

# Standardization of Paku Lindung Extract (*Pneumatopteris callosa* (Blume))

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## ABSTRACT

The standardization of the paku lindung extract was carried out to obtain new data regarding the standardization of the paku lindung extract which had not previously been established. In addition, this determination is carried out to obtain quality preparations in the form of a guarantee process, that the extract obtained has certain parameter values that are constant after the data has been determined. Extract standardization tests include specific parameters including organoleptic, soluble compounds in certain solvents. Non-specific parameter tests include drying shrinkage, microbial contamination, heavy metal contamination, ash content, and moisture content. The chemical content test included chromatogram patterns and determining of total flavonoid content and the yield of the resulting extract was  $13.88 \pm 3.19\%$ . The test results of non-specific parameters include drying shrinkage  $0.47 \pm 0.01\%$ , moisture content  $8.28 \pm 0.16\%$ , total ash content  $18.97 \pm 3.53\%$ , acid insoluble ash content  $1.69 \pm 0.02\%$ , total plate number  $7.8 \times 10^4 \pm 12.91$  colonies/gram, yeast mold number  $1 \times 10^2 \pm 0.24$  colonies/gram, heavy metal contamination of Cd, Cr and Pb negative. The results of the specific parameter test include  $85.55 \pm 4.29\%$  water-soluble compounds and  $31.27 \pm 5.06\%$  ethanol-soluble compounds. The chemical content test results include a total flavonoid content of  $27.1 \pm 14.34$  mgEQ/g, and the Rf value of the extract is the same as the Rf of quercetin with 5 mobile phase variations. The test results were carried out to meet the predetermined determination and provide new data on the standardization of the paku lindung extract.

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## 1. Introduction

Research on the potential of Paku Lindung (*Pneumatopteris callosa* (Blume)) as an ethnobotanical antihypertensive need to be done. This study aims to prove the use of paku lindung as a medicinal plant. The use of paku lindung as an ethnobotanical antihypertensive in Balinese society is made in the form of infusion preparations (Sujarwo et al., 2015).

The infusion dosage form is a traditional dosage form that is still used today because of the ease of preparation, namely by boiling simplicia at  $90^\circ\text{C}$  for 15 minutes. Such a simple infusion preparation causes problems such as being easily contaminated by germs and molds and cannot be stored for a long time, besides that in the process it can cause swelling of cells so that the active substance is strongly bound to the simplicia (Kristianingsih & Setyo Wiyono, 2015). So extract preparations are an option because they can be kept for a long time and can guarantee the availability of more abundant active substances compared to infusion preparations.

Extracts are preparations in dry, viscous, or liquid form which are made by extracting from animal simplicia or vegetable simplicia in a suitable way, and are not affected by sunlight (Kementerian Kesehatan RI, 2009). Extract quality can be influenced by biological factors, chemical factors, and extraction technology used, so it is necessary to test the standardization of extracts that can guarantee the quality of an extract. Parameters and extract standardization tests include non-specific parameters and specific parameters. An extract is said to be of good quality when it is able to meet these parameter standards (Departemen Kesehatan RI, 2000)



## 2. Materials and Methods

The materials used in this research were simplicia-protected nails obtained from Bali, distilled water, and quercetin as a comparison standard.

The tools used in this research are analytical digital scales, rough digital scales, glass equipment, TLC chamber, TLC plate, UV-Vis spectrophotometer, an oven, rotary evaporator, freeze dryer.

### 2.1. Making Extract

The extract was made using aquades solvent maceration method for 24 hours. The paku lindung simplicia solution that has been obtained is filtered through filter paper and then evaporate using a rotary evaporator at a temperature of  $\pm 50$  °C to obtain a concentrated extract. The dry extract was obtained by drying the thick extract using a freeze dryer (Djoko et al., 2020).

### 2.2. Non Specific Parameter

#### 2.2.1. Drying Shrink

One gram of dry extract was put into a weighing bottle whose weight was known. Furthermore, the sample is in the oven at a temp of 100 °C until it reaches a constant weight (Riduana et al., 2021).

#### 2.2.2. Water Content

Determining water content is carried out by the gravimetric method. A total of 10g of extract was added and weighed in a container that had been tared. Then the extract was dried at a temperature of 105°C for 5 hours, weighed with an interval of 1 hour, and weighed until the difference in weight between the 2 samples was not more than 0.25% successively (Manarisip et al., 2020).

#### 2.2.3. Ash Content (Determination of Total Ash Content)

The extract as much as 2g was ground and put into a silicate crucible that had been ignited and then leveled. The extract was incandescent slowly till the charcoal was used up, cooled, and weighed to a constant weight (Ulfah et al., 2019).

#### 2.2.4. Determination of Acid-Insoluble Ash Content

The ash obtained to determine the total ash content was added with 25mL of dilute hydrochloric acid LP (25mL of hydrochloric acid plus 110.6mL of water) for 5 minutes. The acid-insoluble parts were collected and filtered with ash-free filter paper. Then the ash obtained was washed with hot water, ignited, and weighed to a constant weight (Raharjo et al., 2021).

#### 2.2.5. Heavy Metal Contamination

The heavy metal contaminants that were tested for their presence in the extracts were lead (Pb), cadmium (Cd), and chromium (Cr) using the Atomic Absorption Spectrophotometry (AAS) analysis method at the Chemical Laboratory, Gajah mada University, Yogyakarta (Musdalipah et al., 2023).

#### 2.2.6. Total Plate Number (TPN)

One gram of extract was dissolved in 9 mL of NaCl and then diluted to  $10^{-6}$ . The results of each dilution were poured into 1 mL of each petri dish. Media Plate Count Agar (PCA) with a temperature of  $45\pm 1$  °C was poured into each petri dish as much as 15 mL and made in duplicate. The petri dish was shaken until the suspension was evenly distributed. A control test (blank) using NaCl as the medium was made to determine the sterility of the medium and diluent. One cup is only filled with 1 mL of diluent and agar medium, and the other cup is filled with diluent and medium. After the medium was solidified, the Petri dishes were incubated at 37 °C with the cup inverted. The number of colonies that grow at the incubation time of 24 and 48 hours was observed and counted (Syarif et al., 2022).

#### 2.2.7. Yeast Mold Number

The extract was weighed much as 1g and dissolved in 9mL of Agar Distilled Water (ASA). Subsequently, the dilution was carried out to  $10^{-6}$ . Each dilution was pipetted as much as 0.5 mL to be poured on the surface of the PDA medium. The sample is flattened with a triangular rod until it is

evenly distributed and then made in duplicate. To determine the sterility of the medium and diluent, a blank test was performed. All petri dishes were incubated at a temperature of 20-25°C for 3-5 days. After 3 days of incubation, the number of fungal colonies that grown was recorded and the last observation was made on the 5th day of incubation (Rahmawati et al., 2022).

### **2.3. Specific Parameter Test**

#### **2.3.1. Organoleptic**

The organoleptic parameters of the extract were carried out using the five senses to describe the shape, color, scent, and taste. With an initial introduction that is as simple and objective as possible (Andasari et al., 2020).

#### **2.3.2. The Concentration of Water-Soluble Compounds**

A total of 5.0g of the extract was macerated with 100 mL of chloroform LP water for 24 hours using a corked flask while being shaken repeatedly for the first 6 hours and left for 18 hours. Then the extract was filtered, and the filtrate was taken as much as 20mL. Furthermore, the extract was evaporated to dryness in a cup and the residue obtained was heated at temperature of 105°C to a constant weight (Fikayuniar et al., 2021).

#### **2.3.3. The Concentration of Compounds that are Soluble in Ethanol**

An extract of 5.0 g was macerated with 100 mL of 95% ethanol for 24 hours using a corked flask while shaking for the first 6 hours and left for 18 hours, then the extract was filtered rapidly to avoid evaporation of the ethanol. Then 20 mL of the filtrate was evaporated in the cup. The residue formed is heated at a temperature of 105°C to a constant weight (Sadik & Zulfian A. Disi, 2023).

## **3. Results and Discussion**

### **3.1. Extract Making**

The result of fern extract produced was 13.88±3.19%. This result is greater than the yield of other fern extracts, namely sterile male leaves of 10.72% (Suryadini, 2019). The yield results indicate the range of the number of chemical compounds contained in the extract with water-soluble properties.

### **3.2. Non Specific Parameter**

#### **3.2.1. Drying Shrink**

The drying shrinkage value of the protected fern extract obtained was 0.47 ± 0.01%, which was smaller than the results of the drying shrinkage study on the sterile leaves of anchovies, which was 6% (Suryadini, 2019). The drying shrinkage value obtained provides an overview of the purity, contamination value, and the number of compounds lost in the extract drying process. The dry extract obtained in this study made the drying shrinkage value relatively low.

#### **3.2.2. Water Content**

The water content test of the paku lindung extract was 8.28±0.16% lower than the required not more than 10%. Extracts with high water content are at risk of being damaged during the storage process because microorganisms or fungi can grow (Badan Pengawasan Obat dan Makanan, 2019). The results of this measurement provide an overview of the physical quality of the extract such as the remaining water solvent used and the stability of the extract during the storage process. The use of the gravimetric method is based on the simplicity of the equipment, does not damage the contents of the extract, and is safe because it does not use flammable solvents such as the toluene method (Kementerian Kesehatan Republik Indonesia, 2017).

#### **3.2.1. Ash Content**

The value of determining the ash content is divided into total ash content and acid-insoluble ash content. The working principle of determining the ash content is heating the extract at a high temperature where organic compounds and their derivatives are destroyed and evaporated leaving minerals and inorganic elements. The total ash content of the extract from 2 times of replication was 18.96±3.53% and the value of acid insoluble ash content of 2 times of replication was 1.69±0.02%.

The yield was higher than similar plants, namely sterile male leaves with a successive value of 6% for total ash and 1% for acid-insoluble ash (Suryadini, 2019).

The value of total ash content represents the amount of minerals contained in the extract and the large value of the acid insoluble ash content represents the amount of mineral impurities such as sand that may be carried away from the simplicia recovery process (Departemen Kesehatan RI, 2000). So the value of determining the ash content is related to purity and contaminants. Although there is no standardized value of the paku lindung extract in the literature or previous research, the ash content value obtained in this study can provide an illustration that the ash content obtained is quite high when compared to the values of other extract parameters in general in the literature. The value of total ash content of each extract is different in each sample of the tested extract so the result of the ash content can only be compared with the same extract value that has been previously standardized (Kementerian Kesehatan Republik Indonesia, 2017).

**Table 1.** Non-specific parameters test results

Non-Specific Parameter Test	Results
Drying shrink	0.47±0.01%
Water content	8.28±0.16%
Total ash content	18.97±3.53%
Acid-insoluble ash content	1.69±0.02%
Total plate number	7.8x10 <sup>4</sup> ±12.91 colony/g
Yeast mold number	1x10 <sup>2</sup> ±0.24 colony/g
Heavy metal contamination	Cd (negative)
	Cr (negative)
	Pb (negative)

### 3.2.2. Heavy Metal Contamination

The value of heavy metal contamination (Cd, Cr, and Pb) in the paku lindung extract provides information regarding the safety assurance that the extract does not contain heavy metals that can be harmful to health. Identification of heavy metal contamination using atomic absorption spectroscopy (AAS) with 3 replications and testing was carried out at the chemical laboratory of UGM Yogyakarta. The results of the measurement of the metal content of Cd, Cr, and Pb in the extract of paku lindung were not detected at the detection limit of 0.1ppm. The standard limit allowed for Pb is 10 ppm and Cd is 0.3ppm (Badan Pengawasan Obat dan Makanan, 2019).

### 3.2.3. Microbial Contamination

The results of microbial contamination from 2 replications were carried out, for the total plate number value of  $7.8 \times 10^3 \pm 12.91$  colonies/g and yeast mold number  $1 \times 10^2 \pm 0.24$  colonies/g. The total plate number and the number of yeast molds in the paku lindung extract were below the maximum allowable microbial contamination in the extract, namely the total plate number  $10^4$  colonies/gram and for yeast mold numbers  $10^3$  colonies/g (Badan Pengawasan Obat dan Makanan, 2019). This can provide an illustration that the extract obtained has a guaranteed safety from microbial contamination. The solvent used is water which has a susceptibility to microbial contamination more easily than other solvents. In addition, these results can provide an illustration that the raw materials used and the processes used in the manufacture of extracts have been carried out properly to avoid contamination (Badan Pengawasan Obat dan Makanan, 2019).

### 3.3. Specific Parameter

Extract-specific parameter is an extract parameter that explains aspects of qualitative chemical content and quantitative aspects of levels of chemical compounds that are directly responsible for certain pharmacological activities (Departemen Kesehatan RI, 2000). The results of the specific parameter test of the paku lindung extract are written in Table 2.

**Table 2.** Specific parameter test results

Non-Specific Parameter Test	Results
Organoleptic	dry powder, blackish brown color, bitter taste, characteristic smell of early simplicia
Compounds dissolved in water	85.55±4.29%
Ethanol dissolved compounds	31.27±5.06%

### 3.3.1. Organoleptic

The Organoleptic extract of the paku lindung, the extract was obtained in the form of a dry powder with a blackish brown color, bitter taste, and a distinctive odor like the initial simplicia. This test is carried out to provide a simple and as objective initial introduction as possible using the five senses (Dewi et al., 2023).

### 3.3.2. Compounds dissolved in a certain solvent

The results of the test showed that the levels of water-soluble compounds in the paku lindung extract were 85.55±4.29% and the levels of compounds soluble in ethanol were 31.27±5.06%. This result is greater than the value of the water-soluble compound content of sterile kelakai extract of 3.34% and the ethanol soluble content of sterile kelakai leaf extract of 1.80% (Suryadini, 2019).

The results obtained indicate that compounds that dissolve in water are more soluble than compounds that dissolve in ethanol. This is due to the extraction process using water as a solvent (Kementerian Kesehatan Republik Indonesia, 2017).

## 3.4. Extract Chemical Content Test

### 3.4.1. Thin Layer Chromatography (TLC)

This determination was carried out to provide an initial description of the chemical composition based on the chromatogram pattern using thin layer chromatography (TLC) (Cahya et al., 2022). The results of the chromatogram pattern of the extract obtained in 5 variations of the mobile phase were identical to the Rf value of quercetin. So, it can be concluded that the chromatogram pattern obtained indicates that the extract of the paku lindung contains flavonoid compounds (quercetin). This conclusion was drawn from the same Rf value between the extract's Rf value and the comparison's Rf value (quercetin) so that flavonoids could be the identity compounds of the paku lindung extract.

### 3.4.2. Total Flavonoid Levels

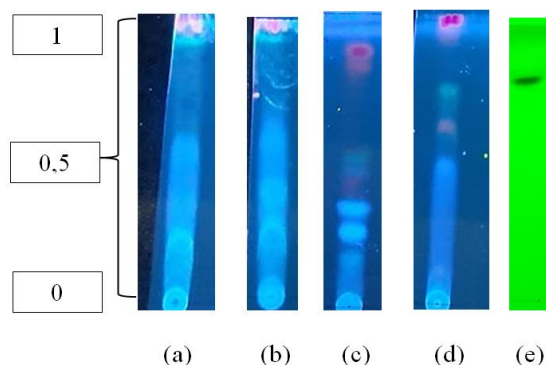
Determining total flavonoid content using the principle of the AlCl<sub>3</sub> method which measured its absorption using a spectrophotometer with a wavelength of 415nm. Total flavonoid level were expressed by the equivalence of the comparison value of quercetin (Dewi, 2020). The comparison of quercetin has a Keto Group on the C4 a hydroxyl atoms and groups on adjacent C3 and C5 atoms so that the reaction that occurs in the addition of AlCl<sub>3</sub> is to form a complex between AlCl<sub>3</sub> and the hydroxyl group, namely OH at C3 or C5 and AlCl<sub>3</sub> with a ketone group at C4. The reaction that occurs is yellow in color by forming a stable complex compound (Sari & Ayuhecaria, 2017).

Examination of total flavonoid levels begins with the calculation of the standard curve of quercetin and obtained the regression equation  $y=0.002x-0.0072$  (Fig 2) with a relation coefficient (r) of 0.9844. The concentration of the extract used was 1000 ppm as the absorbance measurement. Absorbance blank is the absorbance of methanol without AlCl<sub>3</sub> as a blank. The absorbance value of the sample is calculated by subtracting the measured absorbance value from the absorbance of the blank. The value of flavonoid equivalent to quercetin (x) is calculated based on the obtained regression equation where the value of y is the absorbance value of the sample. Then it is converted

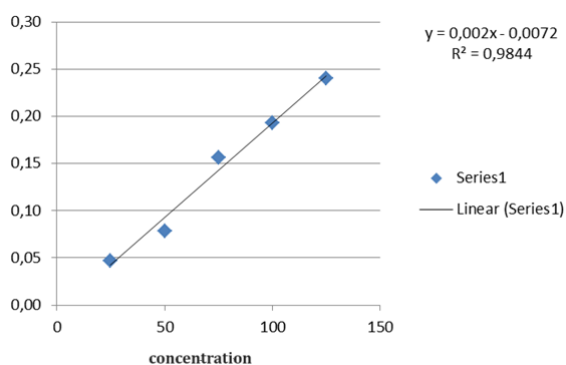
into mg/mL (X). The total flavonoid concentration was calculated by multiplying the volume used to dissolve the quercetin by X and multiplying the dilution factor by 1 because the concentration of the extract used was 1000 ppm. The final result of the research demonstrated that the total flavonoids contents of the paku lindung extract was  $27.1 \pm 14.34$  mgQE/g. These results provide a value for the levels of the paku lindung extract as initial data because there has been no previous research that discusses the quantitative analysis of flavonoids in the paku lindung extract (Daryono & Rhomawati, 2020).

**Table 3.** Rf values of quercetin and paku lindung extract

Mobile phase	Spot sight	Rf	
		Quercetin	Paku Lindung
Toluen P : Ethyl acetat P : formiat acid P (7:2,5:0,5)	Sitroborat and UV <sub>366</sub>	0.86	0.86
Toluen P : Aceton P : acetat acid (10 mL:10 mL:3 tetes)	Sitroborat and UV <sub>366</sub>	0.9	0.9
Toluen P : Aceton : formiat acid (6:6:1)	Aluminium klorida LP and UV <sub>366</sub>	0.38	0.38
chloroform P: Methanol P: aquadest (80:12:12)	Aluminium klorida LP and UV <sub>366</sub>	0.63	0.63
chloroform : Methanol (9:1)	UV <sub>254</sub>	0.78	0.78



**Fig.1.** TLC profile of paku lindung extract, (a) mobile phase toluen P: ethyl acetat P: formiat acid P (7:2.5:0.5); (b) toluen P: aceton P: acetat acid (10 mL:10 mL:3 drops); (c) toluen P: aceton : formiat acid (6:6:1); (d) chloroform P: methanol P: aquadest (80:12:12); (e) chloroform:methanol (9:1) stationary phase : Silica gel GF<sub>254</sub>



**Fig.2.** Quercetin regression graph for calculation of total flavonoid content

**Table 4.** The total flavonoid content of the paku lindung extract

Replication	Absorbance			x (µg/mL)	X (mg/ mL)	TFL (mgQE/ g)	Average (mgQE/g)
	Measurement	Blank	Sample				
1	0.21	0.13	0.08	43.60	0.04	43.60	27.1±14,34
2	0.17	0.14	0.03	17.60	0.02	17.60	
3	0.18	0.14	0.03	20.10	0.02	20.10	

#### 4. Conclusion

The results of the standardization test of the paku lindung extract that has been carried out meet the standardization value of the extract in general. All of these extract standardization data can provide new extract standardization data, considering that there is no standardized data for paku lindung extract in the literature or previous research.

#### Author Contributions

This research was thought up and designed by Muh Fajar Fauzi. Muh fajar fauzi: data analysis,scriptwriter,review,editing. Wahyu widyaningsih:review. All authors have read and agreed to the published version of the manuscript.

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#### Competing Interests

The authors declare no conflict of interest.

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