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. Total flavonoids was also quantified. Methods: Eleutherine bulbosa Urb was extracted with maseration method used ethanol 70 % as solvent. Determination of non-specific includes by determined specific gravity, water content, total ash content, acid insoluble ash content, residual 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. Results: there were no significance difference between nonspecific and specific parameters Eleutherine bulbosa Urb from three different locations. Measurement of total phenol content and total flavonoid content respectively form South Borneo were ; 6,499 ± 0,5248 mg QE/g extract, from central borneo were 7,585 ± 0,0437 mgQE/g extract, and from east borneo were 5,035 mg ± 0,3887 mgQE/g extract. 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 medicine1. Considered herbal medicines have an important role in the health sector, it should to determine the quality and safety standards of medicinal plants extracts2. Standardization of medicinal plant extracts is one of the important stages in the development of natural medicines3.

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 quinones4. Bulbs of this plant had many activities such as immunomodulator5, antiinflammation7, antioxidant8, antihypercholesterol9 and anticancer10. To develop this potential, standardization of extracts were carried out. It consisted of nonspecific and specific parameters4. Beside it, bulbs of Eleutherine bulbosa Urb. were examined for the organoleptic, macroscopic and microscopic parameters11. Standardization of Eleutherine bulbosa Urb. bulbs had been carried out but from three different locations, that were Malang, Bogor, and Purbalingga (Java Island)12 and also the standardization of this plant had been done used different solvent, thus was ethanol 96 % which the plant only from east borneo13. 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.
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 maserat were collected then concentrated used rotavapor at 50°C with 40 rpm. Furthermore evaporated it used waterbath at 50°C until thick extracts were obtained14. 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 plants14.

Microscopic Test

This test used aquabidest reagent. Powder microscopy was also carried out and the specific characteristic were recorded20. Plant parts that can be observed include starch, transport bundles, endodermis, epidermis and parenchyma tissue14.

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 21,20.

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 GF254 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 methanol21.

Determination Non Spesific 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 weighed14.

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 calculated14. Water content is calculated in % v/w14,15.

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/w14,15.

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 14,15.

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 chromatogram pattern14,21.

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 absorption21. 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 calculated21.

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 hours21. 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 recorded21.

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 nm14.
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 at the maximum wavelength 25.

Quercetin Standard Curve

Quercetin was used to make a standard calibration curve. 100 μg 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 shaken to stand for operating time and read the absorbance at the maximum wavelength 25.

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 obtained 25.

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 preparations 15. 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 extract from Melak, West Kutai district, East Kalimantan using 96% ethanol so that was able to attract more than 96% ethanol. This result because the polarity level of 70% ethanol higher than 96% ethanol so that was able to attract more compound, which of these chemical substances can be dissolved or attracted based on their respective polarity. In the Table 2, showed extract from three location were more soluble in ethanol compared water so it can be concluded the attracted compound were semipolar. The results of 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 identification 25. 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 show the presence of chemical compounds present in the sample 19. The results showed there are four spot in TLC plate. The results of specific parameter of TLC profile presented of Figure 3.

Specific parameter describe the identity an extract. The identification process is an important part of quality control of traditional medicine product because ingredients usually come from different cultivated areas, and have many physical similarities with other plants that are still of the same genus. The first parameter determined was extract identity. With the extract identity, it can be a specific clue to differentiate between plant extracts from one another. Then the organoleptic determination of the extract was the second step to check the quality of the extract by observing color, taste and odor. Water soluble content or ethanol soluble content were the next test. Each plant contains different compound, which of these chemical substances can be dissolved or attracted based on their respective polarity. In Table 2, showed extract from three location were more soluble in ethanol compared water so it can be concluded the attracted compound were semipolar.

The results of specific parameter of extract identity, organoleptic and water/ethanol soluble content presented of Table 2.

Table 1: The Yield Extraction of 70% ethanol extract of *Eleutherine bulbosa* Urb. Bulbs from 3 Location.

<table>
<thead>
<tr>
<th>No</th>
<th>Location Unit</th>
<th>Simplicia Gram</th>
<th>Extract weights Gram</th>
<th>Yield % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Banjarbaru city, south kalimantan</td>
<td>500</td>
<td>53.491</td>
<td>10.69</td>
</tr>
<tr>
<td>2</td>
<td>Palangkaraya city, central kalimantan</td>
<td>500</td>
<td>50.573</td>
<td>10.11</td>
</tr>
<tr>
<td>3</td>
<td>Balikpapan city, east kalimantan</td>
<td>500</td>
<td>53.922</td>
<td>10.78</td>
</tr>
</tbody>
</table>

Table 2: Specific Parameter Results of 70% Ethanol Extract of *Eleutherine bulbosa* Urb. Bulbs from 3 Location.

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Bajjarbaru city, south kalimantan</th>
<th>Palangkaraya city, central kalimantan</th>
<th>Balikpapan city, east kalimantan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extract identity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extract name</td>
<td><em>Eleutherine bulbosa</em> extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Latin name</td>
<td><em>Eleutherine bulbosa</em> Urb.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Part of plant</td>
<td>Bucks</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local name</td>
<td>Bawang dayak</td>
<td>Bawang dayak</td>
<td>Bawang tiwai</td>
</tr>
<tr>
<td>2</td>
<td>Organoleptic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Color</td>
<td>Brownish red</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taste</td>
<td>Bitter</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Odor</td>
<td>Faint characteristic odor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Water Soluble Content (% w/w)</td>
<td>33.34 ± 1.78</td>
<td>30.65 ± 1.54*</td>
<td>31.52 ± 0.98*</td>
</tr>
<tr>
<td></td>
<td>Ethanol Soluble Content (% w/w)</td>
<td>83.13 ± 1.67</td>
<td>81.05 ± 1.19*</td>
<td>81.22 ± 1.99*</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>No</th>
<th>Location</th>
<th>Parenchyme with oil drops</th>
<th>Isolated Sclerenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Banjarbaru city</td>
<td><img src="image1" alt="Microscope Image" /></td>
<td><img src="image2" alt="Microscope Image" /></td>
</tr>
<tr>
<td>2</td>
<td>Palangkaraya city</td>
<td><img src="image3" alt="Microscope Image" /></td>
<td><img src="image4" alt="Microscope Image" /></td>
</tr>
<tr>
<td>3</td>
<td>Balikpapan city</td>
<td><img src="image5" alt="Microscope Image" /></td>
<td><img src="image6" alt="Microscope Image" /></td>
</tr>
</tbody>
</table>

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.
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 accound 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. AlCl$_3$ will reacted with C-4 at ketone group and C-3 or C-5 at hydroxyl group from flavonoid structure. The reaction between AlCl$_3$ 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. Eventhough the

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Location</th>
<th>Requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific Gravity (gram/mL)*</td>
<td>Banjarbaru City, Palangkaraya City, Balikpapan City</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Water Content (% w/w)*</td>
<td>9,945 ± 0,04, 9,795 ± 0,04, 9,945 ± 0,03</td>
<td>≤ 10,0 %</td>
</tr>
<tr>
<td>3</td>
<td>Total Ash Content (%)*</td>
<td>5,48 ± 0,01, 5,67 ± 0,04, 7,03 ± 0,13</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Acid Insoluble Ash Content (% w/w)*</td>
<td>0,135 ± 0,04, 0,165 ± 0,00, 0,45 ± 0,00</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Residual Solvent</td>
<td>Negative, Negative, Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Heavy Metal Contamination – Pb (mg/kg)*</td>
<td>1,018 ± 0,04, 2,003 ± 0,04, 1,972 ± 0,00</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>Heavy Metal Contamination – Cd (mg/kg)*</td>
<td>0,142 ± 0,06, 0,144 ± 0,01, 0,148 ± 0,02</td>
<td>0,3 mg/kg</td>
</tr>
<tr>
<td>8</td>
<td>Microbial Contamination (colony/g)*</td>
<td>&lt;01 x 10$^6$, &lt;01 x 10$^6$, &lt;01 x 10$^6$</td>
<td>≤ 10$^6$</td>
</tr>
<tr>
<td>9</td>
<td>Mold and Yeast Contamination (colony/g)*</td>
<td>2,5 x 10$^1$, 0,1 x 10$^1$, 2,0 x 10$^1$</td>
<td>≤ 10$^3$</td>
</tr>
</tbody>
</table>

*Values are means of triplicate determination ± Standard Deviation

<table>
<thead>
<tr>
<th>No</th>
<th>Location</th>
<th>Absorbance</th>
<th>Total Flavonoid Content (mg/g QE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Banjarbaru City</td>
<td>0,101 ± 0,0089</td>
<td>6,499 ± 0,3248</td>
</tr>
<tr>
<td>2</td>
<td>Palangkaraya City</td>
<td>0,115 ± 0,0005</td>
<td>7,585 ± 0,0437</td>
</tr>
<tr>
<td>3</td>
<td>Balikpapan City</td>
<td>0,081 ± 0,0051</td>
<td>5,035 ± 0,3887</td>
</tr>
</tbody>
</table>

*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 similar between each other and that bulbs of *Eleutherine bulbosa* Urb. form central borneo had the highest total flavonoid content.

ACKNOWLEDGEMENTS

The authors would like to acknowledge a research grant from Ministry of Research, Technology and Higher Education Republic of Indonesia (Kemenristekdikti) for the funding support of the research project (Hibah Penelitian Kerja Sama Antar Perguruan Tinggi Nomor SPPK : 191/SP2H/AMD/LT/DRPM/2019) and we would thank to Banjarbaru Industry Standardization and Research Center which was involved in tested the standardization of extract of non-specific parameters.

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Muthia, et al.: Standardization of Eleutherine bulbosa Urb. Bulbs and Total Flavonoid Content from Three Locations in Kalimantan, Indonesia

**GRAPHICAL ABSTRACT**

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