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Standardization Ethanolic Extract of Sungkai (*Paronema canescens*) Leaves

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Abstract

The development of traditional medicinal plants as raw materials for herbal drugs needs to be standardized. The aim of the research was to standardize ethanol extract of *Paronema. canescens* leaves. The extract was determined specific and non-specific parameters. The organoleptic of extract has a thick consistency, the color dark brown has a characteristic non-aromatic odor and has a slightly bitter taste. The soluble content in water and ethanol of 48.1% and 80. 3%, respectively. The extract contains triterpenoid compounds, steroids, flavonoids, and phenols with water content was 8.33%, total ash content of 5.36%, acid insoluble ash content of 2.16%, drying shrinkage of 8.0%, specific gravity of 1.255 g/ml, metal contamination Pb < 0.0628 mg/Kg, and Cd 0.0077 mg/Kg, total bacterial of 0.9 x 10^1 colonies/g and mold contamination with a value of 0.15 x 10^1 colonies/g, respectively. Ethanol extract of *P. canescens* leaves qualified all standard parameters of medicinal plant extract.

Keywords: Paronema canescens, standardized, specific parameter, non-specific parameter

Abstrak (Indonesian)

Pengembangan tanaman obat tradisional sebagai bahan baku obat herbal perlu dilakukan standardisasi. Penelitian ini bertujuan untuk standardisasi ekstrak etanol daun *Paronema canescens*. Ekstrak ditentukan parameter spesifik dan non-spesifik. Organoleptik ekstrak memiliki konsistensi kental, warna coklat tua memiliki ciri khas tidak berbau harum, dan memiliki rasa agak pahit. Kandungan larut dalam air dan etanol masing-masing sebesar 48,1% dan 80,3%. Ekstrak mengandung senyawa triterpenoid, steroid, flavonoid dan fenol dengan kadar air 8,33%, kadar abu total 5,36%, kadar abu tidak larut asam 2,16%, susut pengeringan 8,0%, berat jenis 1,255 g/ml, cemaran logam Pb < 0,0628 mg/Kg, Cd 0,0077 mg/Kg dan Cu 0,00282 mg/g, total cemaran bakteri dan kapang masing-masing 0,9 x 10^1 koloni/g, dan 0.15 x 10^1 koloni/g. Ekstrak etanol daun *P. canescens* memenuhi semua parameter standar ekstrak tumbuhan obat.

Kata kunci: Paronema canescens, standardization, specific parameters, non-specific parameters.

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INTRODUCTION

Research on bioactive compounds from traditional medicinal plants is still being developed. A study of ethnobotanical surveys in various ethnicities, especially in South Sumatra, obtained many plants that have been used by the community for the treatment of diseases but have not been supported by scientific information. One of the plants used in traditional medicine is Sungkai (*Peronema canescens* Jack). Community in South Sumatera and Lampung used *P. canescens* leaves for malaria, fever medicine, hypertension, and high cholesterol drugs [1]. In East Kalimantan, the Dayak community used Sungkai leaves to treat colds, fever, intestinal worms, toothache medicine, and mouthwash [2]. In Malaysia, Sungkai leaves are used to treat ringworm infections [3].

The methanol extract of *P. canescens* leaf has

been reported to contain secondary metabolites of alkaloids, flavonoids, terpenoids-steroids, and tannins [4], while Ramadenti (2017) [5] reported that the ethyl acetate fraction contains alkaloids, flavonoids, tannins and phenolics. *P. canscens* leave extract has been reported to contain sitosterol compounds, phytol, β-amyrin, and seven clerodane-type diterpenoids, while two clerodane compounds have antiplasmodial activity [6]. The methanol extract of *P. canescens* leaves was reported contained acteoside compound and flavonoid glycosides [7] and have antibacterial activity [8, 9]. The ethanol extract of *P. canescens* leaves was also to reported have anticholesterol activity [10]. Many the used of the *P. canescens* in traditional medicine and scientific information about the activity of *P. canescens* leaves extract, it is necessary to standardize the development of the use of *P. canescens* leaf as raw material herbal drug. Standardization of extracts is one of the important stages in the development of extract into medicinal raw materials. Based on the literature study, there is no information of standardized parameter on Sungkai extract. Determination of extract quality standards includes specific and non-specific parameters [11]. The crude extract of *P. canescens* leaves was obtained by maceration using 96% ethanol. Furthermore, to determine the specific parameters include organoleptic, water-soluble extract and ethanol soluble extract content, while the non-specific parameters include water content, ash content, acid insoluble ash content, drying shrinkage, density, metal contamination, and microbial contamination.

MATERIALS AND METHODS

Materials

Sample preparation

P. canescens leaves (2 kg) were collected from Musi Banyuasin district in South Sumatera, Indonesia in November 2021. The sample was identified *Paronema canescens* Jack at Herbarium Bogoriense at Research Center for Biology, Indonesian Institute of Science Bogor, with register number B-134/IV/D1-01/1/2021. The fresh *P. canescens* leaves were sorted, and then the leaves were dried a room temperature until a constant weight was obtained. Furthermore, the dried Sungkai leaves are ground into powder

Methods

Extraction

The *P. canescens* leaves powder (100 g) was macerated using ethanol 96% for 3 x 24 hours, furthermore, filtration. The maceration was carried out with 3 repetitions [10] (Pratiwi et al., 2021). The pooled filtration was concentrated using a rotary evaporator (Buchi®) at a temperature of 70°C until crude extract was obtained. The yield percentage of the extract was calculated by using equation 1.

$$\%\text{Yield} = \frac{\text{Weight Extract}}{\text{Weight Simplicia}} \times 100 \qquad 1)$$

Chemical content analysis

Alkaloid test

Samples (5 g) were crushed in a mortar, a small amount of chloroform was added, then added 5 mL 0.05 N of solution ammonia chloroform in the tube reaction, then the mixture was shaken for several minutes and filtered. The filtered into the test tube and H_2SO_4 2N. was added to the filtrate 5 mL 2 N and shaken regularly until formed two layers. The acid layer (top) is pipetted into 3 test tubes. Into the first test, tube Add 2 drops of Mayer's reagent. In the second reaction tube is added 2 drops of Dragendroff's reagent and the test tube third, add 2 drops of reagent Wagner. The presence of alkaloid compounds was indicated by the formation of a white precipitate on the first test tube, the formation of a precipitate reddish brown on the tube second reaction and brown precipitate redness or orange on the tube third. The alkaloid test is repeated with 3 times replication [12].

Steroid and triterpenoid test

The bottom solution layer at the alkaloid test was separated and dropped into the drop plate, allowed to dry. After drying, 1 mL anhydrous acetic acid was added and then stirred evenly. Subsequently added 3 drops of concentrated sulfuric acid and observed the color that occurred. The Positive reaction if color was formed, blue or green for steroid compounds, brown redness for triterpenoid compounds [11, 13]

Flavonoid test

Powder of sample (1 g) was extracted with 5 mL ethanol, heated for 5 mins. Then filtered and take a small amount of the solution and put it into a plate test, then, 1 m HCl was added, and after that added Mg powder was, after that change, the color of the solution to yellow, orange, and green indicating the presence of flavonoid compounds. The flavonoid test was repeated with replicated 3 times [12].

Phenolic test

The extract was dissolved with ethanol and filtrate, then 5 mL of the filtrate were taken and added 3 drops of 1% $FeCl_3$ reagent. If the changed solution turns green, blue, or purple indicates the presence of phenol compounds. [14]

Saponin test

The sample (500 mg) was added to 10 mL of hot water. Then shake firmly for 15 minutes. Extract positive contained saponin compounds If there was a stable foam as high as 1 - 10 cm [15].

Specific parameter of extract

Organoleptic analysis of the extract

The organoleptic parameters of P. *canescens* leave extract was described in the shape, color, odor, and taste. Shape parameters such as solid, dry powder, and liquid. Color parameters such as yellow and brown. Parameters of aromatic odor or non-odor and taste parameters include sweet, bitter, and others [16].

Water soluble extractive content

The samples (5 g) were added 100 mL of chloroform saturated water (97.5 : 2.5) stirring for the first 6 hours and then let stand for 18 hours. Then filtered and evaporated. The filtrate (20 mL) was heated at a temperature of 105 °C in the steaming dish that has been tera until constant weight. The percentage of the water-soluble extract was calculated by using Equation 2 [16].

$$\%\text{Yield} = \frac{\text{Weight dried filtrate}}{\text{Weight extract}}\ x\ 100 \qquad (2)$$

Ethanol soluble extractive content

The sample (5 g) was added 100 mL of 95% ethanol while shaken during the first 6 hours and left for the next 18 hours. Twenty mL of filtrate was evaporated in the steaming dish that has been tera. The filtrate was heated at 105 °C to a constant weight. The percentage of ethanol soluble extractive content was calculated using equation 3 [16].

$$\%\ \text{Ethanol soluble} = \frac{\text{Weight dried filtrate}}{\text{Weight extrct}}\ \text{x}\ 100 \qquad (3)$$

Non-specific parameter determination of extract

Density of extract

Pycnometer was weighed (W0), then calibration by determining pycnometer weight and water at 25 °C and weighed (W1) The ethanol extract of *P. canescens* leaves was set at temperature 20°C, put into an empty pycnometer, and remove the excess liquid extract and set the temperature of the pycnometer containing extract at 25 °C, then weighed (W2). Subtract the weight of the empty pycnometer from the weight of the filled pycnometer. The density of liquid was calculated based on equation 4 [16].

$$\text{Density} = \frac{\text{W2}-\text{W0}}{\text{W1}-\text{W0}} \qquad (4)$$

Water content

The ethanol extract of *P. canescens* leaves (1 g) was weighed in a raised cup. The extract was dried at 105 °C for 5 hours, then cooled in a desiccator and weighed. Drying was continued and weighing was carried out for 1 hour until the difference between 2 consecutive weighing was not more than 0.25%. Percent water content can be calculated by equation 5.

$$\%\ \text{Water content} = \frac{\text{Initial weight}-\text{Final weight}}{\text{Final weight}} \qquad (5)$$

Drying shrinkage

The extract (1 g) was weighed carefully and put into a covered porcelain crucible which had previously been heated at a temperature of 105 °C for 30 minutes. The sample was flattened in a porcelain crucible by shaking the rate until it was evenly distributed. Put it in the oven, open the lid of the crucible, heat at a temperature of 105 °C, and then weighed. Drying loss was repeated three times the heating until a constant weight is obtained. The percentage of drying loss can be calculated by equation 6 [16].

$$\%\ \text{Drying loss} = \frac{W0-W1}{W0}\ x\ 100 \qquad (6)$$

W0: sample weight before heating (g)
W1: sample weight after heating (g)

Determination of total ash content

The silicate crucible was heated and weighed (W0), then Put 1 g of extract into a silicate crucible and weighed (W1). The extract was put into the furnace with the temperature gradually increasing to 600 °C until the charcoal ran out, left for 4 hours, then cooled in a desiccator and weighed to a constant weight (W2). The total ash content was calculated using equation 7 [16].

$$\%\ \textit{Ash}\ \text{content} = \frac{\text{W2}-\text{W1}}{\text{W2}}\ x\ 100 \qquad (7)$$

Acid insoluble ash content

The ash obtained from the determination of the total ash content was heated in 25 mL of dilute sulfuric acid for 5 minutes. The insoluble part was collected, filtered with ash-free filter paper, washed with hot water and chilled to constant weight. The ash content which is insoluble in acid was calculated using equation 8 [16].

$$\% \text{ Acid in soluble ash content} = \frac{W2-W0}{W1} \; x \; 100 \qquad (8)$$

Microbe contamination

Total plate count

The extract (1 g) was put into a 10 mL volumetric flask and then 0.1% Peptone Dilution Fluid (PDF) was added to 10 mL to obtain a 10-1 dilution and then shaken until mixed. The dilution was continued from 10^{-2} to 10^{-6}. A total of 1 mL of each dilution was pipetted into a sterile petri dish (duple) using a different pipette. 15 mL of Nutrient Agar media was poured into a petri dish and then shaken carefully until the sample was evenly mixed and then allowed to harden until the media hardened, then the petri dish was incubated at a temperature of 35 ^{o}C – 37 °C for 24-48 hours. The number of colonies that grew were counted and recorded. ALT value is calculated in colonies/g sample [16].

Mold and yeast count

P. canescens leaves extract was put into a 10 mL volumetric flask and then added with 0.05% agar (ASA) distilled water (ASA) of 0.05% to 10 mL so that a dilution of 10-1 will be obtained, then shaken until the mixture homogeneous. The dilution was continued from 10-2 to 10-4. Each dilution was pipetted 0.5 mL and then poured on the surface of a petri dish containing 5 mL of 0.05% potato dextrose agar (PDA). The petri dish was shaken while the suspension is spread evenly. The sterility of the media and diluent is known through a blank test which is done by pouring the media into one petri dish and the other petri dish pouring the media and the diluent then allowing the media to solidify. The entire petri dish will be incubated at a temperature of 20 – 25 ^{o}C for 5-7 days. The total number of fungal colonies growing in the media will then be counted and recorded [16].

Metal Contamination Test

Tests to be carried out include levels of Cadmium (Cd), Lead (Pb) and Cu. The sample test was carried out in a porcelain crucible by weighing the extract as much as 1 g added with nitric acid. The sample was ignited until the charcoal ran out and then dissolved in 5 mL of perchloric acid and 10 mL of distilled water until the sample was completely digested. Heavy metal content in the extract can be measured using atomic absorption spectrophotometer (AAS.)

RESULTS AND DISCUSSION

P. canescens leaves powder (100 g) after macerated with 3 replications using 96% ethanol and concentrated, it obtained 4.5 g of crude extract with a yield percentage of 4.5%. The yield percentage was obtained very low compared with yield percentage was reported (20.16%) of Maigoda et al. [17], because in this study using powder sample while Maigola et al. [17] using the fresh sample. The extract was further standardized through the measurement of the parameters relating to quality. Standardization is needed to ensure the quality and safety standards and is achieved by evaluating specific and non-specific parameters, referring to general requirements issued by Indonesian Ministry of Health, (2020) [16]. The standardization data for the ethanol extract of Sungkai leaves are presented in **Tables** 1 and 2. Chemical content analysis of *P. canescens* leaf ethanol extract (Table 1) showed positive content of triterpenoids, steroids, flavonoids and phenolic, but not alkaloids (negative). The results we obtained in accordance with has been reported [17].

Table 1. Chemical content analysis of *P. canescens* leaves ethanol extract.

Compound	Test methods	Observation	Remark	Remark [17]
Alkaloid	Draggendorf	no yellow-orange precipitate	-	-
Triterpenoid	Lieberman Buchard	Brownish red	+	+
Steroid	Lieberman Buchard	Green	+	+
Flavonoid	Shinoda	Yellow	+	+
Phenol	$FeCl_3$	Purple	+	+

Specific parameters

The specific parameters measured here included chemical compounds, organoleptic properties, water solubility, and ethanol solubility. The results showed that the extract was thick, and had a blackish green color, a distinctive non-aromatic stinging odor, and a slightly bitter taste. The chemical content test provides an initial description of the chemical composition of the compound to be analyzed (**Table** 1). The water-soluble extract content (%) was 48.10 ± 3.05, while the ethanol-soluble extract content (%) was 80.3 ± 2.0. The content of water-soluble extracts and ethanol were indicated of the levels of active compounds that can be extracted, both water and ethanol solvents.

Determination of soluble compounds in water and ethanol also aims as a rough estimate of the polar (water-soluble) active compounds and semi-polar to non-polar active compounds (soluble ethanol). The level of the active compound in simplicia were influenced among other by age of the plant, and climate and place of growth [18].

Table 2. Standardization of extract

Parameter	Results	Standard
Specific Parameter		
Organoleptic	Form: thick Color: dark green, distinctive odor: astringent with a slightly bitter taste	
Water soluble extract Content (%)	48.10 ± 3.05	>31%
ethanol soluble extract content (%)	80.3 ± 2.0	>70.5%
Non spesific parameter		
Density	1.255 g/mL	
Water content (%)	8.33± 1.1	< 10%
drying shrinkage (%)	8.00	< 10%
Total ash content (%)	5.36	< 8%
Acid insoluble ash content	2.16% ± 0.001	< 9%
Pb content	< 0,0628 mg/kg	< 10 mg/kg
Cd content	0,0077 mg/kg	< 0.3 mg/kg
Bacterial content	0.9 x 10^1 coloni/g	<1 x 10^4 colony/g
Fungal content	0.15 x 10^1 coloni/g	<1 x 10^3 colony/g

These results to indicate that the active compound in the leaf extract *P. canescens* was many soluble into water and ethanol. Solihah et al. [19] reported ethanolic extract of Tahongai (*Kleinhovia hospita L.*) leaves showed that the extract had 18.30% soluble compound in ethanol and 19.263%, while ethanol extract of Sambiloto show that the content of water-soluble leaf extract is 40.8% and ethanol soluble is 42.0% [20].

Non-specific parameters

The determination of specific gravity provides an overview of the chemical content dissolved in an extract. The density of the Sungkai ethanol extract had a value of 1.255 g/mL (**Table** 2). The Sungkai ethanol extract had a water content (%) of 8.33 ± 1.1, meeting the standard requirements. According to Indonesian Ministry of Health, (2000) [16], the range of moisture content for thick extracts is <10%. The water content is not directly related to pharmacological activity but affects the safety and stability of the extract.

The drying shrinkage value determines the number of compounds contained in the extract (expressed as a percentage) that are lost or easily evaporated during the drying process at a temperature of 105 °C [16]. The mass that can be lost due to heating includes water molecules, essential oil, and ethanol solvents. Drying shrinkage is used as a parameter to ensure the quality of the extract and avoid fungal growth. We found that the ethanol extract used here had a drying shrinkage percentage of 8.00%, meeting the Indonesian Ministry of Health standards (< 10%).

The ash content indicates the quantity of minerals in an extract, which can be organic and inorganic salts. The heating of an extract destroys or evaporates organic compounds and their derivatives, leaving only mineral and inorganic elements. The ethanol extract of *P. canescens* leaves had an ash content of 5.36%, meeting the requirements (< 8.0%). The mineral content in the Sungkai leaf extract is quite low. Minerals are crucial for bodily functions, for example, calcium, phosphorus, and magnesium are needed for bone growth, sodium and chloride for body fluids, and iron for the formation of hemoglobin in the red blood cells [21].

The acid-insoluble ash content represents the amount of silicate derived from sand or silicate soil [22]. The Sungkai extract had a 2.16 ± 0.001 acid-insoluble ash content (%), meeting the specific requirements (< 0.9%). Ethanolic extract of Tahongai (*Kleinhovia hospita L.*) leaves was reported contained total ash content was 15.64% ± 0.75, no meeting the requirements (< 8.0%) and acid insoluble ash content was 8.282% ± 0.28 [19]. The ash content value was depending on the level of inorganic (mineral) contained in the extract and the level of inorganic elements that are insoluble in acid such as silica probably obtained from the soil or sand where the plants grow.

The bacterial contamination test was applied to determine the number of microorganisms and the presence or absence of certain bacteria in the extract [23]. The maximum limit for the number of bacterial colonies is 10^4 cfu/mL. The result of the total plate number test of the Sungkai extract was 0.9×10^1 cfu/g,

meeting the bacterial contamination limit requirements. The maximum limit for the number of fungal colonies is 10^3 cfu/mL. The results of the yeast number test were 0.15 x 10^1 cfu/g, meeting the standard requirements.

The metal contamination test was carried out to assure that the ethanol extract of Sungkai leaves did not contain heavy metals, such as cadmium, lead, and copper, exceeding the specified limits. Heavy metals above the permitted limits, even at low levels, interfere with vital body functions by modifying nucleic acids, enzymes, and structural proteins that can be toxic. Pb and Cd metals are toxic to the body because, in the body, these metals can react to form complex bonds with ligands that contain elements O, S, and N. The results showed that the ethanol extract *P. canescens* leaves included Pb metals of < 0.0628 mg/kg, while the standard requirement is < 10 mg/kg, and Cd contamination had a value of 0.0077 mg/kg, (standard requirement < 0.3 mg/Kg). Therefore, the levels of Pb, and Cd in extract no exceed the predetermined heavy metal residue requirements.

CONCLUSION

Ethanol extract of *P. canescens* leaves qualified all standard parameters of medicinal plant extract base on the parameter that set by Indonesian Ministry of Health, so have the potential to be developed into medicinal raw materials.

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