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Determination of Specific and Non-Specific Standardization Parameters for Ethanol Extract of Purple Leaves *(Graptophyllum Pictum* (L) Griff*)*

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Abstract: this study aims to determine the standardization of specific parameters (organoleptic and chemical content) and non-specific parameters (ash content, water content, drying loss, heavy metal contamination, Yeast Mold Number (AKK), microbial contamination / Total Plate Count (ALT)). The results showed that the specific parameters of the organoleptic properties of 96% ethanol extract of purple leaves (Grapthophyllum pictum (L) Griff) were black, shaped like a paste, a distinctive purple leaf odor and a bitter taste. The results of phytochemical screening of 96% purple leaf ethanol extract contained alkaloids, flavonoids, and tannins. However, the saponin test was negative for saponins. Non-specific parameters of 96% ethanol extract of cherry leaves were water content of 5.96%, total ash content of 16.86%, drying loss of 17.26%, heavy metals As and Cd were not detected, the presence of metals in Pb was 0.12 mg / kg, microbial contamination (Alt) and yeast mold numbers (Akk) were not found colonies. Based on these results, it can be concluded that the standardization test of specific and non-specific parameters, the ethanol extract of purple leaves has met the quality standards of simple drugs.

Keywords: Ethanol extract of purple leaves; Specific and non-specific

Introduction

In Indonesia, there are more than 30,000 types of plants and 1,000 types of medicinal plants are used in the traditional medicine industry. The use of herbal medicinal plants is often not standardized so that the quality and safety of the medicinal ingredients are not guaranteed. Therefore, before being used as medicine, plants must meet the standards set by the Ministry of Health of the Republic of Indonesia, either in the form of simplicia or extract. Extracts are in the form of dry extracts, thick extracts and liquid extracts. The extract must be standardized to ensure its quality and safety (Arnida et al., 2021).

The safety and quality of simplicia is one of the important stages in the development of Indonesian medicine. A simplicia cannot be said to be of good quality if it does not meet the quality requirements stated in the simplicia monograph. The quality requirements stated in the simplicia monograph include drying loss, total ash content, acid-insoluble ash content, water-soluble extract content, ethanol-soluble extract content, and the chemical content of the simplicia including essential oil content and certain compound content. Plants that are efficacious for treatment have long been used by the community for generations. One of them is purple leaves (Putri et al., 2021).

Purple leaves are one of the traditional medicinal plants that can cure various diseases such as treating wounds, liver swelling, and treating gallstones. Purple leaves are known to contain secondary metabolite compounds such as alkaloids, sitosterol, glycosides,

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saponins, steroids, phenolic compounds, tannins, flavonoids such as anthocyanins and leukoanthocyanins (Syukri et al., 2020).

Based on the explanation above, researchers are interested in conducting standardization research on purple leaf extract. This aims to determine the results of standardization of specific parameters (organoleptic and chemical content) and non-specific parameters (ash content, water content, drying loss, heavy metal contamination, Yeast Mold Number (AKK), microbial contamination / Total Plate Count (ALT)) in purple leaf plants (Grapthophyllum pictum (L) Griff).

Method

This study is an experimental laboratory study to determine the results of standardization of specific parameters (organoleptic and chemical content) and non-specific parameters (ash content, water content, drying loss, heavy metal contamination, Yeast Mold Number (AKK), microbial contamination/Total Plate Count (ALT) in purple leaf plants (Grapthophyllum pictum (L) Griff).

Time and Place

This research was carried out in the period of June-July 2024, which was carried out at the Phytochemical Pharmacognosy Laboratory, Microbiology Laboratory, Research Support Installation and Product Provision Tawamangu Surakarta.

Tool

The tools used in this research include a blender (kirin), mesh sieve No. 40, porcelain crucible, evaporating dish, analytical balance, rotary vacuum evaporator, oven, desiccator, furnace, filter paper, glass beaker, petri dish, test tube, micro pipette, volumetric flask, Erlenmeyer, hot plate, blue tip, autoclave, Atomic Absobtion Spectrophotometer (AAS), stir bar, water bath, and tissue processor.

Material

The research material used was the purple leaf plant (Graptophyllum pictum (L.) Griff) obtained from the Pasangkayu area, West Sulawesi Province. Aquadest, aluminum foil, ammonia, 2 N hydrochloric acid, concentrated hydrochloric acid P, dragendorf LP, purple leaf extract, 96% ethanol, FeCl3, handscoon, hematoxylin-eosin, HCl, concentrated H2SO4, concentrated HNO3, cotton, label paper, chloroform , magnesium P, mask, 39 Na CMC 0.5%, NaCl 10%, Plate Count Agar (PCA), and tissue and gloves.

Making Purple Leaf Extract

Making purple leaf ethanol extract was carried out using the maceration method by weighing 2,400 grams of purple leaf simplicia powder and then extracting it using 8 liters of 96% ethanol solvent for 3x24 hours protected from light, while occasionally stirring. The extract is then filtered using filter paper and a filtrate is obtained. Next, it was concentrated using a Rotary Vaccum Evaporator at a temperature of 60°C and followed by evaporation using a water bath until a thick extract is obtained (Ulfah, 2021).

Standardization: Specific Parameters

Organoleptic Testing

The extract was tested using the five senses for shape (solid, thick, liquid), color (greenish, brownish), smell (odorless), and taste (tasteless, bitter, slightly bitter) (Sagita et al., 2021).

Chemical Content

Test Flavanoids

The ethanol extract of purple leaves was weighed as much as 0.5 grams, put into a test tube, then 10 mL of distilled water was added and heated over a water bath then filtered, then dissolved in 1 ml of ethanol (96%) with the addition of magnesium P powder, after which it was dissolved in 10 mL of concentrated hydrochloric acid P, if the purple color occurs, it indicates the presence of flavonoids and if the yellow color occurs, it indicates the presence of flavones, chalcone and aurone (Larasati et al., 2023).

Alkaloid Test

Weigh 0.5 grams of purple leaf ethanol extract, put it in a test tube, then 5 mL of 2 N hydrochloric acid and heat it over a water bath for 2 minutes then add 3 drops of Dragendrof LP reagent. If the results give an orange-yellow to brick red precipitate, then the sample contains alkaloids (Rahmasiahi et al., 2023).

Saponin Test

A total of 0.5 grams of purple leaf ethanol extract was put into a test tube, 10 ml of hot water was added and filtered. The solution or filtrate is taken and put into a test tube, then shaken vigorously for 10 seconds, if stable foam forms in the test tube for no less than 10 minutes with a foam height of 1-10 cm and with the addition of a few drops of hydrochloric acid (Nilamsari et al., 2020).

Tannin Test

Weigh 0.5 grams of purple leaf ethanol extract into a test tube, then add 5 mL of distilled water and boil for 5 minutes. Then filtered, the filtrate was added with 5 drops of 1% FeCl3 (w/v). The dark blue or greenish

black color that forms indicates the presence of tannin compounds (Emilia et al., 2023).

Standardization: Non Specific Parameters
Test Water Level

Carefully weigh approximately 10 g of sample, put it in a container that has been weighed. Dry at 105° for 5 hours, and weigh. Continue drying and weighing at 1 hour intervals until the difference between two consecutive weighings is no more than 0.25% (Andrana, 2023).

$$Water\ level = \frac{\text{Wet simplicia (g)} - \text{Dry simplicia (g)}}{\text{Dry simplicia (g)}} x100\% \quad (1)$$

Drying Shrinkage Test

Accurately weigh 1 to 2 g of simplicia in a shallow weighing bottle with a lid that has previously been heated to the specified temperature and weighed. Flatten the material in the weighing bottle by shaking the bottle, until it forms a layer approximately 5 to 10 mm thick, put it in the drying chamber, open the lid, dry at the specified temperature until the weight remains constant. Before each drying, let the closed bottles cool in a desiccator to room temperature (Karim, 2022).

$$\text{Draying shrinkage} = \frac{\text{Wet simplicia (g)} - \text{Dry simplicia (g)}}{\text{Wet simplicia (g)}} x100\% \quad (2)$$

Total Ash Content Test

Weigh 2-3 g of the ground sample and put it in a crucible that has been tared and dried using an oven at 105°C for 30 minutes (after the crucible has been ground, the crucible is cooled first in a desiccator to room temperature). Incinerate slowly until it turns to ash using a furnace at 600°C, cool the crucible to room temperature, weigh. (If this method cannot remove the charcoal, add hot water, filter through ash-free filter paper. Pivot the remaining paper and filter paper in the same crucible. Put the filtrate in the crucible, evaporate, flammate until it remains at a constant weight, weigh. Calculate the ash content of material that has been dried in air (Dwi Andrana, 2023).

$$Total\ ash\ content = \frac{Ash\ weight\ (g)}{Initial\ weight\ (g)} x\ 100\% \quad (3)$$

Heavy Metal Contamination Test

Determination of As, Pb and Cd levels using the Atomic Absorption Spectroscopy (AAS) method. Determination of the levels of the three heavy metals using wet digestion: 1 gram of extract was weighed and 10 mL of concentrated HNO3 was added, then heated with a hot plate until the volume was half, the thick and cold extract was added with 5 mL of HClO4, then heated until the white smoke disappeared and allowed to cool, then rinsed with distilled water and filtered into a 50 ml measuring flask, add distilled water to 50 ml, the sample is measured using an AAS instrument. Based on the monograph book on medicinal plant extracts, the value of Pb metal is not more than 10 mg/kg, Cd metal is not more than 0.3 mg/kg, while As metal is not more than 5 µg/kg (Andrana, 2023)

Microbial Contamination Test
Media Creation (Depkes, 2000):

Plate count agar (PCA) media was weighed as much as 2.45 grams, mixed with distilled water and heated until the yellow solution was clear, sterilized using an autoclave at a temperature of 121^{0}C.

Sterilization of Tools

Equipment and media used during testing must be sterilized first to prevent contamination by microorganisms. Equipment made of glass and resistant to heat is wrapped in brown paper and put in an iron basket, the wrapped equipment is put into an autoclave with a sterilization process using a temperature of 121^{0}C (after temperature 121^{0}C wait and keep the temperature at 121^{0}C if the temperature rises the fire is turned off and if the temperature falls then the fire is lit again so that the temperature remains 121^{0}C) for 15 minutes, with pressure in the autoclave of 15.

Dilution

Simplicia was weighed as much as 1 gram then mixed with 9 mL of 0.9% NaCl and homogenized to obtain a dilution of 10-1. Next, serial retailing was carried out on the dilutions prepared in four tubes, each filled with 9 mL of 0.9% NaCl. Take 1 mL of the 10-1 dilution and put it into a second tube and then homogenize it using a vortex to get a dilution of 10^{-2}, take 1 mL of dilution 10^{-2}. pipetted and put into the third tube and homogenized using a vortex to obtain 10^{-3} (do the same steps until a dilution of 10 is obtained^{-5}). The results of this dilution are then planted in the media in a petri dish.

Microbial Contamination (Depkes RI, 2000)

Prepare sterilized tools and materials. Pipette 1 mL of each dilution into a sterilized petri dish (using a different pipette for each dilution) pour the melted PCA medium into the petri dish, shake the petri dish containing the media and sample until the sample is evenly mixed (pour plate method). Next, leave it until the media solidifies and wrap the Petri dish again in brown paper. Place the Petri dish in an incubator at a temperature of 35°C for 24 hours. After 24 hours, the growth of bacterial colonies was recorded and counted.

Yeast Mold Number Test

The extract solution was made with a 1:10 dilution by dissolving 1 gram of extract in a 10 mL volumetric flask. Followed by dilutions of 1:100 and 1:1000. The agar medium used is Plate Count Agar (PCA). PCA was melted at 45°C. Then put 15 mL in a petri dish, let it freeze in the cup. A total of 0.5 mL of each dilution of the extract solution was pipetted into a sterile Petri dish (spreader method) using a different, sterile pipette for each dilution. The petri dish is shaken carefully until the sample is evenly distributed in the medium. Then incubate at room temperature (25°C) for 7 days, then determine the number of molds and yeasts/g sample.

Results and Discussion

Standardization in pharmacy is a series of parameters, procedures and a method of measurement whose results are elements related to the quality paradigm pharmaceutical in the sense of meeting standard requirements including guaranteeing stability as product pharmacy on generally. Standardization Also means process guarantee that the final product (drug, extract, extract product) has value parameter certain ones constant and determined (Arnida et al., 2021).

Determination parameter standard quality from extract plant drug need done For ensure quality from extract plant drug Which used as drug contain rate compound active Which constant And can insured answer. Extract quality requirements consist of standard parameters specific and non-specific standard parameters. Specific standard parameters consist of: testing organoleptic, And content chemistry. As for parameter standard non specific consists of determining the ash content, ash content that is insoluble in acid, moisture content, drying shrinkage, contamination metals, and microbial contamination (Syukri et al., 2020).

Results of Standardization Specific and Non-Specific Parameters of Purple Leaf Ethanol Extract

This research used ethanol extract of *Graptophyllum pictum* (L) Griff purple leaves with the maceration method. Maceration is extraction without heating by immersing simplicia powder in a solvent during the soaking process, the liquid will penetrate the cell contents and enter the cell cavity which contains the active substance. The maceration method was chosen because it does not use heating during filtering, thereby preventing the possibility of damaging the bioactive compounds contained in the sample. The solvent used in the maceration process is 96% ethanol. The reason for using 96% ethanol as a solvent is because it has high polarity so it can extract more material than other types of organic solvents (Yuliana et al., 2022).

Table 1. Organoleptic Identification Results

Organoleptic Parameters	Results
Form	Thick like pasta
Color	Black
Smell	Typical purple leaves
Flavor	Bitter

Table 2. Results of Phytochemical Screening of Purple Leaf Extract

Secondary metabolites	Test method	Results	Library (ministry of health RI and BPOM RI)
Flavonoids	Concentrated HCL reagent	+	A yellow color forms
Alkaloids	Dragenrof's reagent	+	An orange red precipitate is formed
Tannin	Reagent 1%	+	A green color is formed
Saponin	Heated	-	No white precipitate was formed

Table 3. Results of Non-Specific Parameters

Parameter	Results	Quality Standards (Ministry of Health RI, BPOM RI)
Water content	5.96 %	Less than 10%
Total ash content	16.85 %	No more from 16.6%
Drying shrinkage	17.26 %	No more from 10%
Heavy metal		
Arsen (as)	Not detected	Approximately 5 mg/kg or mg/L
Lead (pb)	0.12	Approximately 10 mg/kg or mg/L
Cadmium (cd)	Not detected	Approximately 0.3 mg/kg or mg/L
Microbial contamination (ALT)	No colonies found	Less than 10^6 cfu/ml
Yeast mold number (AKK)	No colonies found	Approximately 103 colonies/gr

The thick extract obtained from the maceration of simplicia was 60 grams so that the % yield of *Graptophyllum pictum* (L) Griff purple leaf ethanol extract was 0.025%, obtained from the calculation of the final weight of the extract obtained compared to the weight of the initial extract obtained. When compared with journals (Nurdyansyah, 2019). The yield % yield was 19.97%. The yield obtained was quite high, this was because the compounds in *Graptophyllum pictum* (L) Griff purple leaf simplicia powder which were extracted in ethanol solvent had the same polarity as the polarity of the compounds in the material. Low % yield can be influenced by high temperatures which cause water migration from environmental materials and various other processes. Phytochemical testing was carried out to confirm the secondary metabolites contained in purple leaves. The results of the phytochemical screening test showed that the ethanol extract of

Graptophyllum pictum (L) Griff purple leaves contains alkaloid, flavonoid and tannin compounds. The results of the phytochemical screening test can be seen in table 2.

After obtaining the extract, non-parameters were determined specific. Determination non specific done For know limit maximumcontamination Which allowed, as well as contamination from impurity Which there is in extract so that can ensure security consumer And stability. Non-specific determinations made include determinations rate ash total, determination rate ash No late sour, determination rate water, determination of drying shrinkage, microbial contamination testing as well as testing metal contamination.

Determination of total ash content aims to provide an overview of the levelimpurity by contaminants form compound inorganic like metal alkali (Sodium, Potassium, Lithium) and mineral content. Extract ash process This is done in a furnace using a temperature of 600^{o}C because a temperature of 600^{o}C can cause disappearance content alkali And carbon dioxide on compoundcarbonate. The ashing process is carried out until the compound is destroyed and evaporates until only mineral and inorganic elements remain. On table 3 results k there ash total Which obtained as big as 16.86%. Testing rate Acid insoluble ash aims to determine the level of contamination by sand and land. The acid insoluble ash content obtained was 1.29 %. With standard quality is available ash (Ministry of Health RI. 1989) i.e. no more from 1 2 % and ash content not acid soluble less than 0.7 %. The size total ash content in the extract leaf purple indicates that the mineral content in the extract is got enough Lots contains minerals. The size insoluble ash content acid is not in accordance with the literature that the acid insoluble ash content is less than 0.7%, the height rate ash No late sour This caused Because process washing Which not enough clean so there is Lots impurity.

Determination of water content is carried out to determine the remaining water present on the extract which will then guarantee the quality and storage of the extract. Rate water can determined stability extract and form preparation furthermore. Testing rate water done with moreover formerly dry the porcelain cup to be used at 105^{o}C for 5 hours and placed in desiccator during 5 hours For remove rate water Which there is on cup porcelain the as well as done also warmup sample on temperature 105^{o}C during 5 hours And placed in desiccator for 5 hours to determine the lost water content. In table 3 obtained the results of testing the water content in the leaf extract purple that is 5.96 %. This shows the leaf extract purple has good quality because the water content contained in the extract does not exceed 10%. The water content in the extract is less than 10% to avoid i rapid growth inner fungus extract.

Parameter shrink drying is Wrong One parameter non specific which aims to provide a maximum limit (range) regarding the sizeThe compounds lost in the drying process do not only represent water that is lost but other compounds evaporate. Basically, drying shrinkage is measurement remainder substance after drying on temperature 105^{o}C until constant weight, which is then expressed in percent (DEPKES RI, 2000). On table 4.3 Results shrink drying Which obtained on extract leaf purple that is 17.26 %. Matter This show extract leaf purple No qualify i.e. less from 10% (Department of health RI, 1995).

On determination parameter non specific there is testing contamination microbes, Where testing contamination microbes including Wrong One testing the purity of the extract. This test includes the number of microorganisms which is permitted and for indicates the presence or absence of microbes inside extract the. On extract leaf purple No found exists colony on number total plate and mold and yeast, so the results obtained do not exceed the requirements set, namely 1 x 10^{3} colonies/g by the Agency POM RI. Low bacterial growth on number total plates and mold or yeast in the extract This Can caused Because solvent Which used is solvent ethanol Which Where ethanol can hinder growth bacteria or microbes in extract.

Next, heavy metal contamination in the form of lead, c admium, and arsenic. In table 3 you can see the results of the levels of lead contamination there is content lead in the extract leaf purple , level cadmium obtained results cadmium content of 0.12 ppm, and rate arsenic No there is content arsenic in extract leaf purple so that the results obtained No exceed limit Which has set in parameter extract in a way general. Where maximum residue lead does not exceed 10 ppm extract and cadmium residue does not exceed 0.3 ppm. Heavy metal testing of extracts included This is important because if the levels of heavy metals contained in the extract is high and exceeds the limit which is determined then will have toxic consequences for health (Department of health RI, 2000).

Conclusion

The results showed that the specific parameters of the organoleptic properties of 96% ethanol extract of purple leaves (Grapthophyllum pictum (L) Griff) were black, shaped like a paste, a distinctive purple leaf odor and a bitter taste. The results of phytochemical screening of 96% purple leaf ethanol extract contained alkaloids, flavonoids, and tannins. However, the saponin test was negative for saponins. Non-specific parameters of 96% ethanol extract of cherry leaves were water content of 5.96%, total ash content of 16.86%, drying loss of 17.26%, heavy metals As and Cd were not detected, the presence

of metals in Pb was 0.12 mg / kg, microbial contamination (Alt) and yeast mold numbers (Akk) were not found colonies. Based on these results, it can be concluded that the standardization test of specific and non-specific parameters, the ethanol extract of purple leaves has met the quality standards of simple drugs.

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