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ANTIBACTERIAL ACTIVITY OF BACANG MANGO (*Mangifera foetida* L.) LEAVES ETHANOL EXTRACT IN GEL FORMULATION AS ANTI-ACNE

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Abstract

Acne was a chronic skin disorder that was most often found in adolescents, so it frequently caused psychological effects that made the acne fighters look for ways to treat it quickly. Although topical antibiotics effectively treat acne, excessive long-term use can cause resistance. This has led to an interest in natural antimicrobials as an alternative treatment for acne. The natural antibacterial activity of the ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves has previously been reported to have antibacterial effects against *Propionibacterium acnes*, but only in the form of extracts not formulated into pharmaceutical preparations. Therefore, this study aimed to re-test the antibacterial activity of this extract, which has now been formulated into a gel preparation against the same bacteria. The gelling agent used in the gel formulation was Na-CMC with three different concentrations: 3%, 4%, and 5%. The Agar stab technique tests the antibacterial activity for three different extract concentrations. Those were 10%, 15%, and 20% for optimization of inhibitory activity before being formulated. Based on the results of testing the antibacterial activity of the ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves, an extract concentration of 20% had a more potent inhibitory activity than the others so that this concentration was chosen to be formulated into a gel preparation. After the formulation of the extracted gel, Na-CMC with a concentration of 5% produced a better gel base than other formulations. In addition, the three formulations provided the same inhibitory activity of 10 mm, so it can be concluded that the differences in gelling agent concentrations did not affect the antibacterial activity of the extracts in the preparations. However, a 3 mm decrease in inhibition occurred in the extract after it was formulated into a gel preparation. Even so, the extract gel preparation still has antibacterial activity against *Propionibacterium acnes* with antimicrobial inhibition zone activity, which is in the moderate category.

Keywords: bacang mango leaf extract, formulation, gel**,** Na-CMC, propionibacterium acnes

Introduction

Acne is the most common chronic skin disorder and requires immediate treatment.[1] Acne is one of the most common skin diseases found in adolescents, so it often causes psychological effects such as distress and decreased self-confidence for the ones who suffer it.[1,2] There are several factors for the appearance of acne, one of which is caused by bacteria. It is believed that one of the anaerobic bacteria, *Propionibacterium acnes*, plays a significant role in the pathophysiology of acne.[3] Thus, topical antibiotics are reliable materials in acne therapy.[4]

Topical antibacterial can be easily obtained without a doctor's prescription for minor skin infections such as acne.[5] Excessive long-term use of topical antibacterials can cause resistance, which is one of the increasing public health problems worldwide.[6] The existence of adverse effects from the misuse of antibacterials raises interest in natural antimicrobials as an alternative in treating acne.[7]

One of the plants that grows a lot in tropical areas like Indonesia is the *bacang* mango (*Mangifera foetida* L.). The antibacterial activity of the ethanol extract of *bacang* mango leaves against *Propionibacterium acnes*.[8] The results of this study indicate the potential of *bacang* mango leaves as a natural antibacterial with a minimum inhibitory concentration of 62.5 mg/ml and an inhibition zone of 6.29 mm, which is still classified as a moderate antimicrobial inhibition zone, and an optimum concentration of 2000 mg/ml and the inhibition zone 13.15 mm, which is classified as having a solid inhibition zone activity.[8] The ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves contains various secondary metabolites such as phenols, flavonoids, saponins, tannins and terpenoids.[8] However, extract preparations cannot be used directly to treat acne as a topical antibacterial, so further processes, such as pharmaceutical preparations or formulations, are needed. A gel preparation is one of the topical preparations that are more readily accepted by the skin because of the use of a polar base in its formulation and the ability to diffuse through the skin membrane more effectively than creams.[9] These preparations also have high stability, smooth dosage forms, are easy to use, can maintain skin moisture, and do not irritate the skin.[10] Therefore, this study examined the antibacterial activity of the ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves, formulated into a gel preparation. The use of *Propionibacterium acnes* in antibacterial activity tests, compared to other bacteria such as those from the genera *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Malassezia*, is because the Gram-positive anaerobic bacterium *Propionibacterium acnes* is the primary inhabitant of the normal human skin microbiota and dominates the pilosebaceous unit found on the face.[3]

Method

Instrument

The equipment used was a petri dish, a porcelain cup, a beaker (Pyrex), a spatula, a pipette, sterile cotton, a micropipette (Socorex, Switzerland), a measuring cup (Iwaki), an incubator (Memmert, Germany), a rotary evaporator (Heidolph, Germany).), ose, calliper, Erlenmeyer (Iwaki), spirit lamp, volumetric flask (Iwaki), HVE 50 autoclave (Hirayama, Japan), oven (Memmert, Germany), water bath (Memmert, Germany), test tube (Pyrex), analytical balance (Precisa, Switzerland), five cc syringe.

Material

The materials used were the simplicia from the *bacang* mango (*Mangifera foetida* L.) leaves, 96% ethanol, distilled water, FeCl3 (Merck), Mg powder (Merck), H2SO4 (Merck), HCl (Merck), medium nutrient agar (NA), sodium chloride, *Propionibacterium*

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acnes pure culture, Na-CMC (Bratachem), glycerin (Bratachem), propylenglycol (Bratachem), methylparaben (Bratachem), mango fragrance.

Procedure

Material Collection

Simplicia from *bacang* mango (*Mangifera foetida* L.) leaves were obtained from the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) Bogor City. However, the plant made into simplicia by the Balai has previously been determined at the Biology Research Center, Indonesian Institute of Sciences, Bogor.

Production of Extract

Simplicia from leaves of *bacang* mango was extracted by maceration technique at room temperature using 96% ethanol solvent. The Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) performed the entire extraction process by applying the simplicia extraction standards available at the institution.

Characterization Test and Phytochemical Screening Extract

The characteristic test was carried out on the viscous extract by determining specific parameters only on the organoleptic test and non-specific parameters on the total ash content test. Phytochemical screening was conducted on the component groups of alkaloids, flavonoids, tannins, saponins, phenols, steroids, and terpenoids.

Antibacterial Activity Test of Extract

Testing the antibacterial activity of the ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves against *Propionibacterium acnes* was carried out using the agar stab technique because the method is simple, the equipment is easy to obtain, and it does not require special tools. Three different concentration variations were made in testing the extract samples: 10%, 15%, and 20%. This was done to optimize the inhibitory activity of the extract at a specific concentration as an antibacterial, as indicated by the formation of an inhibition zone in the area around the stabbed Agar. In addition, clindamycin was used as a positive control, and Na-CMC, which was used to dissolve the viscous extract, was used as a negative control. The medium used in this study was Nutrient Agar (NA), which had been dissolved and sterilized in an autoclave at 121℃ with a pressure of 1 atm for 15 minutes. Antibacterial testing was conducted in the Biological Safety Cabinet (BSC) to minimize microbial contamination. The suspension of the test bacteria was inoculated into 0.1 mL of NA medium, then flattened with a hockey stick and left to dry. The stabbed Agar was made using a sterile pipette tip. 40 µL of ethanol extract of *bacang* mango (*Mangifera foetida* L.) was put into the stabbed Agar that had been made and then incubated for 24 hours at 37℃. Clear zone observations were carried out around the wells. The zone of inhibition, the antibacterial activity, was measured using a calliper three times in different positions.[11]

Formulation of Gel

The formulation of the gel preparation from the ethanol extract of the *bacang* mango (*Mangifera foetida* L.) leaves as an anti-acne can be seen in Table 1. The gelling agent used in the gel formulation is Na-CMC because it has good stability in acidic and alkaline conditions (pH 2-10). As a gelling agent, the concentration of Na-CMC used is 3-6%.[12] Therefore, the concentration variations chosen to optimize the gelling agent in the ethanol extract of mango leaf gel formulation (Mangifera foetida L.) are 3%, 4%, and 5%.

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Table 1. Formulation of Gel Preparations with Varying Gelling Agent Concentrations

Materials	Concentration (%) b/v		
	A	B	C
Ethanol extract of *bacang* mango (*Mangifera foetida* L.) leaves	20	20	20
Na-CMC	3	4	5
Trietanolamin (TEA)	1	1	1
Propilenglikol	15	15	15
Gliserin	10	10	10
Metil paraben	0.25	0.25	0.25
Mango fragrance	qs	qs	qs
Aquadest	ad 100	ad 100	ad 100

Production of Gel

Aquadest that had been heated as much as 20 times the weight of Na-CMC was poured into a hot mortar labelled for each formulation. Na-CMC was added to the mortar, covered, and allowed to stand until it swelled. Furthermore, the Na-CMC was crushed until homogeneous and a good gel mass was formed. *bacang* mango (*Mangifera foetida L*.) leaf extract was added to the gel mass formed and then crushed until homogeneous. Propylene glycol was added to the preparation and crushed until homogeneous before adding TEA. The methylparaben, which had been dissolved with aquadest, was then put into the gel mass, and then the aquadest was added up to 100% of the dosage weight. The grinding continued until it was homogeneous and became an excellent gel preparation.[13]

Evaluation of Gel

Organoleptic Test

The organoleptic test observed the physical appearance of the preparation by observing the shape, colour and smell of the ethanol extract gel of *bacang* mango (*Mangifera foetida* L.) leaves.[14]

Homogeneity Test

The homogeneity test was conducted by weighing 0.1 gram of ethanol extract gel from the *bacang* mango leaves, smearing it on a piece of transparent glass, and then observing it. The absence of coarse grains indicated homogeneity.[14]

pH Measurement

One gram of ethanol extract gel of *bacang* mango leaves was weighed, then dissolved in 10 ml of distilled water and stirred until evenly distributed. The pH paper was dipped into the diluted gel and let stand for a few moments, and the results were seen on the pH indicator strip, which compared to the fields of the colour scale on the pH indicator box. The pH of the preparation that met the criteria for skin pH is 4.5 – 6.5.[14]

Spreadability Test

A total of 0.5 grams of ethanol extract gel from the *bacang* mango leaves was placed on a round glass with a diameter of 15 cm, and another glass was placed on top of the gel and left for 1 minute, then measured the diameter of the gel spread. After being measured, add 50 grams of additional weight, let it stand for 1 minute, and then measure the constant diameter. The spreadability of 5 – 7 cm shows a semisolid consistency which was very comfortable to use.[14]

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Stickiness Test

The gel was placed on top of a glass object, and its area had been determined. Another glass object was placed on top of the gel, put a 500 g load for 5 minutes, attached to the adhesiveness test kit, then removed the 80-gram load and recorded the time it took for the glass objects to detach from each other.[14]

Hedonic Test

The hedonic test was carried out by expressing personal likes and dislikes to 10 panellists and their preference levels. The level of preference was also called the hedonic scale. The hedonic scale is transformed into a numerical scale with increasing numbers according to the preference level.[15]

Irritation Test

The skin irritation test of 10 volunteers was carried out utilizing an open patch test (patch test). An open patch test was carried out by applying the preparation to the inner forearm made at the attachment site with a particular area (2.5 x 2.5 cm), keeping it open, and observing what happens. This test was carried out three times daily (morning, afternoon, evening) for three consecutive days. The presence of redness, itching, or swelling of the skin on the treated forearm shows a positive stimulation response.[15]

Stability Test of Gel

The stability of the preparation was carried out using the cycling test method for six cycles, and the gel preparation was stored at a cold temperature of ± 4 ℃ for 24 hours and then removed and placed at a temperature of ± 40 ℃. This process was calculated as one cycle.

Antibacterial Activity Test of Gel

Testing the antibacterial activity of the gel preparations from the ethanol extract of the *bacang* mango (*Mangifera foetida* L.) leaves was carried out on the three gel formulations using the same method as the antibacterial activity test of the extracts. Even though the media and positive control used were the same, the negative control in testing the activity of the extracted gel was different, such as the gel base or gel preparation, which did not contain extract as an antibacterial agent.

Result

Table 2. The Results of Extraction of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Sample Type	Solvent	Sample Weight	Extract Weight	Yield (%) w/w
Bacang mango leaves (Mangifera foetida L.)	Etanol 96%	1000 gram	120.7 gram	12.07%

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Table 3. Phytochemical Screening of the Ethanol Extract of *Bacang* Mango (*Mangifera Foetida* L.) Leaves

Compound Group	Reagents	Result
Fenol	$FeCl_3$ 3%.	+
Flavonoid	HCl 2N	+
Tanin	$FeCl_3$ 1%.	+
Saponin	HCl 2N	+
Steroid/Triterpenoid	*Liebermann-Boucchard*	+
Alkaloid	*Dragendorf*	+
	Mayer	+

Information: (+) = Detected; (-) = Not detected

Table 4. The Test Results of Antibacterial Activity of The Ethanol Extract of *Bacang* Mango (*Mangifera foetida* L.) Leaves against *Propionibacterium acnes*

Sample	Inhibition Zone Diameter (mm)	Antibacterial Inhibitory Power
Extract 10%	10	Medium
Extract 15%	12	Strong
Extract 20%	13	Strong
Positive control	29.5	Very Strong
Negative control	-	-

Information: (-) = Not detected

Table 5. Organoleptic Test Results of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Formula	Form	Colour	Scent
Formula 1	Slightly Thick	Brown	Smells like mango
Formula 2	Slightly Thick	Brown	Smells like mango
Formula 3	Thick	Brown	Smells like mango

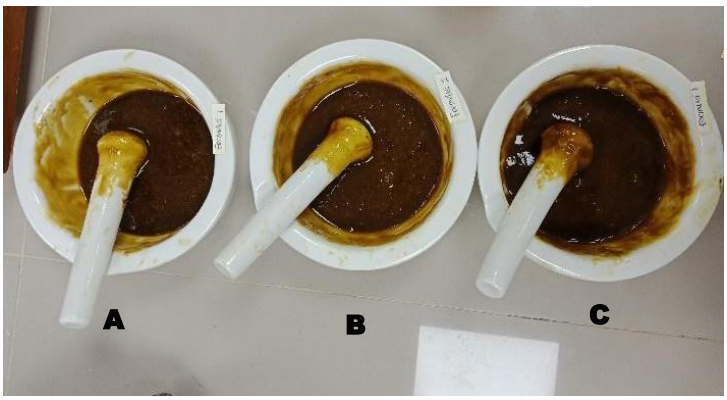

Figure 1. Gel formulation of ethanol extract of *bacang* mango (*Mangifera foetida* L.) Leaves.

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Information: A = Formula A with Na-CMC 3%
B = Formula B with Na-CMC 4%
C = Formula C with Na-CMC 5%

Table 6. Results of Gel Homogeneity Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Formula	Result	Homogeneity
Formula 1	No coarse grain	Homogenous
Formula 2	No coarse grain	Homogenous
Formula 3	No coarse grain	Homogenous

Table 7. Results of pH Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Formula	pH Results
Formula 1	5
Formula 2	5
Formula 3	5

Table 8. Result of Spreadability Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Load	Spreadability Test		
	Formula 1	Formula 2	Formula 3
Glass	5.0 cm	4.8 cm	4.6 cm
Glass + 50 grams of loads	5.6 cm	5.2 cm	5.0 cm

Table 9. Results of Hedonic Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Observation	Formula	Respondents									
		1	2	3	4	5	6	7	8	9	10
Scent	Formula 1	4	4	4	5	4	5	5	3	5	4
	Formula 2	4	4	4	5	4	4	5	3	4	4
	Formula 3	4	3	4	5	4	4	4	3	3	4
Colour	Formula 1	3	4	3	3	4	3	5	2	4	5
	Formula 2	4	4	4	3	4	3	5	2	3	4
	Formula 3	4	4	4	3	4	3	4	2	4	4
Thickness	Formula 1	4	2	1	4	4	2	4	4	3	3
	Formula 2	4	5	4	4	5	3	5	5	4	4
	Formula 3	4	3	5	5	4	5	4	4	5	3
Homogeneity	Formula 1	2	4	3	4	5	2	5	5	4	4
	Formula 2	4	4	4	4	4	3	5	5	4	4
	Formula 3	4	3	4	5	4	5	5	5	4	3

Information: (1) Very Dislike; (2) Dislike; (3) Slightly Like; (4) Like; (5) Very Like.

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Table 10. Results of Irritation Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves

Formula	Respondents									
	1	2	3	4	5	6	7	8	9	10
Formula 1	-	-	-	-	-	-	-	-	-	-
Formula 2	-	-	-	-	-	-	-	-	-	-
Formula 3	-	-	-	-	-	-	-	-	-	-

Information: (+) = Reaction; (-) = No reaction

Table 11. The Results of The Spreadability Test of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L.) Leaves in the Cycling Test

Times	Load	Spreadability Test		
		Formula 1	Formula 2	Formula 3
Cycle 1	Glass	5.0 cm	4.9 cm	4.7 cm
	Glass + 50 gram	5.4 cm	5.2 cm	4.9 cm
Cycle 2	Glass	4.9 cm	4.8 cm	4.6 cm
	Glass + 50 gram	5.2 cm	5.2 cm	5.1 cm
Cycle 3	Glass	5.0 cm	4.9 cm	4.9 cm
	Glass + 50 gram	5.2 cm	5.2 cm	5.1 cm
Cycle 4	Glass	4.9 cm	4.8 cm	4.7 cm
	Glass + 50 gram	5.3 cm	5.2 cm	5.1 cm
Cycle 5	Glass	5.3 cm	5.0 cm	4.9 cm
	Glass + 50 gram	5.6 cm	5.5 cm	5.4 cm
Cycle 6	Glass	5.3 cm	5.1 cm	5.0 cm
	Glass + 50 gram	5.7 cm	5.6 cm	5.5 cm

Table 12. Antibacterial Activity Test Results of Ethanol Extract Gel of *Bacang* Mango (*Mangifera foetida* L) Leaves against *Propionibacterium acnes*

Formula	Inhibition Zone Diameter (mm)	Antibacterial Inhibitory Power
Formula 1	10 mm	Medium
Formula 2	10 mm	Medium
Formula 3	10 mm	Medium
Positive Control	29.5 mm	Very Strong
Negative Control	-	-

Information: (-) = Not detected

Discussion

Determination and Extraction

The determination results showed that the plant used was *bacang* mango (*Mangifera foetida L*.). The extract yield from the *bacang* mango leaves with 96% ethanol solvent looks good, as shown in Table 2, because the yield is > 10%. The higher the yield, the higher the substance content interested in a sample.

Characterization Test and Phytochemical Screening Extract

The organoleptic test was a simple and objective initial introduction. The parameters observed are shape, colour and smell. The organoleptic test of the ethanol

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extract of *bacang* mango (*Mangifera foetida* L.) leaves showed that the extract was thick, concentrated, blackish-green, and had a distinctive smell of *bacang* mango leaves.

The total ash content test was carried out by heating the ethanol extract of the *Bacang* mango (*Mangifera foetida* L.) leaves at a temperature where the organic compounds and their derivatives were destroyed and evaporated. Ash content can describe the presence of mineral content in the extract. The calculation of the total ash content of the extract showed a result of about 4.22%. The size of the ash content indicated the presence of heavy metal contamination that was resistant to high temperatures, so a good ash content must have a small value.[16]

Phytochemical screening showed that in the ethanol extract of the *bacang* mango (*Mangifera foetida* L.) leaves, there were various secondary metabolites, as shown in Table 3. Some of these secondary metabolite compounds were often associated with antibacterial activity.

Antibacterial Activity Test of Extract

Testing the antibacterial activity of the ethanol extract of bacang mango (*Mangifera foetida* L.) leaves was conducted to determine the optimal concentration of the extract with the highest inhibition against *Propionibacterium acnes* bacteria before it was made into a gel preparation. The results of the test show that each concentration has a different diameter of the inhibition zone, as shown in Table 4.

Table 4 showed that the optimal concentration was indicated by the ethanol extract of the *bacang* mango (*Mangifera foetida* L.) leaves with a concentration of 20%. Therefore, the gel was made using the extract with the optimal concentration. The higher the concentration of the extract, the larger the diameter of the inhibition zone against *Propionibacterium acnes* bacteria. This is directly proportional to the results of the antibacterial activity test, which showed that the higher the extract concentration, the larger the inhibition zone.[17] Antimicrobial inhibition zone activity was grouped by David and Stout (1971) into four categories: weak (<5 mm), moderate (5-10 mm), strong (>10-20 mm), very strong (>20-30 mm) activity.[18]

Formulation and Evaluation of Gel

The organoleptic test results for each formulation, as shown in Table 5, showed the same colour in the three formulas, which was brown, as shown in Figure 1. This was caused by using the same extract concentration in each formula, which was 20% extract. The resulting gel preparation produced the same scent: a slightly distinctive smell of roasted mango leaves and smells of mango due to the addition of slight mango scents. Dosage forms differed; formulas 1 and 2 had a relatively thick form, while formula 3 had a thick form. Differences in Na-CMC concentration can cause differences in the form of gel preparations as a gel base; the higher the concentration of Na-CMC, the thicker the resulting gel preparations are.

Based on the homogeneity test results for each formula, as shown in Table 6, all three showed homogeneous results marked by the absence of coarse grains in the gel preparation and had the same colour from the starting point of application to the end point of application.

The pH test results on the ethanol extract gel of *bacang* mango (*Mangifera foetida* L.) leaves showed that the three formulas were at a pH of 5, as shown in Table 7. This followed the pH requirements of topical preparations, namely between 4.5 and 6.5. From these results, it is known that there is no effect of differences in the concentration of Na-CMC in the gel preparation on the resulting pH value.

Based on the results of the spreadability test, each formula showed a difference in the diameter of the spreadability, as shown in Table 8. This could be influenced by

differences in Na-CMC concentration used as a gel base. A spread of 5 – 7 cm shows a semisolid consistency which is very comfortable to use.[14]

The hedonic test results in Table 9 showed that all respondents prefer formula three regarding viscosity and homogeneity. This can be caused by using more Na-CMC than formulas one and two, making the resulting gel thicker and easier to apply to the skin. In addition, all respondents liked the aroma of each formulation because it was given an additional mango scent so that the odour from the extract was covered. As for the colour of the gel, not all respondents liked it because the resulting colour was dark brown, which was influenced by the colour of the ethanol extract of the *bacang* mango (*Mangifera foetida* L.) leaves, thereby reducing the aesthetic colour of the gel preparation.

Based on the results of the irritation test in Table 10, the three formulas did not cause any reactions of redness, itching or redness of the skin in all respondents after applying the gel preparations. No irritation can be caused by a pH of 5 of the gel preparation that meets the pH requirements of topical preparations, between 4.5 and 6.5.

Stability Test of Gel

The stability test was carried out using the cycling test method for six cycles. Furthermore, physical examinations, such as organoleptic, homogeneity, pH, and dispersion tests in each cycle, were carried out. This test was carried out on preparations with different storage temperatures in specific time intervals to accelerate changes that usually occurred under normal conditions.

Observation of the ethanol extract gel preparation of *bacang* mango leaves on organoleptic, homogeneity, and pH tests did not change after six cycles of stability test. The gel preparation was still brown and had a mango aroma. The form of gel formula one and formula 2 was slightly thick, while formula 3 was thick. The three formulas were still homogeneous because there were no coarse grains, the colour was even, and has a pH of 5. Thus, it can be concluded that the ethanol extract gel preparation of *bacang* mango leaves was stable during the test, before and after the cycling test.

The results of the spreadability in the cycling test of the ethanol extract gel preparation of *bacang* mango leaves increased, as shown in Table 11. This could be because the spreadability was strongly influenced by viscosity, so the lower the viscosity value, the higher the spreadability value. Na-CMC was added to water, and Na+ was released into H+ ions, which formed HCMC, increasing the viscosity. The comparison of concentrations between glycerin, propylene glycol, and Na-CMC also determines the stability of the viscosity and spreadability of the gel preparation.[19]

Antibacterial Activity Test of Gel

Based on the results of the antibacterial activity test of the ethanol extract gel preparation of *bacang* mango (*Mangifera foetida* L.) leaves against *Propionibacterium acnes* with an extract concentration of 20%, as well as the difference in the concentration of the use of Na-CMC in each formulation, the inhibition was the same in the three formulas, but a decrease in the diameter of the inhibition zone occurs in gel preparations compared to extracts of 3 mm. In addition, the difference in Na-CMC concentration did not affect the antibacterial activity of the extract in gel preparations.

Conclusion

Based on research results on anti-acne gel formulations, 5% Na-CMC produced the best gel base compared to other formulations. The gel preparation of ethanol extract *bacang* mango (*Mangifera foetida* L.) leaves had antibacterial activity against *Propionibacterium acnes* with an inhibition value of 10 mm. However, compared with the

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antibacterial activity of the 20% extract before it was formulated into a gel preparation, the resulting inhibition value was more significant, about 13 mm.

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