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.

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

Treatment of acne can be done by giving antibiotics such as clindamycin, tetracycline, and erythromycin with the aim of reducing the population of bacteria. It has been reported that acne patients receive clindamycin, tetracycline, and erythromycin as their treatment tended to cause an increased occurrence of upper respiratory tract infections when compared with acne patients without antibiotic therapy [11]. In addition, the use of antibiotics as the first choice of acne treatment may result in antibiotic resistance due to the evolutionary adaptation of bacteria. Antibiotics have limitations with respect to toxicity and side effects also such as skin drying, headache, and nausea [12]. This condition encourages the development of research to explore antimicrobial agents from herbal resources that may provide valuable leads that can be further developed as anti-acne drugs.

Bawang dayak is one of the traditional medicines that used by the local people of Central Kalimantan Province. Empirically, bawang dayak bulb was known to have properties to treat various diseases such as breast cancer, hypertension, diabetes, cholesterol, acne, ulcers, and colon cancer and to prevent stroke, dysentery, dysuria, and colitis. Active compounds contained in bawang dayak bulb that can provide antibacterial activity include alkaloids, glycosides, flavonoids, phenols, steroids, and tannins [13,14]. The previous study reported that bawang dayak ethanol extract gave minimal inhibitory concentration (MIC) at the concentration of 10 mg/ml against the bacteria P. acnes, S. epidermidis, and S. aureus [15,16]. The present study was initiated to investigate the antibacterial activity of bawang dayak ethanol extract obtained by percolation method against acne-causing bacteria namely P. acnes, S. epidermidis, and S. aureus.

MATERIALS AND METHODS

Collection and identification of plant material
Bawang dayak (Eleutherine bulbosa (Mill.) Urb.) were collected from Sei Gohong Village, Bukit Batu Sub-District, Palangka Raya, Central
Preparation of plant extract

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

\[
\text{Yield of the extract} = \left( \frac{\text{Weight of extract}}{\text{Weight of starting plant material}} \right) \times 100\%
\]

Phytochemical screening

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

Preparation of inoculum

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

Determination of antibacterial activity

Antibacterial activity was performed using disc-diffusion technique, where the discs were impregnated with five variations of concentration of 1.25%, 2.5%, 5%, 10%, and 20%. The McFarland 0.5 standard was prepared and 10 mL was put into sterile tubes. Bacterial suspension was made by taking bacterial colonies diluted in sterile normal saline and the turbidity adjusted to 1–2×10^8 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 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 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 extract against acne-causing bacteria, namely P. acnes, S. epidermidis, and S. aureus.

**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) |
|----------------------------------|-------------------|---------------------------------------------|
| 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±0.2| 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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