BUKTI KORESPONDENSI

Lampiran	**:**	Peer review proses korespondensi submit Publikasi Jurnal Ilmiah Internasional Bereputasi
Nama Jurnal	**:**	International Journal of Applied Pharmaceutics Vol. 11 Special Issue 3 2019 Hal: 11-13
Index	**:**	Terindeks pada database internasional bereputasi dan berfaktor dampak (SCOPUS Q3)
Judul Jurnal	**:**	Phytochemical Screening and Antibacterial Activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and Their Combination Against *Propionibacterium acnes*

No	Item	Tanggal	Halaman
1	**Register/Submission pada konferensi internasional ICBMS 2018 (International Conference on Biological and Medical Sciences) di Seoul, Korea Selatan 22-24 Agustus 2018**	15 Mei 2018	1
2	**Artikel accepted pada jurnal AJPCR (Asian Journal of Pharmaceutical and Clinical Research) (M0011)**	04 Juni 2018	2
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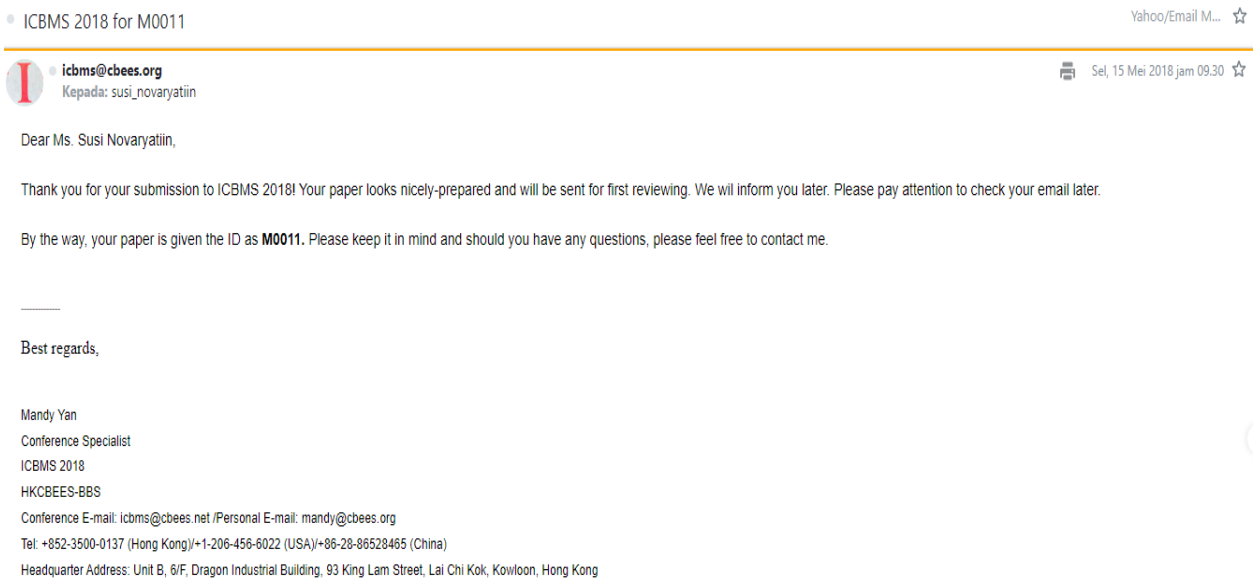
4	**Review process (AJPCR)**	16 Februari 2019	8
5	**Artikel accepted pada jurnal AJPCR namun jurnal tersebut masuk dalam kategori discontinued scopus sehingga panitia konferensi mengganti ke IJAP (International Journal of Applied Pharmaceutics) dengan indeks scopus Q3 (M0011)**	13 Maret 2019	14
6	**Article for final proof (IJAP)**	13 Mei 2019	14
7	**Article Published (IJAP)**	15 Juli 2019	18
8	**Ethical approval**	03 Februari 2018	22

1. **Register/Submission pada konferensi internasional ICBMS 2018 (International Conference on Biological and Medical Sciences) di Seoul, Korea Selatan 22-24 Agustus 2018**



ICBMS 2018 for M0011

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icbms@cbees.org
Kepada: susi_novaryatiin

Sel, 15 Mei 2018 jam 09.30

Dear Ms. Susi Novaryatiin,

Thank you for your submission to ICBMS 2018! Your paper looks nicely-prepared and will be sent for first reviewing. We wil inform you later. Please pay attention to check your email later.

By the way, your paper is given the ID as **M0011.** Please keep it in mind and should you have any questions, please feel free to contact me.

Best regards,

Mandy Yan
Conference Specialist
ICBMS 2018
HKCBEES-BBS
Conference E-mail: icbms@cbees.net /Personal E-mail: mandy@cbees.org
Tel: +852-3500-0137 (Hong Kong)/+1-206-456-6022 (USA)/+86-28-86528465 (China)
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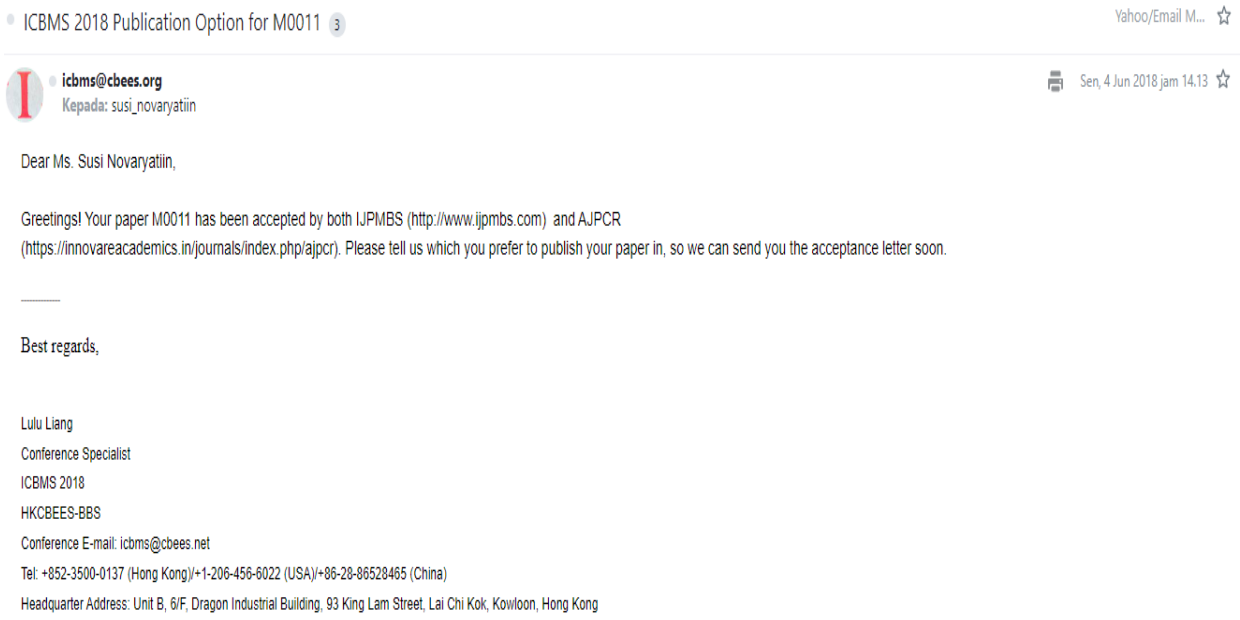
2. **Artikel accepted pada jurnal AJPCR (Asian Journal of Pharmaceutical and Clinical Research) (M0010)**



ICBMS 2018 Publication Option for M0011 3

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Sen, 4 Jun 2018 jam 14.13

Dear Ms. Susi Novaryatiin,

Greetings! Your paper M0011 has been accepted by both IJPMBS (http://www.ijpmbs.com) and AJPCR (https://innovareacademics.in/journals/index.php/ajpcr). Please tell us which you prefer to publish your paper in, so we can send you the acceptance letter soon.

Best regards,

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3. Notification dan Review artikel

ICBMS 2018 Notification for M0011

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Dear Susi,

Congratulations that your paper **M0011** has been accepted by **AJPCR** and also do oral presentation in ICBMS 2018. Please find the attached Notification Letter, Review Form and Registration Form. Please finish the registration according to the Notification Form and send back the revised paper, registration form and payment proof to me before **June 25, 2018**.

Should you have any questions, please feel free to contact me.

Best regards,

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Notification of Acceptance of the ICBMS 2018

Seoul, South Korea, August 22-24, 2018

http://www.icbms.org/

Paper ID : M0011

Paper Title : Phytochemical Screening and Antibacterial Activity of Bawang Dayak (Eleutherine Sp.), Hati Tanah (Angiopteris Sp.) and Their Combination against Propionibacterium Acnes

Dear Susi Novaryatiin,

First of all, thank you for your concern. 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) review procedure has been finished. We are delighted to inform you that your manuscript has been accepted for presentation at 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) in Seoul, South Korea during August 22-24, 2018. Your paper was tripling blind-reviewed and based on the evaluation. The reviewers' comments are enclosed.

The conference received papers from about 10 different countries and regions during the submission period. And there are about 77 papers accepted by our reviewers who are the international experts from all over the world. The selected papers could be published in our conference journal 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 ICBMS conference that would be held on August 22-24, 2018, Seoul, South Korea.

The ICBMS 2018 is co-sponsored by Hong Kong Chemical, Biological & Environmental Engineering Society (HKCBEES) and Biology and Bioinformatics Society (BBS).

Paper of ICBMS 2018 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, SCI mago(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).

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We are looking forward to meet all the authors in our conference. But if you and your co-author(s) could not attend ICBMS 2018 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 ICBMS 2018.

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Again, congratulations. We are looking forward to seeing you in Seoul, South Korea.

Yours sincerely,

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The Evaluation of the paper		
Topic	**The Topic's Conformity**	■Match to the conference topic **very well**; □Match to the conference topic **fairly**; □Match to the conference topic **poorly**;
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	The "literary" presentation	□Totally Accessible ■Mostly Accessible □Partially Accessible □Inaccessible
	The technical depth	□Superficial □Suitable for the non-specialist ■Appropriate for the generally knowledgeable individual working in the field □Suitable only for an expert
Presentation & English	□Satisfactory ■Needs improvement □Poor	
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Recommendation for Publication& Detailed Suggestions	
□Accepted (please chose one)	□Strongly Accept; ■Accept; □weakly Accept **Comments (Please prepare the final version of the paper as per review instructions):** 1. This paper investigates the effect of BD and TU against Propionibacterium acnes. The paper matches the topic very well. Readers can easily catch the theme of the paper through the clear and logic presentation of the abstract. 2. This is a well-written paper containing interesting results, however, it is better to conclude the results in detail and discuss further. 3. Few grammar and formatted errors. E.g. author information's superscript is not required and underline in Table 3 is missing. Please check carefully.
□Rejected	☐ Strongly Reject ☐ Reject ☐ weakly Reject

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4. Review process (AJPCR)

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Sab, 16 Feb 2019 jam 14.32

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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.

8.Insert Table(s) and Figure(s) in Result and Discussion Section at an appropriate place.

9.Headings of the table (s) and figure (s) should be rectified. See the latest issue of AJPCR.

Reviewer Comments for article reference no AJPCR_M0011_RA_20190402_V0

Phytochemical Screening and Antibacterial Activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and Their Combination Against *Propionibacterium acnes*

1. Very general expressions are used in the Introduction section. There is no explanation of the correlation between antibiotics and antibacterial from plants.
2. In this study, what sets it apart from previous research on the antimicrobial properties of these two plants? The introduction should give more attention to this aspect.
3. Which specific plant parts were utilized as specimens from the two plants?
4. What is the specific reason for using ethanol 70% for extraction?
5. Include the reason for choosing clindamycin as the positive control.

Article reference no AJPCR_M0011_RA_20190204_V0

Title **PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND HATI TANAH (*ANGIOPTERIS* SP.) AND THEIR COMBINATION AGAINST *PROPIONIBACTERIUM ACNES***

Name & mail address of corresponding author Susi Novaryatiin, mail: susi_novaryatiin@yahoo.com

Highlighted Corrections

S.No.	Asked query no.	Details
1	1	The correlation was added in the article: 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. This condition encourages the development of research to explore antimicrobial agents from plant origin.
2	2	The difference from previous research was added in the article: The combination of interactions of antimicrobial agents has shown significant effectiveness and two or more compounds interact to produce a joint increase, reinforcing the effects of each antimicrobial agent. Combinations can increase the efficacy of other antimicrobial agents and can be used as an alternative treatment for infections caused by multidrug-resistant microorganism that have not yet had effective therapy. The present study was initiated to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.), and their combination against *Propionibacterium acnes*.
3	3	The bulb part of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were washed thoroughly with tap water, shade dried, powdered using blender and stored.
4	4	The use of 70% ethanol solvents was due to its universal properties that capable of dissolving almost all types of secondary metabolites, non-toxic, and safe to use
5	5	In this study, clindamycin was used as positive controls. *Propionibacterium acnes* was known to
		be sensitive to antibiotics such as clindamycin, tetracycline, quinolones, penicillins, and cephalosporins.

Other corrections

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

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Vol 12, Special Issue 2, 2019

Online - 2455-3891
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Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND HATI TANAH (*ANGIOPTERIS* SP.) AND THEIR COMBINATION AGAINST *PROPIONIBACTERIUM ACNES*

SUSI NOVARYATIIN*

Department of Pharmacy, Faculty of Health Science, Muhammadiyah University of Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia. Email: susi_novaryatiin@yahoo.com

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

ABSTRACT

Objective: The objective of this research was to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *Propionibacterium acnes*.

Methods: The extracts were used for phytochemical screening. Antibacterial activity was performed using disc diffusion technique, with two variations of the concentration of 5% and 10% for each extracts, and combination of both extracts with three combinations: (1) 5%: 5%, (2) 5%: 10%, and (3) 10%: 5%.

Results: Both extracts contained tannins, saponins, and steroids. The antibacterial activity against *P. acnes* showed that the inhibition zones of Bawang Dayak ethanol extract were 6.1±1.5 mm (5%) and 8.7±1.3 mm (10%). On the other hand, the inhibition zones of Hati Tanah ethanol extract were 4.0±1.6 mm (5%) and 9.2±2.5 mm (10%). The inhibition zones produced in combinations 1, 2, and 3 were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively.

Conclusion: In this present study showed the presence of tannins, saponins and steroids in both extracts can be responsible for the antimicrobial properties observed. The best antibacterial activity was produced by combining the two extracts (combination 3).

Keywords: Antibacterial activity, *Eleutherine* sp., *Angiopteris* sp., *Propionibacterium acnes*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12s2.M0011

INTRODUCTION

Acne is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [1]. 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 [2,3]. *Propionibacterium 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 [4]. Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of *P. acnes*. It has been reported that acne patients who 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 [5]. 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. This condition encourages the development of research to explore antimicrobial agents from plant origin.

Kalimantan is the largest island in Indonesia that is famous for its biodiversity. Besides that, there is knowledge of traditional medicine using plants that are passed on orally from generation to generation on indigenous ethnic in Kalimantan. These biodiversities are scattered all over Kalimantan Island, one of which is in Central Kalimantan Province. Central Kalimantan with an area of 15,380,410 hectares where about 70% is considered as forested area. This province has been found to be the home of medicinal plant biodiversity [6,7].

Various medicinal plants that have benefits as traditional medicines and are used by the people of Palangka Raya City, Central Kalimantan Province such as Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.). Empirically, Bawang Dayak (*Eleutherine* sp.) bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne and ulcers, colon cancer, prevent stroke, dysentery, dysuria, and colitis [8]. Active compounds contained in Bawang Dayak bulb that can provide antibacterial activity include flavonoid, phenols, glycosides, triterpenoids, and anthraquinone. Previous research reported that Bawang Dayak ethanol extract gave minimal inhibitory concentrations at concentrations of 10 mg/ml against the bacteria *P. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [9,10]. Hati Tanah (*Angiopteris* sp.) bulb was empirically believed to treat wounds, postpartum sores, malaria, and diarrhea. It was known to contain several active compounds such as flavonoids and tannins, which have antibacterial properties [11]. The previous study showed that Hati Tanah bulb ethanol extract was effective in inhibiting the growth of *S. aureus* with inhibition zone diameter of 15.63±0.15 mm at extract concentration of 1% [12].

The combination of interactions of the antimicrobial agents has shown significant effectiveness and two or more compounds interact to produce a joint increase, reinforcing the effects of each antimicrobial agent. Combinations can increase the efficacy of other antimicrobial agents and can be used as an alternative treatment for infections caused by multidrug-resistant microorganism that has not yet had effective therapy [13,14]. The present study was initiated to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *P. acnes*.

MATERIALS AND METHODS

Plant material

Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were purchased from Kahayan Traditional Market of Palangka Raya, Central Kalimantan, Indonesia. The bulb part of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) was washed thoroughly with tap water, shade dried, powdered using blender, and stored.

Preparation of plant extract

Dried powders of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were extracted with ethanol 70% using Soxhlet's apparatus. The advantage of this system was that instead of many portions of the warm solvent being passed through the sample, just one batch of solvent is recycled [15]. The use of 70% ethanol solvents was due to its universal properties that capable of dissolving almost all types of secondary metabolites, non-toxic, and safe to use [16,17]. The extracts were concentrated and then subjected preliminary phytochemical analysis.

Phytochemical screening

The extracts were used for preliminary screening of phytochemicals such as alkaloids, tannins, flavonoids, triterpenoids, steroids, and saponins [18,19].

Inoculum preparation

P. acnes was grown in brain heart infusion medium for 24 h at 37°C and then grown on the blood agar plate for 24 h at 37°C.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Antibacterial activity was performed using disc diffusion technique [20], where the discs were impregnated with two variations of the concentration of 5% and 10% for each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts. The McFarland 0.5 standard was prepared [21] and 10 mL were put into sterile tubes. Bacterial suspension was made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1-2 × 10^8 CFU/mL (according to McFarland 0.5 standard). A sterile cotton swab was immersed in a standardized bacterial suspension and used to event inoculate on Mueller-Hinton agar plate. Then, all the discs that have been immersed in each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts were placed on the plates. A clindamycin antibiotic was used as positive controls with concentration variations of 5% and 10%. Discs that have been immersed in clindamycin were also placed on the plate. The plate was 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.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination

Antibacterial activity of containing Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination was determined. Solutions containing various concentrations of Bawang Dayak (*Eleutherine* sp.) and various concentrations of Hati Tanah (*Angiopteris* sp.) were prepared. These solutions consisted of the (1) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract, (2) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 10% of Hati Tanah (*Angiopteris* sp.) ethanol extract, and (3) combination of 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% of Hati Tanah (*Angiopteris* sp.) ethanol extract. Antibacterial activity test of these combinations was tested by procedure as described above. The study was repeated in triplicates for each combination.

RESULTS AND DISCUSSION

The results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract are shown in Table 1.

The results showed that both Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts contained tannins, saponins, and steroids. However, alkaloids were found only in Bawang Dayak (*Eleutherine* sp.) ethanol extract, whereas triterpenoids were found only in Hati Tanah (*Angiopteris* sp.) ethanol extract. The flavonoids were not detected in both extracts. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. Tannins can bind to proline-rich proteins and interfere with protein synthesis [22]. 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 [23,24]. Steroids were reported to have antibacterial properties through mechanisms in which steroids can bind to lipid membranes and cause leakage in liposome action [25].

In this study, clindamycin was used as positive controls. *P. acnes* was known to be sensitive to antibiotics such as clindamycin, tetracycline, quinolones, penicillins, and cephalosporins [26]. The diameters of inhibition zones produced by clindamycin with the concentration of 5% and 10% against *P. acnes* were 34.3±2.7 mm and 37.1±2.5 mm, respectively, as presented in Table 2.

The antibacterial activity test of extract against *P. acnes* that was done in triplicates showed the existence of the variation of inhibition zone diameter. The diameters of the inhibition zones of Bawang Dayak (*Eleutherine* sp.) ethanol extract at concentrations of 5% and 10% were 6.1±1.5 mm and 8.7±1.3 mm, respectively. On the other hand, the diameters of the inhibition zones of Hati Tanah (*Angiopteris* sp.) ethanol extract at concentrations of 5% and 10% were 4.0±1.6 mm and 9.2±2.5 mm, respectively.

The antibacterial activity test was also performed by combining Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts and then tested against *P. acnes*. The diameters of inhibition zones produced in combination 1 (5%:5%), combination 2 (5%:10%), and combination 3 (10%:5%) were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively (Table 3).

CONCLUSION

The results of this study showed that both Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract have potentials to inhibit the growth of *P. acnes*. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. The best antibacterial activity was produced by combining the two extracts with 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract (combination 3), wherein the resulting inhibition zone diameter was 15.5±2.8 mm. Clindamycin as a positive control produced a much larger inhibition zone diameter, but there was a high probability of increasing inhibition zone diameter if the concentration of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract is increased. Further, research is needed to obtain minimum inhibitory concentration from both plant extracts and

Table 1: Results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Secondary metabolites	Results	
	Bawang Dayak ethanol extract	Hati Tanah ethanol extract
Alkaloids	+	−
Flavonoids	−	−
Saponins	+	+
Triterpenoids	−	+
Steroids	+	+
Tannins	+	+

+: Detected, −: Not detected

Table 2: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract against *Propionibacterium acnes*

Materials	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
		1	2	3	
Clindamycin (positive control)	5	36.4	35.1	31.3	34.3±2.7
	10	39.9	36.3	35.2	37.1±2.5
Bawang Dayak (*Eleutherine* sp.) ethanol extract	5	5.6	4.9	7.7	6.1±1.5
	10	8.0	7.8	10.2	8.7±1.3
Hati Tanah (*Angiopteris* sp.) ethanol extract	5	2.3	5.5	4.2	4.0±1.6
	10	11.2	6.4	10.0	9.2±2.5

Table 3: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination against *Propionibacterium acnes*

Materials	Combination	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
			1	2	3	
Combination of Bawang Dayak (*Eleutherine* sp.) ethanol extract: Hati Tanah (*Angiopteris* sp.) ethanol extract	1	5:5	5.5	5.8	6.1	5.8±0.3
	2	5:10	12.3	8.5	11.7	10.8±2.0
	3	10:5	16.9	17.3	12.3	15.5±2.8

their combinations so that it can be developed into an antibacterial formulation for acne treatment.

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Author Queries???
AQ1:Kindly provide history details
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Article reference no AJPCR_M0011_RA_20190204_V0
Title **PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND HATI TANAH (*ANGIOPTERIS* SP.) AND THEIR COMBINATION AGAINST *PROPIONIBACTERIUM ACNES***
Name & mail address of corresponding author Susi Novaryatiin, mail: susi_novaryatiin@yahoo.com

Highlighted Corrections

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1	AQ1	*Received: 05 June 2018 , Revised and Accepted: 16 February 2019*
2	AQ2	In this study showed the presence of tannins, saponins, and steroids that might be responsible for antibacterial activity in both extract.
3	AQ3	There is no author initial, his name only "Subeki"
4	AQ4	Warnida H, Sukawaty Y, Mega. Stability and antibacterial activity of ethanol extract bawang tiwai (*Eleutherine americana* (Mill.) Urb.) in anti-acne gel. J Ilmiah Manuntung 2015;1:94-9.

Other corrections

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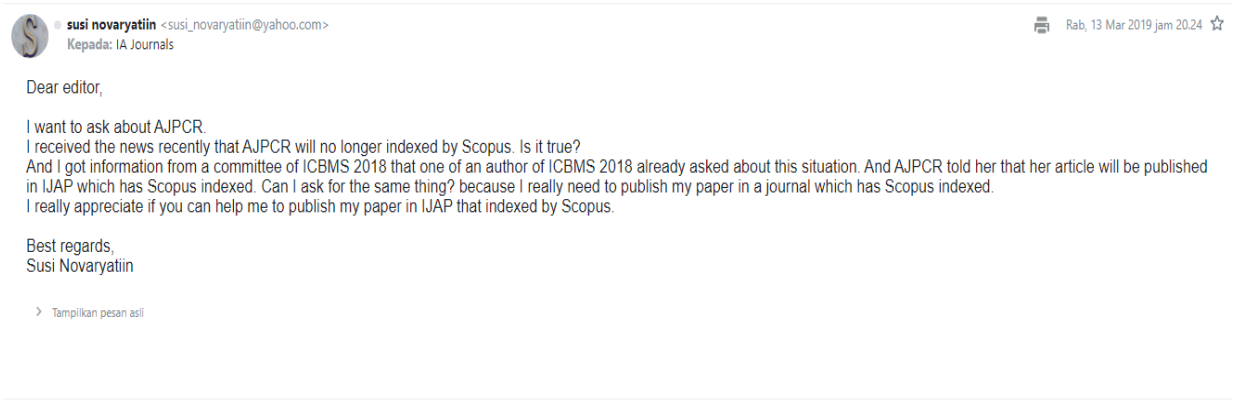

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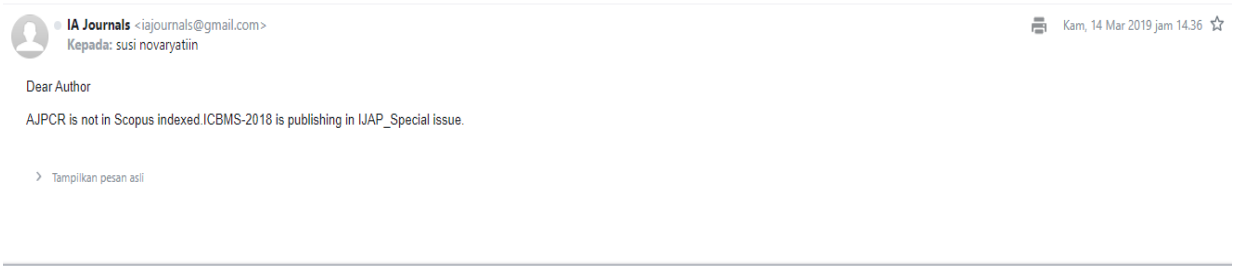

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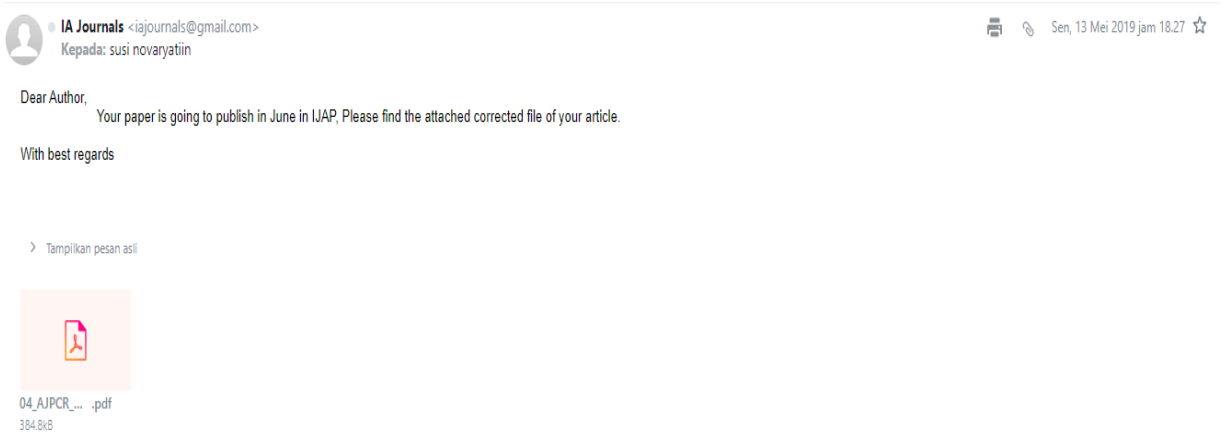

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Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND HATI TANAH (*ANGIOPTERIS* SP.) AND THEIR COMBINATION AGAINST *PROPIONIBACTERIUM ACNES*

SUSI NOVARYATIIN*

Department of Pharmacy, Faculty of Health Science, Muhammadiyah University of 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 preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *Propionibacterium acnes*.

Methods: The extracts were used for phytochemical screening. Antibacterial activity was performed using disc diffusion technique, with two variations of the concentration of 5% and 10% for each extracts, and combination of both extracts with three combinations: (1) 5%: 5%, (2) 5%: 10%, and (3) 10%: 5%.

Results: Both extracts contained tannins, saponins, and steroids. The antibacterial activity against *P. acnes* showed that the inhibition zones of Bawang Dayak ethanol extract were 6.1±1.5 mm (5%) and 8.7±1.3 mm (10%). On the other hand, the inhibition zones of Hati Tanah ethanol extract were 4.0±1.6 mm (5%) and 9.2±2.5 mm (10%). The inhibition zones produced in combinations 1, 2, and 3 were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively.

Conclusion: In this study showed the presence of tannins, saponins, and steroids that might be responsible for antibacterial activity in both extract. The best antibacterial activity was produced by combining the two extracts (combination 3).

Keywords: Antibacterial activity, *Eleutherine* sp., *Angiopteris* sp., *Propionibacterium acnes*.

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INTRODUCTION

Acne is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [1]. 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 [2,3]. *Propionibacterium 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 [4]. Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of *P. acnes*. It has been reported that acne patients who 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 [5]. 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. This condition encourages the development of research to explore antimicrobial agents from plant origin.

Kalimantan is the largest island in Indonesia that is famous for its biodiversity. Besides that, there is knowledge of traditional medicine using plants that are passed on orally from generation to generation on indigenous ethnic in Kalimantan. These biodiversities are scattered all over Kalimantan Island, one of which is in Central Kalimantan Province. Central Kalimantan with an area of 15,380,410 hectares where about 70% is considered as forested area. This province has been found to be the home of medicinal plant biodiversity [6,7].

Various medicinal plants that have benefits as traditional medicines and are used by the people of Palangka Raya City, Central Kalimantan Province such as Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.). Empirically, Bawang Dayak (*Eleutherine* sp.) bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne and ulcers, colon cancer, prevent stroke, dysentery, dysuria, and colitis [8]. Active compounds contained in Bawang Dayak bulb that can provide antibacterial activity include flavonoid, phenols, glycosides, triterpenoids, and anthraquinone. Previous research reported that Bawang Dayak ethanol extract gave minimal inhibitory concentrations at concentrations of 10 mg/ml against the bacteria *P. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [9,10]. Hati Tanah (*Angiopteris* sp.) bulb was empirically believed to treat wounds, postpartum sores, malaria, and diarrhea. It was known to contain several active compounds such as flavonoids and tannins, which have antibacterial properties [11]. The previous study showed that Hati Tanah bulb ethanol extract was effective in inhibiting the growth of *S. aureus* with inhibition zone diameter of 15.63±0.15 mm at extract concentration of 1% [12].

The combination of interactions of the antimicrobial agents has shown significant effectiveness and two or more compounds interact to produce a joint increase, reinforcing the effects of each antimicrobial agent. Combinations can increase the efficacy of other antimicrobial agents and can be used as an alternative treatment for infections caused by multidrug-resistant microorganism that has not yet had effective therapy [13,14]. The present study was initiated to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *P. acnes*.

MATERIALS AND METHODS

Plant material

Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were purchased from Kahayan Traditional Market of Palangka Raya, Central Kalimantan, Indonesia. The bulb part of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) was washed thoroughly with tap water, shade dried, powdered using blender, and stored.

Preparation of plant extract

Dried powders of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were extracted with ethanol 70% using Soxhlet's apparatus. The advantage of this system was that instead of many portions of the warm solvent being passed through the sample, just one batch of solvent is recycled [15]. The use of 70% ethanol solvents was due to its universal properties that capable of dissolving almost all types of secondary metabolites, non-toxic, and safe to use [16,17]. The extracts were concentrated and then subjected preliminary phytochemical analysis.

Phytochemical screening

The extracts were used for preliminary screening of phytochemicals such as alkaloids, tannins, flavonoids, triterpenoids, steroids, and saponins [18,19].

Inoculum preparation

P. acnes was grown in brain heart infusion medium for 24 h at 37°C and then grown on the blood agar plate for 24 h at 37°C.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Antibacterial activity was performed using disc diffusion technique [20], where the discs were impregnated with two variations of the concentration of 5% and 10% for each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts. The McFarland 0.5 standard was prepared [21] and 10 mL were put into sterile tubes. Bacterial suspension was made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1-2 × 10^8 CFU/mL (according to McFarland 0.5 standard). A sterile cotton swab was immersed in a standardized bacterial suspension and used to event inoculate on Mueller-Hinton agar plate. Then, all the discs that have been immersed in each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts were placed on the plates. A clindamycin antibiotic was used as positive controls with concentration variations of 5% and 10%. Discs that have been immersed in clindamycin were also placed on the plate. The plate was 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.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination

Antibacterial activity of containing Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination was determined. Solutions containing various concentrations of Bawang Dayak (*Eleutherine* sp.) and various concentrations of Hati Tanah (*Angiopteris* sp.) were prepared. These solutions consisted of the (1) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract, (2) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 10% of Hati Tanah (*Angiopteris* sp.) ethanol extract, and (3) combination of 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% of Hati Tanah (*Angiopteris* sp.) ethanol extract. Antibacterial activity test of these combinations was tested by procedure as described above. The study was repeated in triplicates for each combination.

RESULTS AND DISCUSSION

The results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract are shown in Table 1.

The results showed that both Bawang Dayak (*Eleutherine sp.*) and Hati Tanah (*Angiopteris* sp.) ethanol extracts contained tannins, saponins, and steroids. However, alkaloids were found only in Bawang Dayak (*Eleutherine* sp.) ethanol extract, whereas triterpenoids were found only in Hati Tanah (*Angiopteris* sp.) ethanol extract. The flavonoids were not detected in both extracts. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. Tannins can bind to proline-rich proteins and interfere with protein synthesis [22]. 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 [23,24]. Steroids were reported to have antibacterial properties through mechanisms in which steroids can bind to lipid membranes and cause leakage in liposome action [25].

In this study, clindamycin was used as positive controls. *P. acnes* was known to be sensitive to antibiotics such as clindamycin, tetracycline, quinolones, penicillins, and cephalosporins [26]. The diameters of inhibition zones produced by clindamycin with the concentration of 5% and 10% against *P. acnes* were 34.3±2.7 mm and 37.1±2.5 mm, respectively, as presented in Table 2.

The antibacterial activity test of extract against *P. acnes* that was done in triplicates showed the existence of the variation of inhibition zone diameter. The diameters of the inhibition zones of Bawang Dayak (*Eleutherine* sp.) ethanol extract at concentrations of 5% and 10% were 6.1±1.5 mm and 8.7±1.3 mm, respectively. On the other hand, the diameters of the inhibition zones of Hati Tanah (*Angiopteris* sp.) ethanol extract at concentrations of 5% and 10% were 4.0±1.6 mm and 9.2±2.5 mm, respectively.

The antibacterial activity test was also performed by combining Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts and then tested against *P. acnes*. The diameters of inhibition zones produced in combination 1 (5%:5%), combination 2 (5%:10%), and combination 3 (10%:5%) were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively (Table 3).

CONCLUSION

The results of this study showed that both Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract have potentials to inhibit the growth of *P. acnes*. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. The best antibacterial activity was produced by combining the two extracts with 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract (combination 3), wherein the resulting inhibition zone diameter was 15.5±2.8 mm. Clindamycin as a positive control produced a much larger inhibition zone diameter, but there was a high probability of increasing inhibition zone diameter if the concentration of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract is increased. Further, research is needed to obtain minimum inhibitory concentration from both plant extracts and

Table 1: Results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Secondary metabolites	Results	
	Bawang Dayak ethanol extract	Hati Tanah ethanol extract
Alkaloids	+	−
Flavonoids	−	−
Saponins	+	+
Triterpenoids	−	+
Steroids	+	+
Tannins	+	+

+: Detected, −: Not detected

Table 2: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract against *Propionibacterium acnes*

Materials	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
		1	2	3	
Clindamycin (positive control)	5	36.4	35.1	31.3	34.3±2.7
	10	39.9	36.3	35.2	37.1±2.5
Bawang Dayak (*Eleutherine* sp.) ethanol extract	5	5.6	4.9	7.7	6.1±1.5
	10	8.0	7.8	10.2	8.7±1.3
Hati Tanah (*Angiopteris* sp.) ethanol extract	5	2.3	5.5	4.2	4.0±1.6
	10	11.2	6.4	10.0	9.2±2.5

Table 3: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination against *Propionibacterium acnes*

Materials	Combination	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
			1	2	3	
Combination of Bawang Dayak (*Eleutherine* sp.) ethanol extract: Hati Tanah (*Angiopteris* sp.) ethanol extract	1	5:5	5.5	5.8	6.1	5.8±0.3
	2	5:10	12.3	8.5	11.7	10.8±2.0
	3	10:5	16.9	17.3	12.3	15.5±2.8

their combinations so that it can be developed into an antibacterial formulation for acne treatment.

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The author would like to express her 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 to the 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018), August 22-24, 2018.

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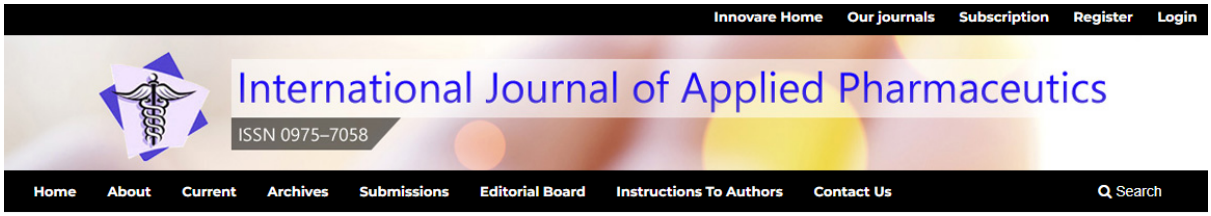

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (ELEUTHERINE SP.) AND HATI TANAH (ANGIOPTERIS SP.) AND THEIR COMBINATION AGAINST PROPIONIBACTERIUM ACNES

SUSI NOVARYATIIN
Department of Pharmacy, Faculty of Health Science, Muhammadiyah University of Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia

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Objective: The objective of this research was to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak
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Methods: The extracts were used for phytochemical screening. Antibacterial activity was performed using disc diffusion technique, with two

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Research Article

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND HATI TANAH (*ANGIOPTERIS* SP.) AND THEIR COMBINATION AGAINST *PROPIONIBACTERIUM ACNES*

SUSI NOVARYATIIN*

Department of Pharmacy, Faculty of Health Science, Muhammadiyah University of 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 preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *Propionibacterium acnes*.

Methods: The extracts were used for phytochemical screening. Antibacterial activity was performed using disc diffusion technique, with two variations of the concentration of 5% and 10% for each extracts, and combination of both extracts with three combinations: (1) 5%: 5%, (2) 5%: 10%, and (3) 10%: 5%.

Results: Both extracts contained tannins, saponins, and steroids. The antibacterial activity against *P. acnes* showed that the inhibition zones of Bawang Dayak ethanol extract were 6.1±1.5 mm (5%) and 8.7±1.3 mm (10%). On the other hand, the inhibition zones of Hati Tanah ethanol extract were 4.0±1.6 mm (5%) and 9.2±2.5 mm (10%). The inhibition zones produced in combinations 1, 2, and 3 were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively.

Conclusion: In this study showed the presence of tannins, saponins, and steroids that might be responsible for antibacterial activity in both extract. The best antibacterial activity was produced by combining the two extracts (combination 3).

Keywords: Antibacterial activity, *Eleutherine* sp., *Angiopteris* sp., *Propionibacterium acnes*.

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INTRODUCTION

Acne is one of the most common and chronic skin infections, affecting almost everyone during his lifetime [1]. 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 [2,3]. *Propionibacterium 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 [4]. Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of *P. acnes*. It has been reported that acne patients who 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 [5]. 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. This condition encourages the development of research to explore antimicrobial agents from plant origin.

Kalimantan is the largest island in Indonesia that is famous for its biodiversity. Besides that, there is knowledge of traditional medicine using plants that are passed on orally from generation to generation on indigenous ethnic in Kalimantan. These biodiversities are scattered all over Kalimantan Island, one of which is in Central Kalimantan Province. Central Kalimantan with an area of 15,380,410 hectares where about 70% is considered as forested area. This province has been found to be the home of medicinal plant biodiversity [6,7].

Various medicinal plants that have benefits as traditional medicines and are used by the people of Palangka Raya City, Central Kalimantan Province such as Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.). Empirically, Bawang Dayak (*Eleutherine* sp.) bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne and ulcers, colon cancer, prevent stroke, dysentery, dysuria, and colitis [8]. Active compounds contained in Bawang Dayak bulb that can provide antibacterial activity include flavonoid, phenols, glycosides, triterpenoids, and anthraquinone. Previous research reported that Bawang Dayak ethanol extract gave minimal inhibitory concentrations at concentrations of 10 mg/ml against the bacteria *P. acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* [9,10]. Hati Tanah (*Angiopteris* sp.) bulb was empirically believed to treat wounds, postpartum sores, malaria, and diarrhea. It was known to contain several active compounds such as flavonoids and tannins, which have antibacterial properties [11]. The previous study showed that Hati Tanah bulb ethanol extract was effective in inhibiting the growth of *S. aureus* with inhibition zone diameter of 15.63±0.15 mm at extract concentration of 1% [12].

The combination of interactions of the antimicrobial agents has shown significant effectiveness and two or more compounds interact to produce a joint increase, reinforcing the effects of each antimicrobial agent. Combinations can increase the efficacy of other antimicrobial agents and can be used as an alternative treatment for infections caused by multidrug-resistant microorganism that has not yet had effective therapy [13,14]. The present study was initiated to investigate the preliminary phytochemical screening and antibacterial activity of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) and their combination against *P. acnes*.

MATERIALS AND METHODS

Plant material

Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were purchased from Kahayan Traditional Market of Palangka Raya, Central Kalimantan, Indonesia. The bulb part of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) was washed thoroughly with tap water, shade dried, powdered using blender, and stored.

Preparation of plant extract

Dried powders of Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) were extracted with ethanol 70% using Soxhlet's apparatus. The advantage of this system was that instead of many portions of the warm solvent being passed through the sample, just one batch of solvent is recycled [15]. The use of 70% ethanol solvents was due to its universal properties that capable of dissolving almost all types of secondary metabolites, non-toxic, and safe to use [16,17]. The extracts were concentrated and then subjected preliminary phytochemical analysis.

Phytochemical screening

The extracts were used for preliminary screening of phytochemicals such as alkaloids, tannins, flavonoids, triterpenoids, steroids, and saponins [18,19].

Inoculum preparation

P. acnes was grown in brain heart infusion medium for 24 h at 37°C and then grown on the blood agar plate for 24 h at 37°C.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Antibacterial activity was performed using disc diffusion technique [20], where the discs were impregnated with two variations of the concentration of 5% and 10% for each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts. The McFarland 0.5 standard was prepared [21] and 10 mL were put into sterile tubes. Bacterial suspension was made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1–2 × 10^8 CFU/mL (according to McFarland 0.5 standard). A sterile cotton swab was immersed in a standardized bacterial suspension and used to event inoculate on Mueller-Hinton agar plate. Then, all the discs that have been immersed in each Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts were placed on the plates. A clindamycin antibiotic was used as positive controls with concentration variations of 5% and 10%. Discs that have been immersed in clindamycin were also placed on the plate. The plate was 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.

Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination

Antibacterial activity of containing Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination was determined. Solutions containing various concentrations of Bawang Dayak (*Eleutherine* sp.) and various concentrations of Hati Tanah (*Angiopteris* sp.) were prepared. These solutions consisted of the (1) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract, (2) combination of 5% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 10% of Hati Tanah (*Angiopteris* sp.) ethanol extract, and (3) combination of 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% of Hati Tanah (*Angiopteris* sp.) ethanol extract. Antibacterial activity test of these combinations was tested by procedure as described above. The study was repeated in triplicates for each combination.

RESULTS AND DISCUSSION

The results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract are shown in Table 1.

The results showed that both Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts contained tannins, saponins, and steroids. However, alkaloids were found only in Bawang Dayak (*Eleutherine* sp.) ethanol extract, whereas triterpenoids were found only in Hati Tanah (*Angiopteris* sp.) ethanol extract. The flavonoids were not detected in both extracts. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. Tannins can bind to proline-rich proteins and interfere with protein synthesis [22]. 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 [23,24]. Steroids were reported to have antibacterial properties through mechanisms in which steroids can bind to lipid membranes and cause leakage in liposome action [25].

In this study, clindamycin was used as positive controls. *P. acnes* was known to be sensitive to antibiotics such as clindamycin, tetracycline, quinolones, penicillins, and cephalosporins [26]. The diameters of inhibition zones produced by clindamycin with the concentration of 5% and 10% against *P. acnes* were 34.3±2.7 mm and 37.1±2.5 mm, respectively, as presented in Table 2.

The antibacterial activity test of extract against *P. acnes* that was done in triplicates showed the existence of the variation of inhibition zone diameter. The diameters of the inhibition zones of Bawang Dayak (*Eleutherine* sp.) ethanol extract at concentrations of 5% and 10% were 6.1±1.5 mm and 8.7±1.3 mm, respectively. On the other hand, the diameters of the inhibition zones of Hati Tanah (*Angiopteris* sp.) ethanol extract at concentrations of 5% and 10% were 4.0±1.6 mm and 9.2±2.5 mm, respectively.

The antibacterial activity test was also performed by combining Bawang Dayak (*Eleutherine* sp.) and Hati Tanah (*Angiopteris* sp.) ethanol extracts and then tested against *P. acnes*. The diameters of inhibition zones produced in combination 1 (5%:5%), combination 2 (5%:10%), and combination 3 (10%:5%) were 5.8±0.3 mm, 10.8±2.0 mm, and 15.5±2.8 mm, respectively (Table 3).

CONCLUSION

The results of this study showed that both Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract have potentials to inhibit the growth of *P. acnes*. The presence of tannins, saponins, and steroids in both extracts can be responsible for the antimicrobial properties observed. The best antibacterial activity was produced by combining the two extracts with 10% Bawang Dayak (*Eleutherine* sp.) ethanol extract and 5% Hati Tanah (*Angiopteris* sp.) ethanol extract (combination 3), wherein the resulting inhibition zone diameter was 15.5±2.8 mm. Clindamycin as a positive control produced a much larger inhibition zone diameter, but there was a high probability of increasing inhibition zone diameter if the concentration of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract is increased. Further, research is needed to obtain minimum inhibitory concentration from both plant extracts and

Table 1: Results of the phytochemical screening of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract

Secondary metabolites	Results	
	Bawang Dayak ethanol extract	Hati Tanah ethanol extract
Alkaloids	+	–
Flavonoids	–	–
Saponins	+	+
Triterpenoids	–	+
Steroids	+	+
Tannins	+	+

+: Detected, –: Not detected

Table 2: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract against *Propionibacterium acnes*

Materials	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
		1	2	3	
Clindamycin (positive control)	5	36.4	35.1	31.3	34.3±2.7
	10	39.9	36.3	35.2	37.1±2.5
Bawang Dayak (*Eleutherine* sp.) ethanol extract	5	5.6	4.9	7.7	6.1±1.5
	10	8.0	7.8	10.2	8.7±1.3
Hati Tanah (*Angiopteris* sp.) ethanol extract	5	2.3	5.5	4.2	4.0±1.6
	10	11.2	6.4	10.0	9.2±2.5

Table 3: Antibacterial activity of Bawang Dayak (*Eleutherine* sp.) ethanol extract and Hati Tanah (*Angiopteris* sp.) ethanol extract in combination against *Propionibacterium acnes*

Materials	Combination	Concentration (%)	Inhibition zone diameters (mm)			Inhibition zone diameter (mm) (mean±SD; n=3)
			1	2	3	
Combination of Bawang Dayak (*Eleutherine* sp.) ethanol extract: Hati Tanah (*Angiopteris* sp.) ethanol extract	1	5:5	5.5	5.8	6.1	5.8±0.3
	2	5:10	12.3	8.5	11.7	10.8±2.0
	3	10:5	16.9	17.3	12.3	15.5±2.8

their combinations so that it can be developed into an antibacterial formulation for acne treatment.

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Sehubungan dengan telah dilaksanakannya sidang Etik Penelitian kepada:

Nama : Susi Novaryatiin
NIK : 14.06.01.015
Judul : Skrining fitokimia dan aktivitas antibakteri Bawang Dayak (*Eleutherine* sp.) dan Hati Tanah (*Angiopteris* sp.) dan kombinasinya terhadap *Propionibacterium acnes*

Telah **DISETUJUI** untuk dilanjutkan penelitiannya.

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

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An. Ketua
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