

The Effect of Drying Method on Potential Antioxidants in Ethanol Extract of *Sungkai* Leaf (*Parenoma Canescens* Jack.) Simplicia from Kalimantan

Pengaruh Pengeringan Simplicia Terhadap Potensi Antioksidan Ekstrak Etanol Daun Sungkai (*Parenoma Canescens* Jack.) Asal Kalimantan

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ABSTRACT

Sungkai (P. canescens) is a medicinal plant whose leaves are used to alleviate fever and strengthen the body's immune system. This study aimed to determine the effect of the simplicia drying technique on the antioxidant potential of the ethanol extract of Sungkai leaves from Kalimantan. The drying method employed was oven drying at 70°C, oven drying at 50°C, sun drying, and wind drying. The study's findings indicated that the antioxidant activity of all drying techniques was in the very active category. The drying method with the lowest antioxidant potential was oven drying at 50°C with IC₅₀ value of 13.340 ppm, wind drying with IC₅₀ value of 14.610 ppm, sun drying with IC₅₀ value of 16.799 ppm, and oven drying at 70°C with IC₅₀ value of 17.034 ppm.

Keywords: *Sungkai* leaf ; IC₅₀; antioxidant

ABSTRAK

Tanaman daun Sungkai (P. canescens) merupakan tanaman obat yang digunakan untuk mengobati demam dan meningkatkan imun tubuh. Tujuan dalam penelitian ini mengetahui pengaruh pengeringan simplicia terhadap potensi antioksidan ekstrak etanol daun sungkai (P. canescens Jack.) dari Kalimantan. Metode pengeringan yang digunakan adalah pengeringan oven 70°C, oven 50°C, matahari dan kering angin. Hasil penelitian didapatkan bahwa aktifitas antioksidan untuk semua metode pengeringan pada tanaman daun sungkai (P. canescens) ini memiliki aktifitas antioksidan termasuk dalam kategori sangat aktif. Metode pengeringan dilihat berdasarkan potensi antioksidan yang paling rendah adalah oven 50°C nilai IC₅₀ 13,340 ppm, kering angin nilai IC₅₀ 14,610 ppm, matahari nilai IC₅₀ 16,799 ppm dan oven 70°C nilai IC₅₀ 17,034 ppm

Kata kunci: daun *Sungkai*; IC₅₀; antioksidan

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1. INTRODUCTION

Sungkai (*P. canescens* Jack.) is a common plant. The Katingan Dayak tribes have traditionally employed the leaves of the *P. canescens* plant to allivate fever and boost overall immunity. Several studies have been conducted to confirm the efficacy of *Peronema canescens* scientifically.

Biologically, antioxidants are substances that can counteract or inhibit the antagonistic activity of oxidant compounds in the body. Antioxidants work by donating electrons to free radical molecules, inhibiting the action of free radical compounds. The balance of oxidants is crucial for the body. Excessive oxidant levels will compromise cell membrane integrity and affect the organelles and activity of body cells. Therefore, antioxidants are crucial for the body (Winarso, 2007). Based on practical use by the community, *P. canescens* leaves have been used to increase body immunity. According to several studies, antioxidants have been shown to increase the body's immunity (Sayuti & Yenrina, 2015).

The drying technique strongly influences the content of active ingredients in plants. Each plant reacts differently. Some plants are susceptible to direct sunlight and excessive heat. Proper drying results in high-quality *simplicia* with a long shelf life and no change in the active components (Manoi, 2006). Based on this description, this study aimed to investigate the effect of the *simplicia* drying technique on *Sungkai* leaf (*Peronema canescens* Jack.) from Kalimantan by employing the oven drying method at 50°C, oven drying method at 70°C, sun drying, and wind drying. This study examined the *simplicia* of *Sungkai* leaf (*P. canescens* Jack.) to determine the ideal drying technique for obtaining the highest antioxidant quality.

2. MATERIALS AND METHODS (Times New Roman 12 pts, bold, all capital letters)

2.1. Materials

The materials used were *Sungkai* leaf (*Peronema canescens* Jack.), filter paper, 96% ethanol, aquadest, DPPH (1.1-diphenyl-1-picrylhydrazil), and methanol (p.a). The tools used were analytical balance, oven, porcelain dish, glass beaker, test tube, macerator, water bath, spectrophotometer, vortex, drying cabinet, and rotary evaporator.

2.2. Material processing and drying of *simplicia*

Samples were taken from the Banjarbaru Botanical Gardens, South Kalimantan Province. The leaves of *P. canescens* taken were mature leaves. The fresh leaves were then sorted to separate other plant parts and impurities. Next, the *P. canescens* leaves were rinsed with running water to keep them clean and dirt-free. *P. canescens* leaves were chopped to reduce the size and facilitate the drying and size reduction process. Samples were dried in an oven at 50°C, 70°C, wind-dried, and under the sunlight. Next, the dried *P. canescens* leaves were sorted to separate the remaining impurities.

2.3. Production of Ethanol Extract from *Sungkai* Leaf (*Peronema canescens* Jack.)

The maceration method was used to extract *P. canescens* leaf using ethanol as the solvent. A total of 35 g of fresh samples of *P. canescens* leaf were dried and ground into *simplicia* powder, then put into a macerator, added with 600 mL of ethanol until the *simplicia* was submerged, and then stirred every 6-8 hours at room temperature. Extraction was carried out for 3 days (re-maceration) with solvent replacement every 1 x 24 hours. The liquid extract was filtered through filter paper to separate the powdered *simplicia* from the liquid extract. The liquid extract of *P. canescens* leaves was concentrated using a waterbath at 50°C to obtain a constant weight

(Leksono et al., 2018). Next, the percentage of extract obtained was calculated. The yield percentage is calculated using the following equation:

$$\text{Yield} = \frac{\text{weight of extract obtained}}{\text{weight of simplicia}} \times 100\%$$

2.4. Moisture Content (Drying Loss)

Two grams of *P. canescens* leaf simplicia powder was weighed in a silica crucible that had been heated at 105°C and calibrated. The simplicia was flattened by shaking the silica crucible. The open silica crucible was put into the oven and then dried at 105°C to a constant weight. The moisture content is calculated using the equation:

$$\text{moisture content} = \frac{\text{initial weight of sample} - \text{final weight of sample}}{\text{initial weight of sample}} \times 100\%$$

2.5. Antioxidant Activity Test

a. Preparation of DPPH solution

A total of 4 mg of DPPH powder was dissolved with 25 mL of methanol (p.a) in a 25 mL flask and stirred until homogeneous to obtain a DPPH solution with a concentration of 0.4 mM (Mardiah et al., 2017).

b. Determination of the maximum wavelength of DPPH

A total of 1 mL of 0.4 mM DPPH solution was added with 4 mL of methanol (p.a). The solution mixture was then vortexed and left in the dark for 30 minutes. The absorption of the solution was measured at a wavelength of 500-550 nm (Mardiah et al., 2017).

c. Preparation of sample solution

A total of 2.5 mg of *P. canescens* leaf ethanol extract was dissolved with sufficient methanol, then put into a 25 mL volumetric flask, and methanol was added until it reached the limit mark. This process produced a sample solution of 100 ppm. The sample solution of 100 ppm was diluted into series with concentrations of 5, 10, 15, 20, and 25 ppm using a 10 mL volumetric flask. A total of 4 mL of each sample solution with concentrations of 5, 10, 15, 20, and 25 ppm

were taken, then added with 1 mL of 0.4 mM DPPH solution. The sample solution was vortexed and allowed to stand in the dark for a predetermined time.

d. Determination of IC₅₀ Value

The antioxidant activity of the ethanol extract of *P. canescens* leaves was expressed in the percentage of inhibition. The % inhibition value was calculated using the equation:

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

The IC₅₀ value was obtained from a linear curve with the equation $y = a + bx$, resulting from the regression between concentration and % inhibition. The formula for calculating the IC₅₀ value is:

$$IC_{50} = \frac{50 - a}{b}$$

2.6. Data Analysis

Data were analyzed using SPSS statistical test for oven drying at 70°C, oven drying at 50°C, wind drying, and sun-drying. The analysis determined the best simplisa drying technique of the four methods employed.

3. RESULTS AND DISCUSSION

3.1. Sample preparation and leaf drying of *P. canescens*

The sample used in this study was the leaf part of the *P. canescens* plant obtained from the Banjarbaru Botanical Garden, South Kalimantan. Samples were gathered from the same location to ensure uniform chemical composition. Furthermore, wet sorting was carried out to separate dirt, or foreign material from *P. canescens* leaf. The material was washed with clean water to eliminate any remaining contaminants, and the simplicia was drained to remove any remaining water. Furthermore, chopping was carried out to increase the surface area to facilitate the drying process. Four ways

of drying were used: oven drying at 50°C, oven drying at 70°C, sun drying, and wind drying.

The drying method employed an oven drying at 50°C for 6 hours, an oven drying at 70°C for 4 hours, sun drying for 3 days (hot conditions), and wind drying for 10 days (hot weather conditions). After drying, the sample was sorted to separate the remaining impurities. After that, the sample was ground using a blender and sieved with a mesh size of 25.

3.2. Extraction process

P. canescens leaf was extracted by cold method, namely maceration. This approach was chosen because it does not involve heating to avoid possible damage to the active compound (Wikanta et al., 2012). Additionally, the procedure is quick, simple, and ideal for the extraction of high amount of samples (Mailoa et al., 2013).

Extraction of *P. canescens* powder was carried out by soaking 35 g of powder from the drying methods in 96% ethanol solvent for 3×24 hours at room temperature and stirring every 6 hours. Maceration was carried out for 3×24 hours. More than three days of maceration might lead to over-saturation of the powder, preventing its compounds from being extracted optimally. *P. canescens* leaf powder obtained by oven drying at 50°C yielded a 6.39 g thick extract with an 18.257% yield. It means that there are 18.257 g of extract in every 100 grams of *P. canescens* leaf. *P. canescens* leaf powder from oven drying at 70°C produced a thick extract of 7.4 g or a yield of 21.142%. *P. canescens* leaf powder from sun drying produced a thick extract of 6.91 g or

a yield of 19.742%. *P. canescens* leaf powder from wind drying resulted in a thick extract of 5.77 g or a yield of 16.485%.

The organic solvent used in the extraction process was 96% ethanol. The resulting extract was filtered through Whatman filter paper to remove any remaining powder grains in the liquid extract. The maceration product was concentrated using a rotary evaporator. The results of the rotary evaporator were re-evaporated using a water bath at 50°C to obtain a thick extract with a constant weight.

The yield value is needed to estimate the amount of extract obtained during the extraction process. In addition, the yield data also has a relationship with the active compounds contained in the sample. So that if the amount of yield increases, the number of active compounds contained in it is also increasing.

3.3. Moisture content (drying loss)

Determination of drying loss aims to provide a limit or range of the amount of water and volatile compounds lost during the drying process. Measurement of residual substances was carried out by drying at 105°C for 30 minutes to constant weight. The results of determining the drying loss of *P. canescens* leaf simplicia with several drying methods are presented in Table 1. From Table 1, it can be seen that from the oven drying method at 70°C, oven drying at 50°C, sun drying, and wind drying, the drying loss values differ, which is not more than 10%. According to the Indonesian Ministry of Health (1995), the drying loss value range is not more than 10%.

Table 1. Moisture content of *P. canescens* leaves simplicia

Method	Drying Loss (%)		
	Replication 1	Replication 2	Replication 3
Oven 50°C	5,5	5,5	5,5
Oven 70°C	5,5	6	6
Sun-dry	6	6,5	6,5
Wind-dry	6	6,5	6,5

It indicates that all the simplicia of the four drying methods meet the requirements. The replication results obtained the same value because the measurements were carried out 3 times in the same solution. The quality of the drying process is said to be getting better if the drying shrinkage value is getting smaller.

3.4. Determination of the maximum wavelength of DPPH

The maximum wavelength is determined to ascertain the wavelength of the substance that delivers the greatest absorbance and sensitivity at each concentration (Robinson et al., 2005). Measurement at the maximum wavelength produces linear findings, increases instrument sensitivity, and minimizes measurement errors (Sadeli, 2016). The DPPH approach was chosen due to the compounds' higher stability than other methods.

The antioxidant activity of *P. canescens* extract was determined using the DPPH radical absorption method on a UV-Vis spectrophotometer, which quantifies the amount of antioxidant activity present in the extract to reduce free radicals. The maximum wavelength used was 516 nm. This maximum wavelength range follows the literature mentioned by Molyneux (2004) between 515 – 520 nm. The maximum range was reached with DPPH in its complementary purple color. The IC₅₀ value expresses the DPPH method. The IC₅₀ value is the concentration of the sample required to inhibit DPPH free radicals by 50%. Antioxidant capabilities are enhanced by a lower IC₅₀ value (Molyneux, 2004).

3.5. Determination of IC₅₀ value of quercetin comparison solution

Determination of the IC₅₀ value of quercetin is needed as a positive control in the antioxidant activity test of the sample. Positive control was used as a comparison to determine the amount of antioxidant activity possessed. Quercetin was chosen due to its high antioxidant activity

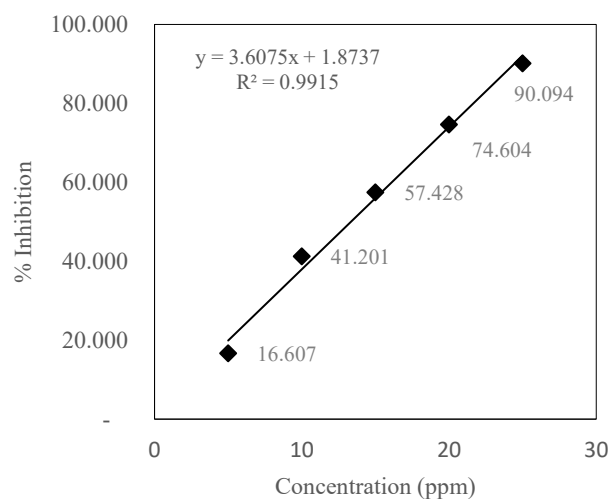
(Adawiyah & Rizki, 2018). According to Yulistati, F.R (2021), a study of the IC₅₀ measurement of quercetin as a positive control used 5 concentrations series (1, 2, 3, 4, 5 ppm). The IC₅₀ value of quercetin obtained was 2.754 ppm. The anticoagulant activity of quercetin was classified as very strong based on its IC₅₀ value.

3.3. Determination of IC₅₀ value of ethanol extract from *P. canescens* leaves

The IC₅₀ value of the ethanol extract of *P. canescens* leaf from oven drying at 70°C, oven drying at 50°C, sun drying, and wind drying was obtained by preparing 5 concentrations, namely 5, 10, 15, 20, 25 ppm in methanol (p.a). One ml of DPPH solution was added to each concentration and incubated for 30 minutes. Furthermore, measurements were taken at a maximum wavelength of 516 nm (Maulina, 2014; Sumaryanto, 2019). The absorbance value was measured to determine the percent inhibition. This percentage of inhibition indicates the free radical activity of each sample (Jatmika et al., 2015). Percent inhibition refers to a sample's ability to block the activity of free radicals in proportion to its concentration. The percentage of inhibition obtained from the difference in absorption between the control absorbance and the sample was measured using a UV-Vis Spectrophotometer (Anggresani et al., 2017). The IC₅₀ value of each sample concentration was calculated using the linear regression equation formula, which states the relationship between the concentration expressed by the x-axis and the percentage of inhibition expressed by the y-axis of the measurement replication series. The results of the percentage inhibition of the ethanol extract of *P. canescens* leaves from oven drying at 70°C, oven drying at 50°C, sun drying, and wind drying are presented in Table 2. The graph of the relationship between concentration and % inhibition of the oven drying at 50°C is presented in Fig. 1.

Table 3. Percentage of inhibition and IC₅₀ of ethanol extract of *P. canescens* leaves from oven drying at 70°C, oven drying at 50°C, sun drying, and wind drying

Concentration (ppm)	Percent Inhibition (%) of Replication			Regression Equation	IC ₅₀ (ppm)
	1	2	3		
Oven drying 50°C	5	12.539	12.539	Y = 2.843 x + 1.5697	17.034
	10	34.562	34.562		
	15	43.414	43.414	R = 0.9831	
	20	59.325	59.325		
	25	71.232	71.232		
	5	16.607	16.607	Y = 3.6075 x + 1.8737	13.340
	10	41.201	41.201		
	15	57.428	57.428	R = 0.9915	
	20	74.604	74.604		
	25	90.094	90.094		
Sun drying	5	11.380	11.380	Y = 3.2898x – 5.2024	16.779
	10	25.289	25.289		
	15	44.573	44.573	R = 0.9782	
	20	66.701	66.701		
	25	72.918	72.918		
Wind drying	5	17.555	17.555	Y = 3.2472x + 2.5583	14.610
	10	34.773	34.773		
	15	54.794	54.794	R = 0.9939	
	20	66.174	66.174		
	25	83.034	83.034		

**Figure 1.** The relationship between concentration and % inhibition of oven drying method at 50°C

Based on the IC₅₀ value, all *P. canescens* leaf extract drying methods were categorized as very active due to their IC₅₀ values ranging between 50 and 100 ppm. The IC₅₀ value is used to measure a sample's antioxidant capacity.

The results indicate that all extracts obtained by the drying technique had good antioxidant activity. The ethanol extract of the oven drying at 50°C has a lower IC₅₀ value than other drying techniques. The best drying technique based on the IC₅₀ was oven drying at 50°C, wind drying, sun drying, and oven drying at 70°C. The smaller the IC₅₀ value, the higher the antioxidant activity. The statistical test showed that the four drying methods were not significantly different. Based on the Uses Harmonic Mean Sample Size = 5,000, less than the significance level (α = 0.05).

4. CONCLUSIONS

From the findings of this study, it can be concluded that ethanol extract from the drying of *Sungkai* leaf (*P. canescens*) by oven drying at 50°C resulted in a lower IC₅₀ value than the oven drying at 70°C, sun drying, and wind drying. The antioxidant potential of the ethanol extract of *Sungkai* leaf (*P. canescens*) based on the drying technique was assessed based on the IC₅₀ value, and the lowest was oven drying at 50°C, wind drying, sun drying, and oven drying at 70°C.

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