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Standardization of *Eleutherine bulbosa* Urb. Bulbs Extract from Lampo, Donggala, Central Sulawesi

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Abstract

Bawang Dayak (*Eleutherine bulbosa* Urb.) (EB) is Indonesian medicinal plant used as a traditional remedy with many biological activities. This study examines the specific and non-specific parameters of the extract of EB from Lampo, Donggala, and Central Sulawesi. The bulbs extracts of EB were obtained from the maceration of the bulbs with 70% ethanol as solvent. The parameters were organoleptic, water/ethanol soluble content, chromatography profile, and total phenolic and flavonoid content. The non-specific factors were examined, including determining specific gravity, water content, total ash content, acid-insoluble ash content, microbial contamination, mold and yeast contamination, and heavy metal contamination. The specific and non-specific characteristics of the extract of EB bulbs have met the required criteria. In conclusion, the extract of EB bulbs from Lampo, Donggala, and Central Sulawesi can be bioactive materials for standardized herbal medicine.

INTRODUCTION

Historically, nature has been an essential source of new bioactive compounds. As reviewed by Newman and Cragg (Newman & Cragg, 2016), from 1981 to 2014, more than half of the drugs approved were derived from natural products. Plants are a traditional source of bioactive compounds, and more than 400 plant species having pharmacological activity have been available in the literature (Bisht, Owais, & Venkatesan, 2006; Choudhury et al., 2017; Malviya N, 2010). Indonesia is considered mega biodiversity covering about 11% of the world's known flowering plant species, of which around half are endemic (Fathurahman, Nursanto, Madjid, & Ramadanil, 2016). Indonesian traditional medicine can be traced back to 1977, with both ethnobotanical and scientific research literature available (Heyne, 1987; Kesehatan, 1977).

Eleutherine bulbosa Urb. (EB). Bawang Dayak (local name) is a well-known plant in Indonesia, particularly in Kalimantan. Dayak tribe uses the plant to cure various illnesses such as cancer, high blood pressure, diabetes mellitus, cholesterol, and ulcers (Kuntorini, Astuti, & Nugroho, 2010). The most common traditional preparation is boiling cloves of EB bulb in three glasses of water until reduced by half. The water is then taken one to three times daily. Eleutherine plant is originally from South America and cultivated in Africa, Malaysia, Indonesia, and the Philippines. This plant can adapt well to grow in various climates and soil types.

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Many studies of pharmacology activities from the Eleutherine plant, such as cytotoxic activity, diabetes mellitus, antibacterial activity, antiviral, anti-inflammatory, antimalarial, antioxidant, and rheumatoid arthritis, were published (Kamarudin, Sayuti, Saad, Razak, & Esa, 2021). Harlita et al. 2018 (Harlita, Oedjijono, & Asnani, 2018) reported the antibacterial activity of EB extract against several pathogenic bacteria. Arung et al. 2009 (Arung, Kusuma, Christy, Shimizu, & Kondo, 2009) reported that EB methanolic extract inhibited melanin production in B16b melanoma cells without significant toxicity. Ieyama et al. 2011 (Ieyama, Gunawan-Puteri, & Kawabata, 2011) reported alpha-glucosidase inhibitory activity of EB extract. The most notable compounds in EB bulbs are naphthalene, anthraquinone, and naphthoquinone. The compounds isolated from the EB bulb extract comprise eleutherin, eleutherol, isoeleutherol, isoeleutherine, elecanacin, eleutherinoside A, eleuthoside B, and four polyketides including (R)-4-hydroxyeleutherin, eleuthone, eleutherinol-8-O-β-D-glucoside and isoeleuthoside C (Bianchi & Ceriotti, 1975; Hara et al., 1997; Zhengxiong et al., 1986). In addition, the study also revealed eight bioactive compounds from *EB* bulb, i.e., eleutherin, gallic acid, chlorogenic acid, quercetin, kaempferol, rutin, epicatechin gallate, and myricetin (Kamarudin, Mohd. Esa, Saad, Sayuti, & Ab. Razak, 2020). Therefore, EB from Lampo, Donggala, Central Sulawesi Indonesia, where the people cultivated and produced it as herbal tea, is needed to examine their quality by measuring the specific and non-specific parameters.

In our effort to develop a standardized herbal-based medicine from Indonesian medicinal plants, the standardization, including specific and non-specific parameters, was performed to ensure the quality of extracts as bioactive materials for herbal medicine.

MATERIALS AND METHODS

Plant Collection

The fresh plant of EB was collected from Lampo, Donggala, Central Sulawesi, Indonesia. The plant was identified as *Eleutherine bulbosa* Urb. (L.) by Prof. Dr. Ramadhanil from the Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, and the voucher specimen was kept in the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University Indonesia.

Plant Material Extraction

The plant material was extracted with the maceration method. The dried and powdered bulb material 500 grams was macerated with 1500 mL 70% ethanol (1:3). The bulb was soaked for the first 6 hours stirring occasionally and stood for 18 hours. The maceration process was carried out twice. The liquid was filtered and then concentrated using rotavapor at 40°C at 40 rpm. Furthermore, the extract evaporated using freeze-drying until thick sections were obtained and weighed.

Determination Specific Parameters of Extract

Organoleptic Extract

An organoleptic test was carried out with the five senses to describe the extract’s shape, color, taste, and odor (Depkes, 2000).

Water/Ethanol Soluble Content

Water/ethanol soluble content was performed by permeating 1.0 g extract with 25 mL water chloroform (39: 1) for 24 hours while shaking it repeatedly during the first 6 hours. The extract was then allowed to stand for 18 hours and filtered. The filtrate obtained was evaporated, and the residue was heated at 105°C until the weight remained constant. The experiment was carried out three times. Ethanol 96% was used as the ethanol-soluble content assay solvent (Kesehatan, 2017; Saifudin, 2011).

Figure 1. *Eleutherine bulbosa* Urb. and bulbs (Rahmi *et al*., 2021)

Chromatography Profile

Thin Layer Chromatography was applied using n-hexane: ethyl acetate (1:1 v/v) as a mobile phase and silica gel 60 GF254 as a stationary phase. The extract was spotted with a concentration of 0.5% on an 8x1.5 cm TLC plate with a distance of 1 cm from the bottom edge and 0.5 cm from the top edge. TLC plate was observed under UV light at 254 nm and 366 nm. A 10% sulfuric acid (H_2SO_4) solution in methanol was used as a spray reagent. (Syariful Anam, 2013).

Total Phenolic Content

Determination of the total phenolic content in the extract using standard gallic acid refers to the previous procedure of Kim et al. (2003) (Kim, Chun, Kim, Moon, & Lee, 2003). The extract was weighed as much as 10 mg, then dissolved in 10 mL of distilled water and homogenized. 0.4 ml of Folin-Ciocelteau reagent was added to 1 ml of the extract solution, shaken, and allowed to stand for 5 minutes. 0.4 mL of 75% $NaCO_3$ was added and shaken until homogeneous. The final solution was made from 10 mL of distilled water, then left for 2 hours at room temperature and repeated three times

Total Flavonoid Content

The total flavonoid extract was measured using quercetin as a reference standard. The extract was weighed as much as 100 mg, then dissolved in 10 mL of ethanol to reach a final concentration of 10000 ppm. To 1 mL of the extract solution, 1 mL of 2% $AlCl_3$ and 120 mM potassium acetate were added. Samples were incubated for 1 hour at room temperature. The UV-Vis spectrophotometry method determined the absorbance with a maximum wavelength of 435 nm. The analysis was performed three times to find the average absorbance value (Rahmi et al., 2021).

Determination of Non-Specific Parameters of Extract

Specific gravity

Accurately 1.0 g of the extract is diluted with 70% ethanol. The empty pycnometer is weighed, added with 25°C water, and weighed. The liquid extract was added, adjusted to 25°C, and weighed. (Saifudin, 2011).

Water content

Water content was performed with the distillation method. A total of 5 g of extract was put into a round bottom flask, and 200 ml of xylol, which had been saturated with water and then heated at a temperature of 110°C for 1 hour. After the layers were separated, the volume of water was read and calculated. Water content is calculated in % v/w (Saifudin, 2011; Syariful Anam, 2013).

Total ash content

Accurately 2.0 g of the extract was put into the silicate crucible and then heated with a hot plate followed by a furnace at 650°C until the charcoal was used. After that, the silicate crucible was weighed after being cooled to room temperature in a desiccator, and then calculated the results, expressed as %w/w (Depkes, 2000).

Acid insoluble ash content

The ash from the previous experiment was boiled with 25 ml of dilute sulfuric acid P for 5 minutes. The insoluble acid was collected and filtered through ash-free filter paper. The ash precipitate is washed with hot water, put into a silicate cup, and heated in a furnace at 650oC until the charcoal runs out. The weight of the material calculates the acid-insoluble ash in %w/w. (Depkes, 2000).

Microbial contamination

A total of 1 mL of the extract resulting from dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} was pipetted using a sterile pipette, then dripped onto NA medium and incubated at 37°C for 24 hours. Colony growth was observed and counted. (Rahmi et al., 2021).

Mold and yeast contamination

A total of 1 mL of the extract resulting from dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} was pipetted using a sterile pipette, then dripped onto PDA media, and incubated at 25°C for three days. Colony growth was observed and counted (Rahmi *et al.*, 2021).

Heavy metal contamination

Determination of heavy metal contamination was carried out using the ICP OES method (Rahmi et al., 2021). a heavy metal standard calibration curve is made with six concentration points. The extract was carefully weighed as much as 0.5-1.0 gram into the vessel, added HNO3(p), and allowed to stand for 15 minutes. Sample digestion was carried out using a microwave. The digestion results were transferred to a volumetric flask, and 50 ml and 100 mg/L internal standards were added. The sample solution was diluted with aquabidest, homogenized, and filtered. The intensity of the test solution is measured in the ICP-OES system. The maximum wavelengths used were Cd: 214.439 nm, Hg: 184.887 nm, and Pb: 220.353 nm. Calculation of metal/mineral content in the sample uses a standard calibration curve with the line equation:

$y = bx + a$, with the following formula:

Concentration of metal (ppm) = (Aspl-a)/b x V x fp

Vspl or Wspl

Which:

Aspl = Sample intensity

a = Intercept from the standard curve

b = Slope from the standard curve

fp = Dilution factor
V = Final volume of sample (mL)
Wspl = Weight of portion test (gram)
Vspl = Volume of portion test (mL)

RESULTS AND DISCUSSION

Bulbs of *Eleutherine bulbosa* Urb. (EB) were macerated with 70% ethanol. The yield extraction of the sample is presented in Table 1. Standardizing medicinal plants is a critical step in researching and developing natural medicines to ensure the quality and safety of drug preparations (Budiastuti, Yusnia Wahyu, Intan Ayu, Riesta, & Sukardiman, 2020). Specific parameters of extract of bulbs of EB tested consist of extract identity, organoleptic extract, water/soluble ethanol content, and chromatography profile. A previous study showed that the yield extract from three locations in Kalimantan used 70% ethanol as solvent producing a yield of about ± 10% w/w (Rahmi *et al*., 2021). Our findings showed products similar to the previous study and confirmed that 70% ethanol yielded a greater yield than 96%. The polarity was the main reason for the results. Thus 70% ethanol was able to extract more compounds.

Table 1. The yield extraction of extract of *Eleutherine bulbosa* Urb.

Sample	Simplicia (g)	Extract weights (g)	Yield (%w/w)
Bulbs extract	500	47	9.40

Table 2. Organoleptic and water/ethanol soluble content

Parameters	Results
Organoleptic	Viscous extract, brownish red color, grass odor, astringent and bitter taste
Water soluble	30.66%
Ethanol soluble	81.33%
Total phenolic content	20,28 ± 3,22 µg/mL
Total flavonoid content	2,026 ± 0,35 µg/mL

Specific parameters describe several parameters, including the extracted identity. The identity is an essential part of ensuring the origin of the extract because the chemical components might differ based on the cultivated area. However, they have physical similarities and the same genus. The organoleptic determination of the extract is also an important step to check the quality of the extract by observing color, taste, and odor. Based on their polarity, water/ethanol soluble content was also quantified to determine the solubility of extract chemical substances in two solvents, water and ethanol. Table 2 showed that extract from Lampo was more soluble in ethanol than water, so it can be concluded that the extracted compound was semipolar. Our results, supported by a previous study by Rahmi et al. 2021 showed that bulb extract from three locations in Kalimantan is also more soluble in ethanol than water. The extract also showed phenolic and flavonoid content using gallic acid and quercetin as standard. The results of a specific parameter of extract identity, organoleptic, water/ethanol soluble content, and total phenolic and flavonoid content are presented in Table 2.

Table 3. Non-specific parameters of *Eleutherine bulbosa* bulbs extract

No.	Parameters	Results	Requirement
1	Specific gravity (g/mL) *	0,90 ± 0,01	-
2	Water content (%w/w) *	15.53 ± 0,81	≤10.0
3	Total ash content (%) *	3.67 ± 0,57	-
4	Acid insoluble ash content (%w/w) *	0,633 ± 0,14	-
5	Heavy metal contamination - Hg (mg/Kg) *	Not detected	
	Heavy metal contamination - Pb (mg/Kg) *	Not detected	10
	Heavy metal contamination - Cd (mg/Kg) *	0.01	0.3
6	Microbial contamination (colony/g)	7 x 10^2	≤ 10^6
7	Mold and yeast contamination (colony/g)	0	≤ 10^4

*Values are means of triplicate determination ± Standard Deviation

One of the essential standardization of extract is the chromatography profile. The Thin Layer Chromatography (TLC) method determined the chromatogram profile, which aimed to separate the compounds in the extract based on observing spot pattern and color under UV light and sulfuric acid as spray reagents. The TLC profile is a qualitative analysis to indicate the presence of chemical components in the extract (Saifudin, 2011; Syariful Anam, 2013). Figure 2 was presented. The results of the TLC profile are shown in Figure 2 and revealed four spots in the TLC plate, mainly when observed under UV 366 nm.

Non-specific parameters of 70% ethanol extract of EB Bulbs represented in Table 3 include specific gravity, water content, total ash content, acid insoluble ash content, residual solvent, and heavy metal contamination (Hg, Pb, and Cd). Specific gravity relates to purity and contamination. Our findings were similar to the previous study by Rahmi *et al*. (2021), with a value of about 0.90 ± 0,01 g/mL. Determining total and acid-insoluble ash content aims to provide an overview of the internal and external mineral content that originated from the initial process until the extract formed (Syariful Anam, 2013). The heavy metal contamination assay proposes to ensure that the extract does not contain certain heavy metals that exceed the specified value and are harmful to health. The three heavy metals tested were mercury (Hg), lead (Pb), and cadmium (Cd). Our results possed that the extract meets the requirements. The extracts also showed no microbial and fungal/yeast contamination.

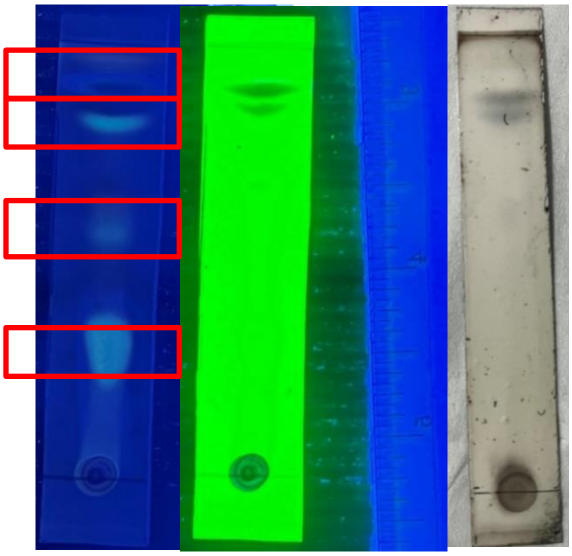

Figure 2. TLC Profile 70% ethanol extract of *Eleutherine bulbosa* Urb Bulbs from Lampo. A. UV 366 nm. B. UV 254 nm. C. Sulfuric acid 10%. Mobile phase: n-hexane: ethyl acetate (1:1). Stationary phase: Silica gel 60 GF254.

CONCLUSION

In conclusion, the bulbs of *Eleutherine bulbosa* Urb. from Lampo Donggala Central Sulawesi Indonesia, on the non-specific and specific parameters, have met requirements and could be used as bioactive materials for herbal medicine.

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