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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. Email: susi_novaryatiin@yahoo.com Received: 05 June 2018, Revised and Accepted: 16 February 2019 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. 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*. Research Article ISSN - 0975 - 7058 Vol 11, Special Issue 3, 2019 © 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ijap.2019.v11s3.M0011> 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) 12 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 $1-2 \times 10^8$ 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 Novaryatiin *Int J App Pharm*, Vol 11, Special Issue 3, 2019, 11-13 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) 13 their combinations so that it can be developed into an antibacterial formulation for acne treatment.

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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

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