PHARMACOGNOSTIC STUDY AND ANTIOXIDANT ACTIVITY OF SUNGKAI (PERONEMA CANESCENS JACK.) METHANOL EXTRACT FROM INDONESIA

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Sungkai (Peronema canescens Jack.) from South Kalimantan, Indonesia, are used to treat fever and increase body immunity. This study aims to establish pharmacognostic standards that include specific and non-specific parameters and determine the antioxidant activity of methanol extract of P. canescens leaves. P. canescens leaves were taken from South Kalimantan, Indonesia, and were processed into simplicia according to the standards. The extraction was carried out using methanol and maceration techniques, then investigated covering specific and non-specific parameters. Antioxidant testing was carried out by TLC and DPPH assay. The results showed that drying shrinkage was 7.43%, total ash content was 4.10%, and acid-soluble ash content was 0.23%. Simplicia of P. canescens leaves was green in color, had a strong taste, and a characteristic odor, as well as some cell parts from microscopic observations. This study provides an overview of P. canescens, including specific and non-specific parameters that meet herbal medicine standards.

Keywords: Antioxidant, Non-specific parameters, Peronema canescens, Pharmacognostic, Specific parameter

INTRODUCTION

Indonesia is known for its enormous natural resources, and the country produces a large number of medicinal plants. The enormous number of medicinal plants can be used as a reference point in the therapeutic world1. Plants are used as a remedy for various diseases based on personal experiences that are then passed down from generation to generation2. One of which is Sungkai (Peronema canescens Jack, Fam. Lamiaecae) which is utilized as a medicinal herb.

Our plant under investigation, P. canescens, can be easily obtained in South Kalimantan, Indonesia, and is frequently used for treatment by locals3-4. The people of Dayak Katingan Tribe from Kalimantan utilize the leaves of P. canescens to treat fever and boost immunity5. According to Ibrahim and Kuncoro6, P. canescens leaves methanol extract contains alkaloid compounds, flavonoids,
terpenoids-steroids, and tannins. The efficacy of P. canescens’ has been scientifically proven in several investigations. It possesses antibacterial action against Streptococcus mutans, Salmonella typhi, Bacillus subtilis, and Staphylococcus aureus, according to Ibrahim and Kuncoro. In another study, Yani and Putranto found that P. canescens leaves extract has an antipyretic effect and improves immunity in mice. In addition, P. canescens leaves methanol extract has the potential to be a natural pesticide, and the n-hexane fraction of P. canescens leaves is potentially antimicrobial.

Different polyphenolic compounds (flavonoids and tannins) are biological antioxidants and strong reducing compounds. Biological antioxidants are substances that can ward off or inhibit the harmful activity of oxidant compounds in the body. Antioxidants work by donating electrons to free radical molecules so that free radical compound activities can be inhibited. The balance of oxidants - antioxidants is pivotal for the body. Too high oxidant levels will result in interrupted cell membrane integrity. This condition will affect organ and body cell activity. Therefore, antioxidants are essential for the body.

Pharmacognostic tests become crucial for plant identification. Besides, pharmacognostic studies are an essential step in standardization to ensure the quality and efficacy of herbal ingredients. Other than pharmacognostic tests, activity tests are also needed, one of which is antioxidant activity tests. The method commonly used to quantitatively measure antioxidant activity is 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH is a purple stable radical compound that will turn yellow when reacted with antioxidant compounds. The DPPH method was chosen because it is more straightforward, sensitive, and fast analysis time. Other studies have also reported the use of a single DPPH assay to determine the antioxidant activity of a medicinal plant.

As one of the endemic plants that grow in Southeast Asia, P. canescens can be found on various islands in Indonesia. Research studies on pharmacognostic and antioxidant activity tests of methanol extract of P. canescens leaves obtained from Sumatra Island have been previously reported by Maigoda et al. However, no one has reported similar studies of these plants from other islands, including Kalimantan (Borneo) Island. Therefore, this study aims to determine the pharmacognostic including specific and non-specific parameters of P. canescens extract as well as their antioxidant activity. The results of this study can be a source of data for further research and the basis for using P. canescens as herbal medicines.

**MATERIALS AND METHODS**

**General Procedures**

**Plants determination**

Determination is held to ascertain the name and type of plant in a more specific way. P. canescens was determined at the Center for Plant Conservation of the Botanical Gardens of the Indonesian Institute of Sciences, Bogor, West Java, Indonesia. The determination process was conducted in March 2021.

![Fig. 1: Plant, simplicia powder, and extract of P. canescens.](image)
Materials collection and simplicia production

The sample collection was carried out in January 2021 in the Special Purpose Forest Area in South Kalimantan, Indonesia. The leaves of *P. canescens* (Fig. 1) were collected as samples were mature, perfectly green leaves. The collected leaves were sorted wet to separate other plant parts and impurities. The collected leaves were washed with running water to keep them clean and free of dirt, and then chopped to reduce the size and facilitate the drying process. Samples were dried at 55°C for 2 x 24 hrs. The dried leaves simplicia were powdered with 25 mesh to simplify the extraction process by reducing the size and increasing the surface area of the sample, thereby increasing the contact of the solvent with the simplicia powder.

Production of *P. canescens* methanol extract

The extraction of *P. canescens* leaves was carried out using methanol (technical, Alfa Kemika Indonesia, Indonesia) as a solvent by the maceration method. The maceration method was chosen because this method is a simple and relatively safe method for plants whose compound stability to heat has not been studied. Methanol was used as a solvent-based on its attractiveness and ease of evaporation. As much as 1 kg of *P. canescens* leaves powder was put into a macerator, and 2.5 L of methanol was added until the simplicia was submerged and stirred every 8 hrs. Extraction was conducted for three days (remaceration) with the replacement of solvent every 24 hrs. The liquid extract of *P. canescens* leaves was concentrated using a rotary evaporator, followed by evaporation with a water bath at a temperature of 55°C to a constant weight. From 250 g of extracted powder, 29.13 g (11.652%) of the extract was obtained, which was dark green in color and bitter in taste (Fig.1).

Non-specific parameter test

Drying shrinkage

About 2 g of *P. canescens* simplicia powder was weighed in a silicate crucible heated at 105°C and customized. The simplicia powder was flattened by shaking the silicate crucible to form a layer with a thickness of approximately 5-10 nm. The silicate crucible was put in an open oven, then dried at 105°C to a constant weight. The porcelain cup is cooled, then weighed.

Total ash content

The methanol extract of *P. canescens* leaves was weighed as much as 2 g and put into a silicate crucible that had been incandescent and customized. The silicate crucible containing the sample was incandescent at a temperature of 800°C until the charcoal ran out. The silicate crucible is cooled, then weighed.

Acid insoluble ash content

The ash obtained from the determination of the total ash content was boiled with 25 mL HCl (pro analytics, Merck, Germany) for 5 minutes. The part that was not soluble in acid was filtered using filter paper. The residue was washed with hot water and ignited in a silicate crucible to a constant weight.

Specific parameter test

Organoleptic examination

Organoleptic examination of *P. canescens* simplicia was carried out using the five senses to examine shape, color, smell, and taste. Five experts held simplicia observations as panelists who could provide an objective assessment.

Water-soluble extract content

Powder simplicia of *P. canescens* weighed as much as 5 g. The weighted simplicia was put into a clogged flask, then 100 mL of chloroform (pro analytics, Merck, Germany) was added, shaken for 18 hrs. Filtered and evaporated 20 mL of the filtrate in a porcelain cup that has been customized and heated at a temperature of 105°C to a constant weight.

Ethanol-soluble extract content

Powder simplicia of *P. canescens* weighed as much as 5 g. It was put into a clogged flask, then 100 mL of 95% ethanol (pro analytics, Merck, Germany) was added, shaken many times for the first six hrs, then left for 18 hrs. Filtered and evaporated 20 mL of the filtrate in a porcelain cup that has been customized and heated at a temperature of 105°C to a constant weight.

Phytochemical screening

Phytochemical screening was carried out on simplicia and methanol extract of *P. canescens* leaves to identify the content of
secondary metabolites. Groups of compounds identified include flavonoids, phenols, alkaloids, tannins, steroids, and saponins. Identification was carried out using specific reagents for these groups of compounds.

**TLC profile and qualitative antioxidant activity test**

A thin-layer chromatographic (TLC) profile of *P. canescens* leaves methanol extract was held to describe the chemical composition based on the chromatogram pattern. The stationary phase used was silica gel plate GF254 (pro analytics, Merck, Germany). The mobile phase used was n-hexane (pro analytics, Merck, Germany)-ethyl acetate (pro analytics, Merck, Germany) (7 : 3, 6 : 4, and 3 : 7) v/v. The spots on the TLC plate were observed at UV 254 and 366 nm. The qualitative antioxidant test was carried out by spraying with a DPPH reagent. Positive results are indicated by the formation of yellow spots on a purple background on the chromatogram.

**Quantitative antioxidant activity test**

*The preparation of 0.4 mM DPPH solution*

A total of 4 mg of DPPH powder (pro analytics, Sigma-Aldrich, US) was dissolved with 25 mL of methanol (pro analytics, Merck, Germany) in a 25 mL volumetric flask, then shaken to mix homogeneously to obtain a DPPH solution with a concentration of 0.4 mM.

*Antioxidant test of quercetin comparison solution*

Quercetin (pro analytics, Sigma-Aldrich, US) was made in a solution with concentrations of 1, 2, 3, 4, 5, and 6 ppm. About 4 mL of each concentration series solution was taken, then 1 mL of 0.4 mM DPPH solution was added. The concentration series solution was vortexed for 30 seconds and left in the dark for a predetermined operating time. The absorbance was read with a UV-Vis Spectrophotometer at the maximum wavelength. The % inhibition can be calculated using the formula:

\[
%\text{ inhibition} = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{sample}}} \right) \times 100\%
\]

Moreover, the concentration required to reduce DPPH radicals by 50% (IC50) value was calculated using the formula:

\[
\text{IC}_{50} = \left[ \frac{50 - a (\text{intercept})}{b (\text{slope})} \right]
\]

**RESULTS AND DISCUSSION**

**Non-specific parameter test**

*Drying shrinkage*

Drying shrinkage was carried out by drying simplicia at a temperature of 105°C for an hr until a constant weight was obtained. Determination of drying shrinkage of simplicia aims to provide a limit on the compounds lost during the drying process. The results of the determination of the drying shrinkage of *P. canescens* leaves are presented in Table 1. The average drying shrinkage of *P. canescens* simplicia was 7.43 ± 0.153%, which met the requirements by not more than 10%. These results are in line with those reported by Latief et al. who also reported non-specific parameters of *P. canescens* from Jambi, Indonesia. The drying process influences the value of drying shrinkage during simplicia processing. The smaller the drying shrinkage value, the smaller the water content of simplicia, and the better the drying process. The significant water content causes the simplicia to be easily overgrown by microbes and causes an enzymatic reaction that will decompose the active compound and reduce the quality of the simplicia.

| Test               | Drying shrinkage (%) | Mean ± SD (%) |
|--------------------|----------------------|---------------|
|                    | 1        | 2        | 3        |           |
| Drying shrinkage   | 7.30     | 7.40     | 7.60     | 7.43 ± 0.153 |
**Total ash and acid insoluble ash content**

Determination of the ash content of the extract aims to provide information on the internal and external mineral content originating from the initial process until the formation of the extract\(^{22}\). Ash is an inorganic substance resulting from the combustion of organic matter. Ash content and composition depend on the type of material and the ashing method\(^{26}\). The determination of the ash content and acid insoluble ash content of *P. canescens* extract are presented in Table 2. The average test results of total ash content and acid insoluble ash content of *P. canescens* methanol extract were 4.10 ± 0.173% and 0.23 ± 0.029%, respectively. The results obtained have met the requirements, in which the total ash content is not more than 13%, and the acid insoluble ash content is not more than 1%\(^{27}\). The total ash content indicates the presence of minerals and inorganic substances in the extract. The value of total ash content can be influenced by several factors such as species, soil nutrients, plant age, climate, growing area, and planting treatment\(^{22}\). Acid insoluble ash content indicates the amount of silicate derived from soil and sand. A high value of acid-insoluble ash content indicates that the material has high impurities (sand and soil)\(^{29}\).

**Specific parameter test**

**Organoleptic examination**

An organoleptic test was carried out with the help of five panelists who were asked to describe simplicia characteristics, including color, smell, and taste. This test aims to determine the physical characteristics of simplicia and visual quality inspection\(^{30}\). The results of an organoleptic examination of *P. canescens* leaves are presented in Table 3. The prepared *P. canescens* simplicia is a course powder with a dark green color, a distinctive odor derived from essential oils, and a bitter taste that is thought to be derived from the content of alkaloids and saponins\(^{7}\).

**Water-soluble and ethanol-soluble extract content**

Determination of the levels of dissolved compounds aims to provide an initial picture of the amount of compound content that the solvent can extract. The solvents that are usually used in this test are water and ethanol\(^{22}\). The results of the determination of the levels of dissolved compounds are presented in Table 4. The average water-soluble and ethanol-soluble extract content of *P. canescens* leaves simplicia were 9.00 ± 0.100% and 10.30 ± 0.100%, respectively. These results meet the requirements, stating that the water-soluble extract content is not less than 5%, while the ethanol-soluble extract content is not less than 3.5%\(^{27}\). Data on the ethanol-soluble was higher than the water-soluble extract content, indicating that the compound content in *P. canescens* leaves simplicia was more soluble in organic solvents\(^{31}\).

Table 2: The total ash content and acid insoluble ash content of *P. canescens* leaves.

| Test                      | Ash content (%) | Mean ± SD (%) |
|---------------------------|----------------|---------------|
|                           | 1   | 2   | 3   |               |
| Total ash                 | 4.00| 4.00| 4.30| 4.10 ± 0.173 |
| Acid insoluble ash        | 0.25| 0.25| 0.20| 0.23 ± 0.029 |

Table 3: Organoleptic examination of *P. canescens* leaves.

| Test                      | Observation |
|---------------------------|-------------|
|                           | Shape  | Colour | Smell | Taste |
| Organoleptic examination  | powder | dark green | typical | bitter |

Table 4: The levels of dissolved compounds of *P. canescens* leaves.

| Test                    | Concentration (%) | Mean ± SD (%) |
|-------------------------|-------------------|---------------|
|                         | 1   | 2   | 3   |       |
| Water-soluble extract   | 8.90| 9.00| 9.10| 9.00 ± 0.100 |
| Ethanol-soluble extract | 10.30| 10.20| 10.40| 10.30 ± 0.100 |
**Phytochemical screening**

Phytochemical screening aims to identify the secondary metabolites contained in simplicia and extracts\(^\text{32}\). The results of phytochemical screening of simplicia and extracts are presented in Table 5. The test results showed that the simplicia and methanol extract of *P. canescens* leaves contain phenolic compounds, flavonoids, steroids, saponins, tannins, and alkaloids. Based on the results obtained, the content of compounds contained in simplicia and extracts remained the same. This shows the consistency of the chemical compound content in the sample, even though it has undergone various previous processing processes\(^\text{33}\).

**TLC profile and qualitative antioxidant activity test**

Identification of TLC profiles was carried out to obtain a specific picture of the chemical content present in the sample. The chromatogram profile obtained can provide specifics for the compounds contained in the methanol extract. This is very important to ensure the characteristics of the sample so that there is no counterfeiting of raw materials for traditional medicines from the *P. canescens*. Several compounds have also been identified as having potential as antioxidants. Our previous study\(^\text{23}\) also reported that compounds with potential as antioxidants show yellow spots on a purple background after the chromatogram is sprayed with DPPH solution. Antioxidant compounds will donate electrons to DPPH, thereby neutralizing the free radical properties of DPPH and causing a yellow color change\(^\text{34}\). The TLC as well as antioxidant potency test results are presented in Fig. 2.

**Table 5:** The phytochemical screening of simplicia and methanol extracts of *P. canescens* leaves.

| Test       | Peronema canescens leaves |
|------------|---------------------------|
|            | Simplicia | Methanol extract |
| Phenolic   | +          | +                 |
| Steroid    | +          | +                 |
| Flavonoid  | +          | +                 |
| Tannin     | +          | +                 |
| Saponin    | +          | +                 |
| Alkaloid   | +          | +                 |

**Fig. 2:** TLC profile (A) and antioxidant potency tests (B) of methanol extracts of *P. canescens* leaves with stationary phase silica gel GF\(_{254}\) and mobile phase n-hexane : ethyl acetate 7 : 3 (a); 6 : 4 (b); and 3 : 7 (c). The detectors used are UV 254 nm (A) and 0.4 mM DPPH (B), with white rings indicating extract profile compounds (A) or compounds with antioxidant activity (B).
Fig. 3: Antioxidant activity of quercetin (A) and P. canescens leaves methanol extracts (B).

Quantitative antioxidant activity test of P. canescens leaves extract
The IC₅₀ value of the quercetin solution was processed using SPSS to obtain a linear regression equation \( y = 13.676x + 3.703 \), with a correlation coefficient value \( r = 0.996 \), which met the requirements greater than 0.98. The IC₅₀ of quercetin was 3.385 ppm (Fig. 3), which is included in the category of a very strong antioxidant less than 50 ppm. Furthermore, the antioxidant activity of P. canescens leaves methanol extract was carried out by reacting the sample solution with 0.4 mM DPPH. The linear regression equation for the antioxidant activity of P. canescens leaves methanol extract was \( y = 0.598x + 11.723 \) with \( r = 0.993 \). The IC₅₀ value of P. canescens leaves methanol extract was 63.977 ppm, included in the strong category between 50–100 ppm. According to Akar et al., samples that can change 50% DPPH color at a 20 g/mL concentration are considered to have good antioxidant activity.

Several groups of compounds that have the potential as antioxidants are flavonoids, tannins, and other phenolic compounds. Antioxidant activity will increase along with the increase in hydroxyl groups. The phenolic compounds and their derivatives, such as flavonoids and tannins, have been widely reported as potent reducing and free radical inhibitors. In addition, flavonoid compounds and tannins, which belong to the group of phenolic compounds and generally have antioxidant activity, will have a mutually reinforcing effect. In addition, P. canescens leaves were reported to have several pharmacological activities, including immunostimulant, anti-inflammatory, antidiabetic, antiplasmodial, and antihyperuricemia. However, further research is needed to prove these activities.

Conclusion
In this study, we aimed to determine the both pharmacognostic specific and non-specific parameters of P. canescens extract, together with antioxidant activity of P. canescens leaves. The results of pharmacognostic tests on non-specific and specific parameters meet the requirements of Indonesian medical materials standards. P. canescens leaves methanol extract has a strong antioxidant activity with an IC₅₀ value of 63.977 ppm and has the potential to be developed as herbal medicine.

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Conflict of Interest
The authors declare no conflict of interest.
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دراسة العقاقير والنشاط المضاد للأكسدة من مستخلص الميثانون لنبات سانجكاي
(برونما كايسينس حاك) من إندونيسيا

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تستخدم سانجكاي (برونما كايسينس حاك) من جنوب كاليمانان، إندونيسيا، لعلاج الحمى وزيادة
مناعة الجسم. تهدف هذه الدراسة إلى وضع معايير عقاقيرية وتشمل معاملات محددة وغير محددة
وتحديد النشاط المضاد للأكسدة لمستقبل الميثانون من أوراق بروما كايسينس. تم أخذ أوراق بروما
كايسينس من جنوب كاليمانان، إندونيسيا، وتم معالجتها في وفقا للمعايير. تم الاستخلاص باستخدام
تقنية الاستقصاء في الميثانون، ثم تم التحقق من تغطية معاملات محددة وغير محددة. تم إجراء اختبار
مضادات الأكسدة بواسطة مقاومة كروماتوغرافيا الطبقة الرقيقة و DPPH. أظهرت النتائج أن انكماش
والتجارب كان 34.8% وحولت الرماد الكلي 10.4% وحولت الرماد الذائب في الأحماض اضافة 33.0%. كانت
أوراق بروما كايسينس خضراء اللون، ولها ضوء قوي ورائحة مميزة، وكذلك بعض أجزاء الخلايا من
الملاحظات المجهرية. تقدم هذه الدراسة لمحبة عامة عن بروما كايسينس، بما في ذلك المعاملات المحددة
وغير المحددة التي تلبي معايير طب الأعشاب.