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Research Article

Formulation of Anti Acne Loose Powder of Bawang Dayak (*Eleutherine bulbosa* (Mill.) Urb.) Ethanol Extract

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Abstract

Bawang dayak (*Eleutherine bulbosa* (Mill.) Urb is one of the notable Iridaceae family, originating from Central Kalimantan, Indonesia. Previous studies have reported that *E. bulbosa* ethanol extract and its cream preparation have antibacterial properties that can inhibit the growth of acne-causing bacteria and cause no significant skin adverse reaction. This study aimed to make a loose powder preparation from *E. bulbosa* ethanol extract and determine its physical evaluation and antibacterial activity. Loose powder formulation was made with various concentrations of *E. bulbosa* ethanol extract, F0 (0%), F1 (5%), F2 (10%), and F3 (15%). Loose powder evaluates for organoleptic, homogeneity, and antibacterial activity by the disc diffusion method. The results show that *E. bulbosa* ethanol extract can produce a loose powder formulation. The color of the formula is rather yellow (F0), brown-ash (F1), and light brown (F2 and F3), which has a typical mint odor, smooth texture, and homogeneous. All formulations inhibited the growth of *Propionibacterium acnes, Staphylococcus epidermidis,* and *Staphylococcus aureus*. This present study showed the potential of Formula 3 (F3) as an anti-acne loose powder due to its organoleptic properties, homogeneity, and antibacterial activity, which has the largest inhibition zone diameter of 17.6 ± 3.1 mm.

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INTRODUCTION

Acne (acne vulgaris) is a skin condition of the sebaceous glands that is characterized by the development of sebaceous papules, cystic acne, inflammatory lesions, and involvement of the follicular canal and sebum production by *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*[1]. *Propionibacterium acnes* was involved in developing inflammatory acne by activating complements and metabolizing sebaceous triglycerides into fatty acids that irritate the follicular wall and surrounding dermis[2]. *Staphylococcus epidermidis* usually involves in superficial infections within the sebaceous unit[3]. Meanwhile, *S. aureus* growth could cause acne lesions[4]. *Propionibacterium acnes*, *S. epidermidis*, and *S. aureus* can be the target sites of anti-acne drugs[5].

The use of antibiotics to treat acne is usually done to reduce the bacterial population. However, overuse of antibiotics can lead to antibiotic resistance. Therefore, it is necessary to explore local medicinal plants to develop anti-acne drugs[6]. Bawang Dayak or *Eleutherine bulbosa* (Mill.) Urb.) is one of the notable Iridaceae family, originating from Central Kalimantan, Indonesia. This plant is also widely cultivated in Southeast Asia. The bulb part has been used traditionally among the Dayak community as folk medicine to treat several diseases[7,8]. *Eleutherine bulbosa* was known to have antibacterial properties against acne-causing bacteria, as reported in our previous studies[9-12]. Our other previous studies[13,14] also reported that cream of *E. bulbosa* ethanol extract could inhibit the growth of *P. acnes*, and it does not cause significant skin adverse reactions[15,16].

However, it is necessary to make a series of anti-acne preparations to increase the effectiveness of using *E. bulbosa* as an anti-acne. Topical products can be directly applied to the affected area, thus decreasing systemic absorption and increasing the exposure of the pilosebaceous units to the acne treatment[17]. One of the preparations for topical application is a loose powder. Loose powder is the original type of face powder that can easily absorb on the skin and free the face from oil[18]. Therefore, this study aims to make innovative loose powder preparations from *E. bulbosa* ethanol extract and to determine its physical evaluation and antibacterial activity. Formulating loose powder of *E. bulbosa* extract is needed as an alternative treatment for acne. So, in the end, it can be produced anti-acne product series from *E. bulbosa* ethanol extract.

MATERIALS AND METHODS

Materials

The materials used were *E. bulbosa* bulbs, peppermint oil, ZnO, menthol, corn starch, sterile talcum, blank antimicrobial susceptibility disc, strains of *P. acnes* ATCC 11827, *S. epidermidis* ATCC 12228, *S. aureus* ATCC 25923, Mueller-Hinton agar, 96% ethanol, NaCl, distilled water, branded loose powder (Wardah acnederm face powder). The main instruments used include an analytical scale, oven, blender, autoclave, incubator, rotary evaporator, hot plate, laminar airflow, and caliper.

Methods

Collection of plant

Fresh bulbs of *E. bulbosa* were collected from Sei Gohong Village, Bukit Batu Sub-District, Palangka Raya, Central Kalimantan, Indonesia. The plant was authenticated by Dr. Joeni Setijo Rahajoe from the Indonesian Institute of Sciences, Research Center for Biology, with specimen voucher 2119.

Preparation of plant extract

The plant materials were prepared by cutting the bulbs and drying them in the sun no later than 10 AM. The dried plant material is ground with a blender. The powdered plant materials were extracted by percolator using 96% ethanol. Then, a rotary evaporator was used to concentrate all extracts[14].

Formulation preparation

The formulation components used are listed in **Table I**. The components include ZnO, menthol, corn starch, sterile talcum, and peppermint oil. The loose powder formulation of *E. bulbosa* ethanol extract was made with three concentrations, 5%, 10%, and 15%. *Eleutherine bulbosa* ethanol extract was weighed and dissolved in ethanol, then some corn starch and sterile talcum were added and grounded until homogeneous. Meanwhile, menthol was dissolved with a bit of ethanol, then some corn starch, sterile talcum, and ZnO were added and grounded until homogeneous. The mixture of *E. bulbosa* ethanol extract was put into a mixture of menthol and ZnO, added peppermint oil, and grounded until homogeneous. The negative control formulation (F0) was prepared in the same procedure without adding *E. bulbosa* ethanol extract. The homogeneous formulation of loose powder was sieved through a 100-mesh sifter and packed[19].

Table I. Formulation of loose powder of *E. bulbosa* ethanol extract

Material	Amount (mg)			
	Negative control or Formula 0 (F0)	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Eleutherine bulbosa ethanol extract	0	500	1000	1500
Peppermint oil	10 drops	10 drops	10 drops	10 drops
ZnO	300	300	300	300
Menthol	100	100	100	100
Corn starch	4000	4000	4000	4000
Sterile talcum ad	10000	10000	10000	10000

Physical evaluation of loose powder

There were two evaluations of physical properties: organoleptic and homogeneity tests[20]:

1. Organoleptic test: Loose powder preparations that have been made were observed in color, odor, and texture.
2. Homogeneity test: The homogeneity test was done by visually observing the mixed color uniformity of the extract and powder base. It was carried out by spreading the powder sample on a white paper.

Antibacterial activity test

A loose powder formulation was tested to determine an antibacterial activity against *P. acnes*, *S. epidermidis*, and *S. aureus* using a disc-diffusion technique with three variations of concentration of 5%, 10%, and 15%. The 0.5 McFarland standard was prepared, and 10 mL was put into sterile tubes. The bacterial suspension was made by diluting the bacterial colonies in sterile physiological saline and adjusting the turbidity to 1-2x10^8 CFU/mL. A sterile cotton swab was dipped in a standardized bacterial suspension and used for uniform inoculation onto Mueller-Hinton agar plates. Then, all the discs were immersed in the solution of loose powder sample placed on the plates. A branded loose powder was used as a control. Discs immersed in a solution of branded loose powder were also placed on the plates. These plates were then incubated for 24 hours at 37°C[19]. The diameter of the inhibition zone was measured in mm using a caliper. The study was repeated three times for each loose powder formulation and control[21].

RESULTS AND DISCUSSION

Physical evaluation of loose powder

Organoleptic test

An organoleptic test was carried out to see the physical appearance of the powder preparations by observing the color, odor, and texture. The result of the organoleptic test showed that F0 had a rather yellow color, F1 had a brown ash color, while F2 and F3 had a light brown color (**Table II**). The color difference is due to differences in *E. bulbosa* ethanol extract concentration in the formulations. All formulations had a typical mint odor and smooth texture based on the odor and texture. Typical mint odor due to the addition of menthol and peppermint oil to the formulation to cover up the pungent odor of *E. bulbosa*. The loose powder formulations of *E. bulbosa* ethanol extract can be seen in **Figure 1**.

Table II. Observations of organoleptic loose powder formulations

Formulation (% concentration of extract)	Texture	Color	Odor
F0 (0 %)	Smooth	Rather yellow	Typical mint
F1 (5 %)	Smooth	Brown ash	Typical mint
F2 (10%)	Smooth	Light brown	Typical mint
F3 (15%)	Smooth	Light brown	Typical mint

Homogeneity test

This study showed that all formulation was homogeneous. The homogeneity test of the loose powder aims to see whether all the content is combined perfectly. Homogeneity is one of the requirements for the preparations of loose powder[22]. The loose powder is said to be homogeneous if all the ingredients that make up the powder are well mixed and there are no palpable ingredients.

Antibacterial activity test

The antibacterial activity was tested in triplicate against three acne-causing bacteria: *P. acnes*, *S. epidermidis*, and *S. aureus*. Based on the zone of inhibition, it could be classified into four categories: weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm)[21,23]. Meanwhile, based on the antibacterial activities of extracts can be classified into three levels: 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)[24].

Figure 1. The loose powder formulations: F0 (**a**), F1 (**b**), F2 (**c**) and F3 (**d**)

The results showed that two loose powder formulations of *E. bulbosa* ethanol extract (F1 and F2) had a weak inhibitory response against *S. aureus*, while F3 showed moderate inhibitory power. F1 and F2 had a moderate inhibitory power against *S. epidermidis*. However, F3 had a strong inhibitory response against *S. epidermidis* with an inhibition zone of 10.8 ± 0.8 mm. Meanwhile, based on the classification of antibacterial activities of extract[24], the three formulations (F1, F2, F3) had a weak activity against *S. epidermidis* and *S. aureus*, with the inhibition zones in the range of 2.9 ± 1.4 to 10.8 ± 0.8 mm. Furthermore, the antibacterial activity of the three formulations can be described as strong against *P. acnes*. The highest zone of inhibition produced by F3 was 17.6 ± 3.1 mm (**Table III** and **Figure 2**). This can occur due to differences in *E. bulbosa* ethanol extract concentration in each formulation. The higher the *E. bulbosa* ethanol extract concentration in the formulation, the higher the inhibition zone produced[21].

The ability to produce the clear zone was presumably dependent on the secondary metabolites possessed by the test sample[25]. This finding was due to flavonoids, alkaloids, saponins, and tannins in *E. bulbosa* ethanol extract[11], which could be responsible for the antibacterial properties observed. Eleutherol A, a flavonoid from *E. bulbosa*, inhibits cell wall synthesis in bacteria[26]. Alkaloids have an antibacterial ability and generally work through efflux pump inhibition activity. Most of the alkaloids are found to be bactericidal rather than bacteriostatic[27,28]. Saponins can cause bacterial cell contents' leakage through cell wall degradation followed by disruption of the cytoplasmic membrane and membrane proteins[29]. Tannins were known to have antibacterial properties against Gram-negative and Gram-positive human pathogens[30,31].

A previous study[32] reported that an anti-acne loose powder of ethanol extract of *Piper betle* leaves had antibacterial activity against one acne-causing bacteria, *S. aureus*. The inhibition zones of loose powder formulation of F1 (0%), F2 (5%), F3 (10%) and F4 (15%) were 1.05 mm, 5 mm, 6.11 mm, and 6.31 mm. The inhibition zones produced in this study were greater on a concentration of 15% of *E. bulbosa* ethanol extract in loose powder formulation (F3) against *S. aureus*, which is 7.9 ± 1.5 mm.

Table III. The inhibition zone of loose powder formulation of *E. bulbosa* ethanol extract and control

Formulation (% concentration of extract)	Zone of inhibition (mm) (mean ± SD; n=3)		
	P. acnes	*S. epidermidis*	*S. aureus*
F0 (0%)	6.0 ± 2.7	7.1 ± 0.4	2.1 ± 0.5
F1 (5%)	12.8 ± 0.1	6.6 ± 1.6	2.9 ± 1.4
F2 (10%)	16.1 ± 1.6	9.1 ± 0.5	4.1 ± 1.2
F3 (15%)	17.6 ± 3.1	10.8 ± 0.8	7.9 ± 1.5
Control	1.1 ± 0.2	1.8 ± 0.5	1.6 ± 0.7

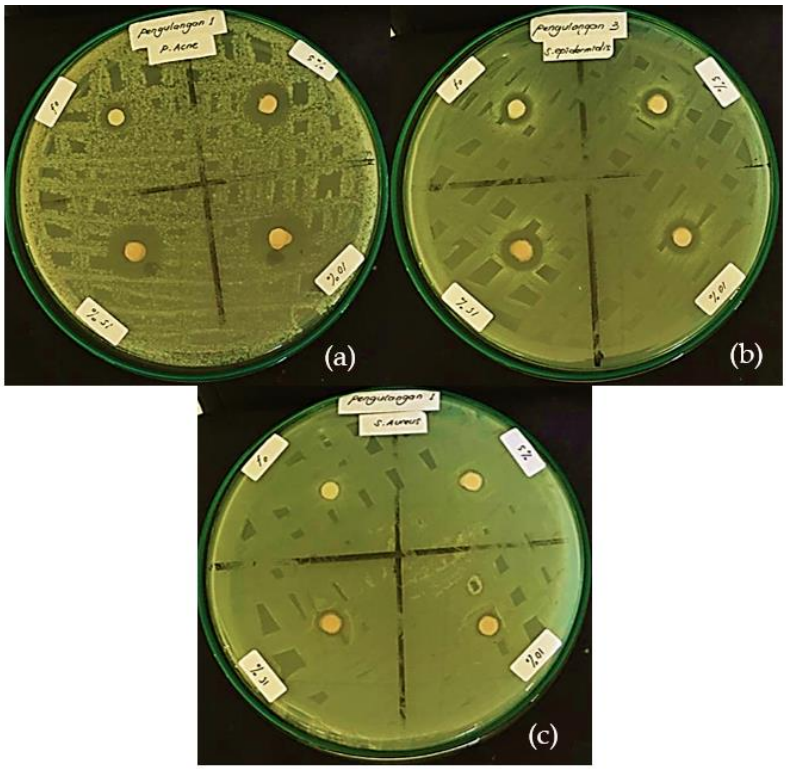

Figure 2. The antibacterial activity of loose powder formulation of *E. bulbosa* ethanol extract against *P. acnes* (**a**), *S. epidermidis* (**b**) and *S. aureus* (**c**)

Negative control (F0) also showed the inhibition zones against three bacterial tested. It can be caused by the presence of zinc oxide. Zinc oxide is known for its antioxidant properties and has been shown to help prevent UV damage. It is used for several dermatological conditions, including infections (warts, leishmaniasis), dermatitis (acne vulgaris, rosacea), pigmentary disorders (melasma), and neoplasias (basal cell carcinoma), and due to its non-toxicity, biocompatibility and antibacterial activity[33].

This study used a branded loose powder (Wardah acnederm face powder) as a control. It contains mica, corn (*Zea mays*) starch, kaolin, silica, zinc stearate, aqua, phenoxyethanol, dimethicone, salicylic acid, ethylhexylglycerin, hydrogen dimethicone, methicone, allantoin, *Epilobium angustifolium* flower/leaf/stem extract, fragrance, aluminum hydroxide, butylene glycol, sodium metabisulfite, *Glycine soja* (soybean) protein, tocopherol. Salicylic acid, *Epilobium angustifolium* flower/leaf/stem extract, and soybean protein are commonly used for acne treatment and have antibacterial activity[34-36].

The antibacterial activity of control was categorized as weak, with the inhibition zones against *P. acnes*, *S. epidermidis*, and *S. aureus* being less than 2 mm. When compared, the inhibition zones resulting from the three formulations of loose powder of *E. bulbosa* ethanol extract were more significant than the inhibition zones of the positive control. Therefore, it can be concluded that the loose powder formulation of *E. bulbosa* ethanol extract has better antibacterial activity against three bacteria that can cause acne.

CONCLUSION

Eleutherine bulbosa ethanol extract can be processed into a loose powder formulation. The color of the formula is rather yellow (F0), brown-ash (F1), and light brown (F2 and F3). Moreover, it has a typical mint odor, smooth texture, and is homogeneous. The highest zone of inhibition produced by F3 (15%) against P. acnes was 17.6 ± 3.1 mm. This present study showed the potential of formulation as anti-acne, but further research is needed to do irritation tests in rabbits and on human skin so it can be developed as an anti-acne loose powder product.

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AUTHORS' CONTRIBUTION

Susi Novaryatiin: conceptualization, funding acquisition, methodology, visualization, writing-original draft, writing-review & editing. **Nursheilla Rizky Amalia**: formal analysis, investigation, project administration, resources. **Syahrida Dian Ardhany**: conceptualization, funding acquisition, methodology, supervision, validation.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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