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

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Original Article

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

Rahmi Muthia[1,*], Helmina Wati[2], Wahyudin Bin Jamaludin[3], Kartini[4], Finna Setiawan[5], Muhammad Fikri[1], Abdul Wahhab[1]

ABSTRACT

Background: Dayak Onion (*Eleutherine bulbosa* Urb.) is a typical plant of Kalimantan which is traditionally used by the Dayak community as medicinal plant. Dayak onion bulbs have been proven had many pharmacology activities. **Objective:** This study aims to determine the nonspesific 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 etanol 70 % as solvent. Determination of non-spesific includes by determined specific gravity, water content, total ash content, acid insoluble ash content, residual sovents, heavy metanol 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 nonspesific 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.

Rahmi Muthia[1,*], Helmina Wati[2], Wahyudin Bin Jamaludin[3], Kartini[4], Finna Setiawan[5], Muhammad Fikri[1], Abdul Wahhab[1]

[1]*Departement of Pharmacognosy and Phytochemstry, Borneo Lestari College of Health Sciences, INDONESIA.*
[2]*Departement of Pharmacology, Borneo Lestari College of Health Sciences, INDONESIA.*
[4]*Departement of Pharmaceuticals, Borneo Lestari College of Health Sciences, INDONESIA.*
[3]*Pharmaceutical Biology Departement, Faculty of Pharmacy, Surabaya University, INDONESIA.*
[1]*Pharmacology Departement, Faculty of Pharmacy, Surabaya University, INDONESIA.*

Correspondence
Rahmi Muthia
Departement of Pharmacognosy and Phytochemstry, Borneo Lestari College of Health Sciences, INDONESIA.
E-mail : rahmimuth@gmail.com

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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[1]. 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[2]. Standardization of medicinal plant extracts is one of the important stages in the development of natural medicines[3].

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[4,5]. Bulbs of this plant had many activites such as immunomodulator[6-8], antiinflamation[9], antioxidant[10], antihypertention[11], antyhipercholesterol[4,12] and anticancer[13].

To develop this potential, standardization of extracts were carried out. It consisted of nonspecific and spesicfic parameters[14]. Beside it, bulbs of *Eleutherine bulbosa* Urb. were examined for the organoleptic, macroscopic and microscopic parameters[15] Standardization of *Eleutherine bulbosa* Urb. bulbs had been carried out but from three different locations, that were Malang, Bogor, and Purbalingga (Java Island)[16] and also the standardization of this plant had been done used different solvent, thas was ethanol 96 % which the plant only from east borneo[17]. 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/If.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 remaseration 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 obtained[18]. Calculated the yield of the thick extract.

Determination spesific 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[14].

Macroscopic and Organoleptic Extract

Observations were carried out with the five senses to describe the shape, color, taste and odor of the extract[14]. 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[15,18].

Microscopic Test

This test used aquabidest reagent. Powder microscopy was also carried out and the specific characteristic were recorded[23]. Plant parts that can be observed include starch, transport bundles, endodermis, epidermis and parenchyma tissue[21].

Water/Ethanol Soluble Content

Determination was done by permeating 1.0 g extract with 25 mL water-chloroform (39: 1) for 28 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 [2,18,20].

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 GF_{254} as a stationary phase. Bottle extract with concentration of 0.5% TLC plate GF254 with 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 (H_2SO_4) solution in methanol[18].

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 25oC weighed by water weight. Liquid extracts at 20°C are introduced, adjusted at 25°C and weighed[14].

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 [14,19]. Water content is calculated in % v/w[20].

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 desiccator then calculated the results, expressed %w/w[14,15].

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, glowed 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 gropus through the similar index and the re resulting cromatogram pattern[14,21].

Heavy metal contamination

The instrument used to perform 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[21]. Weighed 2.5 g of extract and added 20 ml of concentrated HNO_3 and allowed to stand for 24 hours, heated to 100°C for 10 minutes then cooled then added 2 ml of 30% H_2O_2, 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[2,22].

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[2,21]. 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 [2,21].

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 $AlCl_3$, 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[24].

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 $AlCl_3$, 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 minutes[25].

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 dilute standard solutions were pipette out and added with 0.1 mL $AlCl_3$, 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 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 $AlCl_3$ 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[24].

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]. Spesific 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 used 96% ethanol as solvent produced yield 1,49% w/w[16]. Based on these research, the yield used 70% ethanol was greater than 96% ethanol. This result because the polarity level of 70% ethanol higher than 96% ethanol so that was able to atrracted more compounds.

Specific parameter desribe 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 obsercing color, staste and odor. Water soluble content or ethanol soluble content were the next test. Each plant contains different compound, which of these chemical sbstances 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 spesific 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 spesific 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 H_2SO_4 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 spesific parameter of TLC profile presented of Figure 3.

Non spesific 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 extact of *Eleutherine bulbosa* Urb. Bulbs from 3 Location.

No	Location	Simplicia	Extract weights	Yield
	Unit	Gram	Gram	% w/w
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: Spesific 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			
	➔ Extract name		Eleutherine bulbosa extract	
	➔ Latin name		*Eleutherine bulbosa* Urb.	
	➔ Part of plant		Bulbs	
	➔ 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

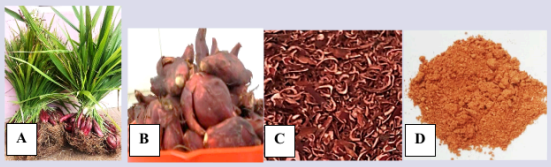


Figure 1: Macroscopic of *Eleutherine bulbosa* Urb. (A) *Eleutherine bulbosa* Urb Plants (B) Bulbs (C) Simplicia of *Eleutherine bulbosa* Urb (D) Powdered Bulbs.

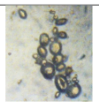
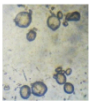
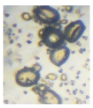
No	Location	Parenchyme with oil drops	Isolated Sclerenchyma
1	Banjarbaru city		
2	Palangkaraya city		
3	Balikpapan city		

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

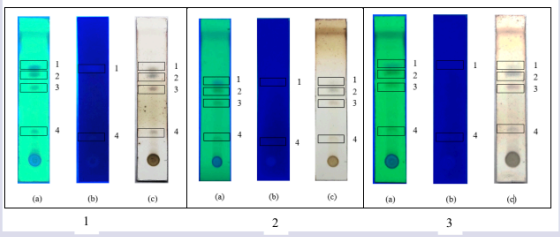


Figure 3: TLC Profile 70% ethanol 30 act of *Eleutherine bulbosa* Urb Bulbs (1) Banjarbaru City (2) Palangkaraya city (3) Balikpapan city. Mobile phase : n-hexane : ethyl acetate (7:3). Stationary phase : Silica gel 60 GF_{254}.

contamination (Pb dan Cd), microbial contamintation and mold yeast contamination. The result showed at Table 3.

Specific gravity relates to purity and contamination. These results spesific gravity from three location almost the same with the result of previous resarch from fridayanti et. al., that was 0,9347 ± 0,00[16]. In this research, determination of water content used destilation method. The results appropriate requirment but its value almost value 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[13]. 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[2]. 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 requirment. 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 requirment because it affects the stability of extract and harmful to healthy[13]. In this determination, the extract also accondace with the requirment.

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[26]. The maximum wavelength accordance with literature that stated the wavelength maximum for quercetin with this method was 415-440 nm[26].

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[28]. 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 3: The Result of Non Spesific Parameter of 70% ethanol extract of *Eleutherine bulbosa* Urb Bulbs from 3 Location.

No	Parameter	Location			Requirment
		Banjarbaru City	Palangkaraya City	Balikpapan city	
1	Specific Gravity (gram/mL)*	0,9140 ± 0,00	0,9155 ± 0,00	0,9126 ± 0,00	-
2	Water Content (% w/w/)*	9,945 ± 0,04	9,795 ± 0,04	9,945 ± 0,03	≤ 10,0 %
3	Total Ash Content (%)*	5,48 ± 0,01	5,67 ± 0,04	7,03 ± 0,13	-
4	Acid Insoluble Ash Content (% w/w)*	0,135 ± 0,04	0,165 ± 0,00	0,45 ± 0,00	-
5	Residual Solvent	Negative	Negative	Negative	Negative
6	Heavy Metal Contamination – Pb (mg/kg)*	1,018 ± 0,04	2,003 ± 0,04	1,972 ± 0,00	10 mg/kg
	Heavy Metal Contamination – Cd (mg/kg).*	0,142 ± 0,06	0,144 ± 0,01	0,148 ± 0,02	0,3 mg/kg
7	Microbial Contamination (colony/g)*	<01 x 10^0	<01 x 10^0	<01 x 10^0	≤ 10^4
8	Mold and Yeast Contamination (colony/g)*	2,5 x 10^1	0,1 x 10^1	2,0 x 10^1	≤ 10^3

*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 ± 0,5248
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

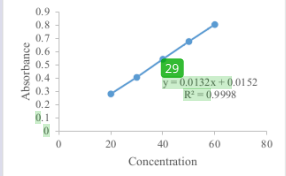


Figure 4: Quercetin Standard Curve Chart.

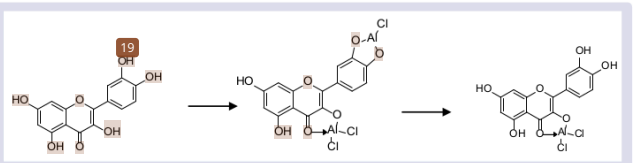


Figure 5: Quercetin – Aluminium Cholide Complex Compound.

sample have the same species, differences in the content of flavonoid compounds can be influenced by several factors such as genetics, the enviroment (climate, soil quality, water quality), the addition of growth support materials and harvest time[2].

CONCLUSION

it can be concluded that bulbs of *Eleutherine bulbosa Urb.* from three locations on the nonspesific 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.

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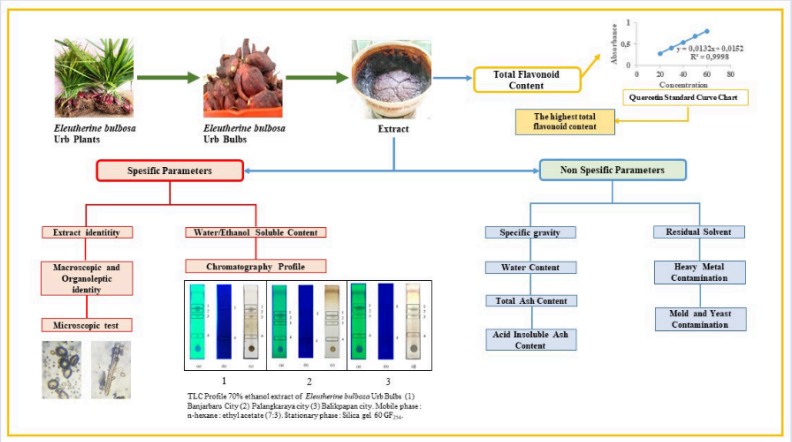

ABOUT AUTHORS

apt. Rahmi Muthia, M.Si. is an Assistant Professor in the Departement of Pharmacognosy and Phytochemistry, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari Banjarbaru, Indonesia. She has completed her magister in Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology. She works on development of natural materials especially simplicia characterization, standardization and in vitro activity test (antioxidant, immunomodulator, antihypertension).

apt. Helmina Wati, M. Sc is an Assistant Professor in The Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari Banjarbaru, Indonesia. she has completed her magister in Clinical Pharmacy, Gadjah Mada University. She work in drug development in the field of pharmacology and clinical pharmacy.

apt. Wahyudin Bin Jamaludin, M.Si. *is lecturer* in the Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari Banjarbaru, Indonesia. he has graduated his magister in Pharmaceutical from School of Pharmacy, Bandung Institute of Technology, Indonesia. He is currently working in projects develop modified delivery system of Indonesian medicinal plants.

Kartini, Ph.D. *is* an Associate Professor in the Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Indonesia. She has completed her Ph.D. in Phytopharmaceutical Sciences from Faculty of Graduate Studies Mahidol University, Thailand. She is currently the Director of Center for Traditional Medicine Information & Development, Faculty of Pharmacy, University of Surabaya. She works on standardization of herbal medicines and its application as wound healing, anticancer, and immunomodulator.

Dr. Finna Setiawan, M.Si. is an Assistant Professor in the Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Indonesia. She has completed her Doctoral Programme in Pharmacology Sciences from Bandung Institute of Technology, Indonesia. She is currently working in bioactivity of herbal medicines especially in effectivity and safety use of herbal medicines.

Muhammad Fikri, S. Farm is an Pharmacist Assistant. He has graduated his bachelor in Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari Banjarbaru, Indonesia. He actively participates in the student creativity program by the Directorate of Higher Education every year and passed humans in 2016. He was a lecturer assistant for quantitative analysis of chemistry, microbiology-parasitology, phytochemistry, human physiological anatomy, and pharmacognosy.

Abdul Wahhab, S. Farm is an Pharmacist Assistant. He has graduated his bachelor in Pharmacy, Sekolah Tinggi Ilmu Kesehatan Borneo Lestari Banjarbaru, Indonesia. He was a lecturer assistant for quantitative analysis of chemistry, microbiology-parasitology, phytochemistry, human physiological anatomy, and pharmacognosy.

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