

## BUKTI KORESPONDENSI

<b>Lampiran</b>	:	Peer review proses korespondensi submit Publikasi Jurnal Ilmiah Internasional Bereputasi
<b>Nama Jurnal</b>	:	International Journal of Applied Pharmaceutics Vol. 11 Special Issue 5 2019 Hal: 22-25
<b>Index</b>	:	Terindeks pada database internasional bereputasi dan berfaktor dampak (SCOPUS Q3)
<b>Judul Jurnal</b>	:	The Antibacterial Activity of Bawang Dayak ( <i>Eleutherine bulbosa</i> (Mill.) Urb.) from Central Kalimantan Against Acne-Causing Bacteria

No	Item	Tanggal	Halaman
1	Register/Submission pada konferensi internasional ICPPS 2019 (International Conference on Pharmacy and Pharmaceutical Science) di Tokyo, Jepang 28-30 Maret 2019	10 November 2018	1
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4	Review process, artikel accepted pada jurnal AJPCR namun jurnal tersebut masuk dalam kategori discontinued scopus sehingga panitia konferensi mengganti ke IJAP (International Journal of Applied Pharmaceutics) dengan indeks scopus Q3	19 Juli 2019	8
5	Article for final proof (IJAP)	24 Juli 2019	15
6	Article Published (IJAP)	15 September 2019	20
7	Ethical approval	11 April 2018	25

### 1. Register/Submission pada konferensi internasional ICPPS 2019 (International Conference on Pharmacy and Pharmaceutical Science) di Tokyo, Jepang 28-30 Maret 2019

• ICPPS 2019: Your Submission to ICPPS 2019 has been Received!

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Kepada: susi\_novaryatiin@yahoo.com

Sab, 10 Nov 2018 jam 18.00 ☆

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icpps <icpps@cbees.net>  
Kepada: susi\_novaryatiin@yahoo.com  
Cc: chass501@gmail.com

Kam, 15 Nov 2018 jam 12.52 ☆

Dear Susi Novaryatiin and Syahrida Dian Ardhany,

Sorry for getting back to you so late. I was on the business trip those days. And thanks for your submission to ICPPS 2019.

Your paper entitled "THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*Eleutherine bulbosa* (Mill.) Urb.) FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA" is good, and it is given the ID as "T0032".

Please keep this ID in mind in case there are any inquiries about it. The paper will be sent to be reviewed and the first review result will be told as soon as possible.

Best regards,

--

Lulu Liang  
Conference Specialist

ICPPS 2019

HKCBEEES-BBS

Conference E-mail: icpps@cbees.net/Personal E-mail: lulu@cbees.org

Tel: +852-3500-0137 (Hong Kong)/+1-206-456-6022 (USA)/+86-28-86528465 (China)

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## 2. Artikel accepted pada jurnal AJPCR (Asian Journal of Pharmaceutical and Clinical Research) (T0032)

### ICPPS 2019 Publication Option of T0032

Dari: icpps (icpps@cbees.net)

Kepada: susi\_novaryatiin@yahoo.com

Cc: chass501@gmail.com

Tanggal: Rabu, 5 Desember 2018 11.14 WIB

Dear Susi Novaryatiin and Syahrida Dian Ardhany,

Congratulations that your paper **T0032** has been both accepted by **AJPCR Journals** (<https://innovareacademics.in/journals/index.php/ajpcr/issue/view/402>), indexed by **Scopus** and **IJPMBBS Journals** (<http://www.ijpmbbs.org/>).

Please let me know in which you would like to publish your paper. Later I'd like to send you a Notification of AJPCR. If you want to publish your paper in IJPMBBS, please let me know.

Best regards,

--

Lulu Liang  
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### 3. Notification dan Review artikel

ICPPS 2019 AJPCR Notification of T0032

Yahoo/Email M... ☆



icpps <icpps@cbees.net>  
Kepada: susi\_novaryatiin@yahoo.com  
Cc: chass501@gmail.com

Sen, 10 Des 2018 jam 14.11 ☆

Dear Susi Novaryatiin and Syahrida Dian Ardhanay,

Sorry for getting back to you so late. Congratulations that your paper T0032 "The Antibacterial Activity of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan Against Acne-Causing Bacteria" has been accepted by AJPCR (<https://innovareacademics.in/journals/index.php/ajpcr>) and also do presentation in the conference. Please find the attached notification form and review form. Please send back your **final paper, payment proof and registration form** to me before **December 20, 2018**.

Should you have any questions, please don't hesitate to contact us!

Best wishes,

--

Lulu Liang

Conference Specialist

ICPPS 2019

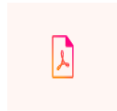
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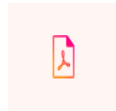
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review form... .pdf  
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## Notification of Acceptance of the ICPPS 2019

Tokyo, Japan, March 28-30, 2019

<http://www.icpps.org/>



Paper ID : T0032

Paper Title : The Antibacterial Activity of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan Against Acne-Causing Bacteria

Dear Susi Novaryati and Syahrida Dian Ardhany,

First of all, thank you for your concern. 2019 4th International Conference on Pharmacy and Pharmaceutical Science (ICPPS 2019) review procedure has been finished. We are delighted to inform you that your manuscript has been accepted for presentation at 2019 4th International Conference on Pharmacy and Pharmaceutical Science (ICPPS 2019) in Meiji University, Tokyo, Japan during March 28-30, 2019. Your paper was tripling blind-reviewed and based on the evaluation. The reviewers' comments are enclosed.

The conference received papers from about 11 different countries and regions during the submission period. And there are about 69 papers accepted by our reviewers who are the international experts from all over the world. The selected papers could be published in our conference proceedings with high quality. According to the recommendations from reviewers and technical program committees, we are glad to inform you that your paper identified above has been selected for publication and oral presentation. You are invited to present your paper and studies during our ICPPS conference that would be held on March 28-30, 2019, Meiji University, Tokyo, Japan.

The ICPPS 2019 is co-sponsored by Hong Kong Chemical, Biological & Environmental Engineering Society (HKCBEEES) and Biology and Bioinformatics Society (BBS).

Paper of ICPPS 2019 will be published in *Asian J Pharm Clin Res (AJPCR, Print ISSN- 0974-2441, Online ISSN- 2455-3891)*, and be indexed by SCOPUS, Google Scholar, Elsevier, EBSCO, EMBASE, SCImago(SJR), CNKI, CAS, CASSI (American Chemical Society), Directory of Open Access Journal (DOAJ), Index Copernicus, ICAAP, Scientific commons, PSOAR, Open-J-Gate, Indian Citation Index (ICI), Index Medicus for WHO South-East Asia (IMSEAR), OAI, LOCKKS, OCLC (World Digital Collection Gateway), UIUC. Impact- 0.40 (SCImago, SJR 2016).

**(Important Steps for your registration): Please do finish all the 5 steps on time to guarantee the paper published in the journal successfully:**

1. Revise your paper according to the Review Comments in the attachment carefully. (Five authors at most each paper)



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2. Format your paper according to the Formatting Instructions carefully.

<https://innovareacademics.in/instructions/sample-submission.pdf> (PDF Format)

3. Download and complete the Registration Form or register through the online system.

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4. Finish the payment of Registration fee. (The information can be found in the Registration form or on the online system)

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5. Send your final papers (both .doc and .pdf format), filled registration form (.doc format), copyright form (.jpg format), and the scanned payment (in .jpg format) to us at [icpps@cbees.net](mailto:icpps@cbees.net). **(December 20, 2018) (Very important)**

ICPPS 2019 will check the format of all the registered papers first, so the authors don't need to upload the paper to the system. After the registration, we will send all qualified papers to the publish house and index organization for publishing directly.

We are looking forward to meet all the authors in our conference. But if you and your co-author(s) could not attend ICPPS 2019 to present your paper for some reasons, please inform us. And we will send you the proceeding in electronic version and the scanned receipt after ICPPS 2019.

Please strictly adhere to the format specified in the conference template while preparing your final paper. If you have any problem, please feel free to contact us via [icpps@cbees.net](mailto:icpps@cbees.net). For the most updated information on the conference, please check the conference website at <http://www.icpps.org/>. The Conference Program will be available at the website in **Middle March, 2019**.

Again, congratulations. We are looking forward to seeing you in Tokyo, Japan.

Yours sincerely,

ICPPS 2019 Organizing Committees



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**Review Form of ICPPS 2019**
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Paper ID : T0032

Paper Title : The Antibacterial Activity of Bawang Dayak (Eleutherine bulbosa (Mill.) Urb.) from Central Kalimantan Against Acne-Causing Bacteria

<b>The Evaluation of the paper</b>	
<b>Topic</b>	<b>The Topic's Conformity</b> <input checked="" type="checkbox"/> Match to the conference topic <b>very well</b> ; <input type="checkbox"/> Match to the conference topic <b>fairly</b> ; <input type="checkbox"/> Match to the conference topic <b>poorly</b> ;
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<b>Contents</b>	<b>Innovation</b> <input type="checkbox"/> Highly Innovate <input checked="" type="checkbox"/> Sufficiently Innovate <input type="checkbox"/> Slightly Innovate <input type="checkbox"/> Not Novel
	<b>Integrity</b> <input type="checkbox"/> Poor <input type="checkbox"/> Fair <input checked="" type="checkbox"/> Good <input type="checkbox"/> Outstanding
	<b>The "literary" presentation</b> <input type="checkbox"/> Totally Accessible <input checked="" type="checkbox"/> Mostly Accessible <input type="checkbox"/> Partially Accessible <input type="checkbox"/> Inaccessible
	<b>The technical depth</b> <input type="checkbox"/> Superficial <input type="checkbox"/> Suitable for the non-specialist <input checked="" type="checkbox"/> Appropriate for the generally knowledgeable individual working in the field <input type="checkbox"/> Suitable only for an expert
<b>Presentation &amp; English</b>	<input checked="" type="checkbox"/> Satisfactory <input type="checkbox"/> Needs improvement <input type="checkbox"/> Poor
<b>Overall organization</b>	<input type="checkbox"/> Satisfactory <input checked="" type="checkbox"/> Could be improved <input type="checkbox"/> Poor
<b>Recommendation for Publication &amp; Detailed Suggestions</b>	
<b>Accepted (please chose one)</b>	<input type="checkbox"/> Strongly Accept; <input checked="" type="checkbox"/> Accept; <input type="checkbox"/> weakly Accept <b>Comments (Please prepare the final version of the paper as per review instructions):</b> The paper is well-structured and I enjoyed reading the paper. Here are two personal opinions on the paper, which may not totally correct, please consider:  1. The conclusion is poorly written. The authors need to summarize the methods adapted, the results found and the challenges still existed; and then put forward some ideas for the future research. Please note that this part should not be a copy of the abstract. 2. Maximum 30 references are enough. Unwanted references have to be removed.



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<input type="checkbox"/> <b>Rejected (please chose one)</b>	<input type="checkbox"/> Strongly Reject	<input type="checkbox"/> Reject	<input type="checkbox"/> weakly Reject
	<input type="checkbox"/> Paper is not of sufficient quality or novelty to be published in the Journal. <input type="checkbox"/> A major rewrite is required, encourage resubmission. <input type="checkbox"/> The topic of the paper does not matches to the conference topic, encourage to submit to another conference: <a href="http://www.cbees.org/events/">http://www.cbees.org/events/</a> .		

Notification



#### 4. Review process, artikel accepted pada jurnal AJPCR namun jurnal tersebut masuk dalam kategori discontinued scopus sehingga panitia konferensi mengganti ke IJAP (International Journal of Applied Pharmaceutics) dengan indeks scopus Q3



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Jun, 19 Jul 2019 jam 13:22 ☆

Dear Authors

Please find your attached file for corrections & please resend the file after filling the table given in the Doc. File.

We believe in quality and publishing on time so please read the mail carefully and send the corrections within 1 day. If you are not sending corrected manuscript on time to us then it would be rejected from the current issue and would be published in the next issue. We would not be responsible for not publishing article and publication of an uncorrected article if you are not submitting in the timeline.

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Important:- The abstract must be up to 250 words in a structured form, consisting of Objective, Methods, Results, and Conclusion in case the not received the structured abstract the article will not be published until the abstract is structured.

Please ensure that you have reviewed your article on the base of the points given below.

**NOTE :- ADD TWO CITATIONS OF AJPCR**

- 1.Format:- Headings and subheading should not be numbered.
- 2.Abbreviations:- At the first appearance in the abstract and the text, abbreviations should be preceded by words for which they stand.
- 3.Abstract: Rewrite Abstract which should be structural (Divide it into- Objective, Methods, Results, and Conclusion).
- 4.Symbol and units: It should be as per the International System of Units (SI). See it in instructions to authors and follow accordingly and strictly.
- 5.Equation tools should be used for formula/equation writing.
- 6.Errors: Grammatical and punctuation errors should be rectified. Authors are suggested to use smart tools like 1 checker, ginger, Grammarly, white smoke, etc.
- 7.Most of the words are stuck, rectify it strictly.

**Reviewer Comments for article reference no IJAP\_T0032\_RA\_20190718\_V0**

**The Antibacterial Activity of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central**

**Kalimantan Against Acne-Causing Bacteria**

1. The introduction section contains overly vague phrases, and it does not provide any clarification regarding why the plant extract was tested against three bacterial strains.
2. What is the reason for using the percolation method for plant extraction?
3. The explanation regarding the extract yield is insufficiently brief. What is the significance of determining the extract yield?
4. Which secondary metabolites present in the tested plant exhibit antibacterial properties?



Article reference no IJAP\_T0032\_RA\_20190718\_V0  
 Title: The Antibacterial Activity of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) from Central Kalimantan Against Acne-Causing Bacteria  
 Name & mail address of corresponding author: Susi Novaryatiin, Syahrída Dian Ardhany, email: susi\_novaryatiin@yahoo.com

**Highlighted Corrections**

S.No.	Asked query no.	Details
1	1	<p>Because the three bacterial strains are acne causing-bacteria. The detailed reason was added in the article:</p> <p>Some of the bacteria that cause acne include <i>Propionibacterium acnes</i>, <i>Staphylococcus epidermidis</i>, and <i>Staphylococcus aureus</i>. <i>Propionibacterium acnes</i> is a Gram-positive bacteria and is a normal flora of the skin that plays a role in the formation of acne. <i>Propionibacterium acnes</i> was involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis. <i>Staphylococcus epidermidis</i> is an aerobic Gram-positive bacteria, which is usually involved in superficial skin infections in sebaceous units. This bacteria is known as pus-forming bacteria that triggers inflammation of acne. In addition, colonization of the skin by <i>Staphylococcus aureus</i>, an opportunistic Gram-positive pathogen, can also cause acne, and these bacteria can also cause superficial skin infections such as ulcers and impetigo.</p>
2	2	<p>The reason for using the percolation method was added in the article:          The advantage of the percolation method that it was easy, simple and the risk of impurity is very small because it uses exhaustive extraction at room temperature.</p>
3	3	<p>The explanation of the significance of determining the extract yield was added in the article:</p> <p>Value yield is related to the number of secondary metabolites that successfully attracted when the extraction process.</p>
4	4	<p>All of the secondary metabolites present in the tested plant had antibacterial activity with their own mechanism. Further study is needed to determine which one of the secondary metabolites gives antibacterial activity to this plant.</p> <p>In the article was added:          The presence of flavonoids, alkaloids, saponins, and tannins in Bawang Dayak extract can be responsible for the antibacterial properties observed. Alkaloids can inhibit bacterial growth by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes bacterial cell death. Flavonoids are known to cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA through inhibition which results in the incorporation of non-crosslinked glucan chains into the cell membrane peptidoglycan so that it becomes a weak structure. Tannins can bind to proline-rich proteins and interfere with protein synthesis. Saponin acts as a chemical barrier in the plant defense system to encounter the pathogens. Saponins can cause leakage of certain proteins and enzymes from bacterial cells.</p>

**Other corrections**

Page No.	Column(left or right )/Section /Paragraph/line no or table or figure	Incorrect text or matter	Corrected text

(Please mention clearly your article reference no.)

Editorial team  
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## Research Article

## THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE BULBOSA* (MILL.) URB.) FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA

SUSI NOVARYATI\*, SYAHRIDA DIAN ARDHANY

Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia. Email: susi\_novaryati@yahoo.com

Received: ???, Revised and Accepted: ???

### ABSTRACT

**Objective:** The objective of this research was to investigate the antibacterial activity of bawang dayak from Central Kalimantan against acne-causing bacteria namely *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

**Methods:** The preliminary phytochemical constituents were qualitatively analyzed. Antibacterial activity of bawang dayak ethanol extract was performed using disc-diffusion technique, with five variations of concentration of 1.25%, 2.5%, 5%, 10%, and 20%.

**Results:** Bawang dayak extract contained flavonoids, alkaloids, saponins, and tannins. The inhibition zones of bawang dayak ethanol extract in five various concentrations (1.25%, 2.5%, 5%, 10%, and 20%) were 7.0±1.3 mm, 8.5±0.6 mm, 9.9±0.3 mm, 11.2±0.1 mm, and 11.9±0.3 mm against *P. acnes*; 18.8±3.3 mm, 21.9±3.3 mm, 20.8±0.6 mm, 22.0±0.2 mm, and 23.1±0.6 mm against *S. epidermidis*; and 14.3±3.1 mm, 13.5±0.9 mm, 14.7±1.5 mm, 16.1±1.0 mm, and 20.1±0.6 mm against *S. aureus*, respectively.

**Conclusion:** This present study showed that bawang dayak ethanol extract was active against all the tested acne-causing bacteria. The highest antibacterial activity was produced by 20% of bawang dayak ethanol extract against *S. epidermidis*.

**Keywords:** Antibacterial activity, *Eleutherine bulbosa* (Mill.) Urb., Acne, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*.

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### 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 is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [2]. Acne occurs mostly during adolescence, namely in girls aged 14-17 years and boy aged 16-19 years. However, it can also arise at 40 years, and this disease can also persist into adulthood [3,4]. In Indonesia, around 95-100% of young men and 83-85% of young woman suffer 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].

This infection was influenced by several internal and external factors such as androgen-mediated stimulation of sebaceous gland activity, follicular hyperkeratinization, hormonal imbalance, inflammation, and external bacterial infection. Some of the bacteria that cause acne include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [6,7].

*P. acnes* is a Gram-positive bacteria and is a normal flora of the skin that plays a role in the formation of acne. *P. acnes* was involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis [8]. *S. epidermidis* is an aerobic Gram-positive bacteria, which is usually involved in superficial skin infections in sebaceous units. These bacteria are known as pus-forming bacteria that trigger inflammation of acne [9]. In addition, colonization of the skin by *S. aureus*, an opportunistic Gram-positive pathogen, can also cause acne, and these bacteria can also cause superficial skin infections such as ulcers and impetigo [10].

Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of bacteria. It has been reported that acne patients receive clindamycin, tetracycline, and erythromycin as their treatment tended to cause an increased occurrence of upper respiratory tract infections when compared with acne patients without antibiotic therapy [11]. In addition, the use of antibiotics as the first choice of acne treatment may result in antibiotic resistance due to the evolutionary adaptation of bacteria. Antibiotics have limitations with respect to toxicity and side effects also such as skin drying, headache, and nausea [12]. 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 is one of the traditional medicines that used by the local people of Central Kalimantan Province. Empirically, bawang dayak bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne, ulcers, and colon cancer and to prevent stroke, dysentery, dysuria, and colitis. Active compounds contained in bawang dayak bulb that can provide antibacterial activity include alkaloids, glycosides, flavonoids, phenols, steroids, and tannins [13,14]. The previous study reported that bawang dayak ethanol extract gave minimal inhibitory concentration (MIC) at the concentration of 10 mg/ml against the bacteria *P. acnes*, *S. epidermidis*, and *S. aureus* [15,16]. The present study was initiated to investigate the antibacterial activity of bawang dayak ethanol extract obtained by percolation method against acne-causing bacteria namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

### MATERIALS AND METHODS

#### Collection and identification of plant material

Bawang dayak (*Eleutherine bulbosa* (Mill.) Urb.) were collected from Jl. Cendrawasih II UPT. km 38 Kelurahan Sei Gohong, Kecamatan Bukit

1 **Batu, Palangka Raya, Central Kalimantan.** The collected plant material  
2 was identified and authenticated by research center for biology of  
3 Indonesian Institute of Sciences.

#### 5 Preparation of plant extract

6 The bulb part of bawang dayak was washed thoroughly with tap  
7 water, shade dried, powdered using a blender, and stored. Dried  
8 powders of bawang dayak were extracted with ethanol 96% using  
9 percolator's apparatus. The advantage of the percolation method  
10 was easy and simple, and the risk of impurity is very small because  
11 it uses exhaustive extraction at room temperature. The use of 96%  
12 ethanol solvents was due to its universal properties that capable of  
13 dissolving almost all types of secondary metabolites that have low  
14 molecular weight such as flavonoids, saponins, and alcohol; nontoxic,  
15 and safe to use [17]. The extract was concentrated and then subjected  
16 to preliminary phytochemical analysis. The percentage yields (w/w) of  
17 the extracts were calculated using the formula below [18]:

$$19 \text{ (Weight of extract - Weight of starting plant material)} \times 100\%$$

#### 21 Phytochemical screening

22 The extract was used for preliminary screening of phytochemical  
23 constituents such as flavonoids, alkaloids, saponins, tannins, and  
24 steroids [19,20].

#### 26 Preparation of inoculum

27 *P. acnes*, *S. epidermidis*, and *S. aureus* were grown in brain heart infusion  
28 medium for 24 h at 37°C and then were grown on the blood agar plate  
29 for 24 h at 37°C.

#### 31 Determination of antibacterial activity

32 Antibacterial activity was performed using disc-diffusion technique,  
33 where the discs were impregnated with five variations of concentration  
34 of 1.25%, 2.5%, 5%, 10%, and 20%. The McFarland 0.5 standard was  
35 prepared and 10 mL was put into sterile tubes. Bacterial suspension  
36 was made by taking bacterial colonies diluted in sterile normal saline  
37 and the turbidity adjusted to  $1-2 \times 10^8$  CFU/mL (according to McFarland  
38 0.5 standard). A sterile cotton swab was immersed in a standardized  
39 bacterial suspension and was used to evenly inoculate on Mueller-Hinton  
40 agar plate. Then, all the discs that have been immersed in bawang dayak  
41 ethanol extract were placed on the plates. A clindamycin antibiotic was  
42 used as positive controls with concentration variations of 0.2%, 0.4%,  
43 0.6%, 0.8%, and 1.0% against *P. acnes* and 0.02%, 0.04%, 0.06%, 0.08%,  
44 and 0.10% against *S. epidermidis* and *S. aureus*. Discs that have been  
45 immersed in clindamycin were also placed on the plates. The plates  
46 were then incubated for 24 h at 37°C. The diameter of the zone of  
47 inhibition formed was measured in mm using a caliper. The study was  
48 repeated in triplicates for each extract and positive control.

#### 50 RESULTS AND DISCUSSION

##### 51 Yield of the extract

52 From the rendement calculation, the extraction of bawang dayak yields  
53 of 5.95%. Value yield is related to the number of secondary metabolites  
54 that successfully attracted when the extraction processes [18].

##### 56 Phytochemical screening

57 The results of preliminary phytochemical screening of bawang dayak  
58 ethanol extract are shown in Table 1.

59 The results showed that bawang dayak ethanol extract contained  
60 flavonoids, alkaloids, saponins, and tannins. The presence of flavonoids,  
61 alkaloids, saponins, and tannins in bawang dayak extract can be  
62 responsible for the antibacterial properties observed. Alkaloids can  
63 inhibit bacterial growth by disrupting the constituent components of  
64 peptidoglycan in bacterial cells so that the cell wall layer is not formed  
65 intact and causes bacterial cell death [21]. Flavonoids are known to  
66 cause damage to the permeability of bacterial cell walls, microsomes,  
67 and lysosomes as a result of interactions between flavonoids and  
68 bacterial DNA through inhibition which results in the incorporation of

1 non-cross-linked glucan chains into the cell membrane peptidoglycan  
2 so that it becomes a weak structure [22]. Tannins can bind to proline-  
3 rich proteins and interfere with protein synthesis [23]. Saponin acts  
4 as a chemical barrier in the plant defense system to encounter the  
5 pathogens. Saponins can cause leakage of certain proteins and enzymes  
6 from bacterial cells [24].

#### 8 Antibacterial activity

9 In this study, clindamycin was used as positive controls. Clindamycin  
10 was known as one of the antibiotics that used for acne treatment [11].  
11 The diameters of inhibition zones produced by clindamycin with  
12 concentration 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% against *P. acnes*  
13 were  $23.0 \pm 0.4$  mm,  $25.4 \pm 0.0$  mm,  $29.0 \pm 0.2$  mm,  $29.9 \pm 0.8$  mm, and  
14  $31.2 \pm 0.1$  mm, respectively as presented in Table 2. The diameters  
15 of inhibition zones of clindamycin at concentrations of 0.02%,  
16 0.04%, 0.06%, 0.08%, and 0.10% against *S. epidermidis* and *S. aureus*  
17 were  $31.7 \pm 0.8$  mm,  $35.6 \pm 1.0$  mm,  $37.1 \pm 1.6$  mm,  $37.7 \pm 0.1$  mm,  
18 and  $39.7 \pm 0.4$  mm and  $27.0 \pm 0.5$  mm,  $29.0 \pm 0.8$  mm,  $30.4 \pm 0.4$  mm,  
19  $30.8 \pm 1.9$  mm, and  $31.5 \pm 0.9$  mm, respectively (Table 2). The diameters  
20 of inhibition zones produced by all tested concentrations of clindamycin  
21 against all tested bacteria were greater than the diameters of inhibition  
22 zones produced by bawang dayak ethanol extract.

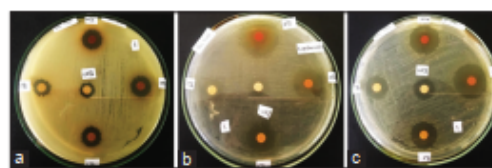
23 Previous studies reported that bawang dayak ethanol extract  
24 obtained by the soxhletation method was able to inhibit the growth  
25 of *S. epidermidis* in all tested concentrations of 1%, 5%, 10%, and  
26 15% [25]. Another previous study also reported that bawang dayak  
27 ethanol extract obtained diameters of inhibition zones against  
28 *P. acnes* at concentrations of 5% and 10% that were  $6.1 \pm 1.5$  mm and  
29  $8.7 \pm 1.3$  mm, respectively [26]. However, this present study showed a  
30 better antibacterial activity of bawang dayak ethanol extract against  
31 acne-causing bacteria, namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

32 The antibacterial activity test of the extract against all tested bacteria  
33 was done in triplicates. Accordingly, bawang dayak ethanol extract was  
34 active against all the tested bacteria, whose inhibition zones were in the  
35 range of  $7.0 \pm 1.3 - 23.1 \pm 0.6$  mm (Fig. 1). The highest antibacterial effect  
36 was found for bawang dayak ethanol extract against *S. epidermidis*, with  
37 the diameters of inhibition zones at concentrations of 1.25%, 2.5%,  
38 5%, 10%, and 20% being  $18.8 \pm 3.3$  mm,  $21.9 \pm 3.3$  mm,  $20.8 \pm 0.6$  mm,  
39  $22.0 \pm 0.2$  mm, and  $23.1 \pm 0.6$  mm, respectively. The diameters of  
40 inhibition zones produced in concentrations of 1.25%, 2.5%, 5%,  
41 10%, and 20% against *S. aureus* were  $14.3 \pm 3.1$  mm,  $13.5 \pm 0.9$  mm,  
42  $14.7 \pm 1.5$  mm,  $16.1 \pm 1.0$  mm, and  $20.1 \pm 0.6$  mm, respectively. On the  
43 other hand, the diameters of inhibition zones of bawang dayak ethanol

47 **Table 1: Results of phytochemical screening of bawang dayak**  
48 **ethanol extract**

Secondary metabolites	Bawang dayak ethanol extract
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Steroids	-

56 +: Detected, -: Not detected



57 **Fig. 1: Antibacterial activity of bawang dayak**  
58 **ethanol extract against Propionibacterium acnes (a)**  
59 **Staphylococcus epidermidis (b) and Staphylococcus aureus (c)**



Table 2: Antibacterial activity of clindamycin against acne-inducing 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	-	31.7±0.8	27.0±0.5
	0.04	-	35.6±1.0	29.0±0.8
	0.06	-	37.1±1.6	30.4±0.4
	0.08	-	37.7±0.1	30.8±1.9
	0.10	-	39.7±0.4	31.5±0.9
	0.2	23.0±0.4	-	-
	0.4	25.4±0.0	-	-
	0.6	29.0±0.2	-	-
	0.8	29.9±0.8	-	-
	1.0	31.2±0.1	-	-

-.: Not tested, *P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

Table 3: Antibacterial activity of bawang dayak ethanol extract against acne-inducing bacteria

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Bawang Dayak ethanol extract	1.25	7.0±1.3	18.8±3.3	14.3±3.1
	2.5	8.5±0.6	21.9±3.3	13.5±1.0
	5	9.9±0.3	20.8±0.6	14.7±1.5
	10	11.2±0.1	22.0±0.2	16.1±1.0
	20	11.9±0.3	23.1±0.6	20.1±0.6

*P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

extract against *P. acnes* at concentrations of 1.25%, 2.5%, 5%, 10%, and 20% were 7.0±1.3 mm, 8.5±0.6 mm, 9.9±0.3 mm, 11.2±0.1 mm, and 11.9±0.3 mm, respectively (Table 3). The antimicrobial activities of extracts can be classified into three levels such as 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) [27].

#### CONCLUSION

The results of this study showed that bawang dayak ethanol extract has potentials to inhibit the growth of acne-causing bacteria. Bawang dayak ethanol extract was active against all the tested 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*, wherein the resulting inhibition zone diameter was 23.1±0.6 mm. The presence of flavonoids, alkaloids, saponins, and tannins in the extract can be responsible for the antimicrobial properties observed. Further research is needed to obtain MIC and to develop an antibacterial formulation for acne treatment from bawang dayak extract.

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15	Author Queries???	15
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**Highlighted Corrections**

S.No.	Asked query no.	Details
1	AQ1	Received: 10 December 2018, Revised and Accepted: 20 July 2019
2	AQ2	Bawang dayak ( <i>Eleutherine bulbosa</i> (Mill.) Urb.) were collected from Sei Gohong Village, Bukit Batu Sub-District, Palangka Raya, Central Kalimantan, Indonesia.
3	AQ3	From the rendement calculation, the extraction of bawang dayak yields of 6,0%.
4	AQ4	Another previous study also reported that the inhibition zones of bawang dayak ethanol extract against <i>P. acnes</i> at concentrations of 5% and 10% were 6.1±1.5 mm and 8.7±1.3 mm, respectively [26]
5	AQ5	Warnida H, Sukawaty Y, Mega M. Stability and activity of bawang tiwai ( <i>Eleutherine americana</i> (Mill.) Urb.) bulbus gel extract as anti acne. J Ilmiah Manuntung 2015;1:94-9.  (There are 3 authors: Warnida H, Sukawaty Y, Mega M)
6	AQ6	Sulastrianah S, Imran I, Fitria ES. The inhibitory test of sirsak leaf extract ( <i>Annona muricata</i> L.) and sirih leaf extract ( <i>Piper betle</i> L.) against <i>Eschericia coli</i> . J Medula 2014;1:76-84.

**Other corrections**

Page No.	Column(left or right )/Section /Paragraph/line no or table or figure	Incorrect text or matter	Corrected text
4	Column right, reference number 26	Novaryatiin S. Phytochemical Screening and Antibacterial Activity of Bawang Dayak ( <i>Eleutherine</i> sp.) and Hati Tanah ( <i>Angiopteris</i> sp.) and Their Combination Against <i>Propionibacterium acnes</i> . Presented on 6th International Conference on Biological and Medical Sciences. Seoul: ICBMS; 2018.	Novaryatiin S. Phytochemical Screening and Antibacterial Activity of Bawang Dayak ( <i>Eleutherine</i> sp.) and Hati Tanah ( <i>Angiopteris</i> sp.) and Their Combination Against <i>Propionibacterium acnes</i> . Int J App Pharm 2019;11:11-3.

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 Name & mail address of corresponding author: Susi Novaryatiin, Syahrida Dian Ardhany, email: susi\_novaryatiin@yahoo.com

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**Other corrections**

Page No.	Column(left or right )/Section /Paragraph/line no or table or figure	Incorrect text or matter	Corrected text
3	Acknowledgment	The authors would like to express their great appreciation to the Overseas Seminar Assistance Program, Directorate General of Research and Development, Kemenristekdikti of Indonesia (program bantuan seminar luar negeri ditjen penguatan riset dan pengembangan, Kemenristekdikti Indonesia) to facilitate the 2019 4th International Conference on Pharmacy and Pharmaceutical Science (ICPPS 2018), March 28–30, 2019.	The authors would like to express their great appreciation to the Overseas Seminar Assistance Program, Directorate General of Research and Development, Kemenristekdikti of Indonesia (program bantuan seminar luar negeri ditjen penguatan riset dan pengembangan, Kemenristekdikti Indonesia) to facilitate the 2019 4th International Conference on Pharmacy and Pharmaceutical Science (ICPPS 2019), March 28–30, 2019.

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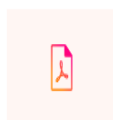
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## THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE BULBOSA* (MILL.) URB.) FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA

SUSI NOVARYATIIN\*, SYAHRIDA DIAN ARDHANY

Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia. Email: susi\_novaryatiin@yahoo.com

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### ABSTRACT

**Objective:** The objective of this research was to investigate the antibacterial activity of bawang dayak from Central Kalimantan against acne-causing bacteria namely *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

**Methods:** The preliminary phytochemical constituents were qualitatively analyzed. Antibacterial activity of bawang dayak ethanol extract was performed using disc-diffusion technique, with five variations of concentration of 1.25%, 2.5%, 5%, 10%, and 20%.

**Results:** Bawang dayak extract contained flavonoids, alkaloids, saponins, and tannins. The inhibition zones of bawang dayak ethanol extract in five various concentrations (1.25%, 2.5%, 5%, 10%, and 20%) were  $7.0 \pm 1.3$  mm,  $8.5 \pm 0.6$  mm,  $9.9 \pm 0.3$  mm,  $11.2 \pm 0.1$  mm, and  $11.9 \pm 0.3$  mm against *P. acnes*;  $18.8 \pm 3.3$  mm,  $21.9 \pm 3.3$  mm,  $20.8 \pm 0.6$  mm,  $22.0 \pm 0.2$  mm, and  $23.1 \pm 0.6$  mm against *S. epidermidis*; and  $14.3 \pm 3.1$  mm,  $13.5 \pm 0.9$  mm,  $14.7 \pm 1.5$  mm,  $16.1 \pm 1.0$  mm, and  $20.1 \pm 0.6$  mm against *S. aureus*, respectively.

**Conclusion:** This present study showed that bawang dayak ethanol extract was active against all the tested acne-causing bacteria. The highest antibacterial activity was produced by 20% of bawang dayak ethanol extract against *S. epidermidis*.

**Keywords:** Antibacterial activity, *Eleutherine bulbosa* (Mill.) Urb., Acne, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*.

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### 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 is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [2]. Acne occurs mostly during adolescence, namely in girls aged 14-17 years and boy aged 16-19 years. However, it can also arise at 40 years, and this disease can also persist into adulthood [3,4]. In Indonesia, around 95-100% of young men and 83-85% of young woman suffer 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].

This infection was influenced by several internal and external factors such as androgen-mediated stimulation of sebaceous gland activity, follicular hyperkeratinization, hormonal imbalance, inflammation, and external bacterial infection. Some of the bacteria that cause acne include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [6,7].

*P. acnes* is a Gram-positive bacteria and is a normal flora of the skin that plays a role in the formation of acne. *P. acnes* was involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis [8]. *S. epidermidis* is an aerobic Gram-positive bacteria, which is usually involved in superficial skin infections in sebaceous units. These bacteria are known as pus-forming bacteria that trigger inflammation of acne [9]. In addition, colonization of the skin by *S. aureus*, an opportunistic Gram-positive pathogen, can also cause acne, and these bacteria can also cause superficial skin infections such as ulcers and impetigo [10].

Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of bacteria. It has been reported that acne patients receive clindamycin, tetracycline, and erythromycin as their treatment tended to cause an increased occurrence of upper respiratory tract infections when compared with acne patients without antibiotic therapy [11]. In addition, the use of antibiotics as the first choice of acne treatment may result in antibiotic resistance due to the evolutionary adaptation of bacteria. Antibiotics have limitations with respect to toxicity and side effects also such as skin drying, headache, and nausea [12]. 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 is one of the traditional medicines that used by the local people of Central Kalimantan Province. Empirically, bawang dayak bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne, ulcers, and colon cancer and to prevent stroke, dysentery, dysuria, and colitis. Active compounds contained in bawang dayak bulb that can provide antibacterial activity include alkaloids, glycosides, flavonoids, phenols, steroids, and tannins [13,14]. The previous study reported that bawang dayak ethanol extract gave minimal inhibitory concentration (MIC) at the concentration of 10 mg/ml against the bacteria *P. acnes*, *S. epidermidis*, and *S. aureus* [15,16]. The present study was initiated to investigate the antibacterial activity of bawang dayak ethanol extract obtained by percolation method against acne-causing bacteria namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

### 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

The bulb part of bawang dayak 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 such as flavonoids, saponins, and alcohol; nontoxic; and safe to use [17]. The extract was concentrated and then subjected to preliminary phytochemical analysis. The percentage yields (w/w) of the extracts were calculated using the formula below [18]:

$$(\text{Weight of extract} + \text{Weight of starting plant material}) \times 100\%$$

#### Phytochemical screening

The extract was used for preliminary screening of phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, and steroids [19,20].

#### Preparation of inoculum

*P. acnes*, *S. epidermidis*, and *S. aureus* were 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 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 bawang dayak ethanol 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 calliper. The study was repeated in triplicates for each extract and positive control.

## RESULTS AND DISCUSSION

#### Yield of the extract

From the rendement calculation, the extraction of bawang dayak yields of 6.0%. Value yield is related to the number of secondary metabolites that successfully attracted when the extraction processes [18].

#### Phytochemical screening

The results of preliminary phytochemical screening of bawang dayak ethanol extract are shown in Table 1.

The results showed that bawang dayak ethanol extract contained flavonoids, alkaloids, saponins, and tannins. The presence of flavonoids, alkaloids, saponins, and tannins in bawang dayak extract can be responsible for the antibacterial properties observed. Alkaloids can inhibit bacterial growth by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes bacterial cell death [21]. Flavonoids are known to cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA through inhibition which results in the incorporation of

non-cross-linked glucan chains into the cell membrane peptidoglycan so that it becomes a weak structure [22]. Tannins can bind to proline-rich proteins and interfere with protein synthesis [23]. Saponin acts as a chemical barrier in the plant defense system to encounter the pathogens. Saponins can cause leakage of certain proteins and enzymes from bacterial cells [24].

#### Antibacterial activity

In this study, clindamycin was used as positive controls. Clindamycin was known as one of the antibiotics that used for acne treatment [11]. 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  $23.0 \pm 0.4$  mm,  $25.4 \pm 0.0$  mm,  $29.0 \pm 0.2$  mm,  $29.9 \pm 0.8$  mm, and  $31.2 \pm 0.1$  mm, respectively as presented in Table 2. 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  $31.7 \pm 0.8$  mm,  $35.6 \pm 1.0$  mm,  $37.1 \pm 1.6$  mm,  $37.7 \pm 0.1$  mm, and  $39.7 \pm 0.4$  mm and  $27.0 \pm 0.5$  mm,  $29.0 \pm 0.8$  mm,  $30.4 \pm 0.4$  mm,  $30.8 \pm 1.9$  mm, and  $31.5 \pm 0.9$  mm, respectively (Table 2). The diameters of inhibition zones produced by all tested concentrations of clindamycin against all tested bacteria were greater than the diameters of inhibition zones produced by bawang dayak ethanol extract.

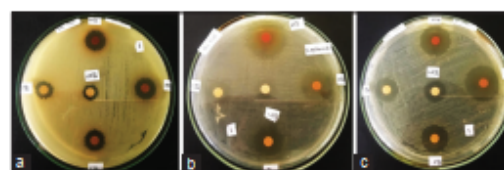
Previous studies reported that bawang dayak ethanol extract obtained by the soxhletation method was able to inhibit the growth of *S. epidermidis* in all tested concentrations of 1%, 5%, 10%, and 15% [25]. Another previous study also reported that the inhibition zones of bawang dayak ethanol extract against *P. acnes* at concentrations of 5% and 10% were  $6.1 \pm 1.5$  mm and  $8.7 \pm 1.3$  mm, respectively [26]. However, this present study showed a better antibacterial activity of bawang dayak ethanol extract against acne-causing bacteria, namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

The antibacterial activity test of the extract against all tested bacteria was done in triplicates. Accordingly, bawang dayak ethanol extract was active against all the tested bacteria, whose inhibition zones were in the range of  $7.0 \pm 1.3$ – $23.1 \pm 0.6$  mm (Fig. 1). The highest antibacterial effect was found for bawang dayak ethanol extract against *S. epidermidis*, with the diameters of inhibition zones at concentrations of 1.25%, 2.5%, 5%, 10%, and 20% being  $18.8 \pm 3.3$  mm,  $21.9 \pm 3.3$  mm,  $20.8 \pm 0.6$  mm,  $22.0 \pm 0.2$  mm, and  $23.1 \pm 0.6$  mm, respectively. The diameters of inhibition zones produced in concentrations of 1.25%, 2.5%, 5%, 10%, and 20% against *S. aureus* were  $14.3 \pm 3.1$  mm,  $13.5 \pm 0.9$  mm,  $14.7 \pm 1.5$  mm,  $16.1 \pm 1.0$  mm, and  $20.1 \pm 0.6$  mm, respectively. On the other hand, the diameters of inhibition zones of bawang dayak ethanol

**Table 1: Results of phytochemical screening of bawang dayak ethanol extract**

Secondary metabolites	Bawang dayak ethanol extract
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Steroids	-

+: Detected, -: Not detected



**Fig. 1: Antibacterial activity of bawang dayak ethanol extract against *Propionibacterium acnes* (a) *Staphylococcus epidermidis* (b) and *Staphylococcus aureus* (c)**

Table 2: Antibacterial activity of clindamycin against acne-inducing 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	*	31.7±0.8	27.0±0.5
	0.04	*	35.6±1.0	29.0±0.8
	0.06	*	37.1±1.6	30.4±0.4
	0.08	*	37.7±0.1	30.8±1.9
	0.10	*	39.7±0.4	31.5±0.9
	0.2	23.0±0.4	*	*
	0.4	25.4±0.0	*	*
	0.6	29.0±0.2	*	*
	0.8	29.9±0.8	*	*
	1.0	31.2±0.1	*	*

\*- Not tested, *P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

Table 3: Antibacterial activity of bawang dayak ethanol extract against acne-inducing bacteria

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Bawang Dayak ethanol extract	1.25	7.0±1.3	18.8±3.3	14.3±3.1
	2.5	8.5±0.6	21.9±3.3	13.5±1.0
	5	9.9±0.3	20.8±0.6	14.7±1.5
	10	11.2±0.1	22.0±0.2	16.1±1.0
	20	11.9±0.3	23.1±0.6	20.1±0.6

*P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

extract against *P. acnes* at concentrations of 1.25%, 2.5%, 5%, 10%, and 20% were 7.0±1.3 mm, 8.5±0.6 mm, 9.9±0.3 mm, 11.2±0.1 mm, and 11.9±0.3 mm, respectively (Table 3). The antimicrobial activities of extracts can be classified into three levels such as 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) [27].

#### CONCLUSION

The results of this study showed that bawang dayak ethanol extract has potentials to inhibit the growth of acne-causing bacteria. Bawang dayak ethanol extract was active against all the tested 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*, wherein the resulting inhibition zone diameter was 23.1±0.6 mm. The presence of flavonoids, alkaloids, saponins, and tannins in the extract can be responsible for the antimicrobial properties observed. Further research is needed to obtain MIC and to develop an antibacterial formulation for acne treatment from bawang dayak extract.

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### THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (ELEUTHERINE BULBOSA (MILL.) URB. FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA

**SUSI NOVARYATIIN**

Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia

**SYAHRIDA DIAN ARDHANY**

Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia

DOI: <https://doi.org/10.22159/ijap.2019.v11s5.T0032>

Keywords: Antibacterial activity, Eleutherine bulbosa (Mill) Urb, Acne, Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus

#### ABSTRACT

**Objective:** The objective of this research was to investigate the antibacterial activity of bawang dayak from Central Kalimantan against acne-causing

bacteria namely Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus.

**Methods:** The preliminary phytochemical constituents were qualitatively analyzed. Antibacterial activity of bawang dayak ethanol extract was

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## THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE BULBOSA* (MILL.) URB.) FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA

SUSI NOVARYATIIN\*, SYAHRIDA DIAN ARDHANY

Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia. Email: susi\_novaryatiin@yahoo.com

Received: 10 December 2018, Revised and Accepted: 20 July 2019

### ABSTRACT

**Objective:** The objective of this research was to investigate the antibacterial activity of bawang dayak from Central Kalimantan against acne-causing bacteria namely *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.

**Methods:** The preliminary phytochemical constituents were qualitatively analyzed. Antibacterial activity of bawang dayak ethanol extract was performed using disc-diffusion technique, with five variations of concentration of 1.25%, 2.5%, 5%, 10%, and 20%.

**Results:** Bawang dayak extract contained flavonoids, alkaloids, saponins, and tannins. The inhibition zones of bawang dayak ethanol extract in five various concentrations (1.25%, 2.5%, 5%, 10%, and 20%) were 7.0±1.3 mm, 8.5±0.6 mm, 9.9±0.3 mm, 11.2±0.1 mm, and 11.9±0.3 mm against *P. acnes*; 18.8±3.3 mm, 21.9±3.3 mm, 20.8±0.6 mm, 22.0±0.2 mm, and 23.1±0.6 mm against *S. epidermidis*; and 14.3±3.1 mm, 13.5±0.9 mm, 14.7±1.5 mm, 16.1±1.0 mm, and 20.1±0.6 mm against *S. aureus*, respectively.

**Conclusion:** This present study showed that bawang dayak ethanol extract was active against all the tested acne-causing bacteria. The highest antibacterial activity was produced by 20% of bawang dayak ethanol extract against *S. epidermidis*.

**Keywords:** Antibacterial activity; *Eleutherine bulbosa* (Mill.) Urb., Acne, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Staphylococcus aureus*.

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### 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 is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [2]. Acne occurs mostly during adolescence, namely in girls aged 14–17 years and boy aged 16–19 years. However, it can also arise at 40 years, and this disease can also persist into adulthood [3,4]. In Indonesia, around 95–100% of young men and 83–85% of young woman suffer 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].

This infection was influenced by several internal and external factors such as androgen-mediated stimulation of sebaceous gland activity, follicular hyperkeratinization, hormonal imbalance, inflammation, and external bacterial infection. Some of the bacteria that cause acne include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [6,7].

*P. acnes* is a Gram-positive bacteria and is a normal flora of the skin that plays a role in the formation of acne. *P. acnes* was involved in the development of inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis [8]. *S. epidermidis* is an aerobic Gram-positive bacteria, which is usually involved in superficial skin infections in sebaceous units. These bacteria are known as pus-forming bacteria that trigger inflammation of acne [9]. In addition, colonization of the skin by *S. aureus*, an opportunistic Gram-positive pathogen, can also cause acne, and these bacteria can also cause superficial skin infections such as ulcers and impetigo [10].

Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of bacteria. It has been reported that acne patients receive clindamycin, tetracycline, and erythromycin as their treatment tended to cause an increased occurrence of upper respiratory tract infections when compared with acne patients without antibiotic therapy [11]. In addition, the use of antibiotics as the first choice of acne treatment may result in antibiotic resistance due to the evolutionary adaptation of bacteria. Antibiotics have limitations with respect to toxicity and side effects also such as skin drying, headache, and nausea [12]. 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 is one of the traditional medicines that used by the local people of Central Kalimantan Province. Empirically, bawang dayak bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne, ulcers, and colon cancer and to prevent stroke, dysentery, dysuria, and colitis. Active compounds contained in bawang dayak bulb that can provide antibacterial activity include alkaloids, glycosides, flavonoids, phenols, steroids, and tannins [13,14]. The previous study reported that bawang dayak ethanol extract gave minimal inhibitory concentration (MIC) at the concentration of 10 mg/ml against the bacteria *P. acnes*, *S. epidermidis*, and *S. aureus* [15,16]. The present study was initiated to investigate the antibacterial activity of bawang dayak ethanol extract obtained by percolation method against acne-causing bacteria namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

### 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

The bulb part of bawang dayak 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 such as flavonoids, saponins, and alcohol; nontoxic; and safe to use [17]. The extract was concentrated and then subjected to preliminary phytochemical analysis. The percentage yields (w/w) of the extracts were calculated using the formula below [18]:

$$(\text{Weight of extract} - \text{Weight of starting plant material}) \times 100\%$$

#### Phytochemical screening

The extract was used for preliminary screening of phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, and steroids [19,20].

#### Preparation of inoculum

*P. acnes*, *S. epidermidis*, and *S. aureus* were 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 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 bawang dayak ethanol 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 extract and positive control.

## RESULTS AND DISCUSSION

#### Yield of the extract

From the rendement calculation, the extraction of bawang dayak yields of 6.0%. Value yield is related to the number of secondary metabolites that successfully attracted when the extraction processes [18].

#### Phytochemical screening

The results of preliminary phytochemical screening of bawang dayak ethanol extract are shown in Table 1.

The results showed that bawang dayak ethanol extract contained flavonoids, alkaloids, saponins, and tannins. The presence of flavonoids, alkaloids, saponins, and tannins in bawang dayak extract can be responsible for the antibacterial properties observed. Alkaloids can inhibit bacterial growth by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes bacterial cell death [21]. Flavonoids are known to cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of interactions between flavonoids and bacterial DNA through inhibition which results in the incorporation of

non-cross-linked glucan chains into the cell membrane peptidoglycan so that it becomes a weak structure [22]. Tannins can bind to proline-rich proteins and interfere with protein synthesis [23]. Saponin acts as a chemical barrier in the plant defense system to encounter the pathogens. Saponins can cause leakage of certain proteins and enzymes from bacterial cells [24].

#### Antibacterial activity

In this study, clindamycin was used as positive controls. Clindamycin was known as one of the antibiotics that used for acne treatment [11]. 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  $23.0 \pm 0.4$  mm,  $25.4 \pm 0.0$  mm,  $29.0 \pm 0.2$  mm,  $29.9 \pm 0.8$  mm, and  $31.2 \pm 0.1$  mm, respectively as presented in Table 2. 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  $31.7 \pm 0.8$  mm,  $35.6 \pm 1.0$  mm,  $37.1 \pm 1.6$  mm,  $37.7 \pm 0.1$  mm, and  $39.7 \pm 0.4$  mm and  $27.0 \pm 0.5$  mm,  $29.0 \pm 0.8$  mm,  $30.4 \pm 0.4$  mm,  $30.8 \pm 1.9$  mm, and  $31.5 \pm 0.9$  mm, respectively (Table 2). The diameters of inhibition zones produced by all tested concentrations of clindamycin against all tested bacteria were greater than the diameters of inhibition zones produced by bawang dayak ethanol extract.

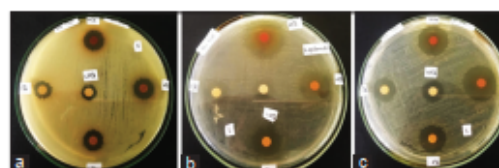
Previous studies reported that bawang dayak ethanol extract obtained by the soxhletation method was able to inhibit the growth of *S. epidermidis* in all tested concentrations of 1%, 5%, 10%, and 15% [25]. Another previous study also reported that the inhibition zones of bawang dayak ethanol extract against *P. acnes* at concentrations of 5% and 10% were  $6.1 \pm 1.5$  mm and  $8.7 \pm 1.3$  mm, respectively [26]. However, this present study showed a better antibacterial activity of bawang dayak ethanol extract against acne-causing bacteria, namely *P. acnes*, *S. epidermidis*, and *S. aureus*.

The antibacterial activity test of the extract against all tested bacteria was done in triplicates. Accordingly, bawang dayak ethanol extract was active against all the tested bacteria, whose inhibition zones were in the range of  $7.0 \pm 1.3$ – $23.1 \pm 0.6$  mm (Fig. 1). The highest antibacterial effect was found for bawang dayak ethanol extract against *S. epidermidis*, with the diameters of inhibition zones at concentrations of 1.25%, 2.5%, 5%, 10%, and 20% being  $18.8 \pm 3.3$  mm,  $21.9 \pm 3.3$  mm,  $20.8 \pm 0.6$  mm,  $22.0 \pm 0.2$  mm, and  $23.1 \pm 0.6$  mm, respectively. The diameters of inhibition zones produced in concentrations of 1.25%, 2.5%, 5%, 10%, and 20% against *S. aureus* were  $14.3 \pm 3.1$  mm,  $13.5 \pm 0.9$  mm,  $14.7 \pm 1.5$  mm,  $16.1 \pm 1.0$  mm, and  $20.1 \pm 0.6$  mm, respectively. On the other hand, the diameters of inhibition zones of bawang dayak ethanol

**Table 1: Results of phytochemical screening of bawang dayak ethanol extract**

Secondary metabolites	Bawang dayak ethanol extract
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Steroids	-

+: Detected, -: Not detected



**Fig. 1: Antibacterial activity of bawang dayak ethanol extract against *Propionibacterium acnes* (a) *Staphylococcus epidermidis* (b) and *Staphylococcus aureus* (c)**



Table 2: Antibacterial activity of clindamycin against acne-inducing 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	-	31.7±0.8	27.0±0.5
	0.04	-	35.6±1.0	29.0±0.8
	0.06	-	37.1±1.6	30.4±0.4
	0.08	-	37.7±0.1	30.8±1.9
	0.10	-	39.7±0.4	31.5±0.9
	0.2	23.0±0.4	-	-
	0.4	25.4±0.0	-	-
	0.6	29.0±0.2	-	-
	0.8	29.9±0.8	-	-
	1.0	31.2±0.1	-	-

◁ Not tested, *P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

Table 3: Antibacterial activity of bawang dayak ethanol extract against acne-inducing bacteria

Materials	Concentration (%)	Inhibition zone diameter (mm) (mean±SD; n=3)		
		<i>P. acnes</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Bawang Dayak ethanol extract	1.25	7.0±1.3	18.8±3.3	14.3±3.1
	2.5	8.5±0.6	21.9±3.3	13.5±1.0
	5	9.9±0.3	20.8±0.6	14.7±1.5
	10	11.2±0.1	22.0±0.2	16.1±1.0
	20	11.9±0.3	23.1±0.6	20.1±0.6

*P. acnes*: *Propionibacterium acnes*, *S. epidermidis*: *Staphylococcus epidermidis*, *S. aureus*: *Staphylococcus aureus*, SD: Standard deviation

extract against *P. acnes* at concentrations of 1.25%, 2.5%, 5%, 10%, and 20% were 7.0±1.3 mm, 8.5±0.6 mm, 9.9±0.3 mm, 11.2±0.1 mm, and 11.9±0.3 mm, respectively (Table 3). The antimicrobial activities of extracts can be classified into three levels such as 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) [27].

#### CONCLUSION

The results of this study showed that bawang dayak ethanol extract has potentials to inhibit the growth of acne-causing bacteria. Bawang dayak ethanol extract was active against all the tested 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*, wherein the resulting inhibition zone diameter was 23.1±0.6 mm. The presence of flavonoids, alkaloids, saponins, and tannins in the extract can be responsible for the antimicrobial properties observed. Further research is needed to obtain MIC and to develop an antibacterial formulation for acne treatment from bawang dayak extract.

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Komite Etik Penelitian  
STIKES Sari Mulia Banjarmasin

Jl. Pramuka No.02 Banjarmasin Telp. 0511-3268105 Fax.0511-3270134

Banjarmasin, 11 April 2018

No. SK : 004.4/KE-LPPM/STIKES-SM/IV/2018  
Lampiran : -  
Perihal : Rekomendasi Penelitian

Sehubungan dengan telah dilaksanakannya sidang Etik Penelitian kepada:

Nama Ketua : Susi Novaryatiin  
NIK : 14.06.01.015  
Anggota : Syahrída Dian Ardhany  
Judul : Uji aktivitas antibakteri Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) asal Kalimantan Tengah terhadap bakteri penyebab jerawat

Telah **DISETUJUI** untuk dilanjutkan penelitiannya.

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

Menyetujui

An. Ketua

Sebagai Ketua Komite Etik Penelitian



Ali Rakhman Hakim, M.Farm., Apt  
NIP. 1944.2015.100