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Standardization of Ethanol Extract of *Dioscorea alata* L. Variety of Paoateno from Banggai Islands District, Central Sulawesi

(*Standarisasi Ekstrak Etanol Dioscorea alata L. Varietas Paoateno Asal Kabupaten Banggai Kepulauan Sulawesi Tengah*)

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ABSTRACT

Background: Standardizing herbal medicines is crucial for assessing their quality and effectiveness. **Objectives:** This study aims to determine non-specific and specific standardization parameters for medicinal plants. Non-specific parameters include drying shrinkage, water content, total ash content, acid insoluble ash content, total bacteria, total mold, and metal limits (Pb, Hg, and Cd). Specific parameters involve organoleptic examination, identification of chemical content in extracts, dissolved compounds in specific solvents, total flavonoid content, and qualitative testing of anthocyanin content in the extract. **Methods:** An experimental laboratory approach was employed to extract *Dioscorea alata* L using 96% ethanol, which was later concentrated through a rotary evaporator to obtain a viscous extract. Specific and non-specific parameters were determined using standardized techniques. **Results:** The extract yielded 3.32% of the initial substance. Drying shrinkage was 19.7769±1.2682%, and specific gravity was 0.6192±0.1476 gram/ml for 5% m/v and 0.5101±3.9591 gram/ml for 10% m/v. Ash content was 0.6793%, with acid insoluble ash content at 0.1895%. Heavy metal contamination for Hg was 0.47 ppm, but undetected for Pb & Cd). The total plate number was 27 x 10^3 colonies/g, and negative *Escherichia coli* contamination. The mold count was 39 x 10^1 colonies/g. Additional specific parameters included the organoleptic properties of the viscous extract (purple color, characteristic odor, and sweet taste), water-soluble compound content (15.63%), ethanol-soluble compound content (14.73%), and a flavonoid content of 76.669 mg EQ/100 mg. **Conclusions:** The ethanol extract of *Dioscorea alata* complies with the standard requirements established by the Food and Drug Supervisory Agency. It presents as a viscous extract with a distinct odor, purple-black color, and sweet taste. The extract contains alkaloids, flavonoids, saponins, tannins, steroids, and phenolic compounds, with a total flavonoid content of 76.669 mg. Furthermore, it tested positive for anthocyanins.

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INTRODUCTION

The source of herbs every region in the world has its own culture. Herbal enhancement as a treatment has long been used by humans. Herbal products have been used by humans since 4000 years ago and the use of herbs as medicine is the most ancient medical culture where science has led researchers to find various drugs with synthetic and semi-synthetic sources with active medicinal compounds, treatment mechanisms, and guaranteed safety, and efficacy. and quality (Dixit VK & Yadav NP, 2008). Disadvantages of the use of herbs as drugs in terms of standardization, many plants are empirically known to have useful pharmacological effects but are not standardized so that the safety profile, Standardization according to the American Herbal Product Association is information and control that is certain and carried out to obtain a product with a consistent composition of sustainable results and guaranteed safety, quality, and efficacy (Calixto JB & Barz J, 2000). according to WHO parameters standardization needed for herbal medicines includes organoleptic standardization, microscopic, standardization, physical standardization, chemical standardization, and biological standardization. (WHO, 2005). Organoleptic standardization includes color, taste, texture, and smell. then microscopic standardization. Physical standardization includes ash content, acid-soluble ash content, water-soluble extract content, ethanol-soluble extract content, and humidity. Furthermore, chemical standardization includes fingerprint determination, biological standardization includes microbial contaminants, mold and yeast numbers, and heavy metal limit tests. All of them have their respective standard values for each standardization parameter.

Indonesia is one of the countries where many herbal plants are found, one of which is *Dioscorea alata* L. This plant has many species and varieties with varying characteristics (Yusuf, 2016). *Dioscorea alata*L. has a specific characteristic, namely purple. The purple color of these plants is caused by the presence of natural dyes called anthocyanins which belong to the class of flavonoid compounds. Anthocyanins are a group of pigments that cause a reddish color, located in the cell fluid which is soluble in water. Anthocyanin components are mono or diacetyl 3-(2-glucosyl) glucosyl-5-glucosyl peonidin and cyanidin derivatives. Anthocyanin compounds function as antioxidants and free radical scavengers so they play a role in preventing aging, cancer, and degenerative diseases. In addition, anthocyanins also have the ability as antimutagenic and anticarcinogenic, prevent liver function disorders, antihypertensives, and lower blood sugar levels (Nida., et al, 2013). In addition, the methanol extract of *Dioscorea alata* L. butylated hydroxyanisole and α-tocopherol (Lubag et al. 2008), so it is necessary to standardize the test to determine the safety profile, efficacy, and source of quality of these herbs.

Based on the description above, this study aims to determine the specific standardization parameters of the ethanolic extract of *Dioscorea alata* L. which include organoleptic examination, dissolved compounds in certain solvents, identification of the chemical content of the extract, total flavonoid

content and qualitative testing of anthocyanin content in the extract and determination of non-standardized parameters. specific ethanol extract of *Dioscorea alata* L. which includes drying shrinkage, determination of water content, determination of ash content, determination of acid insoluble ash content, determination of total bacteria, determination of total mold, determination of metal limits (Pb, Hg, and Cd) and determination of specific gravity.

MATERIAL AND METHODS

Sample Extraction

The dried Simplicia *Dioscorea alata* L. weighed as much as 502 grams, then a container was prepared and the simplicia was put in and 96% ethanol was added into a 3-liter container until the simplicia was completely submerged. Immersion was carried out for 3x24 hours and stirred every 24 hours. Stored in a place protected from direct sunlight and then filtered and accommodated in one container to get the filtrate. The resulting filtrate was then evaporated using a rotary evaporator at a temperature of 60°C at a speed of 100 rpm to evaporate the solvent to obtain a thick extract of *Dioscorea alata* L.

Drying Loss

In a cup that had previously been heated to 105^{o} C, for thirty minutes, a total of one gram of extract was weighed. Dry to a constant weight at the determination temperature, flatten to a thickness of 5-10 mm, close the cup, cool to room temperature in a desiccator, and then record the obtained constant weight.

Specific Gravity

Using a pycnometer, the results of diluting the extract (5% and 10%) in a solvent containing 96% ethanol were used to determine the extract's specific gravity. At the 5% dilution, the extract was carefully weighed to 0.5 grams and dissolved in up to 10 milliliters of 96 percent ethanol; at the 10% dilution, the extract was carefully weighed to 1 milliliter and dissolved in up to 10 milliliters of 96 percent ethanol. Use a dry, clean, fat-free pycnometer that has been calibrated by setting the pycnometer weight and water weight in an oven for three hours at 105°C. The pycnometer was filled with the liquid extract. Weigh the mixture until it reaches a constant weight, then discard any excess liquid extract. Divide the weight of the filled pycnometer by the weight of the empty one. By dividing the weight of the liquid extract by the weight of the water in the pycnometer, the result is the specific gravity of the liquid.

Water content

In a calibrated container, a total of 0.5 grams of extract were weighed. Using a Moisture Analyzer, dry for less than three minutes at 105°C. The moisture content, expressed as a percentage of the initial sample weight, was calculated from the value that was obtained.

Ash Level

In a crucible that has been calibrated and is slowly incandescent, carefully weigh 1 gram of extract. After that, the temperature was gradually raised to 600 to 250^{o}C until the extract turned white, indicating that it was carbon-free. The extract was then cooled in a desiccator, and the weight of the ash was weighed. The weight of the initial sample is used to determine the percent ash content.

Acid Insoluble Ash Content

The parts that are insoluble in acid were boiled for five minutes with 25 ml of dilute HCl P, collected, filtered with ash-free filter paper, washed with hot water, weighed, and the acid insoluble ash content was determined in units of a percent of the initial sample weight using the ash results from the ash content determination.

Metal Contaminants

Using the AAS (Atomic Absorption Spectroscopy) apparatus, heavy metals were measured sequentially in the blank solution, standard series solution, and test solution at a wavelength of 253.7 nm with 0.2% NaBH4 reducing agent and 3% HCl carrier fluid.

Microbial contamination

Total Plate Number (TPN)

The sample was aseptically weighed as much as 10 grams into a suitable sterile container, then 9 ml of Lactose broth (LB) was added, and shaken homogeneously so that a suspension with a dilution of 10^{-1} was obtained. Two tubes each filled with 9 ml LB were prepared for dilution. The suspension of the 10^{-1} dilution homogenized in the preparation of the sample was pipetted as much as 1 ml into the first LB tube, and shaken homogeneously until a dilution of 10^{-2} was obtained.

The dilution was continued to 10^{-5} using a new sterile pipette for each dilution. The suspension from each dilution was pipetted 1 ml into a petri dish and made in duplicate. Into each petri dish, 15-20 ml of PCA+1% TTC media was poured at 450C. The petri dish was immediately shaken and rotated in such a way that the suspension was evenly distributed. To determine the sterility of the media and diluent, a control test (blank) was made. One cup was filled with PCA media. After the media solidified, the cup was incubated at 32.5^{0}C for 48 hours with the position being turned upside down. The number of growing colonies was observed and counted.

Test *Escherichia coli*

Using an aseptic method, 10 ml of the homogenized suspension was then inoculated on 90 ml of Tryptic Soy Broth (TSB) and incubated at 370C for 18-24 hours. From the enrichment culture, 1 loop was inoculated on the surface of the Eosin Methylene Blue Agar (EMBA)

and incubated at 35-370C for 24-48 hours, with the plate position reversed. Specific colonies that grew were observed with the characteristics of round colonies, 2-3 mm in diameter, purplish red color with a metallic luster. Selected two or more specific colonies on EMBA to be inoculated on Nutrien Agar or Tryptic Soy Agar oblique followed by IMVic test.

Mold/Yeast Contamination

Make three tubes and fill each one with 9 milliliters of LB. From the results of sample homogenization, 1 ml was pipetted into the first LB tube, and shaken homogeneously until the next dilution was obtained up to 10-1. From each dilution, 0.5 ml was pipetted onto the surface of the PDA media plate, each was made in duplicate, immediately the plate was shaken and rotated in such a way that the suspension was evenly distributed. To determine the sterility of the media and diluent, a blank test was performed. On one PDA plate, 0.5 ml of diluent was evenly distributed and for the media test, another PDA plate was used. All PDA plates were incubated for 7 days at a temperature of 250C, with the position not being reversed, observations and calculations of mold/yeast colonies were carried out starting on day 2. Yeast colonies are small, white, and almost like bacteria.

Organoleptic

The organoleptic determination of the extract of the proud *Dioscorea alata* L. was tested including the shape, color, smell, and taste (Depkes RI, 2000).

Soluble Compounds in Certain Solvents

The concentration of water-soluble compounds

A stopper flask was used to macerate 1 g of extract (W1) with 25 ml of chloroform water for 24 hours, shaking it several times for the first six hours. After that, it was filtered and left to stand for 18 hours. After heating the residue to a constant weight (W2) at 105°C, 5 ml of the filtrate was evaporated in a shallow dish with a flat bottom (W0). The residue remained after the solvent evaporated. Find out how much of the compound's concentration is water-soluble.

$$\text{Soluble Compounds in Certain Solvents} = \frac{W2-W0}{W1} \times 100\%$$

Information:

W0 = Weight of empty cup

W1 = Extract weight

W2 = Weight + oven residue

Content of compounds soluble in ethanol

A stopper flask was used to macerate 1 g of extract (W1) for 24 hours with 25 ml of 95 percent ethanol. The flask was shaken several times for the first six hours and then left for 18 hours. Evaporate 5 milliliters of the filtrate to dryness in a shallow dish with a flat base that has been tapered (W0), then heat the residue to a constant weight (W2) by avoiding ethanol evaporation. Determine the concentration of ethanol-soluble compounds in percent (95%).

$$\text{Content of compounds soluble in ethanol} = \frac{W2-W0}{W1} \times 100\%$$

Information:

W0 = Weight of empty cup

W1 = Extract weight

W2 = Weight + oven residue

Identification of Compound Group

Alkaloid Test

Simplicia powder was precisely weighed 0.5 g, added 1 ml of 2 N HCl and 9 ml of refined water, warmed over a water shower for 2 minutes, cooled then separated. The following is an analysis of the remaining filtrate:

a. A white or yellow precipitate is produced by combining two drops of Mayer's reagent with three drops of filtrate.
b. A dark brown precipitate is produced when three drops of filtrate and two drops of Bouchard reagent are combined.
c. Approximately three drops of the filtrate and two drops of Dragendorff reagent were combined to produce a brick-red precipitate.

It is possible to conclude that it contains positive alkaloids if it displays the results of a white precipitate from the two or three analyses mentioned earlier (Marjoni, M. R., 2016).

Flavonoid Test

10 grams of *Dioscorea alata* extract add to100 milliliters of hot distilled water, weigh it carefully, boil it for about 5 minutes, and then filter it when it is hot.0.1 g of magnesium powder, 1 ml of concentrated HCl, and 2 ml of amyl alcohol were added to 5 ml of the filtrate, shaken, and allowed to separate. Positive for containing Flavonoids, if it happens a red, yellow, and orange tone is shaped on the amyl liquor layer (Marjoni, M. R, 2016).

Saponin Test

A test tube containing 0.5 g of the extract was carefully weighed, 10 ml of hot distilled water was added, and the tube was cooled and vigorously shaken for 10 seconds. If foam formed for at least 10 minutes with a height of 1-10 cm after adding 1 drop of 2 N HCl solution and the foam did not disappear, this indicated the presence of saponins (Marjoni, M.R, 2016).

Tannin Test

0.5 g of concentrate was weighed cautiously, then, at that point, disintegrated with 10 ml of refined water. The results of the extraction were filtered, the filtrate was diluted with distilled water until it had no color. After this dilution, a volume of up to 2 milliliters is added, along with one or two drops of $FeCl_3$. If a blue or green-black color develops, this is a positive sign that tannins are present. (Marjoni, M. R, 2016).

Steroid Test

After being macerated for two hours in 20 ml of n-hexane, 1 g of the extract was filtered. In a cup, the filtrate is evaporated. The remaining ingredients are two drops of concentrated H_2SO_4 and two drops of anhydrous CH_3COOH. The color changes from purple or red to positive blue or green when steroids or triterpenoids are present. (Marjoni, M. R, 2016).

Determination of Flavonoid Level

Determination of total flavonoid levels by chlorometric method referring to the procedures of Chang et al., (2002) and Ahmad et al., (2014) with some modifications with quercetin (QE) as the standard.

Preparation of quercetin standard solution

10 mg of standard quercetin standard dissolved in 10 ml of methanol pa for 1000 ppm. From a standard solution of 1000 ppm quercetin, then 1 ml pipetted and dissolved in 10 ml of methanol pa for 100 ppm, then made several concentrations, namely 1 ppm, 5 ppm, 10 ppm, 20 ppm, and 40 ppm. From each concentration of the standard solution of quercetin added 3 ml of methanol, 0.2 AlCl3 10%, 0.2 ml of 1M potassium acetate, and made up with distilled water to 10 ml. The mixture was shaken and allowed to stand for 30 minutes at room temperature and measure the absorbance on UV-Vis spectrophotometry.

Maximum wavelength determination

The concentration of the mother liquor was taken to measure its absorption in the wavelength range of 400-800 nm. The wavelength that shows the high absorption value is the maximum wavelength.

Determination of total flavonoid levels in *Dioscorea alata*

100 mg of ethanol extract of Uwi Banggai dissolved it in 10 ml of methanol. 1 ml solution add 3 ml of methanol, add 0.2 $AlCl_3$ 10%, add 0.2 ml of potassium acetate, and distilled water to 10 ml, store for 30 minutes in a dark place at room temperature, the absorbance is measured by UV-spectrophotometry. VIS with a wavelength of 442 nm. The sample solution was made in three replications so that the flavonoid content obtained was equivalent to quercetin.

Identification of Anthocyanin Compounds in Extract

Identification of anthocyanin compounds using the thin layer chromatography (TLC) method which refers to the Adrianta, KA (2016) procedure with several modifications.

Preparation of Butanol Acetic Acid eluent solution

5 ml of the solution was made by mixing n-butanol -acetic acid-water in a ratio of 4: 1: 5. The eluent solution was then put into the chamber and then closed for saturation.

Separation by Thin layer chromatography (TLC) method

TLC utilized a G60F254 8.5 cm x 1 cm silica gel plate. Eluent BAA = n-butanol was the mobile phase used to separate the components: acid acetic: water (4:1:5). A capillary tube was used to spot the *Dioscorea alata* L. extract 1 cm from the bottom edge of a silica gel plate G60F254, after which it was dried in the air and eluted for 7 cm. Spots that range in color from red to purple are a sign of a positive reaction.

RESULTS AND DISCUSSION

Non Specific Parameters

The initial process carried out in this study was to change the shape of the tubers processed into simplicia in the form of dry powder for the extraction process using the maceration method with 96% ethanol solvent. Maceration is the process of extracting a material using a solvent with stirring at room temperature (Dianita, 2010). The maceration method is used because the process is easy, produces a fairly high yield, and the possibility of damage to chemical compounds contained in the sample can be avoided because it is not accompanied by heat treatment (Sundari T, 2010). Immersion was carried out for 3×24 hours using 96% ethanol as a solvent because it was able to dissolve almost all substances, both polar, semi-polar and non-polar (Harborne, JB, 1987; Voight, R., 1994). In this case, ethanol itself also has the advantage that it is able to extract more chemical compounds than water and methanol and is commonly used for sample extraction (Azizah and Salamah, 2013). Furthermore, the liquid extract from the simplicia was filtered and evaporated using a rotary vacuum evaporator until a extract was formed. The extract obtained was 30.2 grams with a yield of 3.32% (Ukieyanna, 2012).

Table 1. Non-specific results of *Dioscorea alata*

No	Parameter	Results +/- SD	Requirements
1	Drying shrink	19.7769±1.27%	Fixed weight < 0.5 mg (FI edition III)
2	Specific Weight:		
	a. 5% m/v	0.6192±0.15 gram/ml	-
	b. 10 % m/v	0.5101±3.96 gram/ml	-
3	Water content	6,6753±2.21%	<10% (BPOM, 2014)
4	Ash content	0.6793±0.53%	<10%
	Acid insoluble ash content	0.1895±0.094%	<10% (BPOM, 2014)
5	Heavy metal contamination		
	a. Hg	0.471 ppm	0.5 ppm
	b. Pb	Negative	10 ppm
	c. CD	Negative	0.3 ppm (BPOM, 2014)

The results of non-specific parameter tests can be seen in Table 1. Test on drying shrinkage was 19.7769±2.2397%. A set weight is one of the drying shrinkage requirements. Fixed weight is the weight on weighing after the substance has been dried for 1 hour does not differ by more than 0.5 mg from the weight of the substance in the previous weighing (FI edition III), Then the determination of the specific gravity at each 5% dilution is 0.6192 ±0.1476 gram/ml and 10% dilution is 0.5101±3.9591 gram/ml. The goal of specific gravity determination is to give a general overview of the dissolved chemical composition in an extract. Results for the water content determination were 6.67532.2108%. Water content The less water present in the extract, the less likely it is to be contaminated by bacteria and to retain the extract's quality over time. The maximum water content that is allowed must not be more than 10% (BPOM, 2005). Ash content and acid insoluble ash content testing is the following non-specific parameter test. The goal of this determination is to give a general overview of the mineral composition of the both inside and externally. Both internal and exterior minerals can come in the form of silica compounds made from sand or plant nutrients (MOH, 2000). This is significant because the amount of ash in an extract can indicate how well it will work as a raw material for pharmacological preparations. The ash content was 0.67930.5293% weight-for-weight, while the acid-insoluble ash content was 0.18950.0938%. The extract's ash level and acid insoluble ash content shouldn't be higher than 10% (BPOM, 2005). Determination of heavy metal contaminants (Hg, Pb and Cd) in the extract of *Discorea alata* is aimed at ensuring that the extract does not contain metals exceeding the specified limit because it can be toxic to the body. The method used to identify the metal content is using the Atomic Absorption Spectroscopy (AAS) tool because it is more selective in determining the metal content of the sample. The results of heavy metal contamination adjusted to the requirements of BPOM obtained results from metal Hg (Mercury) which was 0.471 ppm and the conditions that had been determined were 0.5 ppm. If the level of Hg metal exceeds the required limit it will cause poisoning so that when this metal is

heated it will form a vapor that is corrosive to the skin, eyes, mouth and digestive tract. The results obtained from metal in Pb (Lead) are negative with the condition 10 ppm and if this metal exceeds the required limit it will be dangerous for human health that lasts a lifetime, because it accumulates in the human body which affects urine and lacks hemoglobin in the blood. The results obtained from Cd (Cadmium) metal are negative with the condition 0.3 ppm and for this metal if it exceeds the requirement limit it will affect humans in the long term because of the high risk of blood vessels and then it can accumulate in the body, especially the liver (Lu, 2006)

Table 2 The results of contamination of bacteria *Dioscorea alata*.

No	Test	Yield (colonies/gram)	Requirements (colonies/gram)
1	Total plate number	$27x10^3$colony/g	104 colonies/g (BPOM, 2014)
2	*Escherichia coli*	Negative (-)	Negative (-) (BPOM, 2014)
3	Mold/yeast number	$39x10^1$ colony/g	103 colonies/g (BPOM, 2014)

Determination of microbial contamination (Table 2) includes total plate number (ALT), *Escherichia coli* bacteria and determination of mold/yeast number (AKK). The results of microbial contamination adjusted to BPOM requirements obtained results from ALT testing, namely $27x10^3$ colonies/g (Table IV.2 No. 1) and the specified conditions were 10^4 colonies/g. The large amount of microbial growth is obtained from the storage process, namely a less sterile container that triggers microbial growth. Then the storage temperature can also affect because in general microbes can grow well at temperatures between 200C to 600C. Extract storage conditions carried out at room temperature affect the microorganisms that grow and cause damage. Temperature is one of the most important environmental factors that affect the growth of microorganisms (Perko, 2011). In this test, if it passes the requirements, it can be dangerous for humans, especially for breastfeeding mothers and their babies because they can produce toxins for various diseases including diarrhea, vomiting, and fever and can be infected (Saifudin, et al. 2011). The results obtained from the test of Escherichia coli bacteria are negative, this is in accordance with predetermined conditions and if the test results do not meet the requirements, it will be able to cause absorption in the intestinal wall to decrease and cause diarrhea due to infection that arises so that the movement of the solution in large quantities can damage the electrolyte balance in the mucous membrane (Moody, 2005)

Specific Parameters

Table 3 Organoleptic test of ethanolic extract *Dioscorea alata*

Sample	Organoleptic Test			
	Form	**Color**	**Smell**	**Flavor**
Dioscorea alata L.	Thick	black purple	Typical	Sweet

Based on the results of the identity of the organoleptic extract, it was obtained a thick form, blackish purple color, distinctive odor and sweet taste which was carried out on 10 people (Table 3). The purpose of extract identity is to provide objectivity from the name and specifications of the plant, while the organoleptic observation of the extract aims as an initial introduction using the five senses by describing the shape, color, smell, and taste (Depkes RI, 2000).

Table 4 Quantitative data on levels of water-soluble compounds

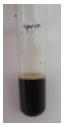
Replication	Initial Extract Weight (mg)	Final Extract Weight (mg)	Content of Water Soluble Compounds (%)	Average Content of Water Soluble Compounds (%)
I	1005.2	150.2	14.94	
II	1009.9	150.2	14.87	15.63
III	1009.4	172.6	17.09	

Table 5 Quantitative data on the concentration of ethanol-soluble compounds

Replication	Initial Extract Weight (mg)	Final Extract Weight (mg)	Content of Soluble Compounds in Ethanol (%)	Average Content of Soluble Compounds in Ethanol (%)
I	1003.5	134.6	13.41	
II	1008.1	154.4	15.31	14.73
III	1003.2	155.2	15.47	

The consequences of the trial of mixtures disintegrated in specific solvents involving water and ethanol as solvents, from the ethanol concentrate of Banggai yam (*Dioscorea alata* L.) it was observed from table 4 and 5, that the outcomes were broken up in water, in particular 15.63% while 14.73% broke up in ethanol. This shows that the concentrate is more dissolvable in water than in ethanol dissolvable. The test for deciding the degrees of mixtures broke down in water and ethanol plans to decide how much synthetic substance contained in the concentrate as a good guess of the substance of dynamic mixtures that are polar (water solvent) and dynamic mixtures that are semi-polar-non-polar (dissolvable in ethanol). (Saifudin, A., Rahayu, & Teruna, 2011).

Table 6. The result of compound groups identification

Test Parameters	Picture		Test result
	Before	After	
Alkaloids			(+)
Flavonoids			(+)
Saponins			(+)
Tannins			(+)
Steroids			(+)

Identification of groups of chemical compounds in the ethanol extract of *Dioscorea alata* L. to determine the group of compounds (Table 6). The identification test for the class of alkaloid compounds in Banggai sweet potato with Dragendorff reagent obtained positive results indicated by the presence of brick red precipitate (Marjoni, M. R, 2016). The brick red produced because Dragendorff is able to precipitate alkaloids, namely in alkaloid compounds there is a nitrogen group which has one free electron pair causing the alkaloid compound to be nucleophilic (base). Therefore, alkaloid compounds are able to bind heavy metal ions (dragendorff) which have a positive charge so that the color is formed. Alkaloids are characterized by the formation of a precipitate. The identification test of the flavonoid compound group in Banggai sweet potato by adding 0.5 magnesium powder and 2 drops of concentrated HCl obtained positive results as indicated by a red color (Marjoni, M. R, 2016). In flavonoids, there is a reduction reaction of the carbonyl group on the d-lacttone ring to form an alcohol group to form a colored hydroxy compound depending on the functional group attached to the A or B ring, the color that occurs is drawn by the amyl alcohol. The identification test for the class of saponin compounds in Banggai sweet potato with the addition of hot water obtained positive results indicated by the presence of foam or foam after vigorous shaking (Marjoni, M. R, 2016). Saponins when hydrolyzed will produce glycon (sugar) and aglycone (non-sugar) parts. With vigorous agitation, glycosides are capable of forming foam or foam in water which is hydrolyzed to sugars and other compounds. The foam does not disappear when added with hydrochloric acid because the acid cannot bind to aglycones and glucose.

The test for identification of the steroid/triterpenoid group of sweet potatoes with Lieberman-Burchard reagents obtained positive results which are shown in red (Marjoni, M. R, 2016). The reagent contains anhydrous acetic acid and concentrated sulfuric acid. Anhydrous acetic acid forms acetyl derivatives of steroids which are soluble in chloroform or ether. The chloroform or ether used does not contain water molecules because the presence of water can convert anhydrous acetic acid into acetic acid so that acetyl is not formed. The concentrated sulfuric acid functions to oxidize acetyl from steroids/triterpenoids. The steroid and triterpenoid compounds are similar compounds because they are composed of isoprene. The identification test for the class of tannin compounds in Banggai sweet potato with iron (III) chloride reagent obtained positive results indicated by a blackish green color (Marjoni, M. R, 2016). Tannin compounds are polar compounds due to the presence of an OH group, when added with 10% FeCl3 there will be a color change such as blackish blue or blackish green. Tannin compounds are included in polyphenol compounds which means compounds that have a phenolic part. Identification of compound groups is a qualitative test of the content of chemical compounds in plant parts, especially the number of secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, and other substances. Identification of compound groups must meet several requirements including simple, fast, and can be done with minimal equipment (Septyaningsih, 2010).

Table 7 Total Flavonoid Etanolic Exrract of *Dioscorea alata*.

Replication	Absorbance Value	Concentration mg/100 mg	Average Total flavonoid concentration (mg QE/100 mg)
1	0.106	86,939	
2	0.105	86.041	76.669±17,015
3	0.073	57.028	

The total flavonoid content of EEDA was calculated from calibration plot of the regression equation y=0.011 x + 0.010, R = 0,997. The average content of total flavonoids produced by the ethanolic extract of sweet potato was 76,669 mg QE in 100 mg of extract (Table 7).

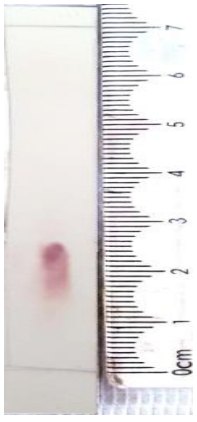


Figure 1. Identification of anthocyanin compounds *Dioscorea alata* L

Identification of anthocyanin compounds in the ethanolic extract of yam Banggai using TLC plate (Silica gel G60F254) as the stationary phase with eluent n-butanol:acetic acid:water (BAA) (4:1:5) as the mobile phase. Banggai (*Dioscorea alata* L.) is purplish red which means it contains anthocyanin with an Rf value of 0.34 with a medium category (0.10-0.40) (Figure 1) (Supriyanti, et al., 2010). It can be said that the color of anthocyanins will follow the color of the type of aglycone. The color of the pelargonidine aglycone itself is red. The color difference may also be influenced by the bonding of the aglycone with the glucoside of anthocyanins. The presence of impurities during preparation, storage of materials and tools and during extraction may also affect color differences (Harborne,1987).

CONCLUSION

Determination of non-specific parameter tests including water content, ash content, acid insoluble ash content, determination of heavy metal contamination, bacterial contamination and mold/yeast count (AKK) has met the standard requirements of the Food and Drug Supervisory Agency (BPOM), drying shrinkage 19.7769 ±1.2682%, determination of specific gravity which has been diluted 5% and 10% using 96% ethanol solvent 0.6192 ± 0.1476 gram/ml and 0.5101 ± 3.9591 gram/ml and determination of specific standard includes organoleptic with viscous extract form, characteristic odor, blackish purple color, and sweet taste, found alkaloids, flavonoids, saponins, tannins, steroids, and phenolic compounds. %, The total flavonoid content of the extract was 76.669 mg, and positively contained anthocyanins.

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CONFLICT OF INTEREST

All authors declare there is no conflict of interest with the research, authorship, and/or publication of this article.

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