INTRODUCTION

Averrhoa bilimbi is known in Indonesia as Belimbing Wuluh or Asam Sunti, a plant that is often used for cooking spices and traditional medicine in Indonesia. The purpose of this study was to obtain a gel formula and measure its activity in inhibiting the growth of Staphylococcus aureus and Propionibacterium acnes.

METHODS

The gel formulation was tested organoleptically, homogeneity, pH, dispersibility test, and skin irritation test. Antibacterial activity test using agar diffusion method, inhibition of bacterial growth was compared with clindamycin as a positive control.

RESULTS: All gel formulations showed good homogeneity, pH of the preparations ranged from 4.5 to 6.1, the dispersion test was 5.5-6.5 cm, and none of them caused irritation to the skin. The results of the inhibitory activity against the growth of Propionibacterium acnes bacteria on concentrations 1.0, 1.5, and 2.0% were 16.67 + 0.40, 22.70 + 0.32 and 28.10 + 0.36 and Staphylococcus aureus bacteria were 18.53 + 0.22, 24.16 + 0.29 and 30.40 + 0.4. The inhibitory activity of clindamycin against Propionibacterium acnes and Staphylococcus aureus bacteria were 35.33 +0.29 and 36.30 +0.37.

CONCLUSION: Ethyl acetate extract gel Averrhoa bilimbi had good activity in inhibiting the growth of Propionibacterium acnes and Staphylococcus aureus bacteria and has the potential to be used as an herbal anti-acne drug.

Keywords: Antibacterial activities, Averrhoa bilimbi, Gel formulation, Propionibacterium acnes, Staphylococcus aureus
The irritation test was carried out on 10 volunteers who each affixed an obtained. The spreadability of 5-7 cm indicates a semisolid addition of the load is continued until a constant diameter is recorded. The number shown by the pH meter is the pH of the preparation. The pH of gel must be in accordance with the value until it is constant. The tool is allowed to show the pH value. Then the electrode is washed with distilled water, then dried with a tissue. 1 gram of gel to be examined was diluted with 100 ml of distilled water. The electrodes are immersed in the solution being examined, the tool is allowed to show the pH value until it is constant. The number shown by the pH meter is the pH of the preparation. The pH of gel must be in accordance with the skin pH, specifically 4.5-6.5 [12, 13].

Gel formula evaluations

The evaluation of the formula included organoleptic test, homogeneity, pH value; surface disperse ability, and skin irritation test. Organoleptic test was carried out by direct observation to the shape, color, and smell of the gel made. The characterization of the gel formula was studied in more depth after refining the formula in further research. The gel is usually clear with a semi-solid consistency. The homogeneity test is carried out by applying the gel sample to a piece of glass or other suitable transparent material, the preparation must show a homogeneous structure and no coarse grains are visible [12, 13]. The pH meter was first calibrated using a standard neutral buffer solution (pH 7.01) and an acid buffer solution (pH 4.01) until the tool shows the pH value. Then the electrode is washed with distilled water, then dried with a tissue. 1 gram of gel to be examined was diluted with 100 ml of distilled water. The electrodes are immersed in the solution being examined, the tool is allowed to show the pH value until it is constant. The number shown by the pH meter is the pH of the preparation. The pH of gel must be in accordance with the skin pH, specifically 4.5-6.5 [12, 13].

0.5 grams of gel is weighed and placed in the middle of a round glass. The round glass cover is weighed first, then placed over the gel mass and left for 1 minute. The length of the diffused gel diameter was measured, then 150 grams of additional weight was added, left for 1 minute and the diameter of the gel spread was recorded. The addition of the load is continued until a constant diameter is obtained. The spreadability of 5-7 cm indicates a semisolid consistency, which is very comfortable in use [12, 13].

The irritation test was carried out on 10 volunteers who each affixed the test material, namely F0 (without extract), F1 (10% extract), F2 (15% extract), F3 (20% extract), the gel was applied to the back of the volunteer’s hands 3 times a day in an interval of 8 h for three consecutive days. Look for changes that occur, in the form of irritation with characteristics the skin becomes rough, itchy, and reddish [12, 13]. This irritation test has been approved by Health Research Ethical Committee of North Sumatera/RSUP H Adam Malik c/o Medical School, Universitas Sumatera Utara No 105/Komet/FK USU/2019.

Antibacterial activity test

All tools used for the antibacterial test were washed with clean water, then wrapped in paper. Tools made of glass (heat resistant) are sterilized in the oven at a temperature of 160-170 °C for 1-2 h, the bacterial growth media are sterilized in autoclave at 121 °C for 15 min, and round loop or loop needles are sterilized on flame fire [13, 14].

The nutrient agar (NA) is dissolved with distilled water (23 g/1000 ml) in an erlenmeyer then cover the erlenmeyer tightly using a cotton pad covered with paper and tie it with a rope. Then homogenize it on a water bath until it boils. Then sterilize it in an autoclave for 15 min at 121 °C [15].

Each bacterial culture was taken from the media to slant using a round loop then put into a test tube containing 10 ml of 0.9% NaCl, stirring until homogeneous. The level of the suspension solution was adjusted to the turbidity of the Mc Farland solution [16, 17]. Prepare a sterilized petri dish. Put 0.1 ml of the bacterial suspension into a petri dish. Then 20 ml of NA media were added, stirring until homogeneous and allowed to solidify. Furthermore, a pet is made using a metal holder. Then the well that has been made is inserted with a gel preparation as much as 0.05 g. Then it is incubated in an incubator for 24 h at a temperature of 35-37 °C and measured the diameter of the resistance zone (clear zone) that is formed [13].

Data analysis

The data obtained is presented in tabular form, then the data obtained from the research results are processed by statistical analysis of variance (ANOVA) tests.

RESULTS AND DISCUSSION

Gel Formula evaluations

Organoleptic test results of Averrhoa bilimbi L. fruit ethyl acetate extract gel are the resulting gel had a semi-solid form, brown to blackish-brown in color and the resulting odor was the distinctive smell of the extract. The pH of each formula is in the range 4.5-6.1. The pH of gel must be in accordance with the skin pH, specifically 4.5-6.5 [12, 13]. The diameter of the surface dissipation power is 5.5-6.5 cm. Neither formula caused skin irritation in the study process. The results of the organoleptic test can be seen in table 2. The results of the homogeneity, pH, and dispersion can be seen in table 3, and the results of skin irritation test in table 4.

Table 2: Organoleptic test results

| Formulas | Consistence | Color | Odor |
|----------|-------------|-------|------|
| F0       | Semisolid   | Transparent | Odorless |
| F1       | Semisolid   | Brown transparent | the distinctive smell of the extract |
| F2       | Semisolid   | Blackish brown transparent |
| F3       | Semisolid   | Blackish brown transparent |

Table 3: The result of homogeneity, pH, and disperse of gel

| Formula | Homogeneity | pH Value | Disperse Value |
|---------|-------------|---------|---------------|
| F0      | Homogeneous | 6.05±0.03 | 6.53±0.03 |
| F1      | Homogeneous | 4.86±0.03 | 6.06±0.03 |
| F2      | Homogeneous | 4.70±0.03 | 5.76±0.03 |
| F3      | Homogeneous | 4.53±0.03 | 5.46±0.03 |

The data of pH and disperse value presented were the average pH value±SEM where each group was repeated three times.
There was no significant difference between the test groups in physical appearance and the value of pH and disperse measurements \( (p>0.05) \), indicates that each formula has the same characteristics. The physical test results prove that the gel preparation meets the gel quality test parameters, namely the organoleptic test, the form is semi-solid, the color and smell are in accordance with the concentration of the extract it contains. Based on these results, it was concluded that the more extracts that were added to each formula, the color would be darker. The results of homogeneity examinations of each preparations made had a homogeneous structure \[18, 19\].

The pH value shows that the higher the extract concentration contained in the gel, the lower the pH value. The change in pH value is not significantly different \( (p>0.05) \). The pH value of a topical pharmaceutical product should correspond to a skin pH of 4.5–6.5. Based on table 4.3, the pH measurement results of each formula show the pH value of each gel formula according to the skin’s pH range so it is safe to use \[20–22\]. The dispersion test also did not show any significant difference for each formula \( (p>0.05) \); however, based on the smaller the dispersion value as the extract concentration increased, it showed that there was a slight change inconsistency when the dispersion was carried out. The gel formula that was formed met the dispersion test parameters \( (5–7 \text{ cm}) \). The test was carried out on all the gel formulas. The additional load on the extract will also increase the spread area of the gel. Increasing the load illustrates a dispersive characteristic of semi-solid preparations. The more the gel spreads due to the additional load, the conclusion is that its ability to distribute the drug is more evenly distributed. The test is carried out to see the consistency of the preparation, as well as to observe the dispersion of the preparation when applied to the skin so that it is hoped that the area on the skin will get the active substance with the same dose evenly. The test results show that the gel has consistency and results in good surface dispersion \[23–25\].

### Table 4: The result of skin irritation test

| Observations    | Formulas | Volunteer                   |
|-----------------|----------|-----------------------------|
|                 | 1        | 2                           | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Reddish Skin    | F0       | -                           | - | - | - | - | - | - | - | - |
|                 | F1       | -                           | - | - | - | - | - | - | - | - |
|                 | F2       | -                           | - | - | - | - | - | - | - | - |
|                 | F3       | -                           | - | - | - | - | - | - | - | - |
| Rough Skin      | F0       | -                           | - | - | - | - | - | - | - | - |
|                 | F1       | -                           | - | - | - | - | - | - | - | - |
|                 | F2       | -                           | - | - | - | - | - | - | - | - |
|                 | F3       | -                           | - | - | - | - | - | - | - | - |
| Itchy Skin      | F0       | -                           | - | - | - | - | - | - | - | - |
|                 | F1       | -                           | - | - | - | - | - | - | - | - |
|                 | F2       | -                           | - | - | - | - | - | - | - | - |
|                 | F3       | -                           | - | - | - | - | - | - | - | - |

Explanation+: Irritation Symptoms occur (reddish, rough, and itchy). -: There are no symptoms or irritation

### Table 5: Antibacterial activity of A. bilimbi gel

| Formulas  | Propionibacterium acnes+SEM | Staphylococcus aureus+SEM |
|-----------|-----------------------------|---------------------------|
| Positive Control | 35.333\pm0.2905*9          | 36.300\pm0.37859*         |
| F0 (Negative Control) | 0000\pm0.000            | 0000\pm0.000             |
| F1 (10%)  | 16.667\pm0.2557*          | 18.533\pm0.21858*        |
| F2 (15%)  | 22.700\pm0.32146*         | 24.166\pm0.29059*        |
| F3 (20%)  | 28.100\pm0.36056*         | 30.400\pm0.40415*        |

The data presented is the value of inhibition diameter \( (\text{mm})\)\+SEM. * significantly different to negative control \( (p<0.05) \).

The skin irritation test was carried out by applying a gel preparation on the backs of the volunteers’ hands for three consecutive days, the results showed that all volunteers had a negative effect on the parameters of the irritation reaction. From the results of the irritation test, it can be concluded that the gel preparation is safe for topical use \[26–28\].

### The results of antibacterial activity

The results of the antibacterial activity test of Averrhoa bilimbi ethylacetate extract gel against Propionibacterium acnes and Staphylococcus aureus can be seen in table 5. MIC values were not calculated from this study. This study was limited to the ratio of the inhibition zone diameter of the gel to the growth of bacteria in nutrient agar media.

The positive control used was a gel containing clindamycin (Mediklin®) and the negative control used was a gel base without any active substances. Inhibition value resulted from the test showed that positive control had a very strong ability compared to other groups, and negative control did not have inhibitory power against bacterial growth. The formula containing the test sample showed significant inhibition \( (p<0.05) \) compared to the negative control. Based on the results data, it can be concluded that the higher the extract concentration in a given preparation, the greater the antibacterial activity of the gel. This is in accordance with the theory put forward by Pelczar and Chan that the greater the concentration of antimicrobial compounds being tested, the greater the antimicrobial activity of these compounds \[29, 30\].

The clear zone around the well is caused by the active substance content of Averrhoa bilimbi fruit ethyl acetate extract gel, which contains flavonoids, alkaloids, saponins, terpenoids and tannins which can function as antibacterial \[3, 31\]. Flavonoid compounds have the ability to form complexes with bacterial cell proteins through hydrogen bonds. The structure of the cell wall and bacterial cytoplasmic membrane, which contains protein, becomes unstable because the protein structure of bacterial cells is damaged due to hydrogen bonds with flavonoids, so that the bacterial cell protein loses its biological activity, as a result, the function of bacterial cell permeability is disrupted and results in bacterial cell death \[32–34\]. Alkaloids have antibacterial abilities and inhibitory mechanisms by interfering with the components of the peptidoglycan composition...
in bacterial cells so that the wall layer is not formed completely and causes bacterial death [35-37].

CONCLUSION

Gel with active ethyl acetate extract of *Averrhoa bilimbi* has good formula characteristics and fulfills the requirements in all formula evaluations. The gel shows no symptoms of skin irritation and meets simple basic requirements (organeleptic, homogeneity and pH). This gel also has good antibacterial activity so that it can inhibit the growth of the *Propionibacterium acnes* and *Staphylococcus aureus* bacteria which are generally a problem on the skin such as wounds and acne. Based on the research results, it can be considered as the cause of herbal remedies to treat wounds and acne. However, it is necessary to develop a more in-depth formula and test.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All the authors had no to declare.

REFERENCES

1. Xu E, Wijaya CH, Faridah DN. Characterisation of aroma compounds in traditional Indonesian seasoning (asam sunti) made from *Averrhoa bilimbi*. *Emerates J Food Agric Sci*. 2017;29:376-80.
2. Samuel AJS, Kalasalingam A, Chellapan DK, Gopinath R, Radhamani S, Husain A, et al. Ethnomedical survey of plants used by the orang asli in kampung bawong, perak, West Malaysia. *Ethnobiol Etnomedi*. 2006;1:6-10.
3. Alhassan MA, Ahmed QU. *Averrhoa bilimbi* limon: a review of its ethnomedicinal uses, phytochemistry, and pharmacology. *J Pharm Bioallied Sci*. 2016;8:265-71.
4. Miraj AJ, Kabir A, Mamun Y, Akhter S, Ahammad MS, Sultana S, et al. Evaluation of the analgesic and anti-inflammatory activities of methanolic extracts of the leaves of *averrhoa bilimbi*. *Discovery Phytochem*. 2019;6:12-5.
5. Yulinangtyas A, Kusmartono B. Optimization of solvent volume and maceration time on extraction of flavonoids from *averrhoa bilimbi* leaves. *J Tek Kim*. 2016;10:68-44.
6. Rahman M, Habib R, Hasan A, Al Amin M, Saha A, Mannan A. Comparative assessment on *in vitro* antioxidant activities of ethanol extracts of *averrhoa bilimbi*, gynnema sylvestre and capiscum frutescens. *Pharmacognosy Res*. 2014;6:36-41.
7. Seebeck Sandoz R, Lall N, Fribich B, van Staden AB, Saleem H, Mamoudally MF. Antimicrobial, antioxidant and cytotoxic evaluation of two underutilised food plants: *averrhoa bilimbi* and *gymnema sylvestre*. *Biocatal Agric Biotechnol*. 2019;18:100998.
8. Ikhlasudin A, Mardiyah S. Formulasi dan uji antijerawat gel ekstrak etanol 70% buah belimbing wuluh (*Averrhoa Bilimbi*). *J Tek Kim*. 2017;5:416-26.
9. Hasim, Rifin YY, Andrianto D, Faridah DN. Ethanol extracts of *averrhoa bilimbi* leaf demonstrated antioxidative and anti-inflammatory activity. *Appl Teknol Pangan*. 2019;8:86-93.
10. Kusuma SAF, Abdassah M, Valas BE. Formulation and evaluation of anti-acne gel containing citrus aurantium fruit juice using carbopol as gelling agent. *Int J Appl Pharm*. 2018;10:147-52.
11. Iyothi D, Kolan M. Formulation and evaluation of an herbal anti-inflammatory gel containing trigonella foenum graecum seed extract. *Int J Pharm Pharm Sci*. 2016;8:41-4.
12. Kaur D, Raina A, Singh N. Formulation and evaluation of carbopol 940 based glibenclamide transdermal gel. *Int J Pharm Pharm Sci*. 2014;6:434-40.
13. Pertwii D, Hafiz I, Salma R. Antibacterial activity of ethanol extract of papaya leaves (*Carica papaya* L) gel against *p. acnes*. *Indones J Pharm Clin Res*. 2019;2:1-6.
14. Saising J, Hinnrat A, Mahabusarakam W, Ongkalud K, Voravuthikunchate SP. Rhodomyrtone from rhodomyrtus tomentosa (*Alston*) hassk as a natural antibiotic for staphylocooccal cutaneous infections. *J Heal Sci*. 2008;54:589-95.
15. Widiandito BK, Elimasa RV, Rahmawati MD. Effectivity *in vitro* of *averrhoa bilimbi* ethanolic extract against escherichia coli and *staphylococcus aureus* growth. *Prosiding Seminar Nasional dan Internasional Universitas Muhammadiyah Semarang*. 2017; p. 140-8.
16. Mc Farland J. *The nephelometer: an instrument for estimating the number of bacteria in suspension used for calculating the opsonic index and for vaccines*. *JAMA*. 1907;XLIX:1176-8.
17. Jak 1, Orjala J, Bürgi HR, Sticher O. Biological screening of cyanobacteria for antimicrobial and molluscicidal activity, brine shrimp lethality, and cytotoxicity. *Pharm Biol*. 1999;37:138-43.
18. Draget KL, Ostgaard K, Smidt H. Homogeneous alginate gels: a technical approach. *Carbohydrate Polym*. 1990;1:149-57.
19. Schnepz Z, Yang Z, Hollamby MJ, Paw B, Tanaka M, Matsushita Y, et al. Doped-carbon electrocatalysts with trimodal porosity from a homogeneous polypeptide gel. *Mater Chem A*. 2013;4:13576–81.
20. Hanatana T, Morikita S, Aiba T, Katayama K, Koizumi T. Effect of pH on the skin permeability of a zwitterionic drug, cephalaxin. *Int J Pharm*. 1995;125:195–203.
21. Schmaljohann D. Thermo- and pH-responsive polymers in drug delivery. *Adv Drug Delivery Rev*. 2006;58:1655–70.
22. Martinez PA J, Martín Biosca Y, Sagra S, Villalaveya Canamas RM, Medina Hernandez MJ. Evaluation of the pH effect of formulations on the skin permeability of drugs by partitioning micellar chromatography. *J Chromatogr A*. 2004;1047:2255–62.
23. Souto EB, Wissing SA, Barbosa CM, Muller RH. Evaluation of the physical stability of SN and NLC before and after incorporation into hydrogel formulations. *J Eur Pharm Biopharm*. 2004;58:89–90.
24. Edityaningrum CA, Kintoko K, Zulien F, Widyastuti L. Optimization of water fraction gel formula of binahong leaf (*Anredera cordifolia* (Tenn.) Steen) with gelling agent of sodium alginate and carboxymethyl chitosan combination. *Tradit Med J*. 2018;23:97–105.
25. Piau JM. Carbopel gels: elastoviscoplastic and slippery glasses made of individual swollen sponges: meso- and macroscopic properties, constitutive equations and scaling laws. *J Non-Newtonian Fluid Mechanic*. 2007;144:1–29.
26. Verschoore M, Ponct E, Czernielewski J, Sorba V, Clucas A. Antimicrobial, anti-inflammatory and HPTLC fingerprint of *averrhoa bilimbi* (L.) fruits. *J Pharm Bioallied Sci*. 2013;5:145–50.
27. Soliman SM, Abdel Malak NS, El Gazayerly ON, Abdel Rahim AA. Formulation of microemulsion gel systems for transdermal delivery of cephalexin: *in vitro* permeation, anti-inflammatory activity and skin irritation tests. *Drug Discovery Ther*. 2018;14:459-71.
28. Dreher F, Walde P, Luis PL, Elsner P. Human skin irritation studies of a lecitin microemulsion gel and of lecithin liposomes. *Skin Pharmacol Physiol*. 1996;9:124–9.
29. Peczkar MJ, Chan ECS, Krieg NR. *Microbiology. New York: McGraw-Hill*. 1993; p. 578.
30. Masoodi MH, Ahmed B, Zargar IM, Khan SA, Khan S, Singh P. Antibacterial activity of whole plant extract of *marrubium vulgare*. *Afr J Biotechnol*. 2008;74:100998.
31. Draget KL, Ostgaard K, Smidt H. Homogeneous alginate gels: a technical approach. *Carbohydratre Polym*. 1990;14:159-78.
32. Alhassan MA, Ahmed QU. *Averrhoa bilimbi* limon: a review of its ethnomedicinal uses, phytochemistry, and pharmacology. *J Pharm Bioallied Sci*. 2016;8:265-71.
33. Norren K, Van Leeuwen PAM. Flavonoids: a review of probable mechanisms of action and potential applications. *Am J Clin Nutr*. 2011;94:170–71.
34. Verschoore M, Ponct E, Czernielewski J, Sorba V, Clucas A. Antimicrobial, anti-inflammatory and HPTLC fingerprint of *averrhoa bilimbi* (L.) fruits. *J Pharm Bioallied Sci*. 2013;5:145–50.
35. Soliman SM, Abdel Malak NS, El Gazayerly ON, Abdel Rahim AA. Formulation of microemulsion gel systems for transdermal delivery of cephalexin: *in vitro* permeation, anti-inflammatory activity and skin irritation tests. *Drug Discovery Ther*. 2018;14:459-71.
36. Dreher F, Walde P, Luis PL, Elsner P. Human skin irritation studies of a lecitin microemulsion gel and of lecithin liposomes. *Skin Pharmacol Physiol*. 1996;9:124–9.
37. Peczkar MJ, Chan ECS, Krieg NR. *Microbiology. New York: McGraw-Hill*. 1993; p. 578.
35. Kumar P, Sharma B, Bakshi N. Biological activity of alkaloids from solanum dulcamara l. Nat Prod Res 2009;23:719–23.
36. Raji P, Samrot AV, Keerthana D, Karishma S. Antibacterial activity of alkaloids, flavonoids, saponins and tannins mediated green synthesized silver nanoparticles against pseudomonas aeruginosa and bacillus subtilis. J Clust Sci 2019;20:881–95.
37. Mohanan S, Nabeela R, Bimal RKS. Formulation and evaluation of antimicrobial gels for the treatment of paronychia. Int J Appl Pharm 2018;10:161-7.