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Research Article

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

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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±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, tetracyclin, 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]:

(Weight of extract÷Weight of starting plant material)×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 1–2×10^8 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±0.4 mm, 25.4±0.0 mm, 29.0±0.2 mm, 29.9±0.8 mm, and 31.2±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±0.8 mm, 35.6±1.0 mm, 37.1±1.6 mm, 37.7±0.1 mm, and 39.7±0.4 mm and 27.0±0.5 mm, 29.0±0.8 mm, 30.4±0.4 mm, 30.8±1.9 mm, and 31.5±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±1.5 mm and 8.7±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±1.3–23.1±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±3.3 mm, 21.9±3.3 mm, 20.8±0.6 mm, 22.0±0.2 mm, and 23.1±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±3.1 mm, 13.5±0.9 mm, 14.7±1.5 mm, 16.1±1.0 mm, and 20.1±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

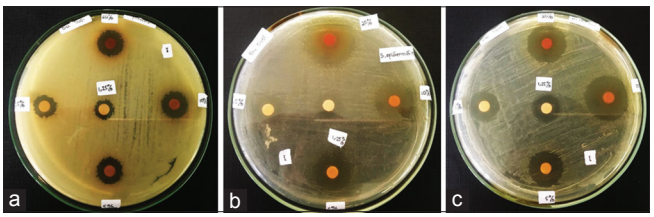

Fig. 1: Antibacterial activity of bawang dayak ethanol extract against *Propionibacterium acnes* (a) *Staphylococcus epidermidis* (b) and *Staphylococcus aureus* (c)

Table 2: Antibacterial activity of clindamycin against acne-inducing bacteria

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