Standardization of *Eleutherine bulbosa* Urb. Bulbs and Total Flavonoid Content from Three Locations in Kalimantan, Indonesia

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**ABSTRACT**

**Background:** Dayak Onion (*Eleutherine bulbosa* Urb.) is a typical plant of Kalimantan which is traditionally used by the Dayak community as a medicinal plant. Dayak onion bulbs have been proven had many pharmacology activities. **Objective:** This study aims to determine the nonspecific and specific parameters of 70% ethanol extract of *Eleutherine bulbosa* Urb. **Methods:** Total flavonoids was also quantified. **Results:** Extract was carried out with maseration method using ethanol 70% as solvent. Determination of non-specific includes by determined specific gravity, water content, total ash content, acid insoluble ash content, residue solvents, heavy metal contamination, microbial contamination, mold and yeast contamination. Determination of specific parameters included extract identity, organoleptic extract, water/ethanol soluble content, chromatography profile. Total flavonoid content were quantified with colorimetric method. **Conclusion:** it can be concluded that bulbs of *Eleutherine bulbosa* Urb from three locations have characters to similar between each other and bulbs of *Eleutherine bulbosa* Urb form central borneo had the highest total flavonoid content. **Key words:** *Eleutherine bulbosa* Urb., Standardization, Non-specific parameters, Specific parameters, Flavonoids.

**INTRODUCTION**

The use of traditional medicines which has not been tested in the efficacy and safety of herbal medicines, cannot be used like modern medicine. Considered herbal medicines have an important role in the health sector, it should be to determine the quality and safety standards of medicinal plants extracts. Standardization of medicinal plant extracts is one of the important stages in the development of natural medicines.

One of potential plants as medicine is the dayak onion (*Eleutherine bulbosa* Urb.). This plants contained secondary metabolites such as phenols, flavonoids, saponins, alkaloids, tannins and quinones. Bulbs of this plant had many activities such as immunomodulator, anti-inflammation, antioxidant, antihyperglycemia, antihypercholesterol and anticancer. To develop this potential, standardization of extracts were carried out. It consisted of nonspecific and specific parameters. Beside it, bulbs of *Eleutherine bulbosa* Urb. were examined for the organoleptic, macroscopic and microscopic parameters. Standardization of *Eleutherine bulbosa* Urb. bulbs had been carried out but from three different locations, that were Malang, Bogor, and Purbaingga (Java Island) and also the standardization of this plant had been done used different solvent, thus was ethanol 96% which the plant only from east borneo. Therefore this research needed to complete the standardization data for 70% ethanol extract of *Eleutherine bulbosa* Urb. bulbs and also to determined the total flavonoid content.

**MATERIALS AND METHODS**

**Plant collection**

Adult specimens of *Eleutherine bulbosa* Urb. plants were collected from three different location. The locations were Banjarbaru city, south borneo; palangkaraya city, central borneo and balikpapan city, east borneo. The sample were collected in the morning around 7-10 a.m. at Desember 2019. The collected plants were determinated at the Herbarium Bogoriense, Biology Research Center, Indonesian Institute of Sciences (LIPI), Bogor with number 2242/IPH.1.01/II.07/XII/2019. Manufacture of simplicia started with collected the bulbs as part of the *Eleutherine bulbosa* Urb. plants will be used, then sample will sorted and washed with running water. Then chopped and dried the sample under the sun at 7-10 a.m. The sample which had been dried, mashed with blender and sieved with mesh no. 16.

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**Extraction**

The plant material was extracted with maceration method. The each dried and powdered plant material from three different location 500 grams was macerated with 1500 mL 70% ethanol (1:3). Soak for the first 6 hours, stirring occasionally. Then let stand for 18 hours. Repeat the remaceration process twice. All mazerat were collected then concentrated used rotavapor at 50°C with 40 rpm. Furthermore evaporated it used waterbath at 50°C until thick extracts were obtained. Calculated the yield of the thick extract.

**Determination specific parameter of 70% ethanol extract of Eleutherine bulbosa Urb. Bulbs**

**Extract Identity**

Determination by doing nomenclature description includes extract names, Latin names of plants (botanical systematic), parts of plants used and names of local plants.

**Macroscopic and Organoleptic Extract**

Observations were carried out with the five senses to describe the shape, color, taste and odor of the extract. The statements "odorless", "practically odorless", "a faint characteristic odor", or variations there of, were determined by observation after the material has been exposed to the air for 15 minutes. Freshly opened package of apportion of about 25 g of the article to an open evaporating dish of about 100 ml capacity.

**Microscopic Test**

This test used aquabidest reagent. Powder microscopy was also carried out and the specific characteristic were recorded. Plant parts that can be observed include starch, transport bundles, endodermis, epidermis and parenchyma tissue.

**Water/Ethanol Soluble Content**

Determination was done 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. Then allowed to stand for 18 hours and filtered. The filtrate is evaporated, the residue was heated at 105°C until the weight remained. Replicated 3 times. For Ethanol soluble content, the solvent used 96% ethanol.

**Chromatography Profile**

The method used Thin Layer Chromatography used n-hexane: ethyl acetate (7: 3 v/v) as a mobile phase and silica gel 60 GF₅₀ as a stationary phase. Bottle extract with a concentration of 0.5% TLC plate GF254 with a size of 8 x 1.5 cm with a distance of 1 cm from the bottom edge and 0.5 cm from the top edge. Spotted on UV light of 254 nm and 366 nm. Sprayed with 10% sulfuric acid (H₂SO₄) solution in methanol.

**Determination Non Spefic Parameter of 70% ethanol extract of Eleutherine bulbosa Urb. Bulbs**

**Specific gravity**

The 1 g extract was diluted by 5% with 70% ethanol. Empty pycnometer is weighed then added with water at 25°C weighed by water weight. Liquid extracts at 20°C are introduced, adjusted at 25°C and weighed.

**Water content**

Determination is done by distillation. 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 separate completely, the volume of water is read and calculated. Water content is calculated in % v/w.

**Total ash content**

Accurately 2 g of the extract was put into the silicate crucible then heated with a hot plate followed by a furnace at 650°C until the charcoal was used up. After that, the silicate crucible weighed after cooled to room temperature in a desicator then calculated the results, expressed %w/w.

**Acid insoluble ash content**

The ash obtained as directed under Total Ash Content was boiled with 25 ml of dilute sulfuric acid P for 5 minutes, the acid insoluble part was collected, the filtered ash was filtered with ash-free filter paper, washed with hot water, put into a silicate crucible, glazed with a furnace at a temperature of 650°C to charcoal was gone. Acid insoluble ash content was calculated to the material weight in %w/w.

**Residual solvent**

Concentrated extract was diluted to a concentration of 0.1% with methanol as a solvent. Samples were injected into the GC-MS at temperatures of 70°C to 200°C. Analysis of the presence of ethanol groups through the similar index and the re resulting cromatogram pattern.

**Heavy metal contamination**

The instrument used to perform this test was Atomic Absorption Spectrophotometry (AAS) with the calibration curve method. Create a standard curve for lead (Pb) and Cadmium (Cd) with a concentration of 1000 ppm. Dilution was carried out gradually until a contentration of 1 ppm was obtained. Series levels of 1, 5, 10 and 15 ppm for lead (Pb) and 0.2; 0.4; 0.6 and 1 ppm for Cadmium (Cd) were made. Concentration of the sample solution was measured after absorption. Weighed 2.5 g of extract and added 20 ml of concentrated HNO₃ and allowed to stand for 24 hours, heated to 100°C for 10 minutes then cooled then added 2 ml of 30% H₂O₂, heated until a clear yellow solution and filtered to a 50 volumetric flask and added aquadest until border mark. Samples were measured by means of AAS then heavy metal content was calculated.

**Microbial contamination**

Pipette 1 ml from each dilution into a sterile (duplo) petri dish. Plate Count Agar (PCA) media was poured as much as 5 ml into each petri dish which had been melted at 45°C. Leave it until the mixture is frozen and put in an incubator cabinet at 37°C for 48 hours in an upside down position. Colony growth was recorded after 24 hours. Observed and counted the number of colonies that growth on petri dish.

**Mold and yeast contamination**

In a sterile (duplo) petri dish, 5 ml of diluted Potato Dextrose Agar (PDA) media was poured at 45°C, then 1 ml was pipetted from each dilution. Leave to freeze in a saucer and incubated at room temperature or 25°C for 7 days. Results recorded.

**Total Flavonoid Content**

Total flavonoid content was determined by aluminium chloride spectrophotometric method.

**Determination of The Maximum Quercetin Wavelength**

0.5 mL of a quercetin solution with concentration 60 µg/mL added to the vial. Then added 0.1 mL AlCl₃, 0.1 mL of sodium acetate 1 M and 2.8 mL aquadest, shaken and read the absorbance at a wavelength of 400-600 nm.
Determination of Operating Time

0.5 mL of a quercetin solution with concentration 60 µg/mL added to the vial. Then added 0.1 mL AlCl₃, 0.1 mL of sodium acetate 1 M and 2.8 mL aquadest, shaken and read the absorbance continuously at intervals 3 minutes for 60 minutes²⁵.

Quercetin Standard Curve

Quercetin was used to make a standard calibration curve. 100 mg quercetin was dissolved in 100 mL of ethanol (1000 µg/mL) and then diluted to get the concentration 20, 30, 40, 50, 60 µg/mL. 0.5 mL of each solution diluted standard solutions were pipette out and added with 0.1 mL AlCl₃, 0.1 mL sodium acetate 1 M and 2.8 mL aquadest then shake it to stand for operating time and read the absorbance at the maximum wavelength²⁵.

Determination of Total Flavonoid Content

0.5 mL extract solution with concentration 1000 µg/mL was added to the vial, added with 0.1 mL AlCl₃, 0.1 mL sodium acetate 1 M and 2.8 mL aquadest, then shaken and allowed to stand during operating time and read the absorbance at the maximum wavelength obtained²⁶.

RESULTS AND DISCUSSION

In this study, bulbs of Eleutherine bulbosa Urb. extracted with maseration method used 70% ethanol. The yield extraction of sample from three locations presented at Table 1. Standardization of medicinal plants is an important step in conducting research and development of natural medicines to ensure the quality and safety of drug preparations³⁵. Specific parameter of 70% ethanol extract of bulbs of Eleutherine bulbosa Urb. tested consist of extract identity, organoleptic extract, microscopic test, water/ethanol soluble content and chromatography profile.

Previous research results, the yield extraction from Melak, West Kutai district, East Kalimantan used 96% ethanol as solvent produced yield greater than 96% ethanol. This result because the polarity level of 70% ethanol higher than 96% ethanol so that was able to attract more compounds. The specific parameter of extract identity, organoleptic and water/ethanol soluble content presented of Table 2. Macroscopic and microscopic characters are one of the important criteria for identification²⁵. Bulbs of Eleutherine bulbosa Urb between three location Kalimantan have the save from. The sample have whole bulbs in groups, each group consists of several bulb, part of bulb base is hard, the bulb surface is smooth, pointed ends and have oval form. At microscopic characters between three location have similarity, their have parenchyma with oil drops and isolated schlerencyma. The results of specific parameter of macroscopic and microscopic presented of Figures 1 and 2.

The next parameter in extract standardization is chromatography profile. The determination of the chromatogram pattern was carried out by the TLC method which aimed to separated the compounds in the extract based on spot pattern and color after being observed on UV light and H₂SO₄ as spray reagents. The TLC profile is a qualitative analysis to extract based on spot pattern and color after being observed on UV light by the TLC method which aimed to separated the compounds in the extract. The determination of the chromatogram pattern was carried out by the TLC method which aimed to separated the compounds in the extract based on spot pattern and color after being observed on UV light and H₂SO₄ as spray reagents. The TLC profile is a qualitative analysis to show the presence of chemical compounds present in the sample. The results showed there were four spot in TLC plate. The results of specific parameter of TLC profile presented of Figure 3.

Non specific parameter of 70% ethanol extract of Eleutherine bulbosa Urb bulbs tested consist of specific gravity, water content, total ash content, acid insoluble ash content, residual solvent, heavy metal

| Table 1: The Yield Extraction of 70% ethanol extract of Eleutherine bulbosa Urb. Bulbs from 3 Location. |
|---|---|---|---|
| No | Location | Simplicia | Extract weights | Yield |
|---|---|---|---|---|
| 1 | Banjarbaru city, south kalimantan | 500 | 53.491 | 10.69 |
| 2 | Palangkaraya city, central kalimantan | 500 | 50.573 | 10.11 |
| 3 | Balikpapan city, east kalimantan | 500 | 53.922 | 10.78 |

| Table 2: Specific Parameter Results of 70% Ethanol Extract of Eleutherine bulbosa Urb. Bulbs from 3 Location. |
|---|---|---|---|
| No | Parameter | Banjarbaru city, south kalimantan | Palangkaraya city, central kalimantan | Balikpapan city, east kalimantan |
|---|---|---|---|---|
| 1 | Extract identity | Eleutherine bulbosa extract | Eleutherine bulbosa Urb. | Eleutherine bulbosa Urb. |
| | Extract name | | | |
| | Latin name | | | |
| | Part of plant | | | |
| | Local name | Bawang dayak | Bawang dayak | Bawang tiwai |
| 2 | Organoleptic | | | |
| | Color | Brownish red | | |
| | Taste | Bitter | | |
| | Odor | Faint characteristic odor | | |
| 3 | Water Soluble Content (% w/w) | 33.34 ± 1.78 | 30.65 ± 1.54* | 31.52 ± 0.98* |
| | Ethanol Soluble Content (% w/w) | 83.13 ± 1.67 | 81.05 ± 1.19* | 81.22 ± 1.99* |

*Values are means of triplicate determination ± standard deviation
Figure 1: Macroscopic of *Eleutherine bulbosa* Urb. (A) *Eleutherine bulbosa* Urb Plants (B) Bulbs (C) Simplicia of *Eleutherine bulbosa* Urb (D) Powdered Bulbs.

| No | Location          | Parenchyme with oil drops | Isolated Sclerenchyma |
|----|-------------------|---------------------------|-----------------------|
| 1  | Banjarbaru city   | ![Image](image1.png)      | ![Image](image2.png)  |
| 2  | Palangkaraya city | ![Image](image3.png)      | ![Image](image4.png)  |
| 3  | Balikpapan city   | ![Image](image5.png)      | ![Image](image6.png)  |

Figure 2: Microscopic of *Eleutherine bulbosa* Urb. Bulbs Powdered.

Figure 3: TLC Profile 70% ethanol extract of *Eleutherine bulbosa* Urb Bulbs (1) Banjarbaru City (2) Palangkaraya city (3) Balikpapan city. Mobile phase: n-hexane : ethyl acetate (7:3). Stationary phase: Silica gel 60 Gf 254+.
contamination (Pb and Cd), microbial contamination and mold yeast contamination. The result showed at Table 3.

Specific gravity relates to purity and contamination. These results of specific gravity from three location almost the same with the result of previous research from fridayanti et. al., that was 0.9347 ± 0.0036. In this research, determination of water content used distillation method. The results appropriate requirement but its value almost very standard. One of the reason it can be happen because the solvent used was 70% ethanol which contains a high water content.

Next determination were total ash content and acid insoluble ash content. This determination aims to provide an overview of the internal and external mineral content originated from the initial process until the extract formed. At this stage the extract was heated until the organic compounds and their derivatives are destructed and evaporated until only the mineral and inorganic elements remain. Another nonspecific parameter was determined the residual solvent. If the residual solvent still high in the extract, it is possible to enter the body and give the side effect. This method used GC-MS for analyze. Based on chromatogram pattern, the sample from three location proven negative.

Heavy metal contamination determination aims to ensure that the extract does not contain certain heavy metal exceeding the specified values which are harmful to health. Two heavy metals tested were lead (Pb) and cadmium (Cd). Based on the result, the extracts accordance with the requirement. And the last non specific parameter were microbial contamination, mold and yeast contamination. This parameter aims to provide assurance that the extract does not contain microbes, mold and yeast exceed the requirement because it affects the stability of extract and harmful to healthy. In this determination, the extract also accocnade with the requirement.

Based on metabolit secondary and activity from *Eleutherine bulbosa* Urb, total flavonoid content was determined. In this method used quercetin as standard. The results for maximum wavelength was 435 nm, with operating time 30 minutes. The maximum wavelength accordance with literature that stated the wavelength maximum for quercetin with this method was 415-440 nm. Quercetin standard curve have regression $y = 0.0132x + 0.0152$, $R^2 = 0.9998$. Quercetin standard curve showed at Figure 4. Total flavonoid content used aluminium chloride as reagent. AlCl₃ will reacted with C-4 at ketone group and C-3 or C-5 at hydroxyl group from flavonoid structure. The reaction between AlCl₃ and quercetion showed at Figure 5. Furthermore determination of total flavonoid content for *Eleutherine bulbosa* Urb from three location. The result for total flavonoid content presented at Table 4 showed the highest total flavonoid content from palangkaraya city as 7.585 ± 0.0437 mg QE/g extract. Even though the

### Table 3: The Result of Non Specific Parameter of 70% ethanol extract of *Eleutherine bulbosa* Urb Bulbs from 3 Location.

| No | Parameter                              | Location          | Requirement |
|----|----------------------------------------|-------------------|-------------|
| 1  | Specific Gravity (gram/mL)*            | Banjarbaru City  | 0.9140 ± 0.00 | ≤ 10.0%    |
|    |                                        | Palangkaraya City| 0.9155 ± 0.00 |            |
|    |                                        | Balikpapan city  | 0.9126 ± 0.00 | -          |
| 2  | Water Content (% w/w)*                 | Banjarbaru City  | 9.945 ± 0.04  | -          |
|    |                                        | Palangkaraya City| 9.795 ± 0.04  | -          |
|    |                                        | Balikpapan city  | 9.945 ± 0.03  | -          |
| 3  | Total Ash Content (%)*                 | Banjarbaru City  | 5.48 ± 0.01   | -          |
|    |                                        | Palangkaraya City| 5.67 ± 0.04   | -          |
|    |                                        | Balikpapan city  | 7.03 ± 0.13   | -          |
| 4  | Acid Insoluble Ash Content (% w/w)*    | Banjarbaru City  | 0.135 ± 0.04  | -          |
|    |                                        | Palangkaraya City| 0.165 ± 0.00  | -          |
|    |                                        | Balikpapan city  | 0.45 ± 0.00   | -          |
| 5  | Residual Solvent                       | Banjarbaru City  | Negative      | Negative   |
|    |                                        | Palangkaraya City| Negative      | Negative   |
|    |                                        | Balikpapan city  | Negative      | Negative   |
| 6  | Heavy Metal Contamination – Pb (mg/kg)* | Banjarbaru City  | 0.018 ± 0.04  | -          |
|    |                                        | Palangkaraya City| 2.003 ± 0.04  | -          |
|    |                                        | Balikpapan city  | 1.972 ± 0.00  | -          |
|    | Heavy Metal Contamination – Cd (mg/kg)*| Banjarbaru City  | 0.142 ± 0.06  | -          |
|    |                                        | Palangkaraya City| 0.144 ± 0.01  | -          |
|    |                                        | Balikpapan city  | 0.148 ± 0.02  | -          |
| 7  | Microbial Contamination (colony/g)*    | <01 x 10⁶         | -            |
|    |                                        | <01 x 10⁶         | -            |
|    |                                        | <01 x 10⁶         | -            |
| 8  | Mold and Yeast Contamination (colony/g)*| 2.5 x 10⁵         | -            |
|    |                                        | 0.1 x 10⁵         | -            |
|    |                                        | 2.0 x 10⁵         | -            |

*Values are means of triplicate determination ± Standard Deviation

### Table 4: Total Flavonoid Content 70% ethanol extract of *Eleutherine bulbosa* Urb Bulbs from 3 Location.

| No | Location      | Absorbance | Total Flavonoid Content (mg/g QE) |
|----|---------------|------------|-----------------------------------|
| 1  | Banjarbaru City| 0.101 ± 0.0069 | 6.499 ± 0.3248                  |
| 2  | Palangkaraya City| 0.115 ± 0.0005 | 7.585 ± 0.0437                  |
| 3  | Balikpapan City| 0.081 ± 0.0051 | 5.035 ± 0.3887                  |

*Values are means of triplicate determination ± Standard Deviation
sample have the same species, differences in the content of flavonoid compounds can be influenced by several factors such as genetics, the environment (climate, soil quality, water quality), the addition of growth support materials and harvest time.  

CONCLUSION

it can be concluded that bulbs of Eleutherine bulbosa Urb. from three locations on the nonspecific and specific parameters have characters to similarity between each other and that bulbs of Eleutherine bulbosa Urb. form central borneo had the highest total flavonoid content.

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GRAPHICAL ABSTRACT

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