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52 Potential Anti-acne: Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) from Central Kalimantan-Indonesia Susi Novaryatiin1,*, Syahrida Dian Ardhany1 INTRODUCTION Acne is a disease of the pilosebaceous unit that causes noninflammatory lesions (open and closed comedones), inflammatory lesions (papules, pustules, and nodules), and varying degrees of scarring.1 Acne can be caused by several factors such as androgen-mediated stimulation from sebaceous gland activity, follicular hyperkeratinization, hormonal imbalances, inflammation, and bacterial infections.2,3 Some of the bacteria that cause acne include P. acnes, S. epidermidis, and S. aureus. 4,5 In Indonesia, around 95-100% of young men and 83-85% of young women suffering from acne. The prevalence of acne in adult women is around 12% and 3% in adult men.

In another study, it was found that acne is a skin problem until past adolescence with a higher prevalence of women than men in the age range of 20 years or more.5,6 Giving antibiotics is one alternative acne treatment that aims to reduce the bacterial population. However, giving antibiotics to acne patients was reported to increase the occurrence of upper respiratory tract infections when compared with acne patients without antibiotic therapy.

Besides, it can also cause antibiotic resistance because of the evolutionary adaptation of bacteria. 7,8 This condition encourages the development of research to explore antimicrobial agents from herbal resources that may provide valuable leads that can be further developed as anti-acne drugs. Bawang dayak was known to have antibacterial properties. Previous studies reported that the bawang dayak ethanol extract obtained using the soxhlet method was able to inhibit the growth of S. epidermidis and S. aureus at all concentrations tested namely 1%, 5%, 10%, and 15%.

9,10 Bawang dayak extracted using 70% ethanol by the soxhlet method, was also able to inhibit the growth of P. acnes at concentrations of 2.5%, 5% and 10%.11,12 Other studies have shown that the bawang dayak ethanol extract obtained by the percolation method has better antibacterial activity. This can be seen from the diameter of inhibition zones produced bigger when tested against P. acnes, S. epidermidis, and S. aureus. 13 The study of bawang dayak as anti-acne in Indonesia was limited so that it becomes one of the reasons why this study should be developed.

This study is a continuation study to find out the Minimum Inhibitory Concentration (MIC) of bawang dayak ethanol extract and to determine the antibacterial activity of the chloroform fraction and the ethyl acetate fraction of bawang dayak extract. These additional data are needed to produce a good formulation of bawang dayak as an alternative treatment for acne. So, in the end, it can be produced an anti-acne product that has a sale value and high quality. ABSTRACT Background: Research development has been carried out by exploring antimicrobial agents from herbal sources that can be further developed as anti-acne drugs. Some previous studies reported that bawang dayak has antibacterial properties.

However, the study of bawang dayak as anti-acne in Indonesia was limited so that it becomes one of the reasons why this study should be developed. Objective: This study was aimed to determine the minimum inhibitory concentration (MIC) of bawang dayak ethanol extract and to determine the antibacterial activity of the chloroform fraction and the ethyl acetate fraction of bawang dayak extract. Methods: The MIC value was determinate by measured initial absorbance and final absorbance of ten variations of concentration of extract using a UV-Vis spectrophotometer.

The antibacterial activity of chloroform and ethyl acetate fraction was performed using the disc diffusion technique, with five variations of concentration against P.acnes, S. epidermidis, S. aureus. Results: The decrease in absorbance value occurred at a concentration of 0.19%, 1.56% to 100%, which means that at that concentration can inhibit bacterial growth. The antibacterial activity showed that both the chloroform fraction and ethyl acetate fraction of bawang dayak extract were active against all the tested bacteria, whose inhibition zones were in the range of 5.8 ± 0.9 - 23.6 ± 2.3 mm.

However, the ethyl acetate fraction of bawang dayak extract showed better antibacterial activity than chloroform fraction of bawang dayak extract. Conclusion: In this study, it was found that the concentration of 0.19% is the MIC of bawang dayak extract against P. acnes. The highest antibacterial activity was produced by 20% of ethyl acetate fraction of bawang dayak extract against S. aureus. Key words: Minimum inhibitory concentration, Antibacterial activity, Eleutherine bulbosa (Mill.) Urb., Acne-causing

bacteria.

Susi Novaryatiin1,*, Syahrida Dian Ardhany1 1Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, INDONESIA. Correspondence Susi Novaryatiin Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, INDONESIA. E-mail: susi_novaryatiin@yahoo.com History • Submission Date: 17-10-2019; • Review completed: 30-10-2019; • Accepted Date: 04-11-2019. DOI : 10.5530/pj.2020.12.9 Article Available online http://www.phcogj.com/v12/i1 53 MATERIALS AND METHODS Collection and identification of plant material Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) were collected from Sei Gohong Village, Bukit Batu Sub-District, Palangka Raya, Central Kalimantan, Indonesia. The collected plant material was identified and authenticated by Research Center for Biology of Indonesian Institute of Sciences. Preparation of plant extract and fraction The bulb part of bawang dayak was used in this study. Bawang dayak bulb was washed thoroughly with tap water, shade dried, powdered using a blender, and stored.

Dried powders of bawang dayak were extracted with ethanol 96% using percolator's apparatus. The advantage of the percolation method was easy and simple, and the risk of impurity is very small because it uses exhaustive extraction at room temperature. The use of 96% ethanol solvents was due to its universal properties that capable of dissolving almost all types of secondary metabolites that have low molecular weight, nontoxic, and safe to use. 14 The ethanolic extract of bawang dayak was concentrated then added water to form a homogeneous suspension.

The suspension is moved into the separating funnel and the solvent is added based on its level of polarity, which is from a non-polar (chloroform) and semi-polar (ethyl acetate) solvent. The solvent layer was evaporated to dryness to yield chloroform fraction and ethyl acetate fraction. Preparation of inoculum P. acnes, S. epidermidis and S. aureus was grown in brain heart infusion medium for 24 h at 37°C and then were grown on the blood agar plate for 24 h at 37°C.

Determination of minimum inhibitory concentration MIC test is done by taking a bacterial suspension that has been equalized with McFarland 0.5 standard as much as 0.5 ml, then put into a tube containing bawang dayak extract then measured the initial absorbance value using a UV-Vis spectrophotometer. There are ten variations of extract

concentrations which are 100%; 50%; 25%; 12.5%; 6.25%; 3.13%; 1.56%; 0.78%; 0.39%; 0.19% made in a ratio of 1:2 (w/v). All test tubes were then incubated in an incubator for 24 hours. After 24 hours, all test tubes were measured for their absorbance using a UV-Vis spectrophotometer as the final absorbance value.

The study was repeated in triplicates. MIC determined from the smallest extract concentration in the test tube which begins to inhibit the growth of P. acnes bacteria. Determination of antibacterial activity Antibacterial activity was performed using disc diffusion technique, where the discs were impregnated with five variations of concentration of 1.25%, 2.5%, 5%, 10%, and 20%. The McFarland 0.5 standard was prepared and 10 mL was put into sterile tubes. Bacterial suspension was made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1-2x10 CFU/mL (according to McFarland 0.5 standard).

A sterile cotton swab was immersed in a standardized bacterial suspension and was used to evenly inoculate on Mueller- Hinton agar plate. Then, all the discs that have been immersed in each of chloroform fraction and ethyl acetate fraction of bawang dayak extract were placed on the plates. A clindamycin antibiotic was used as positive controls with concentration variations of 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% against P. acnes and 0.02%, 0.04%, 0.06%, 0.08%, and 0.10% against S. epidermidis and S. aureus. Discs that have been immersed in clindamycin were also placed on the plates. The plates were then incubated for 24 h at 37°C.

The diameter of the zone of inhibition formed was measured in mm using a caliper. The study was repeated in triplicates for each fraction and positive control. RESULTS AND DISCUSSION Minimum inhibitory concentration In this study, the absorbance value was measured using a UV-Vis spectrophotometer. UV-Vis spectrophotometer has a wavelength range of 200-800 nm according to the wavelength of visible light. The wavelength that can be absorbed by a sample in this study was 343 nm. The results of spectrophotometric measurements can be seen in Table 1.

MIC test by measuring the absorbance value using UV-Vis spectrophotometer showed that at a concentration of 0.19% bacterial growth inhibition began to occur. The decrease in absorbance value occurs at a concentration of 0.19%, 1.56% to 100%, which means that at that concentration can inhibit bacterial growth. The higher the concentration of the extract used, the bacterial growth activity can be reduced, because the content of compounds that are as antibacterial in the extract is greater.

Statistical tests on the absorbance values were performed using the IBM SPSS Statistics 20. In this study, the Wilcoxon Signed Rank Test was used to measure the significant

difference in absorbance values before and after incubation. Results of the Wilcoxon Signed Rank Test showed that negative rank 7 samples, positive ranks 0 samples, and ties 3 samples. The asymptotic p-value obtained 0.018 ($p<0.05$), it indicates that there is a significant difference between the absorbance values before and after incubation. Increased absorbance values occur at concentrations of 0.39% and 0.78%.

This is not entirely caused by bacteria but can be influenced by other particles in the solution in the form of residual extracts that are not homogeneous with solutions that can absorb light, causing an increase in absorbance value. It also can be caused by the concentration that occurs at higher concentrations, thus affecting the absorption of light by dead bacterial cells in the solution. One of the weaknesses of Materials Concentration (%) Absorbance value (mean; n=3) Information Before incubation After incubation Bawang dayak ethanol extract 100 3.582438 2.547119 Decrease 50 1.757690 1.708049 Decrease 25 0.694906 0.693644 Decrease 12.5

0.637044 0.601440 Decrease 6.25 0.323364 0.289592 Decrease 3.13 0.225627 0.187703 Decrease 1.56 0.164388 0.134277 Decrease 0.78 0.130086 0.130168 Increase 0.39 0.108154 0.109863 Increase 0.19 0.102620 0.087280 Decrease Table 1: Absorbance values of bawang dayak extract against P. acnes measured by using a UV-Vis spectrophotometer. 54 the UV-Vis spectrophotometer is its selectivity to distinguish samples from other particles or contaminants that absorb light in the same wavelength.15 Based on the results of measurements using a UV-Vis spectrophotometer it can be stated that the concentration of 0.19% is the MIC of bawang dayak extract against P. acnes . MIC result in this study was in line with previous studies.

In the previous study, the antibacterial activity test was done by using disc diffusion technique on P. acnes, where it was known that bawang dayak extract was able to inhibit the growth of P. acnes in the concentration range of 1.25% - 20%, where the smallest concentration of 1.25% produced diameter of inhibition zone of 7.0 ± 1.3 mm. 13 So there was a high possibility that bawang dayak extract can inhibit the growth of P. acnes at concentrations of less than 1.25%. The antibacterial activity of bawang dayak extract is due to the presence of certain chemical compounds that are as antibacterial.

In previous studies, it was known that the presence of chemical compounds in bawang dayak extract were flavonoids, alkaloids, saponins, and tannins.13 Antimicrobial action of flavonoids may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Flavonoids also may demonstrate the antibacterial activity by reducing membrane fluidity of bacterial cells and causing cell fluid imbalance.

16,17 Alkaloids inhibit bacterial growth by changing the nature of cell protein (denaturation), thus increasing the permeability of cell membranes of the bacteria. The cell membrane causes loss or leakage of the contents of a cell of bacteria to the outside or through a direct link membrane of cell bacteria, causing the demise of a polar membrane of bacteria, which leads to the death of a cell bacteria gradually.18 Saponins act as chemical barriers in plant defense systems to deal with pathogens. Saponins can cause leakage of certain proteins and enzymes from bacterial cells. 19 While tannins can bind to proline-rich proteins and interfere with protein synthesis.20 Antibacterial activity In this study, antibacterial activity was done against chloroform fraction and ethyl acetate fraction of bawang dayak extract, and clindamycin.

Clindamycin was used as a positive control because it's known as one of the antibiotics used for acne treatment. 7 The diameters of inhibition zones produced by clindamycin with concentration 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% against P.acnes were 21.8 ± 0.9 mm, 24.4 ± 0.6 mm, 25.1 ± 0.9 mm, 26.6 ± 0.5 mm, and 27.6 ± 1.0 mm, respectively. The diameters of inhibition zones of clindamycin at concentrations of 0.02%, 0.04%, 0.06%, 0.08%, and 0.10% against S. epidermidis and S.aureus were 27.5 ± 1.3 mm, 32.5 ± 0.5 mm, 34.2 ± 1.4 mm, 35.3 ± 0.7 mm, 35.0 ± 1.2 mm, 30.5 ± 1.1 mm, 34.3 ± 0.6 mm, 36.2 ± 1.6 mm, 38.1 ± 1.1 mm, and 38.7 ± 1.9 mm, respectively (Table 2).

The previous study was done to investigate the antibacterial activity of bawang dayak ethanol extract with concentration 1.25%, 2.5%, 5%, 10%, and 20% against acne-causing bacteria, namely P. acnes, S.epidermidis, and S. aureus. The results reported that bawang dayak ethanol extract was active against acne-causing bacteria, whose inhibition zones were in the range of 7.0 ± 1.3 - 23.1 ± 0.6 mm. The highest antibacterial activity was produced by 20% of bawang dayak ethanol extract against S. epidermidis.13 This study is a continuation of previous studies, by testing the chloroform fraction and ethyl acetate fraction of bawang dayak extract against acne-causing bacteria.

This study showed that both the chloroform fraction and ethyl acetate fraction of bawang dayak extract were active against all the tested bacteria, whose inhibition zones were in the range of 5.8 ± 0.9 - 23.6 ± 2.3 mm (Figures 1 and 2). However, the ethyl acetate fraction of bawang dayak extract showed better antibacterial activity than chloroform fraction of bawang dayak extract. In this study, it was found that the ethyl acetate fraction of bawang dayak extract had better antibacterial activity against P.acnes and S.

aureus compared to bawang dayak ethanol extract tested in Materials Concentration (%) Inhibition zone diameter (mm) (mean ± SD; n=3) P. acnes S. epidermidis S. aureus Clindamycin (positive control) 0.02 - 27.5 ± 1.3 30.5 ± 1.1 0.04 - 32.5 ± 0.5 34.3 ± 0.6

0.06 - 34.2 ± 1.4 36.2 ± 1.6 0.08 - 35.3 ± 0.7 38.1 ± 1.1 0.10 - 35.0 ± 1.2 38.7 ± 1.9 0.2 21.8 ± 0.9 - - 0.4 24.4 ± 0.6 - - 0.6 25.1 ± 0.9 - - 0.8 26.6 ± 0.5 - - 1.0 27.6 ± 1.0 - - Table 2: Antibacterial activity of clindamycin against acne-causing bacteria. * -: Not tested Materials Concentration (%) Inhibition zone diameter (mm) (mean ± SD; n=3) P. acnes S. epidermidis S.

aureus Chloroform fraction of bawang dayak extract 1.25 5.8 ± 0.9 6.9 ± 1.9 9.5 ± 1.0 2.5 7.3 ± 0.5 7.9 ± 2.1 10.4 ± 0.6 5 9.4 ± 1.3 13.1 ± 2.1 12.0 ± 0.8 10 9.8 ± 1.7 13.2 ± 0.6 15.5 ± 0.2 20 10.6 ± 1.9 15.7 ± 0.9 18.4 ± 0.2 Ethyl acetate fraction of bawang dayak extract 1.25 12.0 ± 2.1 13.6 ± 1.8 13.7 ± 2.3 2.5 13.5 ± 1.8 17.0 ± 1.4 16.9 ± 1.7 5 13.0 ± 1.1 17.7 ± 0.5 18.3 ± 2.5 10 13.8 ± 0.3 17.9 ± 0.4 20.7 ± 2.2 20 17.3 ± 2.1 20.4 ± 1.4 23.6 ± 2.3 Table 3: Antibacterial activity of chloroform fraction and ethyl acetate fraction of bawang dayak extract against acne-causing bacteria. 55 A B B B C Figure 1: Antibacterial activity of chloroform fraction of bawang dayak extract against P.

acnes (A), S. epidermidis (B) and S. aureus (C). A B C B C Figure 2: Antibacterial activity of ethyl acetate fraction of bawang dayak extract against P. acnes (A), S. epidermidis (B) and S. aureus (C). 56 previous studies. The highest antibacterial effect was found for ethyl acetate fraction of bawang dayak extract against S. aureus, with the diameters of inhibition zones of concentration of 1.25%, 2.5%, 5%, 10%, and 20% being 13.7 ± 2.3 mm, 16.9 ± 1.7 mm, 18.3 ± 2.5 mm, 20.7 ± 2.2 mm, and 23.6 ± 2.3 mm, respectively (Table 3).

The antimicrobial activities of extracts can be classified into three levels, weak activity (inhibition zone lower than 12 mm), moderate activity (inhibition zone between 12 and 20 mm), and strong activity (inhibition zone higher than 20 mm).21 Therefore it can be concluded that the 20% ethyl acetate fraction of bawang dayak extract has strong activity against S. aureus. CONCLUSION The results of this study showed that bawang dayak has the potential to developed as anti-acne. It was found that the concentration of 0.19% is the MIC of bawang dayak extract against P. acnes. Chloroform fraction and ethyl acetate fraction of bawang dayak extract were active against all the tested acne-causing bacteria, whose inhibition zones were in the range of 5.8 ± 0.9 - 23.6 ± 2.3 mm.

The highest antibacterial activity was produced by 20% of ethyl acetate fraction of bawang dayak extract against S. aureus, wherein the resulting inhibition zone diameter was 23.6 ± 2.3 mm. This study is a continuation of previous studies to get additional data to produce a good formulation of bawang dayak as an alternative treatment for acne. Further research is needed to develop a formulation of bawang dayak so it can be produced an anti-acne product that has a sale value and high quality.

ACKNOWLEDGEMENT The authors would like to thank the Universitas Muhammadiyah Palangkaraya through Institute for Researches and Community Services Universitas Muhammadiyah Palangkaraya for the financial grants (PKDI Grant). CONFLICTS OF INTEREST The authors declare no conflicts of interest. ABBREVIATIONS MIC: Minimum Inhibitory Concentration, P. acnes : Propionibacterium acnes, S. epidermidis : Staphylococcus epidermidis, S. aureus: Staphylococcus aureus, CFU: Colony Forming Units. REFERENCES 1. Tan AU, Schlosser BJ, Paller AS. A review of diagnosis and treatment of acne in adult female patients. Int J Womens Dermatol. 2018;4(2):56-71. 2. Toyoda M, Morohashi M.

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57 GRAPHICAL ABSTRACT ABOUT AUTHORS Cite this article: Novaryatiin S, Ardhany SD. Potential Anti-acne: Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) from Central Kalimantan-Indonesia. Pharmacog J. 2020;12(1):52-7. Susi Novaryatiin: Lecturer at the Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Central Kalimantan, Indonesia. Syahrida Dian Ardhany: Lecturer at the Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Central Kalimantan, Indonesia.

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