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INTRODUCTION One of the plants known to be efficacious as medicine is purun danau (Lepironia articulata (Retz.) Domin), especially the rhizome. Our previous study showed that the ethanolic extract of the rhizome of L. articulata contained alkaloids, flavonoids, tannins, saponins, and anthraquinone compounds1. The rhizome of L. articulata has antimalarial and antioxidant activity2. Traditional medicinal raw materials from plants are often constrained by scientific research in clinical trial research and standardization of these traditional medicinal raw materials3. The L.

articulata plant is generally used as raw material for making hands woven by utilizing its stems. The plants are widespread in several countries such as Sri Lanka, India, China, Indonesia, Malaysia, Micronesia, Australia, New Caledonia, and Fiji4. The plant can live in a swamp or brackish land, which is included in the Cyperaceae group so that L. articulata may contain chemical compounds of alkaloids, flavonoids, steroids, and tannins5. The dominant plant part of L. articulata widely studied because it is considered efficacious is the rhizome. Lepironia articulata, emergent aquatic to approximately 200 cm tall, grow in Esk River, New South Wales, Australia.

Lepironia articulata (grey-green plant to left-hand side foreground) growing with Machaerina articulata (right-hand side) and naturalized Nymphaea caerulea (mid-foreground). Pseudo-lateral inflorescence at the functionally female stage, past functionally male phase with spider and grey mold and fully mature, with most fruits and bracts fallen6. The development of Indonesian traditional medicines continues to be carried out towards obtaining standardized herbal medicinal products and phytopharmaceuticals, so the raw materials for these medicines must meet the quality standards of materials expected to guarantee their safety and efficacy7. The thing that can be done to meet the quality standards of medicinal raw materials is to standardize the raw materials of the drug.

Standardization is a series of procedures or tests carried out physically, chemically, and biologically to ensure the quality of drugs and the quality of raw materials is obtained8. This study aims to determine the standardization of simplicia and ethanol extract of L. articulata rhizome consisting of specific and non-specific parameters. Specific parameters include organoleptic, microscopic, ethanol-soluble extract content, water-soluble extract content, description of extracts, yield, and phytochemical screening.

Non-specific parameters include drying shrinkage, total ash content, acid insoluble ash content, and metal contamination. MATERIALS AND METHODS Materials The tools used were furnace (Ney-Vulcan D-550), hot plate stirrer (Stuart), atomic absorption spectrophotometer, microscope, oven (Vinco), silica gel plate 254, analytical balance

(Pioneer), water bath (SMIC). The materials used in this study were fresh rhizome of L. articulata (Retz.)

Domin (Figure 1) obtained from three sites in South Kalimantan: Guntung Manggis in the city of Banjarbaru (A), Haur Gading in the Hulu Sungai Tengah Regency (B), and Halat in the Balangan Regency (C), as presented in Figure 2. The determination of this plant was carried out at the Indonesian Institute of Sciences, Bogor, Indonesia, with specimen number B-43/IV/DI.01/2021. The reagent used was hydrochloric acid, nitric acid, sulfuric acid, ethanol, ethyl acetate, FeCl3, 10% gelatin, potassium hydroxide, chloroform, n-hexane, sodium hydroxide, methanol, Dragendorff's reagent, Liebermann Burchard's reagent, Mayer's reagent, magnesium powder, and toluene. / A B C Figure 1. Stem (A), flower (B), and rhizome (C) of L. articulata Methods Simplicia preparation Lepironia articulata was obtained from swamp area by purposive sampling method.

The determination of the three selected locations was based on the consideration of the size of the growing L. articulata population and the condition of the geographical area. The fresh L. articulata rhizomes were sorted and cleaned, and then chopped. The rhizome of L. articulata was dried using an oven at a temperature of 55°C until the sample was dry with a water content of less than 10%. This temperature range was ideal for drying herbal raw materials, because higher drying temperatures could destroy the active components in simplicia. Furthermore, the simplicia was powdered with a number 25 sieve until a fine powder was obtained9.

Lepironia articulata rhizome simplicia obtained weighing about 600 g from 1000 g of fresh samples. / A / B / C Figure 2. Map of sampling areas in Guntung Manggis (A), Haur Gading (B), and Halat (C) Ethanol extract preparation As much as 400 g of L. articulata simplicia powder was put into the macerator, then 96% ethanol solvent was added until all simplicia was submerged. The filtering process was carried out for five days, in which every 24 hours, the solvent was changed and continuously stirred every six hours. The filtrate was then evaporated using a water bath until a thick extract was obtained.

The method used for solvent evaporation is rotary evaporation. The extract obtained was weighed, and the yield was expressed in percentage w/w10,11. Simplicia standardization (Specific parameters) Organoleptic test Organoleptic tests were carried out using the five senses to determine the form, color, smell, and taste of the L. articulata simplicia. Observations were made after the simplicia came into contact with air for 15 minutes12. Microscopic test Microscopic tests were carried out using a microscope with a magnification of 10x, with transverse and longitudinal preparations.

Ethanol-soluble extract content As much as 5 g of L. articulata simplicia powder was

macerated with 100 mL of 96% ethanol for 18 hours. The 20 mL filtrate was evaporated until dried in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant. Water-soluble extract content As much as 5 g of L. articulata simplicia powder was macerated with 100 mL of chloroform saturated distilled water in Erlenmeyer for 18 hours. The 20 mL filtrate was evaporated to dryness in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant.

Simplicia standardization (Non-specific parameters) Drying shrink The porcelain crucible was heated at 105°C for 30 minutes then added with 1-2 g of L. articulata simplicia. The silicate crucible containing simplicia was left open in the oven at 105°C for 30 minutes until the weight remained constant. Total ash content Simplicia powder (2 g) in a crucible that had been tared was put at incandescent at 800°C for 6 hours, then cooled and weighed. Total ash content was calculated to the initial powder weight in %w/w.

Acid-insoluble ash content Five grams of simplicia powder was macerated with 100 mL of 96% ethanol for 18 hours. The 20 mL filtrate was evaporated to dryness in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant. Metal contamination of Pb, Hg, and Cd Thirty grams of simplicia powder was placed in a porcelain cup then ignited in a furnace. The temperature was gradually increased to 100°C every 30 minutes until the temperature reached 450°C and allowed to stand until the ash turned white. The ash is then cooled at room temperature.

After cooling, 5 mL of 6 M HCl was added while shaking until the ash dissolved. The solution was evaporated on a hotplate at 100°C until dried. The sample was added with 10 mL of 0.1 M HNO3 and then cooled for an hour. The solution was added 0.1 M HNO3 to 100 mL, then filtered with Whatman paper, and the filtrate was read using an atomic absorption spectrophotometer (AAS)13. Extract standardization (Specific parameters) Description of extract The description of the extract used the five senses in terms of describing color, smell, and taste11. Yield The yield was the percentage of the extract obtained from simplicia.

Phytochemical screening Harborne developed the standard procedure to analyze biologically active compounds, including alkaloids, saponins, flavonoids, tannins, anthraquinones, phenolics, glycosides, triterpenoids, and steroids14. Alkaloids As much as 0.2 g of the thick extract of the rhizome of L. articulata was dissolved in 2 mL of ethanol and then dripped with 2 N HCl then divided into two test tubes of 1 mL each. In the first tube, ten drops of Mayer's reagent were added, in which a positive result was indicated by the formation of a yellow or white precipitate.

In the second tube, ten drops of Dragendorff's reagent were added, in which the

presence of an orange precipitate indicated alkaloid's existence15. Triterpenoids and steroids As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol, then ten drops of Liebermann Burchard reagent were added. A color change indicated the positive results, as triterpenoids would appear red-purple while steroids would appear blue-green16. Saponins As much as 0.1 g of the thick extract of the rhizome of L. articulata was added with 10 mL of hot water, then two drops of 2 N HCl were added. Saponins were indicated by the formation of stable foam as high as 1-10 cm for not less than 10 minutes17.

Flavonoids As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol. The solution was then added with Mg powder and a drop of concentrated HCl. A change in color to orange, red, or yellow indicated positive results18. Tannins As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol. The test solution was added with 1 mL of 10% gelatin. The appearance of a white precipitate indicated positive results19. Anthraquinone As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol, then added with ten drops of 10% KOH (in methanol).

A change in color to yellow or yellow-brown indicated the presence of anthraquinone20. Phenolic As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol, then added with five drops of 10% FeCl3. A change in color to blue-black indicated phenolics presence21. Glycoside As much as 0.1 g of the thick extract of the rhizome of L. articulata was dissolved in 1 mL of ethanol, then added with ten drops of chloroform and concentrated sulfuric acid. A change in color to brown indicated positive results22. Extract standardization (Non-specific parameters) Water content Measurement of water content was carried out using toluene distillation. Five grams of L.

articulata thick extract was placed in a round bottom flask and then added toluene solvent that had been saturated with 200 mL of water. The solution was heated for 100 minutes until the toluene boiled. After it was boiled, then the solution was cooled to room temperature. The volume of water was read after the toluene and water had separated. The water content was determined in percentage. Total ash content Two grams of thick extract in a crucible ignited at 800°C for six hours, then cooled and weighed. Total ash content was calculated to the initial powder weight in %w/w.

Acid-insoluble ash content The ash obtained from the total ash content test was boiled with 25 mL of dilute HCl for five minutes. The ash was then filtered using ash-free filter paper and then washed with hot water to collect acid-insoluble ash, then ignited with a porcelain crucible in a furnace to obtain ash with a constant weight. The content was

calculated from the initial weight of the powder, expressed in %w/w23. Data Analysis The results obtained showed in qualitative and quantitative data. Qualitative data were the results of organoleptic, microscopic, and phytochemical screening.

In contrast, quantitative data resulted from drying shrinkage testing, water content, total ash content, acid-insoluble ash content, water-soluble extract content, ethanol-soluble extract content, and heavy metal contamination levels. The data was made in tabular form along with the documentation and analyzed descriptively. RESULTS AND DISCUSSION Ethanol extract preparation The results obtained from each growing place were different, as presented in Table I. This result was more than our previous study, in which from 400 g of L. articulata rhizome powder using the maceration method for 3x24 hours and the solvent used was 96% ethanol, the yield was 8.232%1.

Sampling was carried out in three different places with different environmental factors that affected the yield. Metabolism in plants is influenced by environmental factors such as soil, altitude, light, temperature, humidity, organic compounds, and inorganic compounds. Specimens of the same plant species grown under different environmental conditions showed significant differences in the production and accumulation of primary and secondary metabolites.

Chemical interactions between plants and their environment are mediated primarily by the biosynthesis of secondary metabolites, which carry out their biological roles, as a plastic adaptive response to their environment24. Table I. Yield of L. articulata rhizome ethanol extract Area _Powder weight (g) _Thick extract weight (g) _Yield (%) _ _Guntung Manggis _400 _44.90 _11.23 _ _Haur Gading _400 _37.81 _9.45 _ _Halat _400 _32.18 _8.05 _ _ Simplicia standardization (Specific parameters) Organoleptic test The organoleptic test was carried out with the help of five panelists who explained the shape, color, smell, and taste of the simplicia, as presented in Table II.

Lepironia articulata simplicia powder has a characteristic odor, with a chelate taste and reddish-brown color. The simplicia of L. articulata had a chelate taste with a reddish-brown color because it contains tannins and polyphenols25. Table II. Organoleptic properties of L. articulata simplicia Area _Smell _Taste _Color _Form _ _Guntung Manggis _Specific _Chelate _Reddish-brown _Coarse powder _ _Haur Gading _Specific _Chelate _Reddish-brown _Coarse powder _ _Halat _Specific _Chelate _Reddish-brown _Coarse powder _ _ Microscopic test Microscopic tests were carried out using an optical microscope with a magnification of 10 times to observe the constituent elements of a sample. Microscopic observations on the rhizome of L. articulata were carried out transversely (cross) and longitudinally.

The results of cross-sections of L. articulata rhizomes from the three locations showed the same results covering the epidermis, cortex, endodermis, cortical parenchyma, and bundle vessels (Figure 3). The cross section of L. articulata rhizome from the three locations also showed similar results, consisting of parenchyma cells and scaliform vessels (Figure 4), which results were also found in longitudinal sections. / Figure 3. Cross section of L. articulata rhizome / Figure 4. Longitudinal section of L. articulata rhizome Ethanol and water-soluble extract content The results obtained in determining the levels of dissolved compounds are relatively small, as presented in Table III.

The results were different because the samples used in this study were taken from three different places. Differences in plant growth area affect the percentage composition of chemical compounds in a plant26. The water solvent used aims to dissolve polar compounds, and ethanol solvent is used to dissolve polar-nonpolar compounds27. Table III. Determination of water-soluble and ethanol-soluble extract content of L. articulata simplicia Area _Water-soluble extract content (%) _Ethanol-soluble extract content (%) _ _Guntung Manggis _10.87 ± 0.15 _12.66 ± 0.05 _ _Haur Gading _8.80 ± 0.10 _10.73 ± 0.15 _ _Halat _8.03 ± 0.15 _10.00 ± 0.10 _ _ Simplicia standardization (Non-specific parameters) The results of non-specific parameter testing of L. articulata rhizome simplicia were presented in Table IV. The results of the drying shrinkage test of L.

articulata rhizome simplicia meet the standards set by the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI), which is less than 10%28. Dry shrinkage is generally related to the water content contained in simplicia, so the smaller the drying loss, the better the results obtained. The smaller the water content contained in simplicia, it can reduce the growth of molds and fungi as well as enzymatic reactions that can damage the quality of simplicia29.

Data on total ash content and acid-insoluble ash content in the simplicia rhizome of L. articulata did not differ. The higher the total ash content obtained, the higher the mineral content contained in the simplicia. Humans need minerals such as calcium, phosphorus, and magnesium for bone formation, sodium, and chloride for body fluids, and iron to form hemoglobin and red blood cells. Minerals can also be harmful if they accumulate in the human body over a long time, which can interfere with the circulatory, nervous, and kidney systems such as mercury, lead, copper, cadmium, and strontium30.

The acid-insoluble ash content reflects the contamination of minerals or metals that are not acid soluble in the simplicia. The high content of acid-insoluble ash in simplicia indicates the presence of impurities such as soil or sand, metallic elements silver, lead, and mercury15,23. Measurement of metal content ensures that the samples do not

contain heavy metals that can endanger health if they exceed the specified requirements12. Based on the results obtained, the Cd levels from the three sites did not meet the specified requirements31.

The results obtained can be influenced by genetic factors and environmental factors. The simplicia processing process can also cause high levels of cadmium metal from sampling to sample processing, such as in the sample washing process, which may be less clean because the sample used itself is a rhizome so that the risk factor is greater. The use of metal materials during sample processing can affect the results obtained—in the sample processing, chopping the rhizome using a machete made of iron so that metal contamination is possible.

Therefore, it can be reviewed more carefully in terms of simplicia processing. Table IV. Non-specific parameter testing of L. articulata simplicia Standarization Parameters _Result _Requirements28 _ _ _Guntung Manggis _Haur Gading _Halat _ _ _Drying shrinkage (%) _7.78 _7.10 _7.33 _<10 _ _Total ash content (%) _2.52 _2.03 _2.05 _- _ _Acid-insoluble ash content (%) _0.43 _0.33 _0.42 _- _ _Pb contamination (mg/kg) _9.989 _5.698 _6.297 _=10 _ _Cd contamination (mg/kg) _0.499 _0.500 _0.300 _=0.03 _ _Hg contamination (mg/kg) _0.070 _0.079 _0.090 _=0.5

_ _ Extract standardization (Specific parameters) Description of extract Extract description is the initial stage in the introduction, which is carried out as simply and objectively as possible by using the five senses with the help of panelists who are asked to describe the shape, color, smell, and taste of the L. articulata rhizome extract, as presented in Table V. Based on the results presented by the panelists, the ethanolic extracts of L. articulata rhizomes in three places all had a thick extract form, a characteristic odor, and a chelating taste.

The data obtained from the panelists are collected and converted into quantitative data (quantification) using 'coding' so that conclusions can be drawn from the organoleptic results. Towaha reported that the taste of chelates in plants is because it contains tannins and polyphenol compounds32. The smell of the L. articulata rhizome ethanol extract from the three places obtained a distinctive and more pungent odor than simplicia because the compounds contained in the L. articulata rhizome had been extracted. The color of the L.

articulata rhizome ethanol extract from the three places was reddish-brown which was more concentrated than the liquid extract due to the concentration process. Table V. Organoleptic examination of L. articulata etanol extract Area _Parameters _Extract profile _ _ _Form _Color _Smell _Taste _ _ _Guntung Manggis _Thick Extract _Reddish-brown

_Specific _Chelate _/ _ _Haur Gading _Thick Extract _Reddish-brown _Specific _Chelate _/ _ _Halat _Thick Extract _Reddish-brown _Specific _Chelate _/ _ _ Phytochemical screening Screening of phytochemical content of ethanolic extract of L. articulata rhizome aims to determine the content of active ingredients, which are secondary metabolites in plants.

These active ingredients can function as a plant's self-defense against the environment, disease, and insects33. The results of phytochemical screening of L. articulata rhizome extract at three locations indicated the presence of alkaloids, flavonoids, saponins, anthraquinones, glycosides, tannins, and phenolics, as presented in Table VI. This is consistent with our previous study, which also reported the presence of alkaloids, flavonoids, tannins, saponins, and anthraquinones1. Table VI. Phytochemical screening of L.

articulata etanol extract Phytochemicals _Guntung Manggis _Haur Gading _Halat _ _Alkaloids _+ _+ _+ _ _Terpenoids/steroids _- _- _- _ _Flavanoids _+ _+ _+ _ _Saponins _+ _+ _+ _ _Anthraquinones _+ _+ _+ _ _Glycosides _+ _+ _+ _ _Tannins _+ _+ _+ _ _Phenolics _+ _+ _+ _ _Notes: (+) presence of the phytochemicals; (-) absence of the phytochemicals Extract standardization (Non-specific parameters) The results of non-specific parameter testing of L. articulata rhizome ethanol extract included water content, total ash content, and acid-insoluble ash content, as presented in Table VII. The total ash content and acid-insoluble ash obtained from the three sites did not show a significant difference.

This value has met the requirements set by BPOM RI, which states that the water content contained in the extract should not be more than 10%. Table VII. Non-specific parameter testing of L. articulata etanol extract Standarization Parameters _Results _Requirements28 _ _ _Guntung Manggis _Haur Gading _Halat _ _ _Water content (%) _8.50 _7.10 _7.60 _=10 _ _Total ash content (%) _1.67 _1.58 _1.63 _- _ _Acid-insoluble ash content (%) _0.33 _0.22 _0.23 _- _ _ CONCLUSION The results of specific and non-specific parameters of simplicia and ethanol extract of L. articulata rhizome at three different places (Guntung Manggis, Haur Gading, and Halat) can be used as a standard for using L.

articulata rhizome as raw material for traditional medicine.

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