Formulation Of Anti-Acne Extract Aloe Vera (*Aloe vera* (L.) Burm.f.) In Hibiting The Activity Of *Propionibacterium acnes*

Mohd. Bilal[1], Minda Sari Lubis[1*], Rafita Yuniarti[1], Haris Munandar Nasution[1]

[1]Department of Pharmacy, Universitas Muslim Nusantara Al Washliyah., Jalan Garu II Medan Amplas 20147, North Sumatra, Indonesia
*Corresponding author:
Email : mindasarilubis@umnaw.ac.id

Abstract
Aloe vera (Aloe vera (L.) Burm.f.) is widely used as an alternative medicine to treat various types of diseases and is now widely used as an antibacterial, one of which is against the acne bacteria Propionibacterium acnes. The purpose of this study was to determine the gel preparation of aloe vera extract (Aloe vera (L.) Burm.f.) which has activity against Propionibacterium acnes and to see if the gel preparation produced has good and stable quality. Aloe vera ethanol extract was made by maceration method, then the extract was made in a gel preparation and then tested for antibacterial activity against Propionibacterium acnes by the agar diffusion method using several concentration variations: 2.5%, 5%, and 10%. Furthermore, the inhibitory power of 2.5% has a medium category, the inhibition of 5% has a medium category, and the inhibition of 10% has a strong category. then tested for organoleptic, homogeneity test, pH dispersion and physical stability of the preparation. The results of the study showed that the gel preparation of aloe vera extract had antibacterial activity against Propionibacterium acnes, namely a concentration of 2.5% inhibition zone of 8.8 mm including the medium category, 5% concentration of the inhibitory zone of 9.8 mm including the medium category, the concentration of 10 % with an inhibition zone of 12.9 mm including the strong category. The conclusion of this study is that the gel preparation of 10% aloe vera ethanol extract concentration has the best inhibition. Then the stability test was then carried out, which included, the organoleptic did not change significantly, the homogeneity test showed good results, while the dispersion test showed good results, and the pH test showed good results according to skin pH.

Keywords: *Aloe vera, gel, Propionibacterium acnes, extract and physical quality.*

I. INTRODUCTION

Acne is a skin disease that usually affects teenagers or at puberty which is generally caused by several factors such as genetics, hormones, food, and bacterial infections. One of the bacteria that causes acne is Gram-positive bacteria, namely Staphylococcus aureus. These bacteria are commonly found in the respiratory tract, skin surface, and inner skin tissue from purulent ulcers and wound infections [1].

Acne, whose medical language is called acnes vulgaris, is a condition in which the pores of the skin are blocked so that red spots and inflamed abscess on the skin. If that happens, it will mix with make-up, sweat, and pollution and can grow into blackheads. If blackheads are infected by bacteria, they will stick to the skin [2]. The main factors involved in the formation of acne are increased sebum production, sloughing of keratinocytes, bacterial growth and inflammation. Microorganisms such as Staphylococcus epidermidis and Propionibacterium acnes play a role in the pathogenesis of this disease by producing metabolites that can react with sebum, thereby increasing the inflammatory process [3]. Aloe vera (*Aloe vera* (L.) Burm.f.) is a type of plant that has been known for thousands of years, commonly used as hair fertilizer, wound healing, and skin care. This plant is useful as a raw material for the pharmaceutical and cosmetic industries, as well as as a raw material for traditional medicines, food, and health drinks [4].

Aloe vera extract also has an acidity (pH) that is similar to the pH of the skin, so the use of aloe vera is very appropriate to maintain the acidity of our skin [5]. Aloe vera extract contains anthraquinone, aloin, aloe emodin, barbaloin, isobarbaloin, and saponins. Aloin and aloe-emodin are the main anthraquinones in the aloe vera plant. It has a polyphenolic structure, which can inhibit bacterial cell protein synthesis, so it has strong antibacterial and antiviral activity. Saponins contained in aloe vera are soapy substances that have cleansing and antiseptic properties [6].

II. METHOD

Phytochemical Screening Test

1. Alkaloid Examination

Ethanol extract of Aloe vera was weighed 0.5 g added 1 ml HCl 2 N added 9 ml distilled water, then heated on

a water bath for 2 minutes, cooled and filtered the filtrate was used for the examination of alkaloids:

1. 3 drops of filtrate are added with 2 drops of Mayer reagent, a white or yellow lumpy residue will be formed.
2. 3 drops of filtrate are added with 2 drops of Bouchardat reagent, a brown to black residue will be formed.
3. 3 drops of filtrate are added with 2 drops of Dragendroff reagent to form brown or orange.

If there is a residue or turbidity in at least 2 test tubes in the above experiment, the alkaloid is positive [7].

2. Flavonoid Examination

As much as 10 g of ethanol extract of aloe vera was weighed and then added 100 ml of hot distilled water, boiled for 5 minutes, and filtered in a hot state, into 5 ml of the filtrate added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the presence of a red, yellow, or orange color on the amyl alcohol layer.

3. Saponin Examination

As much as 0.5 g of ethanol extract of aloe vera was put into a test tube, then added 10 ml of hot distilled water and cooled, then shaken vigorously for 10 minutes. If the foam is formed with a height of 1-10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl, it indicates the presence of saponins.

4. Tannin Examination

As much as 1 g of ethanol extract of aloe vera with 10 ml of distilled water and then filtered the filtrate was diluted with distilled water until it was colorless. 2 ml of the solution was taken and 1-2 drops of 1% iron (III) chloride reagent were added. If a blue-black or green-black color occurs, it indicates the presence of tannins.

5. Steroid/Triterpenoid Examination

As much as 1 g of aloe vera . Simplicia powder and ethanol extract were each macerated with 20 mL of n-hexane for 2 hours and then filtered. The filtrate is evaporated in a vaporizer cup. To the remainder, a few drops of Liebermann Buchard reagent were added. The appearance of blue or green-blue indicates the presence of steroids, red, pink, or purple colors indicate the presence of triterpenoids [8].

Antibacterial Activity Test

1 Equipment Sterilization

The tools used in this antibacterial activity test were sterilized before being used. Glass utensils are sterilized in the oven at 170˚C for 1-2 hours. Bacterial growth media were sterilized in an autoclave at 121ºC for 15 minutes. While the ose needles are sterilized by burning them in a spirit lamp until they glow.

2. Making Tilt Media

The MHA media was weighed as much as 38 grams, then 1000 ml of distilled water was added. Furthermore, the MHA medium was stirred and heated using a *hot plate*. Furthermore, the MHA media was *autoclaved* for 15 minutes at a temperature of $121^{oC}$to sterilize the media [9].

3. NaCl solution 0.9%

Weighed as much as 0.9 grams of sodium chloride then dissolved in sterile distilled water little by little in a 100 mL volumetric flask until completely dissolved. Sterile distilled water was added up to the marked line, put in a sterile Erlenmeyer with a lid, and then sterilized in an autoclave at 121˚C pressure at 1 atm for 15 minutes.

4. McFarland Standard Suspension

Preparation Method: 9.95 ml of 1% sulfuric acid solution is mixed with 0.05 ml of Bacl2 solution in an Erlenmeyer. Then vortex until the solution is cloudy. If the turbidity of the bacterial suspension test is the same as the turbidity of the standard Mac Farland 0.5 solution, the concentration of the bacterial suspension is $1.5x10^8$ CFU/ml [10].

5. Preparation of Bacterial Suspension

From the stock culture of *Propionibacterium acnes* that has been overgrown, a sterile ose needle is taken and then suspended in a tube containing 10 ml of 0.9% sodium chloride solution until the turbidity of the bacterial suspension is the same as the turbidity of the Mc.Farland standard solution, the weight of the concentration bacteria is 10^8 CFU/ml. After that, the dilution was carried out by pipetting 0.1 ml of bacterial suspension, put into a sterile tube and added 9.9 ml of 0.9% sodium chloride and shaken homogeneously. From here obtained

$10^{6\cdot}$ CFU/ml [11].

6. Antibacterial Activity Testing

bacteria *Propionibacterium acnes* that have been suspended with a 0.9% NaCl solution are inserted into a sterile cotton swab, then rubbed over the solidified Mueller-Hinton Agar media. Then leave it for 30 minutes on the surface of Mueller-Hinton Agar that has been smeared with bacterial suspension. Place a paper backing (paper disc) on the media which had previously been left for 20 minutes in each concentration of aloe vera gel. Left temporarily at room temperature, then incubated in an incubator at 37ºC for 24 hours. After that, the diameter of the area of inhibition of bacterial growth around the paper backer was measured using a caliper. The smallest concentration that still provides obstacles is determined as the minimum level of inhibition [12]

Gel dosage formulation

Table 3.2 Gel dosage formula used

No.	Name of Ingredients	Formula			
		F0	**FI**	**FII**	**FIII**
1	Aloe vera extract	0	2.5	5	10
2	Methyl paraben	0.18	0.18	0.18	0.18
3	Glycerin	1	1	1	1
4	Propylene glycol	1	1	1	1
5	Gel base	30	30	30	30
6	Aquadest ad	100	100	100	100

Information: F0 = Blank
F1 = 2.5% gel preparation
F2 = 5% gel preparation
F3 = 10% gel preparation

7. Preparation of Gel Preparation

0.18 g Methyl paraben dissolved in 5 ml of distilled water and 1 g of propylene glycol was added and stirred until homogeneous (Mass I). 2.5% extract was added; 5%; and 10% was added to the gel base (Mass II), Mass I was added to Mass II was added to Glycerin 1 g and the remaining crushed aquadest was added until it was homogeneous with a pH (4.5-6.5) [13].

8. Physical Quality Test of Aloe Vera Gel Preparation

9. Organoleptic Test The

Gel was observed organoleptically (shape, color, smell and taste).

10. Homogeneity Test

Performed by applying the preparation on a transparent glass, the test preparation must show a homogeneous arrangement [14]

11. Spreadability Test The

Ethanol extract of aloe vera leaf was weighed 0.5 grams and placed in the middle of a round glass that was given a millimeter block, weighed the other glass first, placed the glass on top of the gel mass and left for 5 minutes. After that, add 50 grams of weight, then count again to 5 minutes. Then add a load of up to 150 grams for up to 5 minutes. The dispersion that meets the requirements is 5-7 cm.

12. pH test

The gel preparation is dipped with a pH meter which is still in a neutral state. The pH criteria needed are 4.5-6.5, according to the pH of the skin.

III. RESULTS

Table 1 . Results of Phytochemical Screening of Powder and Ethanol Extract of Aloe vera

No.	Group of Chemical Compounds	Identification
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponin	+
5.	Steroids/Triterpenoids	+

Description:
(+) = contains the substance being examined
(-) = does not contain the substance examined

Table 2 The result of inhibition of aloe vera ethanol extract gel against *Propionibacterium acnes.*

Bacteria	Extract concentration	I	II	III	Average (mm)
Propionibacterium acne	2.5%	8.5	8.9	9.0	8.8
Propionibacterium acne	5%	9.0	9.9	10.5	9.8
Propionibacterium acne	10%	12.3	12.9	13.7	12.9

Graph of Minimum Inhibitory Concentration Test of Aloe Vera Ethanol Extract against *Propionibacterium acnes* can be seen in Figure 3.1:

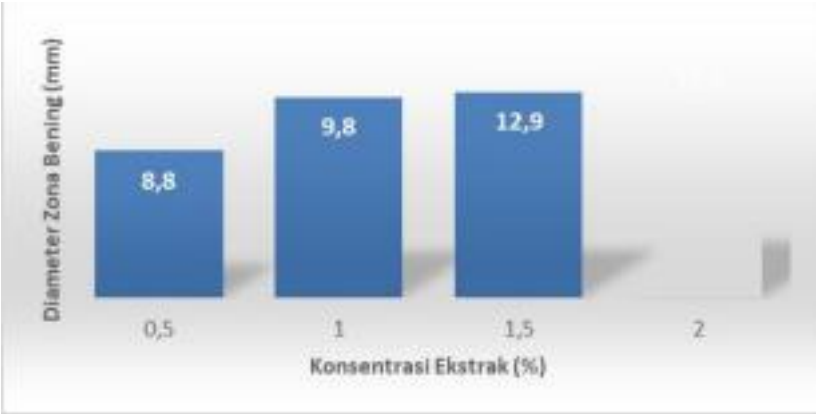

Fig 1. Graph of inhibition of aloe vera ethanol extract against *Propionibacterium acnes*

The results of the study show that aloe, aloe vera has antibacterial activity against *Propionibacterium acnes*, namely a concentration of 2.5% inhibition zone of 8.8 mm including the medium category, 5% concentration of inhibition zone of 9.8 mm including moderate category, and 10% concentration with an inhibition zone of 12.9 mm included in the strong category.

In table 2 it can be observed that the concentration of 10% aloe vera ethanol extract indicates that with this concentration it has the greatest inhibitory power which means it is better than the other concentrations. Where it can be concluded that the higher the concentration, the greater the resulting inhibition.

Table 3 Results of Organoleptic Tests for Organoleptic

Time Observation Formulas	Viscous Cycle	for						
		0th Cycle	1st Cycle	2nd Cycle	3rd Cycle	4th Cycle	5th Cycle	6th Cycle
F0	Forms	Viscous	Viscous	Viscous	Viscous	Thick	Thick	Thick
	Color	Clear Bening	Clear	Clear	Clear	Clear	Clear	Clear
	Odor	Characteristic base	Typical base	Typical base	Typical base	Typical base	Typical base	Typical base
FI	Shape	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous
	Color	Light green green	green green	green green	Light green	Light green	Light Odor	Light Characteristic
	extract	Typical Light	Light extracts	Typical extracts	Typical extracts	Typical extracts	Typical _	_ extracts
FII	Forms	Viscous	Condensed	Viscous	Condensed	Viscous	Viscous	Viscous
	Color	green light	green light	green light	green light	green light	green light	green light
	odor	characteristic extract	typical extract	typical extract	typical extract	typical extract	typical extract	typical extract
FIII	Form	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Dark
	Dark	green green	green green	green green	green green	Dark Dark	Dark Odor	Typical extrac
	Typical	extract K	Dark Dark	Darkhas extract	Typical extract	Typical extract	Typical extract	The shaped

Organoleptic observation of the gel preparation in the table shows that before and after storage of the preparation for 6 cycles where one cycle was 1 x 24 hours, the preparation did not experience significant changes, namely with a dark green color,thick and distinctive smell of aloe vera extract.

This shows that the parameters of the preparation are said to be both before and after storage, or the components in the preparation during storage do not experience a reaction between one material and another, so that there are no signs of a reaction from a color change.

So far, the viscosity is still stable because the *gelling agent* keeps the preparation stable.

Previous researchers [15] said organoleptically stable preparations, namely preparations that had been stored did not experience significant changes.

Table 4. Results of Homogeneity Testing

Formula	Results
F0	Homogeneous
FI	Homogeneous
FII	Homogeneous
FIII	Homogeneous

Description:
= preparations without extract
FI = preparations with 2.5% extract
FII = preparations with 5% extract
FIII = preparations with 10% extract

Based on homogeneity examination of Aloe vera extract gel preparations showed that all preparations did not show the presence of coarse grains when the preparations were smeared on transparent glass. This indicates that the preparation does not contain coarse grains when the preparation is smeared on transparent glass which results in a homogeneous arrangement.

It is said [16] observation of homogenity is must doing to look after formulation of gel mixed well and homogenity remark with no small particles within gel formulation.

Table 5. Spreadability Test Results of Gel Preparations

Formula	Spreadability
	Average
F0	6.5
FI	6.6
FII	6.7
FIII	7

Information:
F0 = preparation without extract
FI = preparation with extract 2.5%
FII = preparation with extract 5 %
FIII = preparation with 10% extract

Based on the results obtained by testing the spreadability of the gel preparation, it was obtained that the diameter of dispersion was that the F0 preparation had an average dispersion of 6.5 cm, the gel preparation of 2.5% F1 had an average dispersibility of 6, 6 cm, 5% FII gel preparations had an average dispersion of 6.7 cm, and 10% FIII gel preparations had an average dispersibility of 7 cm.

From the results obtained, it can be concluded that the preparation meets the dispersion requirements. The dispersion test of the preparation was 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.

According to previous researchers [17] the requirements are like a gel preparation if a semi-solid preparation

that is good for topical use has a spreadability range of 5-7cm.

Table 6. Results of pH Size Preparations Gel

Formula	Mean
	I
F0	6.7
FI	6.7
FII	6.2
FIII	6.3

Information:
F0 = preparations without extract
FI = preparations with 2.5% extract
FII = preparations with extract 5 %
FIII = preparation with 10% extract

Based on Table 4.7 the results of determining the pH of the gel preparation have an average F0 pH value of 6.7 and an FI pH value of 6.2.
Where the pH values of F0,F1,F2, and F3 have met the requirements such as skin pH, which is 4.5-6.5. This pH measurement test is carried out because the preparations made must follow the resistance of human skin so that irritation or redness does not occur and even avoids skin allergies if the pH is too acidic.
In accordance with previous researchers [18] a good pH value for gel preparations with conditions such as the pH of the skin in general is in the range of 4.5-6.5.

IV. CONCLUSION

1. Aloe vera ethanol extract gel preparation has an inhibitory power against *Propionibacterium acnes* where the diameter of the inhibition produced from each concentration is 2.5% concentration, the inhibition zone is 8.8 mm including the medium category, 5% concentration inhibition zone is 9, 8 mm
is in the medium category, and the concentration is 10% with an inhibition zone of 12.9 mm.
2. Aloe vera extract gel preparations 2.5%, 5%;, and 10% can be formulated into stable gel preparations, which based on the observed physical stability tests include, organoleptic (shape, smell, color) did not change significantly , the homogeneity test showed good results, while the dispersion test showed the average dispersion was 6.6 cm, 6.7 cm, and 7 cm, respectively. (which means the results are good), and from the pH test the average sequentially is 6.7;, 6.2;, 6.3;. (according to skin pH).

REFERENCES

[1] Jawetz, E., Melnick, J. L. Dan Adelberg, E. A., 2005, Mikrobiologi Kedokteran, diterjemahkan oleh Mudihardi, E., Kuntaman, Wasito, E. B., Mertaniasih,

[2] Sampelan, M. G. Pangemanan, D. Dan Kundre, R. M.(2017). Hubungan Timbulnya Acne vulgaris Dengan Tingkat Kecemasan Pada Remaja Di SMP N 1 Likupang Timur. ***E-Journal Keperawatan (e-Kp),*** 5(1), 2.

[3] Widiawati, W. (2014). Perbedaan Hasil Penyembuhan Kulit Wajah Berjerawat Antara Masker Lidah Buaya Dengan Masker Non Lidah Buaya. E- Journal Tata Rias, 3(1), 218.

[4] Natsir, N.A. (2013). Pengaruh Ekstrak Daun Lidah Buaya (Aloe vera) sebagai Penghambat Pertumbuhan Bakteri Staphylococcus aureus. Prosiding FMIPA Universitas Pattimura 2013.

[5] Soviati, Iceu. 2008. Olahan dari Lidah Buaya. Tangerang: PT Panca Anugerah Sakti

[6] Fani, M; Kohanteb, J. (2012). Inhibitory Activity of Aloe vera Gel on Some Clinically Isolated Cariogenic and

Periodontopathic Bacteria. ***Journal of Oral Science***, Vol. 54, No. 1, 15-21.
[7] Ditjen POM. 1979. Farmakope Indonesia. Edisi III. Jakarta : Departemen Kesehatan RI.
[8] Depkes RI. 1989. Materia Medika Indonesia. Edisi V. Jakarta : Kementrian Kesehatan RI.
[10] Mahmudah, F. L., & Sri, A. (2017). Uji aktivitas Antibakteri dari Ekstrak Etanol Temukunci (Boesenbergia pandurata) terhadap Bakteri Streptococcus mutans. ***Jurnal Penelitian Saintek***, 22(1), 59-66.
[11] Borges, M.T., dan Bresson, W. (2004). Delivery Metodhs for Introducing Endhopitic Bacteria. Journal Internasional : Biocontrol. Hal 315-322
[12] Silaban L.W. (2009). Skrining Fitokimia dan Uji Aktivitas Antibakteri dari Kulit Buah Sentul (Sandaricum koetjae) Terhadap Beberapa Bakteri Secara inVitro. Medan : Universitas Sumatera Utara. Hal 34
[13] Esterina. 2018. Uji Aktivitas Antibakteri Sediaan Gel Ekstrak Etanol 70% Daun Bangun-Bangun (Plectranthus amboinicus (Lour.) Spreng.) terhadap Bakteri Staphylococcus aureus Dan Pseudomonas aeruginosa. Jurnal Penelitian Farmasi : Vol 1, No 2.
[14] Borman, Ikaolivia. 2015. Gel Anti Jerawan Ekstrak Daun Buta Buta (Excoecaria agallocha L.) dan Pengujian Antibakteri Staphylococcus epidermidis. ***GALENIKA Jurnal Ilmiah Farmasi***, Vol. 1 (2) : 65-72.
[15] Iramie, D.K.I., Purwanto. Marwan, T.M. 2020. Aktivitas Antibakteri dan Uji Sifat Fisik Sediaan Gel Dekokta Sirih Hijau (Piper betle L.) Sebagai Alternatif Pengobatan Mastitis Sapi. ***Majalah Farmausetik*** : Vol. 16, No. 2.
[16] Astuti, D. P., Husni, P., dan Hartono, K. 2017. Formulasi dan uji stabilitas fisik sediaan gel antiseptik tangan minyak atsiri bunga lavender (Lavandula angustifolia Miller). Farmaka, 15(1), 176-184.
[17] Minda, S. L., Khoyrill, M. Formulasi Dan Aktivitas Antibakteri Sediaan Gel Ekstrak Etanol Herba Rumput Bambu (Lopatherum gracile Brongn) Terhadap Bakteri Propionibacterium acnes. ***Farmasainkes*** No.1 Vol.1 Agustus 2021. 6-7.
[18]Garg, A. K., Kim, J. K., Owens, T. G., Ranwala, A. P., Do Choi, Y., Kochian, L. V., dan Wu, R. J. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proceedings of the National Academy of Sciences, 99(25): 15898-15903.
[19] Wardiyah, S. 2015. Perbandingan sifat fisik sediaan krim, gel, dan salep yang mengandung etil p-metoksisinamat dari ekstrak rimpang kencur (Kaempferia galanga linn.). Bachelor's thesis. UIN Syarif Hidayatullah Jakarta: Fakultas Kedokteran dan Ilmu Kesehatan.