne spot treatment* gel preparations of miana leaf extract (*Coleus atropurpureus*) carried out by the agar diffusion method or paper disc method, by measuring the diameter of the growth resistance of *Propionibacterium acnes* bacteria. This method was chosen because it is simple, easy, and fast because it only uses paper discs (Jayanti et al., 2017). (Jayanti et al., 2017). The data obtained in the form of antibacterial activity of preparations with various concentration series and preparation stability results.

Table 4.10 Observation Results of Antibacterial Activity Test of Miana Leaf Extract Gel Preparations (*Coleus atropurpureus*)

Results	Inhibition zone diameter (mm)	Category
F1	27 mm	Very Strong
F2	29 mm	Very Strong
F3	27 mm	Very Strong
K+	28 mm	Very Strong
K-	-	-

Description: F1 : Formulation with 5% extract concentration
F2 : Formulation with 10% extract concentration
F3 : Formulation with 20% concentration
(K+) Positive Control
(K-) Negative Control

This test was conducted by comparing the concentration of extracts from formulas I, II and III as well as positive and negative controls. The positive control used was Medi-Klin with the active ingredient *Clindamycin phosphate* 1.2%, while the negative control used was the gel base. The diameter of the inhibition zone produced was F1: 27mm, F2: 29mm, F3: 27mm, K+: 28mm, K-: 27mm.

Table 4.11 Anova Test Table of Miana Leaf Extract (*Coleus atropurpureus*)

ANOVA

form_kPo_kNe

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12192.000	2	6096.000	.495	.633
Within Groups	73926.000	6	12321.000		
Total	86118.000	8			

The results showed that miana leaf extract (*Coleus atropurpureus*) has the activity to inhibit the growth of bacteria that cause acne. Proven by the results of statistical analysis using SPSS 22 where the significant value for the data normality test using shapiro-wilk is 1.000 so that it can be said to be normally distributed because the sig value (p>0.05). Data is said to be normally distributed if the sig value (p>0.05) (SEARLE et al., 1989). Furthermore, the homogeneity test was carried out with a significant value on each data> 0.05 which means it has a homogeneous value. *One Way Anova* test obtained p=0.633,

because the value obtained >0.05, it can be interpreted that the data on the diameter of the inhibition zone of *acne spot treatment* gel preparations of miana leaf extract (*Coleus atropurpureus*) against *Propionibacterium acnes* bacteria do not have significant differences.

Acne spot treatment preparations of miana leaf extract have very strong antibacterial activity comparable to miana leaf extract (*Coleus atropurpureus*) this is because *Coleus atropurpureus* miana leaves) contain flavonoids, saponins, tannins, alkaloids which are antibacterial compounds. Flavonoids have antibacterial properties where the mechanism of action is to form complex compounds with extracellular and soluble proteins so as to damage the bacterial cell membrane and followed by the release of intracellular compounds (Darmawati et al., 2015). (Darmawati et al., 2015). Based on research (Intan & Asngad, 2019)Based on research (Intan & Asngad, 2019), flavonoid compounds at low concentrations can damage the cytoplasmic membrane and can cause cell nucleus leakage while at high concentrations flavonoid compounds coagulate with cellular proteins. Flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA.

From the results of the research conducted, the best *acne spot treatment* preparations for physical stability, irritation and hedonic tests are F1 and F2 preparations with F1 5% and F2 10% concentrations, as well as the best antibacterial activity is in F2 with 10% extract concentration.

4. Conclusions and Suggestions

4.1 Conclusions

Based on the research that has been done, the following conclusions are obtained:

1. In testing the physical stability of the acne spot treatment gel of miana leaf extract (*Coleus atropurpureus*) which includes organoleptic, homogeneity, viscosity, spreadability, pH, irritation and hedonic tests, the most stable preparation results are obtained in Formulas I and II for each test carried out in accordance with the preparation evaluation requirements. Whereas in Formula III, the preparation did not meet the requirements for viscosity and spreadability testing after storage for 4 weeks.
2. In testing the antibacterial activity of miana leaf extract (*Coleus atropurpureus*), each formula with 5% extract concentration: 27mm, 10%: 29mm and 20%: 27mm is effective as an antibacterial to inhibit the growth of Propionibacterium acnes bacteria that cause acne with the diameter of the inhibition zone in the very strong category.

4.2 Suggestions

For further research, it is hoped that researchers can continue this research with different methods so that it can provide better information to the community regarding the utilization of miana leaves.

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