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Judul Jurnal	:	Potential Anti-Acne: Bawang Dayak (<i>Eleutherine bulbosa</i> (Mill.) Urb.) from Central Kalimantan-Indonesia

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1. Submission article

Manuscript Received Successfully & Peer Review Acceptance 3 Yahoo/Email M... ✉

 **Pharmacognosy Journal** <editor.phcogi@gmail.com>
Kepada: susi novaryatiin, chass501@gmail.com 📧 Kam, 17 Okt 2019 jam 12.07 ☆

Dear Dr. Susi Novaryatiin,

Thank you very much for your submission to Pharmacognosy Journal. We have successfully received your article titled "Potential Anti-acne: Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia". The article is now under our PRELIMINARY EVALUATION and we will keep you updated on the article status accordingly.

Upon article acceptance, corresponding authors are required to pay article processing charge of 700 USD. Please confirm your acceptance. Article processing charges (APCs) are associated with only accepted articles to cover the costs of making the final version of the manuscript freely available via open access.

The charges are to cover the cost of the following:

- Manuscript submission systems
- In-house copy editing and linguistic support
- Pagination & typesetting
- Inclusion in Crossref, giving a permanent DOI and enabling easy citation in other journals.
- Archiving, where appropriate, in an internationally recognized, open access repositories.

We thank you for submitting your valuable work to the Pharmacognosy Journal.

Do let me know if you have any questions.

Have a great day.

Best regards,
Managing Editor
Pharmacognosy Journal
ISSN : 0975-3575

2. Review Process (Manuscript Revision)

Author Revision Notification 3

Yahoo/Email M... ☆



Pharmacognosy Journal <editor.phcogi@gmail.com>
Kepada: susi_novaryatiin@yahoo.com

Rab, 30 Okt 2019 jam 12.20 ☆

Susi Novaryatiin, Syahrída Dian Ardhaný:

We have reached a decision regarding your submission to Pharmacognosy Journal, "Potential Anti-acne: Bawang Dayak (Eleutherne bulbosa (Mill.) Urb.) from Central Kalimantan-Indonesia".

Our decision is: Revisions Required

Reviewer Comments

For author and editor

This article is interesting for anti-acne drugs.

I have some comments. In Table 1, the results showed that bawang dayak extract decreased the absorbance P. acnes. Which statistical analysis did you use? If you use the statistics, please show P value between before incubation and after incubation.

For editor only

Accept with minor revisions

This article is interesting for anti-acne drugs.

I have some comments. In Table 1, the results showed that bawang dayak extract decreased the absorbance P. acnes. Which statistical analysis did you use? If you use the statistics, please show P value between before incubation and after incubation.

Emily Flora

Comments to the Author

- The rationale and significance of the problem are not clear. I am not sure what is the originality of this work in the context of the previous work. The author should give more detail about the previous work.
- Which statistical analysis did the author use in Table 1? Please show the P value between before incubation and after incubation.
- The author should give a deep discussion on the obtained result, not just report the value from the experimental work. For example for 0.39% and 0.78%, the absorbance value increased, while in the other concentrations, the absorbance value decreased. Why?
- The standard deviation of the mean value should be provided in Table 2 and Table 3
- The quality of the Table needs to be improved.

Potential Anti-acne: Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia

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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 determined 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

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.

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 $1-2 \times 10^8$ 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

Table 1: Absorbance values of bawang dayak extract against *P. acnes* measured by using a UV-Vis spectrophotometer.

Materials	Concentration (%)	Absorbance value (mean; n=3)		Information
		Before incubation	After incubation	
Bawang dayak	100	3.582438	2.547119	Decrease
ethanol extract	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

* The asymptotic p-value was 0.018 ($p < 0.05$) between the absorbance values before incubation and after incubation

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 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).

Table 2: Antibacterial activity of clindamycin against acne-causing bacteria.

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
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	-	-

* -: Not tested

Table 3: Antibacterial activity of chloroform fraction and ethyl acetate fraction of bawang dayak extract against acne-causing bacteria.

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
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

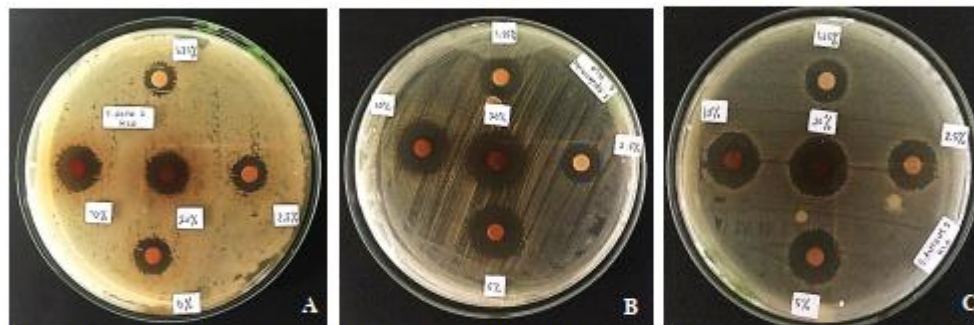


Figure 1: Antibacterial activity of chloroform fraction of bawang dayak extract against *P. acnes* (A), *S. epidermidis* (B) and *S. aureus* (C).

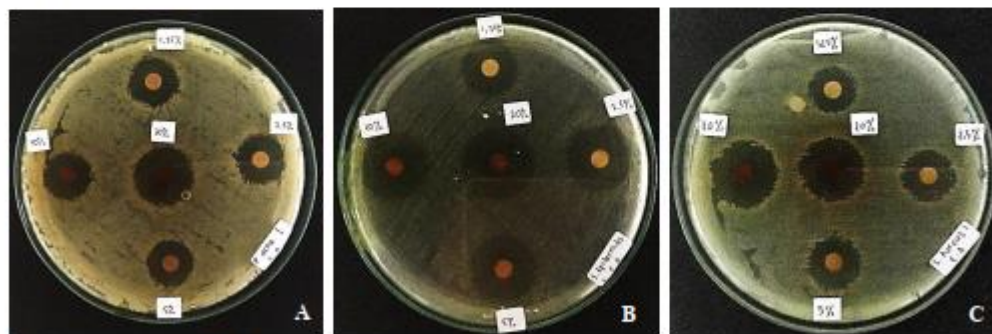


Figure 2: Antibacterial activity of ethyl acetate fraction of bawang dayak extract against *P. acnes* (A), *S. epidermidis* (B) and *S. aureus* (C).

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 (Figure 1 and Figure 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 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).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS USED

MIC: Minimum Inhibitory Concentration, **P. acnes:** *Propionibacterium acnes*, **S. epidermidis:** *Staphylococcus epidermidis*, **S. aureus:** *Staphylococcus aureus*, **CFU:** Colony Forming Units.

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3. Acknowledgement of revision

Response to the reviewer:

1. The previous work was written in the introduction of the manuscript.
 "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."
2. In this study, the Wilcoxon Signed Rank Test was used to measure the significant difference in absorbance values before and after incubation.
 "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."
3. The deep explanation of obtained result was provided in the discussion section of the manuscript.
 "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 the UV-Vis spectrophotometer is its selectivity to distinguish samples from other particles or contaminants that absorb light in the same wavelength."¹⁵
4. The standard deviation was provided in Table 2 and Table 3.
5. Table quality was improved in the whole manuscript.

 **susi novaryatiin** <susi_novaryatiin@yahoo.com>
Kepada: Pharmacognosy Journal

 Kam, 31 Okt 2019 jam 10.26 ☆

Dear editor,

Thank you for your revision.

In this study, in Table 1, the Wilcoxon Signed Rank Test was used to measure the significant difference in absorbance values before and after incubation. The asymptotic p-value was 0.018 ($p < 0.05$) between the absorbance values before and after incubation.


I already submit revisions with the explanation above.

I'll wait for your great response.

Best regards,
Susi Novaryatiin

> Tampilkan pesan asli



 **Pharmacognosy Journal** <editor.phcogi@gmail.com>
Kepada: susi novaryatiin

 Kam, 31 Okt 2019 jam 12.05 ☆

Dear Dr. Susi Novaryatiin,

Thanks for your email. Final decision will be notified to you shortly.

Have a great day!

Regards,
Emily Flora

4. Acceptance of manuscript

Manuscript Accepted for Publication 3

Yahoo/Email M... ☆

 **Pharmacognosy Journal** <editor.phcogi@gmail.com>
Kepada: susi novaryatiin

 Sen, 4 Nov 2019 jam 14.39 ☆

Susi Novaryatiin, Syahrida Dian Ardhanj:

We have reached a decision regarding your submission to Pharmacognosy Journal, "Potential Anti-acne: Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia".

Our decision is to: Accept Submission

Publication charges – 700 USD: Please let me know your mode of remittance 1) Wire/Bank transfer or 2) Credit Card. Invoice will be raised accordingly.

Let me know if you have any questions.
Congratulations and have a great day!

Emily Flora

> Tampilkan pesan asli

--

Regards,
Emily Flora

5. Article Published

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Potential Anti-acne: Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) from Central Kalimantan-Indonesia

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Potential Anti-acne: Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) from Central Kalimantan-Indonesia

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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

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Potential Anti-acne: Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia

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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 determined 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.

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.^{6,4}

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.

Cite this article: Novaryatiin S, Ardhaný SD. Potential Anti-acne: Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan-Indonesia. Pharmacogn J. 2020;12(1):52-7.



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 1-2x10⁸ 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

Table 1: Absorbance values of bawang dayak extract against *P. acnes* measured by using a UV-Vis spectrophotometer.

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

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

Table 2: Antibacterial activity of clindamycin against acne-causing bacteria.

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean \pm SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
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	-	-

* -: Not tested

Table 3: Antibacterial activity of chloroform fraction and ethyl acetate fraction of bawang dayak extract against acne-causing bacteria.

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean \pm SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
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

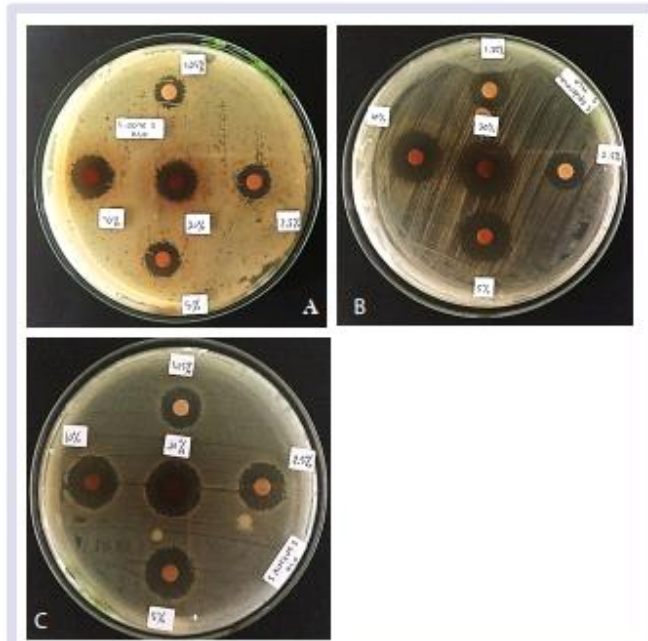


Figure 1: Antibacterial activity of chloroform fraction of bawang dayak extract against *P. acnes* (A), *S. epidermidis* (B) and *S. aureus* (C).

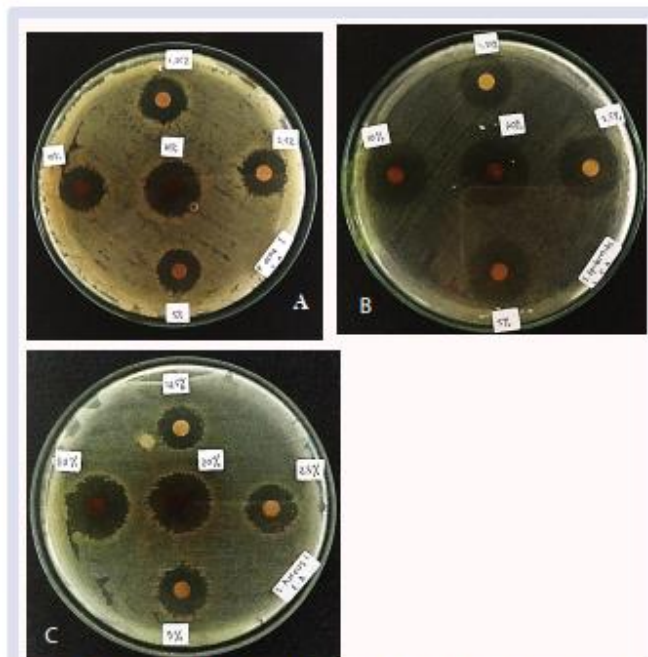


Figure 2: Antibacterial activity of ethyl acetate fraction of bawang dayak extract against *P. acnes* (A), *S. epidermidis* (B) and *S. aureus* (C).

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).²⁴ 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

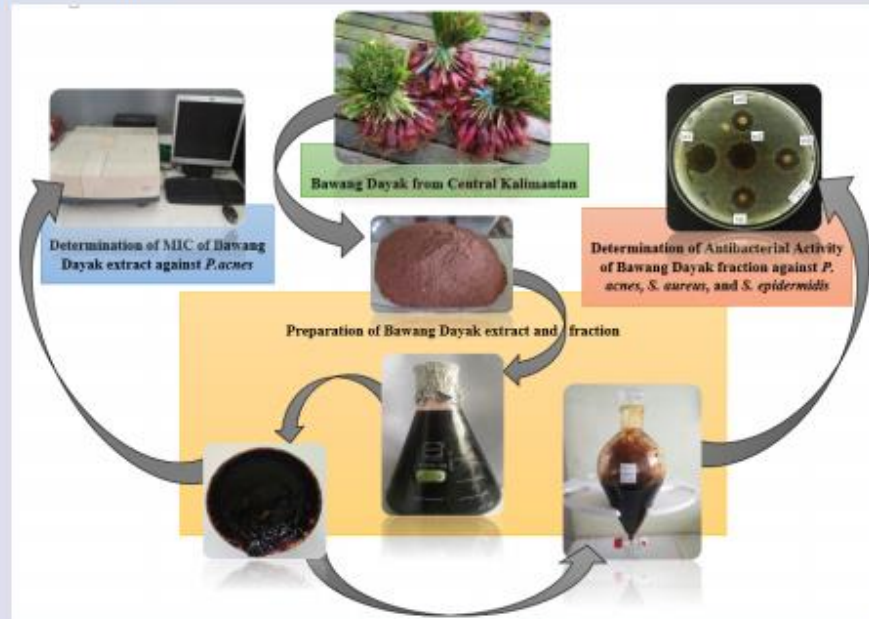
MIC: Minimum Inhibitory Concentration, *P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, CFU: Colony Forming Units.

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GRAPHICAL ABSTRACT



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LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
DEWAN KOMITE ETIK PENELITIAN

Jl. Pramuka No.02 Banjarmasin Tlp. (0511) 3268105

Banjarmasin, 25 Februari 2019

No. SK : 001.12/KE-LPPM/UNISM/II/2019
Lampiran : -
Perihal : Rekomendasi Penelitian

Peneliti yang disebutkan dibawah ini :

Ketua Peneliti : Susi Novaryatiin
NIP/NIK/NIM : 14.06.01.015
Anggota Peneliti : Syahrída Dian Ardhany
Judul Penelitian : Uji Minimum Inhibitory Concentration (MIC) ekstrak Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) asal Kalimantan Tengah terhadap *Propionibacterium acnes*

Berdasarkan pertimbangan Dewan Komite Etik Penelitian diputuskan bahwa Peneliti yang disebutkan telah **DISETUJUI** untuk melanjutkan penelitiannya.

Demikian surat persetujuan ini diterbitkan untuk dipergunakan dengan penuh tanggung jawab.

Menyetujui,

An. Ketua
Sekretaris Dewan Komite Etik Penelitian



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