

Pharmacognostic Study And Antioxidant Activity Of Sungkai (*Peronema canescens* Jack.) Methanol Extract From Indonesia *by Sutomo Sutomo*

Submission date: 04-Feb-2023 10:22AM (UTC+0800)

Submission ID: 2005938471

File name: kai_Peronema_canescens_Jack._Methanol_Extract_From_Indonesia.pdf (968.43K)

Word count: 5040

Character count: 27825



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

Sutomo Sutomo^{1,2}, Arnida Arnida¹, Fachrida Rahmah Yulistati³, Normaidah Normaidah⁴ and Mohammad Rizki Fadhil Pratama^{5,6*}

¹Center for Study of Natural Medicine, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

²Department of Pharmaceutical Biology, Pharmacy Study Program, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

³Pharmacist Professional Education Program, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

⁴Department of Pharmaceutical Chemistry, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia

⁵Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia

⁶Department of Pharmacy, Universitas Muhammadiyah Palangkaraya, Palangka Raya, Central Kalimantan, Indonesia

1
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. Lamiaceae) 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 locals^{3,4}. 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,

Received in 2/6/2022 & Accepted in 8/8/2022

*Corresponding author: Mohammad Rizki Fadhil Pratama, E-mail: mohammadrizkifadhilpratama@umpr.ac.id

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 Kuncoro⁶. In another study⁸, 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 antimicrobial⁹.

Different polyphenolic compounds (flavonoids and tannins) are biological antioxidants and strong reducing compounds⁷. 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 inhibited¹⁰. 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 body¹¹.

Pharmacognostic tests become crucial for plant identification. Besides, pharmacognostic studies are an essential step in standardization to ensure the quality and efficacy of herbal ingredients¹². 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 time¹³. Other studies have also reported the use of a single DPPH assay to determine the antioxidant activity of a medicinal plant^{14&15}. As one of the endemic plants that grow in Southeast Asia, *P. canescens* can be found on various islands in Indonesia¹. 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*¹⁶. 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*.

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 evaporation¹⁷. 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 weight¹⁸. 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 weighed¹⁹.

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 weighed²⁰.

6

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 weight¹⁹.

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 assessment¹⁹.

Water-soluble extract content

Powder simplicia of *P. canescens* weighed as much as 5 g. The weighted simplicia was put into a corked flask, then 100 mL of chloroform (pro analytics, Merck, Germany) saturated water was added, shaken several 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 weight¹⁹.

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 weight¹⁹.

Phytochemical screening

Phytochemical screening was carried out on simplicia and methanol extract of *P. canescens* leaves to identify the content of

secondary metabolites. **1** Groups of compounds identified include flavonoids, phenols, alkaloids, tannins, steroids, and saponins. Identification was carried out using specific reagents for these groups of compounds²¹.

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 pattern²². 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 chromatogram²³.

Quantitative antioxidant activity test

The preparation of 0.4 mM DPPH solution

4 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 mM¹³.

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 **3** concentration series solution was taken, then 1 mL of 0.4 mM DPPH solution was added. The concentration series solution was vortexed and allowed to stand in the dark for a predetermined operating time. The absorbance was read with a UV-Vis Spectrophotometer at the maximum wavelength¹³.

Antioxidant test of *P. canescens* extract

The antioxidant activity of *P. canescens* extract was carried out by DPPH assay, as

reported by Maigoda *et al*¹⁶. The extract of *P. canescens* was made in a solution with 20, 40, 60, 80, and 100 ppm concentrations. Each sample solution with concentrations of 20, 40, **3**, 80, and 100 ppm was taken at 4 mL, then 1 mL of 0.4 mM DPPH solution was added. The 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 wavelength¹³. The % inhibition can be calculated using the formula²³:

$$\% \text{ inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{sample}}} \times 100\%$$

Moreover, the concentration required to reduce DPPH radicals by 50% (IC₅₀) value was calculated using the formula²³:

$$IC_{50} = \frac{[50 - a(\text{intercept})]}{b(\text{slope})}$$

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 process²⁴. 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 **m2** the requirements by not more than 10%¹⁹. These results are in line with those reported by Latief *et al.*²⁵ 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 simplicia²⁶.

Table 1: The drying shrinkage of *P. canescens* leaves.

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 extract²². 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 method²⁶. 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 $4.10 \pm 0.173\%$ and $0.23 \pm 0.029\%$, 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^{Error! Reference source not found.}. 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 inspection³⁰. The results of an organoleptic examination of *P. canescens* leaves are presented in Table 3. The prepared *P. canescens* simplicia is a coarse 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 saponins⁷.

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 ethanol²². 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 $9.00 \pm 0.100\%$ and $10.30 \pm 0.100\%$, 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 solvents³¹.

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

Test	Ash content (%)			Mean \pm 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 \pm 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 extracts³². 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 processes³³.

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 study²³ 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 change³⁴. 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	<i>Peronema canescens</i> leaves	
	Simplicia	Methanol extract
Phenolic	+	+
Steroid	+	+
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Alkaloid	+	+

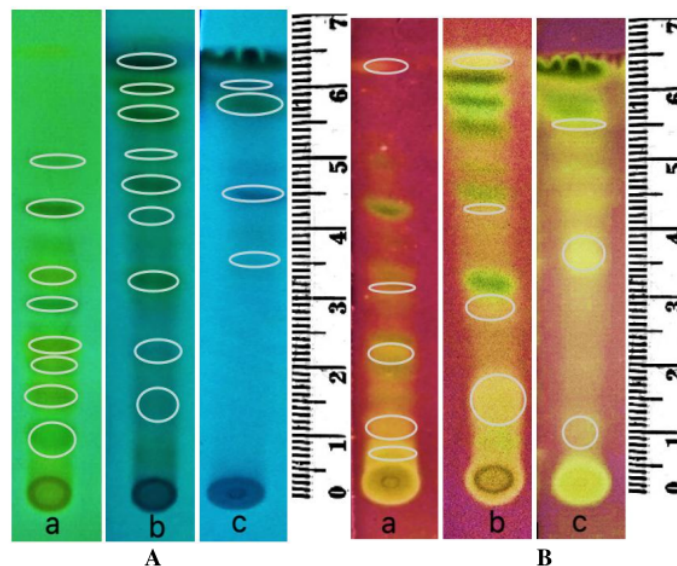


Fig. 2: TLC profile (A) and antioxidant potency tests (B) of methanol extracts of *P. canescens* leaves with stationary phase silica gel GF₂₅₄ and mobile phase n-hexane : ethyl acetate 7 : 3 (a); 6 : 4 (b); and 3 : 7 (c). The detectors used are UV 254 nm (A) and 0.4 mM DPPH (B), with white rings indicating extract profile compounds (A) or compounds with antioxidant activity (B).

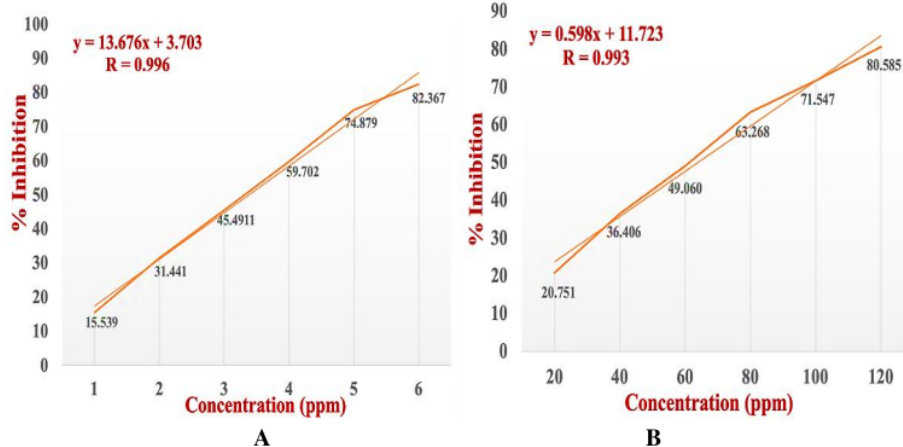


Fig. 3: Antioxidant activity of quercetin (A) and *P. canescens* leaves methanol extracts (B).

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 0.98⁴. The IC₅₀ of quercetin was 3.385 ppm (Fig. 3), which is included in the category of a very strong antioxidant less than 50 ppm³⁵. 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 ppm³⁵. According to Akar *et al.*³⁶, 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 groups³⁷. The phenolic compounds and their derivatives, such as flavonoids and tannins, have been widely reported as potent reducing and free radical inhibitors³⁸. 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^{39&40}. In addition, *P. canescens* leaves were reported to have several pharmacological activities, including immunostimulant⁴¹, anti-inflammatory⁴², antidiabetic²⁵, antiplasmodial⁴³, and antihyperuricemia⁴⁴. 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.

Acknowledgments

The researchers are grateful to Lembaga Penelitian dan Pengabdian Kepada Masyarakat Universitas Lambung Mangkurat for the funding of this research with grant number 010.3/UN8.2/PL/2021. All researchers have an equal contribution to this research and have agreed to the entire contents of this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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دراسة العقاقير والنشاط المضاد للأكسدة من مستخلص الميثانول لنبات سانجكاي (برونما كانسينس جاك) من إندونيسيا

سوتومو سوتومو^١ - ارنيدا ارنيدا^١ - فاخريدا راهماه يولستاتي^٣ - نورمايداه نورمايداه^٤ -
محمد رزكي فضيل براتاما^{٥,٦*}

^١ مركز دراسة الطب الطبيعي، جامعة لامبونج مانجكورات، بانجاربارو، كاليمانتان الجنوبية، إندونيسيا

^٢ قسم البيولوجيا الصيدلانية، برنامج دراسة الصيدلة، جامعة لامبونج مانجكورات، بانجاربارو، جنوب
كاليمانتان، إندونيسيا

^٣ برنامج التعليم المهني للصيدلة، جامعة لامبونج مانجكورات، بانجاربارو، كاليمانتان الجنوبية، إندونيسيا

^٤ قسم الكيمياء الصيدلانية، جامعة لامبونج مانجكورات، بانجاربارو، كاليمانتان الجنوبية، إندونيسيا

^٥ برنامج الدكتوراه في العلوم الصيدلانية، كلية الصيدلة، جامعة إيرلانجا، سورابايا، جاوة الشرقية، إندونيسيا

^٦ قسم الصيدلة، جامعة محمديه بالانجكا راي، بالانجكا راي، وسط كاليمانتان، إندونيسيا

تستخدم سانجكاي (برونما كانسينس جاك) من جنوب كاليمانتان، إندونيسيا، لعلاج الحمى وزيادة
مناعة الجسم. تهدف هذه الدراسة إلى وضع معايير عقاقيرية و التي تشمل معاملات محددة وغير محددة
وتحديد النشاط المضاد للأكسدة لمستخلص الميثانول من أوراق برونما كانسينس. تم أخذ أوراق برونما
كانسينس من جنوب كاليمانتان، إندونيسيا، وتم معالجتها في وفقا للمعايير. تم الاستخلاص باستخدام
تقنيات النقع في الميثانول، ثم تم التحقق من تغطية معاملات محددة وغير محددة. تم إجراء اختبار
مضادات الأكسدة بواسطة مقايسة كروماتوجرافيا الطبقة الرقيقة و DPPH. أظهرت النتائج أن انكماش
التجفيف كان ٧.٤٣٪ ومحتوى الرماد الكلي ٤.١٠٪ ومحتوى الرماد الذائب في الأحماض ٠.٢٣٪. كانت
أوراق برونما كانسينس خضراء اللون، ولها طعم قوي ورائحة مميزة، وكذلك بعض أجزاء الخلايا من
الملاحظات المجهرية. تقدم هذه الدراسة لمحة عامة عن برونما كانسينس، بما في ذلك المعاملات المحددة
وغير المحددة التي تلي معايير طب الأعشاب.

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