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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*

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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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