

## Formulation And Activity Test Of Acnes Serum Bidara Leaf Extract (Ziziphus Mauritiana) Against Propionibacterium Acne Bacteria

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### Abstract

Bidara leaf (*Ziziphus mauritiana*) is a plant that has antibacterial activity, one of which is against *Propionibacterium acne* bacteria. Therefore, it can be formulated as an active ingredient in serum preparations. This study aims to formulate Bidara leaf extract and test its antibacterial activity against *Propionibacterium acne* bacteria using the disc diffusion method. In this study, 4 formulations were prepared with different concentrations of Bidara leaf extract (*Ziziphus mauritiana*). Then physical stability test was conducted with test parameters including organoleptic, homogeneity, spreadability, pH, irritation. The results of the study based on physical stability test showed that organoleptic, pH, spreadability, irritation, for 4 formulations were stable during storage. Furthermore, based on the antibacterial test of acne serum against *Propionibacterium acne*, it was found that the concentration of Bidara leaf extract affects the inhibition of the growth of *Propionibacterium acne* bacteria with an inhibition of 13,3 mm for a concentration of 2.5%, 14,5 mm for a concentration of 5%, and 14,83 mm for a concentration of 7.5%.

Keywords: Acnes serum, Bidara leaf, *Ziziphus mauritiana*

### 1. Introduction

Acne is a skin disease that often occurs in adolescence to adulthood which is characterised by the presence of blackheads, papules, pustules, nodes, and cysts on the face, neck, upper arms, chest, and back (Wahdaningsih, Untari, and Fauziah, 2014). The bacteria that cause acne include *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* (Pelen et al., 2016). Treatment of acne can be done by administering antibiotics. Antibiotics such as clindamycin, erythromycin, tetracycline, doxycycline are often used in acne treatment because they can inhibit inflammation and kill bacteria (Anggraini et al., 2013).

One of the plants that can be used as traditional medicine is bidara leaf (*Ziziphus mauritiana*), which has potential as a natural antioxidant. Bidara contains phenolics and flavonoids that are efficacious as antioxidants, anti-inflammatory, antimicrobial, antifungal and inhibit tumour growth (Kaur et al., 2015). The bidara plant itself has a maximum zone to inhibit microbial growth at a concentration of 2%. The excellent inhibition zone is good for inhibiting antibacterial activity against *Propionibacterium acnes* bacteria at an interval of 24 hours and 72 hours, namely at a concentration of 60% (22.2 mm and 23.1 mm) (Gerung et al., 2021). Due to the presence of tannins, saponins, flavonoids, the *Ziziphus mauritiana* plant has antimicrobial activity (Najafi, 2013).

One of the cosmetic products that has developed today is facial serum. Serum is a cosmetic preparation that contains a high concentration of active substances with low viscosity and more effectively treats facial skin (Thakre, 2017). Serum is effective for treating

skin problems such as dark spots, fine lines on the skin, dry skin, and fading acne scars (Pratiwi et al., 2021).

## 2. Methodology

This research was conducted from September to October at the Microbiology Laboratory, Faculty of Health Sciences, University of Muhammadiyah Palopo.

### Research Variables

Independent variables: The independent variable that will be investigated in this study is bidara leaf (*Ziziphus mautiana*). The dependent variable: These dependent variables that will be investigated in this study are organoleptic test, homogeneity test, pH test, spreadability test, viscosity test, and irritation test and antibacterial test.

### Tools and materials

The tools used are the tools used were beakers, dropper, blender, stirring rod, porcelain cup, paper disc, ose needle, parchment paper, tweezers, mortar, oven, petri dish, watch glass, aluminium foil, universal pH.. The materials used were bidara leaf extract (*Ziziphus mauritiana*), 70% ethanol, glycerin, NA benzoate, carbomer, sodium EDTA, TEA (Triethanolamine), NA (Nutrient agar) media, *Propionibacterium acnes* bacterial isolate, distilled water.

### Sample processing (*Rhizophora mucronata*)

The bidara leaves were picked manually by hand, the leaves that were picked were old leaves. Then the bidara leaf samples that have been collected are wet sorted, then washed thoroughly with running water. After washing, the bidara leaves were cut into pieces and then dried without direct sunlight. Then dry sorting is carried out to be free from unwanted particles. The samples were then powdered using a blender and sieved to obtain fine powder. Bidara leaf samples that have become powder are stored in a tightly closed container. (Umar et al., 2012).

### Preparation of Bidara Leaf Extract (*Ziziphus mauritiana*)

The dried bidara leaves were pulverised and sieved with a 40 mesh sieve. Furthermore, the powder obtained was extracted by maceration method using 70% ethanol solvent, and 700 grams of bidara leaf powder was dissolved in 7 L of 70% ethanol solvent and stirred for 1 hour. Then cover and store for 24 hours in a place protected from sunlight at room temperature. The result of maceration was filtered and the remaining pulp and filtrate were separated into separate containers. The precipitate was extracted two more times with the same amount using the same method. The whole maceration results were concentrated using a rotary evaporator (Umar et al. 2012).

### Preparation of Positive Control and Negative Control

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

### Preparation of Serum

Preparation of serum begins with dissolving the ethanol extract of bidara leaves in enough water, dissolving sodium EDTA, dissolving sodium benzoate, then heating the water until it dissolves carbomer. After boiling the water, put the carbomer into the mortar, then add 15 ml of boiling water and mix well. Add EDTA dissolved in water and mix well. Add

sodium benzoate and mix well. Add triethanolamine and stir until smooth, add bidara leaf extract little by little stir until homogeneous and finally add glycerin stir until homogeneous (Dedhi, 2018).

### **Evaluation of Deodorant spray preparation**

#### a. Organoleptic Test

Organoleptic testing is done visually by observing the changes that occur including colour, odour and consistency (Allen and Ansel, 2013).

#### b. Homogeneity Test

The serum preparation was applied to a piece of glass, and then the immiscible part was observed. Homogeneity is indicated by the absence of coarse grains (Kumesan et al., 2013).

#### c. pH Test

Measurement of the pH of the preparation is carried out using a pH-universal. The pH of the serum preparation must be in accordance with the pH of the skin, which is 4.5-6.5 (Zhelsiana, et al., 2016).

#### d. Spreadability Test

A total of 0.5gram of preparation was placed in the centre of a round glass scale, then covered with another round glass. Measurement of the diameter of the spread of the preparation longitudinally and transversely, and carried out every additional load of 50 grams to a total weight of 150 grams. The spreadability that meets the requirements is 5-7 cm (Yusuf et al., 2017).

#### e. Viscosity Test

The viscosity test was carried out using a lamy rheology viscometer by inserting the preparation into a 50 mL beaker glass then spindle 4 was lowered at a speed of 50 rpm (A. H. Kusumawati et al., 2022). Good viscosity standards in serum preparations are in the range of 230-1150 cPs (Hairunnisa et al., 2022).

#### f. Irritation Test

The irritation test was conducted in vivo on one rabbit using the Draize method. The rabbits used were adult male rabbits whose back fur had been shaved. This shaving was done 24 hours before treatment. Before applying the test preparation, each rabbit received a parallel epidermal abrasion using a sterile needle. The test material was applied by rubbing on the test area. After applying the test material, the test area was then covered with a non-reactive bandage. After 24 hours, the bandage was removed and the test area was cleaned with water to remove any remaining test material. At 24, 48 and 72 hours after application of the test substance, the test area was examined and observed for changes in the skin reaction to the test substance and scored from 0 to 4 depending on the severity of the skin reaction (Draize, 1959).

#### g. Bacteria Rejuvenation

Test the antibacterial activity of bidara (*Ziziphus mauritiana*) leaf extract against *Propionibacterium acnes* bacteria using the disc diffusion method. This method is done by placing paper discs that have been soaked in the test solution on solid media that has been inoculated with bacteria and then dipping the solution in the test media until the entire surface of the disc is wet. Observations were made after the bacteria were inoculated, bacterial growth was observed to see a clear zone around the disc. The selection of this method is because it is

easy and simple to determine the antibacterial activity of the sample being tested. The disc paper used is 0.5 cm in diameter (Mulyadi et al., 2017).

#### h. Zone of Inhibition Measurement

Antibacterial activity can be said to be positive if a clear zone of inhibition is formed around the disc paper. The part that is calculated with a caliper or ruler is the diameter of the inhibition zone formed. According to Davis and Stout (1971) in (Harita, 2019) the criteria for the strength of antibacterial power, namely the diameter of the inhibition zone of 5 mm or less, the inhibitory activity is categorized as weak, the diameter of the inhibition zone of 5-10 mm is categorized as moderate, the diameter of the inhibition zone of 10-20 mm is categorized as strong and if the diameter is 20 mm or more, the inhibitory activity is categorized as very strong. The formation of the inhibition zone in the antibacterial activity test is influenced by several factors including the concentration of the extract, the content of antibacterial compounds and the type of bacteria (Harita, 2019).

### 3. Result and Discussion

#### 3.1. Result

**Table 1. Result of Maceration of Ethanol Extract of Bidara Leaf**

Plants	Powder (gram)	Viscous Extract (gr)	Soaking (%)
Mangrove Leaf	700	98,2	18,8%

**Table 2. Results of Determination of Bidara Leaf Water Content**

Parameters	Water content	standard
Water content test	6,5 %	≤10 %

**Table 3. Mangrove Leaf Drying Shrinkage Determination Results**

Parameters	Drying Shrinkage	Standard
Drying Shrinkage Test	3,37%	≤10%

**Table 4. Organoleptic Test Results of Acne Serum**

Formula	F1 (2,5%)	F2 (5%)	F3 (7,5%)	F0 (Basis)	Standar
Colour	Brown	Brown	Dark brown	Clear white	Corresponds to the extract colour
Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid (Serum)
Scent	Greentea	Greentea	Greentea	Greentea	Essense

#### Description

A = FO Concentration without extract Dosage base

B = F1 Concentration of 2.5% bidara leaf ethanol extract

C = F2 Concentration of 5% bidara leaf ethanol extract

D = F3 Concentration of 7.5% bidara leaf ethanol extract

**Table 5. Homogeneity Test**

Formula	F1(2,5%)	F2 (5%)	F3 (7,5%)	F0 (Basis)	Standar
Homogenitas	Homogen	Homogen	Homogen	Homogen	Homogen

#### Description

A = FO Concentration without extract Dosage base  
 B = F1 Concentration of 2.5% bidara leaf ethanol extract  
 C = F2 Concentration of 5% bidara leaf ethanol extract  
 D = F3 Concentration of 7.5% bidara leaf ethanol extract

**Table 6. pH Test**

Formula	F1 (2,5%)	F2 (5%)	F3 (7,5%)	F0 (Basis)	Standar
pH	4	5	5	6	4,5 – 6,5

Description

A = FO Concentration without extract Dosage base  
 B = F1 Concentration of 2.5% bidara leaf ethanol extract  
 C = F2 Concentration of 5% bidara leaf ethanol extract  
 D = F3 Concentration of 7.5% bidara leaf ethanol extract

**Table 7. Spreadability Test**

Formula	F1 (2,5%)	F2 (5%)	F3 (7,5%)	F0 (Basis)	Standar
Spreadability Test	6,5 cm	6,1 cm	6,3 cm	6,6 cm	5-7 cm

Description

A = FO Concentration without extract Dosage base  
 B = F1 Concentration of 2.5% bidara leaf ethanol extract  
 C = F2 Concentration of 5% bidara leaf ethanol extract  
 D = F3 Concentration of 7.5% bidara leaf ethanol extract

**Table 8. Viskosity Test**

Formula	F1 (2,5%)	F2 (5%)	F3 (7,5%)	F0 (Basis)	Standar
Viscosity Test	605,25 cps	644,5 cps	509,5 cps	419,25 cps	230-1150 cps

Description

A = FO Concentration without extract Dosage base  
 B = F1 Concentration of 2.5% bidara leaf ethanol extract  
 C = F2 Concentration of 5% bidara leaf ethanol extract  
 D = F3 Concentration of 7.5% bidara leaf ethanol extract

**Table 9. Irritation Test**

Time	Terjadinya Eritema				Terjadinya Edema			
	F0	F1	F2	F3	F0	F1	F2	F3
24 Hours	-	-	-	-	-	-	-	-
48 Hours	-	-	-	-	-	-	-	-
72 Hours	-	-	-	-	-	-	-	-

Description:

(-) = No irritation

**Table 10. Bacteria Test**

Serum Type	Diameter Of Inhibition Zone (mm)				Zone Of Inhibition Ability
	Perlakuan I	Perlakuan II	Perlakuan III	Average	
K-	0	0	0	0	Nothing
K+	13,5	14,5	14	14	Strong
F1	12,5	13,5	14	13,3	Strong
F2	14,5	13,5	15,5	14,5	Strong
F3	16,5	12,5	15,5	14,83	Strong

#### Description

F0 = Concentration without extract) preparation base

F1 = 2.5% concentration of bidara leaf extract

F2 = 5% concentration of bidara leaf extract

F2 = Concentration of 7.5% bidara leaf extract

K+ = Positive Control

#### **Antibacterial Activity Test Results of *Acne* serum Bidara Leaf Extract (*Ziziphus mauritiana*)**

Antibacterial testing on the preparation of bidara leaf anti-acne serum in F1 has a clear zone value of 13.3 mm inhibition zone shows entry into the strong category, F2 has a clear zone value of 14.5 mm with a strong category and F3 has a clear zone value of 14.83 mm shows entry into the strong category and FO has an inhibition zone value of 14 mm shows entry into the strong category, seen from the clear zone of the disc hole area. From the three formulas, it can be concluded that the best clear zone produced in F3 is considered in the strong category, the higher the concentration of bidara leaf extract, the higher the inhibition formed. The clear zone produced by the positive control is fairly strong, having a clear zone value of 14 mm, the clear zone around the well is caused by the presence of active compounds of bidara leaf extract, namely flavonoids and saponins. Flavonoid compounds have antibacterial properties with the mechanism of action of damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA (Ganiswarna, 1995). Meanwhile, saponins can disrupt the permeability of microbial cell membranes resulting in cell membrane damage and cause the release of important components from inside microbial cells (Robinson, 1991).

#### **3.2. Discussion**

Bidara leaves (*Ziziphus mauritiana*) were taken in the city of Palopo. The bidara leaves were picked manually by hand, the leaves that were picked were old leaves. Then the bidara leaf samples that have been collected are wet sorted, then washed thoroughly with running water. After washing, the bidara leaves were cut into pieces and then dried without direct sunlight. Then dry sorting is carried out to be free from unwanted particles. The samples were then powdered using a blender and sieved to obtain fine powder. Bidara leaf samples that have become powder are stored in a tightly closed container. (Umar et al., 2012).

The powder obtained was then extracted by maceration method using 70% ethanol solvent, 700 grams of bidara leaf powder was dissolved with 70% ethanol solvent as much as 7 L and stirred for 1 hour. Then it was covered and allowed to stand for 24 hours in a place

protected from sunlight at room temperature. The results of maceration were filtered and separated between the pulp and filtrate in a separate container. The dregs were re-extracted in the same way and amount for 2 times. The results of the whole maceration were concentrated using a rotary evaporator (Umar et al. 2012).

Determination of water content aims to determine the percentage of water content left in simplisia. This is important to know the maximum limit of water content in simplisia because if the amount of water contained is too high, it becomes a medium for the growth of bacteria and fungi which can damage the quality of simplisia (Depkes RI, 2000). The test results of determining the water content of bidara leaf (*Ziziphus mauritiana*) simplisia powder are 6.5%, this meets the requirements of water content in general, which is not more than 10% (Depkes RI, 2017).

Drying shrinkage is one of the non-specific parameters that aims to provide a maximum limit (range) of the amount of compounds lost in the drying process. Basically, drying shrinkage is the measurement of substances after drying at a temperature of 105°C until constant weight is then expressed in percent (Ministry of Health, 2000). The results of the drying shrinkage test on simplisia showed that the drying shrinkage of bidara leaves reached 3.37%.

Organoleptic test was conducted to determine the physical form of the finished preparation where observations were made directly including the shape, colour, and smell of the serum preparation using the five senses (Widia *et al.*, 2012).

The results of the organoleptic test of the four serum formulations produced were semi-solid dark brown in colour with different viscosity, and a distinctive smell of greentea. Good serum quality parameters are semi-solid dosage form, serum smells typical of the extract used and is coloured like the extract (Ministry of Health, 2000).

Based on the observations that have been made, the texture of the three dosage formulations is semi-solid and the distinctive smell of greentea is obtained from the addition of flavouring. From observations for 4 weeks the four formulas did not experience changes in either shape or aroma, while the colour formed from the four formulations had differences, namely formula 0 had a clear colour, formulas 1 and 2 had a brown colour while formula 3 had a dark brown colour, this was due to differences in the concentration of extracts used in each formula.

The homogeneity test was carried out by applying the preparation using an object glass, the preparation was crushed with two object glasses by ensuring that the preparation was homogeneous with no visible coarse grains (Ditjen POM, 1985). The homogeneity test results show a homogeneous composition characterised by the absence of lumps or coarse grains in the serum preparation and evenly distributed (Ojha *et al.*, 2019).

Based on the pH stability test for 28 days, all formulas showed a pH that met the requirements for topical use on the skin. The addition of carbomer concentration as a gelling agent affects the pH of the formula, the higher the concentration of carbomer, the more carboxyl groups are ionised and carbomer will be easily hydrolysed, so that the pH value of the preparation will increase (Mappa *et al.*, 2013). the increase in pH value in each formulation, occurs due to differences in the concentration of active substances used, the

greater the concentration used, the smaller the resulting pH value. The pH range in each formulation meets the requirements of 4.5-6.5.

Testing the spreadability of the preparation is carried out to determine the ability to spread the preparation. Spreadability is an important characteristic in the formula because it affects the transfer of active ingredients (Darwis *et al.*, 2011). The results of the spreadability test show that the higher the concentration of the active substance used, the higher the spreadability value, the wider the active substance is well distributed, the spreadability range is 5-7 cm (Garg *et al.*, 2002).

The viscosity test aims to determine the viscosity value of the serum preparation. The higher the consistency value the harder the drug is applied to the skin, the lower the viscosity value the easier the drug is used (Febrianto *et al.*, 2020). Viscosity is carried out using a lamy rheology viscometer by putting the preparation into a 50 mL beaker glass then spindle 4 is lowered at a speed of 50 rpm (A. H. Kusumawati *et al.*, 2022). Good viscosity standards in serum preparations are in the range of 230-1150 cPs (Hairunnisa *et al.*, 2022) set at a speed of 50 rpm (Hasrawati *et al.*, 2020). Good viscosity standards in serum preparations are in the range of 230-1150 cPs (Hairunnisa *et al.*, 2022) at a speed of 50 rpm (Hasrawati *et al.*, 2020).

The purpose of the irritation test is to fulfil skin sensitivity and prevent adverse effects on the skin. The irritation test is carried out by observing the presence or absence of erythema and oedema. Erythema is a reddish reaction on the skin that arises as a result of the side effects of using topical preparations. This redness is also characterised by the appearance of blotches. Meanwhile, edema is a swelling reaction on the skin that arises as a result of the side effects of using topical preparations (Hakim, 2018). The results of the irritation test in this study, acnes serum preparations from ethanol extract of bidara leaves (*Ziziphus mauritiana*) in formula 0, formula 1, Formula 2, Formula 3, did not occur irritation in rabbits. In this irritation test, it can be concluded that acnes serum preparations applied to the skin do not show any skin irritation effects such as erythema and edema.

Based on table 10, Measurement of inhibition zone can be seen by the formation of inhibition zone around the disc paper (Kurniawati, 2015). The principle of the disc diffusion method is that the serum preparation contained on the disc paper will diffuse into the media that has been inoculated with test bacteria, namely *Propionibacterium acnes* (Balouri *et al.*, 2016). This method was chosen because the process is easy to do and does not require special equipment. Antibacterial testing on the preparation of bidara leaf anti-acne serum in F1 has a clear zone value of 13.3 mm inhibition zone shows entry into the strong category, F2 has a clear zone value of 14.5 mm with a strong category and F3 has a clear zone value of 14.83 mm shows entry into the strong category and FO has an inhibition zone value of 14 mm shows entry into the strong category, seen from the clear zone of the disc hole area. From the three formulas, it can be concluded that the best clear zone produced in F3 is considered in the strong category, the higher the concentration of bidara leaf extract, the higher the inhibition formed. The clear zone produced by the positive control is fairly strong, having a clear zone value of 14 mm, the clear zone around the well is caused by the presence of active compounds of bidara leaf extract, namely flavonoids and saponins. Flavonoid compounds have antibacterial properties with the mechanism of action of damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between

flavonoids and bacterial DNA (Ganiswarna, 1995). Meanwhile, saponins can disrupt the permeability of microbial cell membranes resulting in cell membrane damage and cause the release of important components from inside microbial cells (Robinson, 1991).

Based on the research of David and Strout (1971) in (Harita, 2019) the criteria for the strength of antibacterial inhibition are the diameter of the inhibition zone of 5 mm or less, the inhibitory activity is categorized as weak, the diameter of the inhibition zone of 5-10 mm is categorized as moderate, the diameter of the inhibition zone of 10-20 mm is categorized as strong and if the diameter is 20 mm or more, the inhibitory activity is categorized as very strong diameter of 5-10 mm is categorized as moderate, the diameter of the inhibition zone of 10-20 mm is categorized as strong and if the diameter is 20 mm or more then the inhibitory activity is categorized as very strong.

Data from the antibacterial activity of several concentrations of bidara leaf extract (*Ziziphus mauritiana*) were analysed using SPSS 24 which aims to see whether the acnes serum preparation of bidara leaf extract (*Ziziphus mauritiana*) is able to inhibit the growth of *Propionibacterium acne* bacteria. The normality test basically compares the test data with normally distributed data which has a comparison between the test data with normally distributed data which has the same mean and standard deviation as the data from the homogeneity test using Levene statistic, aims to determine whether the distribution of data comes from the same population or not.

The results of the normality test of antibacterial activity of bidara leaf serum acnes (*Ziziphus mauritiana*) obtained normality test values can be seen in (attachment 15). F1  $p > 0.637$ , F2  $p > 1,000$ , F3  $p > 0.463$ , KP  $p > 1,000$ , KN  $p > 0$  which is greater than  $p > 0.05$ . This shows that the data on the diameter of the inhibition zone of the serum acnes preparation of bidara leaf extract (*Ziziphus mauritiana*) against *Propionibacterium acne* bacteria are normally distributed. Homogeneity test results can be seen in (Appendix 15). Based on the homogeneity test shows that the test has a homogeneous value because the value of  $p > 0.05$ . Data that are normally distributed and homogeneous are then analysed with One-Way Anova. The results of the One-Way Anova test obtained a result of 0.000. The value obtained is smaller than  $p < 0.05$  (Trisia *et al.*, 2018). This shows that the data on the diameter of the inhibition zone of the serum acnes preparation of bidara leaf extract (*Ziziphus mauritiana*) has a significant difference.

Tukey test to determine the effect of concentration series in killing the growth of test bacteria. The results of the tukey test on the total number of colonies produced on *Propionibacterium acne* bacteria. The results of the analysis showed that the concentration series had an effect on the inhibition of the growth of test bacteria, where the increase in concentration showed a difference in inhibitory activity against the growth of *Propionibacterium acne* bacteria.

#### 4. Conclusion

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

- a. Based on the research that has been done, it is concluded that bidara leaf (*Ziziphus mauritiana*) can be formulated into acnes serum because it can meet good physical stability standards including organoleptic tests, which do not experience changes in

aroma, and shape during testing. The pH test of F0, F1, F2, F3 from before and after testing for 4 weeks met the requirements of the skin. In the homogeneity test, the results were obtained, namely preparations that were clear and free of foreign particles.

- b. Antibacterial activity test of bidara leaf serum (*Ziziphus mauritiana*) acnes preparation using different concentrations, namely 2.5% has an inhibition zone of 13.3 mm, 5% has an inhibition zone of 14.5 mm, and 7.5% has an inhibition zone of 14.83 mm. It can be concluded that the concentration of the maximum activity effect of bidara leaf antibacteri (*Ziziphus mauritiana*) is at a concentration of 7.5%..

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