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Physicochemical Analysis, Antioxidant Activity, and Sensory of Sungkai (*Peronema canescens* Jack.) Leaf Extract

Analisis Fisikokimia, Aktivitas Antioksidan, dan Sensoris dari Ekstrak Daun Sungkai (Peronema canescens Jack.)

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ABSTRACT

Sungkai leaves were widely used as an immunomodulatory agent during the COVID-19 pandemic. This research aimed to determine the color, phytochemical characteristics, vitamin C content, polyphenol content, antioxidant activity, and sensory of sungkai leaves extracts from various types of leaves (shoot, young, and old) as well as the condition (fresh and dry). The extraction process included boiling the sample with water at a temperature of 90^{0}C for 10 minutes. The parameters observed in this study were color, phytochemicals screening, vitamin C, polyphenols, antioxidant activity and sensory. The results showed that the extract color was influenced by the types of leaves. These included red-purple for the shot leaves and yellow for young and old leaves. The extract contained flavonoids, tannins, and saponins. The fresh leaves extract produced a higher total vitamin C, polyphenols, and antioxidant activity compared to the dry counterparts. Furthermore, the highest chemical content of sungkai leaves extract was discovered in fresh shoot leaves with total vitamin C, polyphenols, and IC50 values of 172.77 μg/ml, 280.77 μg/ml, and 28.33 μg/ml (strong antioxidant activity), respectively. Sensory characteristics of color, aroma, taste, and overall taste in each extract differed in liking score. Overall, panelists preferred the extract from dried older leaves.

INTISARI

Daun sungkai banyak digunakan sebagai immunomodulator dalam masa pandemi Covid-19. Penelitian ini bertujuan untuk menentukan karakteristik warna air rebusan, skrining fitokimia, kandungan vitamin C, kandungan polifenol, aktivitas antioksidan dan sensoris dari air rebusan daun sungkai dari berbagai jenis daun (pucuk, daun muda, dan daun tua) serta kondisi daun (segar dan kering). Daun sungkai direbus dengan air mendidih pada suhu 90^{0}C selama 10 menit. Parameter yang diamati adalah warna, skrining fitokimia, kandungan vitamin C, kandungan polifenol, aktivitas antioksidan, dan sensoris. Hasil penelitian menunjukkan bahwa warna air rebusan dipengaruhi oleh jenis daun. Air rebusan daun pucuk berwarna merah keunguan sedangkan daun muda dan tua cenderung berwarna kuning. Air rebusan daun sungkai mengandung flavonoid, tanin, dan saponin. Semakin muda daun yang digunakan, maka semakin tinggi total vitamin C dan total polifenol, serta aktivitas antioksidan semakin kuat. Kandungan vitamin C, total polifenol dan aktivitas antioksidan daun kering lebih rendah dari daun segar. Kandungan kimia air rebusan yang paling tinggi terdapat pada sampel daun pucuk segar dengan nilai total vitamin C 172,77 μg/ml; total polifenol 280,77 μg/ml; dan nilai IC50 28,33 μg/ml (aktivitas antioksidan sangat kuat). Karakteristik sensoris warna, aroma, rasa, dan keseluruhan rasa pada masing-masing sampel menghasilkan tingkat kesukaan yang berbeda-beda. Secara keseluruhan panelis menyukai sampel daun tua kering.

Introduction

Indonesia has rich biodiversity and great potential for researching herbal medicinal plants. According to the Ministry of Forestry in Badiaraja (2014), the country is home to 30,000 plant species out of a global total of 40,000, with 940 of them possessing medicinal properties. Sungkai, a medicinal plant, is commonly used by the public as an immuno-modulator during the COVID-19 pandemic. It is abundant around the University of Bengkulu and serves as an ethnobotanical source for traditional medicine (Latief et al. 2021). Yani et al. (2013) recorded the use of boiled water from sungkai leaves as a fever remedy by the Lembak Eight tribe in Bengkulu. Additionally, the Dayak tribe in East Kalimantan uses sungkai for fevers, colds, and worms, as a mouthwash to prevent toothache, and in postpartum care for women (Ningsih & Ibrahim 2013).

Extensive research has provided significant scientific insight into the properties of sungkai leaves. Ningsih and Ibrahim (2013) found that sungkai leaves exhibit antibacterial activity. Moreover, the leaf extract shows promise in enhancing health by increasing the number of leukocytes in mice, thus boosting immunity. Phytochemical analyses have revealed the presence of beneficial compounds such as steroids, triterpenoids, and phenolics. Muharni et al. (2021) have identified various biological activities in the phenolic compounds, including antioxidant, antibacterial, antidiabetic, anticancer, antihyper-tensive, and antihyperlipidemic properties. Kusriani et al. (2015) reported that sungkai leaf extract contains various secondary metabolites, including phenolic compounds, alkaloids, flavonoids, tannins, saponins, and steroids.

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The hues of sungkai leaves are significantly correlated to their age, and understanding this correlation could lead to significant advancements in plant biochemistry. The tops of newly grown leaves are purplish, while young leaves are green and gradually darken as they mature, indicating different compositions at each stage of growth. According to Winkel-Shirley (Badiaraja 2014), flavonoids in young leaves are primarily responsible for the formation of the purple pigment. It has also been suggested that the process of drying before boiling can influence the characteristics and contents of sungkai leaves. Widarta & Wiadnyani (2019) have reported that the aging and drying process significantly influences the bioactive compounds and antioxidants contained in the leaves. Traditionally, sungkai leaves are consumed by boiling them in water and drinking the extract. However, information about the characteristics and contents of the extract has yet to be documented. This research aimed to analyze the color, vitamin C content, total polyphenols, antioxidant activity, and sensory of sungkai leaf extracts at various stages of maturity (shoots, young, and old) and under different conditions (fresh and dry).

Methods

Materials and Tools

The research utilized sungkai leaves (*Peronema canescens* Jack) along with various materials such as water, ammonia, sulfuric acid, *Mayer's reagent*, magnesium powder, concentrated hydrochloric acid, acetic anhydride, concentrated sulfuric acid, methanol, $FeCl_3$, NaOH, DPPH, starch, standard iodine, standard solution of gallic acid, *folin-ciocalteu*, and Na_2CO_3. The equipment involved laboratory glassware, a YSD-UNIB 12 solar dryer, thermometers, water baths, a UV-VIS spectrophotometer (Thermo Scientific Genesys IOS), and an Android device.

Research methods

The study applied a Completely Randomized Design (CRD) with two factors. The first factor involved the positions of the leaves: shoot, young, and old. The second factor was the pretreatment of fresh (S) and dry (K) boiling leaves, with three repetitions (Table 1).

Fresh Leaves Extraction

The fresh leaves were carefully weighed, and 25 g of each sample was then boiled in 1000 mL of water. Following this, the sungkai leaves were added to the boiling water and left to simmer for 15 minutes at a temperature ranging between 70℃ to 75℃ until a color change was observed. The water was then filtered and utilized as a fresh, boiled Sungkai leaf water sample.

Dry Leaves Extraction

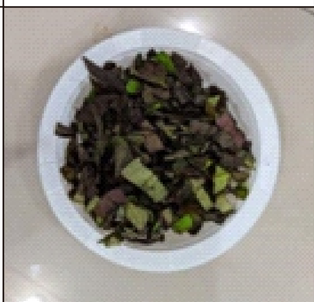
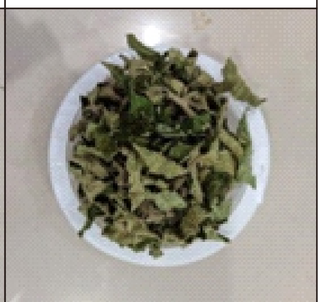
At 35℃ to 45℃, 25 g of fresh sungkai leaves were dried using a YSD-UNIB 12 solar dryer until the moisture content reached ≤10%. Subsequently, approximately 1000 milliliters of water were boiled, then the heat was reduced between 70℃ and 75℃. The sungkai leaves were added to the water and simmer until the color changed. Afterward, the mixture was filtered.

Research parameters

Color Measurement

Color measurements were carried out on sungkai leaf extract using the Color Grab application. The method was straightforward and involved placing the sample in a clear bottle and capturing an image from a

Table 1. Sungkai leaves samples

Sungkai leaf			
	Shoot	Young	Old
Fresh			
	Shoot	Young	Old
Dry			
	Shoot	Young	Old

distance of approximately 20 cm with adequate lighting using a smartphone camera. Color Grab then detected the color of the digital image and expressed it in terms of RGB values, indicating the color intensity in the red (R), green (G), and blue (B) space.

Phytochemical Screening

Phytochemical compound screening was conducted by qualitatively testing sungkai boiled leaves water samples with the color reactor to identify the secondary metabolites (Vifta & Advistasari 2018). The bioactive compounds were tested for alkaloids, flavonoids, steroids/terpenoids, tannins, and saponins.

Alkaloids

Approximately 2 ml of $CHCl_3$ and 5 ml of 10% CH_4OH were mixed with 4 ml of sungkai leaf extract. Subsequently, ten drops of 2 M H_2SO_4 were added to clarify the formation of 2 phases. After the upper phase was formed, *Mayer's reagent* was added, and the formation of a red precipitate indicated the presence of alkaloids in the sample (Rumagit et al. 2015).

Flavonoids

Sungkai leaf extract (1 ml) was mixed with an adequate amount of Mg powder and ten drops of concentrated HCl. The formation of an orange, reddish black, or yellow color indicated the presence of flavonoids in the sample (Rumagit et al. 2015).

Steroids and Triterpenoids

The procedure involved adding 1 ml of sungkai leaf extract to two drops of CH_3COOH anhydride and two drops of concentrated HCl. The formation of a green or blue color confirmed the presence of steroids in the positive sample, while orange or purple indicated triterpenoids (Puspitasari 2018).

Tannin

Approximately 1 ml of sungkai leaf extract was combined with 2 ml of methanol before undergoing filtration. Subsequently, 2 drops of $FeCl_3$ were introduced into the mixture. The presence of tannins was identified through the observation of color formation, which manifested as a chocolate-reddish or black-green hue in the sample (Puspitasari 2018).

Saponin

Approximately 2 ml of methanol was combined with sungkai leaf extract before heating to nearly boiling point. Subsequently, the sample was allowed to cool and shaken for approximately 10 seconds. Static foam manifestation indicated saponin's presence in the positive sample (Sarjono et al. 2018).

Total Vitamin C Test

The total Vitamin C content in sungkai leaf extract was quantitatively tested using a standardized iodine solution following the method outlined by 'Rahman et al. (2015). In a 125 mL Erlenmeyer flask, 10 mL of boiled water sample from sungkai leaves, 2 ml of 1% starch solution, and 20 mL of distilled water were combined. The mixture was then titrated with 0.01 N iodine solution until a dark blue color appeared. The 1 ml of 0.01 N iodine solution was equivalent to 0.88 mg of ascorbic acid.

Total Polyphenol Content Test

The total polyphenol content was assessed using the *Folin-Ciocateu* method (Hartanto et al. 2018). A standard 100 ppm gallic acid solution was prepared and dissolved in concentrations of 10, 20, 30, 40, and 50 ppm. These solutions were used as standards for creating a calibration curve using a UV-Vis spectrophotometer. The 0.1 ml of 10 ppm gallic acid was mixed with 0.8 ml of 7.5% Na_2CO_3 solution and 0.1 mL of 10% *Folin-Ciocateu* reagent in an Erlenmeyer flask, stirred, and allowed to stand for 30 minutes. Absorbance was then measured using a UV-Vis spectrophotometer in the 400 to 800 nm wavelength, with the optimal wavelength being the one with the highest absorbance. The absorbance of standard solutions at the determined optimal wavelength was measured to establish an absorbance standard. The absorbance data from the standard solution measurements was then used to create a calibration curve in Microsoft Excel. Next, the absorbance of sungkai leaves boiled in water was measured at the optimal wavelength and used to determine the polyphenol content of the sample.

Antioxidant Activity Test

The antioxidant properties of boiled sungkai leaf extract were analyzed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals method (Maesaroh et al. 2018). In a volumetric flask, 4.0 mg of DPPH reagent was dissolved in 25 ml of methanol to create a solution with a concentration of 160 mg/l. The resulting solution was then stored in a dark space and protected with aluminum foil. A standard curve for DPPH was generated by diluting the 160 mg/L solution to obtain concentrations of 4, 8, 16, and 32 mg/l. The maximum wavelength of DPPH in methanol was determined by measuring the absorption of a solution using a UV-VIS spectrophotometer. Subsequently, the absorption of each concentration was measured at the maximum wavelength. The sungkai leaf extract was diluted to concentrations of 5, 10, 15, 20, and 25 mg/l. All the samples were placed into reaction tubes and incubated at ambient temperature for 30 minutes after adding the DPPH solution. The absorption was then measured with a UV-VIS spectrophotometer at the wavelength of 517 nm. Finally, the inhibitory power of each standard was expressed as % inhibition, indicating the antioxidant properties of the boiled sungkai leaf extract.

$$\%\ inhibition = \left(1 - \frac{As}{Ak}\right) \times 100\%$$

Note: Ak = control absorbance; As = sample absorbance.

Based on the data, a linear regression equation curve was plotted between the sample concentration and the percent inhibition of the sample to determine the IC 50 value. The linear regression equation obtained in the form of the equation y = a + bx, is used to find the value of lC50. The lC50 value states the concentration of the sample solution required to reduce DPPH free radicals by 50%.

Organoleptic Test

The organoleptic test followed ISO8587-2006 and involved a sensory analysis with a ranking test based on hedonic preferences. This test is determined based on the level of panelist acceptance (hedonic) of the sample. There are 6 samples of boiled sungkai leaf water served, the panelists are asked to make a ranking by sorting the samples based on their level of acceptance of the sample on each attribute, starting from the least preferred sample (ranking 1) to the most preferred sample (ranking 6). The attributes observed are appearance (color), taste, aroma, and overall assessment. The panelists involved in this test were 35 untrained panelists.

Data Analysis

The data from the color, phytochemical screening (Alkaloids, Flavonoids, Steroids, Tannin, dan Saponin), total polyphenol and antioxidant activity tests obtained were analyzed descriptively. The data from the total vitamin C test were analyzed using the 2-Way ANOVA (Analysis of Variance) data analysis test at a 5% confidence level. For the treatment that has a significant effect, then a further DMRT (Ducan's Multiple Range Test) test was carried out. While the data from the organoleptic test were analyzed using the Friedman test. The data were processed using IBM SPSS statistics 25 software.

Results and Discussion

Color

The image's color index was specified in terms of RGB (Red, Green, and Blue) values (Table 2). Different indices resulted in variations in the colors of objects, including red, yellow, purple, green, and blue. The higher and lower RGB values indicated brighter and darker objects, respectively (Bustomi & Dzulfikar 2014). The color of sungkai leaf extract was affected by the maturity and condition of the leaves. The RGB values of shoots, young, and old leaves showed an increase in red and green and a decrease in blue, respectively. The boiled fresh sungkai leaf water had a lighter color than the dried leaves. Additionally, the color contrast varied based on the maturity of the leaves. Boiling young sungkai leaves produces an orange-brown hue, while boiling old leaves produces a bright yellow color. The leaves of the shoots yielded a reddish-purple color in boiled water, indicating a higher concentration of flavonoids than the young and old leaves. Badiaraja (2014) and Vifta & Advistasari (2018) noted that the flavonoid content largely influences the presence of red/purple pigments in plants.

Phytochemical Screening

Phytochemical screening was an initial phase to gain insight into the natural ingredients by identifying the secondary metabolite compounds (Vifta & Advistasari 2018). A qualitative test with color reagent was conducted to screen sungkai leaf extract for active phytochemical compounds. The formation

Table 2. Color test results based on RGB

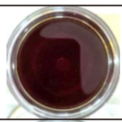
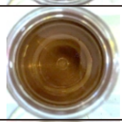
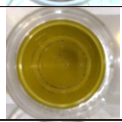
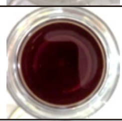
Sungkai leaf extract		Color	RGB value			Interpretation
			Red	Green	Blue	
Fresh	Shoot		90	55	59	*Dark Brown: Red*
	Young		130	88	51	*Brown: Orange*
	Old		150	122	12	*Dark Yellow: Orange*
Dry	Shoot		93	53	44	*Dark Brown: Red*
	Young		126	78	49	*Dark Brown: Orange*
	Old		149	105	16	*Dark Brown: Yellow*

Table 3. Phytochemical screening of Sungkai leaf extract

Sungkai leaves boiled water		Alkaloids	Flavonoids	Steroids	Terpenoids	Tannin	Saponin
Fresh	Shoot	-	++	-	-	++	+++
	Young	-	+	-	-	++	++
	Old	-	+	-	-	++	+
Dry	Shoot	-	+	-	-	++	++
	Young	-	+	-	-	++	++
	Old	-	-	-	-	+	+

Description: (+++) = a lot, (++) = moderate, (+) = a little

of a reddish, yellow, or orange color upon adding magnesium powder indicated the presence of flavonoids. The extract from dry old leaves exhibited a dark brown-yellow color, making it challenging to observe color changes. As a result, a qualitative test was required to determine the bioactive compound content.

This research screened alkaloids, flavonoids, steroids/terpenoids, tannins, and saponins (Table 3). The results indicated that each treatment's boiled sungkai leaves water sample exhibited positive secondary metabolites (flavonoids, tannins, and saponins). Flavonoids have been found in all parts of plants, including the leaves, flowers, and seeds, marked by red, blue, and purple pigments (Badiaraja 2014). A test was performed by adding magnesium powder and concentrated hydrochloric acid to each sample. The two substances reduced the benzopyrone core in the flavonoid structure, forming falvilium salts with a red or orange color (Vifta & Advistasari 2018). The test results indicated the presence of flavonoids in the boiled Sungkai leaf water of each sample, except for the dried old leaves. Meanwhile, the shoot contained the most of these compounds than others. According to Al-Mamary & Moussa (2016), flavonoid compounds, with various activities, act as anti-oxidants, functioning as free radical scavengers due to the presence of hydroxyl groups.

Tannin is a phenolic compound that imparts a bitter and astringent taste in plants. Additionally, it can react with and coagulate proteins or other organic compounds containing alkaloids and amino acids (Julianto 2019). Tannin analysis was carried out by introducing $FeCl_3$, which acted as a central atom source and formed a stable complex by reacting with a hydroxyl group, resulting in a green color (Souhoka et al. 2021). In the study, all samples contained tannin compounds, with the dried older samples exhibiting lower concentrations than the others. According to Sarjono et al. (2018), tannins also have the potential to be antioxidants, with efficacy depending on the presence of hydroxy and phenolic groups and the degree of hydroxylation of the aromatic ring.

Saponins, which are glycosides of sapogenin, were tested by hydrolyzing in water and shaking until a stable foam was formed (Endarini 2016).These compounds consist of polar and nonpolar groups that form micelles. The structure of the micelles results in the polar groups facing outward and the nonpolar groups facing inward (Prayoga et al. 2019).In this study, all samples contained saponin, with the highest concentration in the fresh shoot leaves.

Comparing the phytochemical screening result with previous research by Ibrahim & Kuncoro (2012), Latief et al. (2021), and Prasiwi et al. (2018), alkaloids and terpenoids/steroids were also confirmed positive due to the use of ethanol and methanol fractions as solvents. According to Endarini (2016), alkaloid and terpenoid/steroid compounds were non-polar compounds and were difficult to dissolve in water. It is important to note that the adoption of an inappropriate solvent led to the failure of bioactive compound extraction (Vifta & Advistasari 2018). Consequently, alkaloid and terpenoid/steroid compounds were not identified in this research.

Total Vitamin C

Vitamin C, or ascorbic acid, is an antioxidant commonly used to boost the immune system. It is generally non-toxic and seldom causes significant side effects when consumed (Rahman et al. 2015). Total Vitamin C was analyzed using the iodimetric titration method, with iodine as the oxidizer and starch as the indicator (Alquraisi et al. 2021). The highest vitamin C was obtained from fresh shoots as much as 172.77 mg/100 ml. In contrast, the lowest total vitamin C was recorded at 80.67 mg/100 ml from old dry leaves (Figure 1). Young leaves produce a higher total amount of vitamin C compared to mature leaves. In addition, fresh leaves contain a higher concentration of vitamin C compared to dry leaves, because the vitamin is susceptible to damage from oxygen, light, and heat during processing and storage (Techinamuti & Pratiwi 2018).

The ANOVA indicated that the maturity level and condition significantly affected total vitamin C at a level ($p<0.05$). However, the interaction did not significantly affect the total vitamin C ($p<0.05$). Subsequently, the significantly different sample data were further analyzed using DMRT (Duncan's Multiple Range Test) at a 5% significance level. The results indicated that the total vitamin C content in the shoot leaves differed significantly from that in the young and old leaves. Additionally, there was a significant difference in total vitamin C content between the fresh and dried leaves.

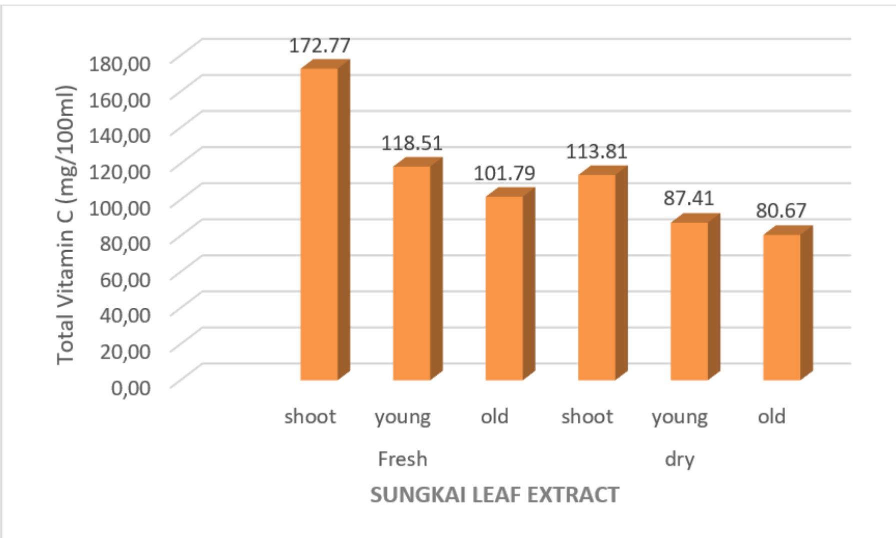


Figure 1. Total vitamin C of Sungkai leaf extract

Table 4. Relationship between leaves maturity (left) and leaves condition (right) with total vitamin C (DMRT)

Leaves Maturity	Total Vit C (mg/100 ml)		Leaves Condition	Total Vit C (mg/100 ml)	
	a	B		a	B
Shoot	143.29		Fresh	131.02	
Young		91.23	Dry		93.96
Old		102.96			

Note: The different columns at a and b show a significant difference at $p<0.05$.

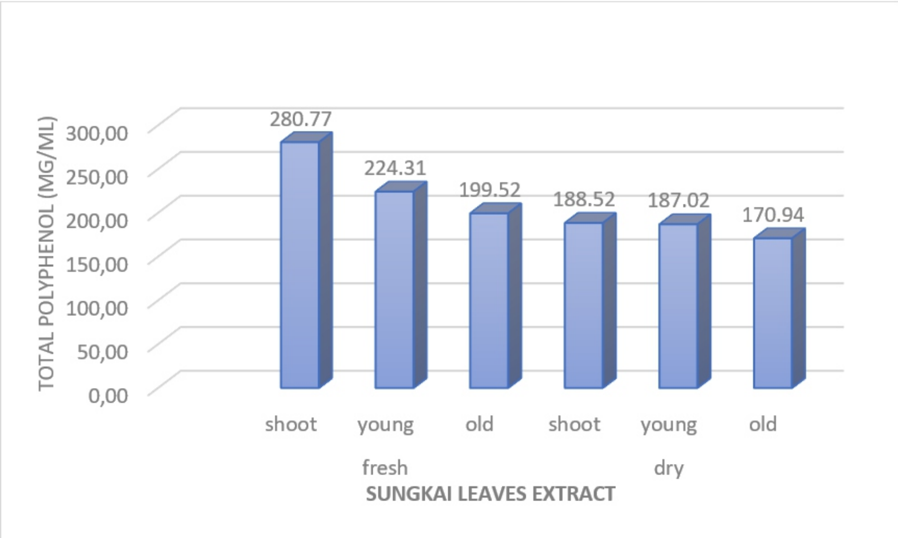


Figure 2. Total polyphenols of sungkai leaf extract

Total Polyphenols

Polyphenols, secondary metabolites derived from plants, are formed from phenol compounds containing more than one hydroxyl group and tend to be polar. These compounds act as antioxidants by donating hydroxyl atoms to free radicals (Tsao 2010).The total polyphenols of the boiled water samples ranged from 280.77 to 170.94 µg/ml (Figure 2). The highest and lowest total polyphenols were found in the fresh shoots and dried old leaves, respectively. When comparing the maturity levels, the shoot leaves contained a higher total polyphenol than the young and old leaves. These results aligned with the phytochemical results, which showed that the shoot contained more flavonoids and tannins than its young and old counterparts. Fresh leaves contained more total polyphenols than the dried samples. This result was consistent with the research conducted by Widarta & Wiyadnyani (2019), which suggested that sunlight degradation and long, intensive drying can reduce the total polyphenol content in the sample. A prolonged open drying process can lead to more significant enzymatic damage by polyphenol oxidase and intermolecular condensation reactions.

Antioxidant Activity

Antioxidants are bioactive compounds that prevent the formation of free radical reactions by acting as electron donors. They play a crucial role in protecting the body from damage caused by reactive oxygen compounds. They can help inhibit degenerative diseases such as immune disorders, cancer, diabetes, tissue inflammation, heart infarction, and premature aging (Najoan et al. 2016; Sarjono et al. 2018). The antioxidant activity value is typically determined using the DPPH method, which captures radicals with a compound exhibiting antioxidant activity.

Results of percent inhibition from sungkai leaves extract can be seen in Table 5. The percentage of inhibition and the concentration of the solution obtained from each sample were then made into a relationship curve between the percentage of antioxidant activity and the concentration of fresh (Figure 3) and dry (Figure 4) sungkai leaf extract, after which the equations of the lines were obtained (Table 6). The IC50 value can be obtained by replacing the y value with 50.

Table 5. Percent inhibition of sungkai leaves extract

Sample	Concentration	Blanko	Adsorbance Sample			Mean	% Inhibition
			1	2	3		
Fresh Shoot	5	0.376	0.066	0.081	0.081	0.076	79.787
	10		0.091	0.097	0.093	0.094	75.089
	15		0.121	0.122	0.129	0.124	67.021
	20		0.140	0.168	0.157	0.155	58.777
	25		0.160	0.171	0.170	0.167	55.585
Fresh Young	5	0.376	0.066	0.063	0.056	0.062	83.599
	10		0.071	0.076	0.072	0.073	80.585
	15		0.098	0.101	0.091	0.097	74.291
	20		0.109	0.113	0.111	0.111	70.479
	25		0.115	0.134	0.123	0.124	67.021
Fresh Old	5	0.376	0.061	0.050	0.069	0.060	84.043
	10		0.064	0.064	0.080	0.069	81.560
	15		0.080	0.081	0.091	0.084	77.660
	20		0.091	0.082	0.100	0.091	75.798
	25		0.108	0.096	0.145	0.116	69.060
Dry Shoot	5	0.376	0.056	0.066	0.066	0.063	83.333
	10		0.079	0.072	0.072	0.074	80.230
	15		0.120	0.122	0.123	0.122	67.642
	20		0.133	0.123	0.123	0.126	66.401
	25		0.147	0.150	0.123	0.140	62.766
Dry Young	5	0.376	0.049	0.052	0.052	0.051	86.436
	10		0.062	0.068	0.066	0.065	82.624
	15		0.076	0.082	0.078	0.079	79.078
	20		0.085	0.108	0.090	0.094	74.911
	25		0.103	0.110	0.097	0.103	72.518
Dry Old	5	0.376	0.049	0.052	0.052	0.051	86.436
	10		0.062	0.068	0.066	0.065	82.624
	15		0.076	0.082	0.078	0.079	79.078
	20		0.085	0.108	0.090	0.094	74.911
	25		0.103	0.110	0.097	0.103	72.518

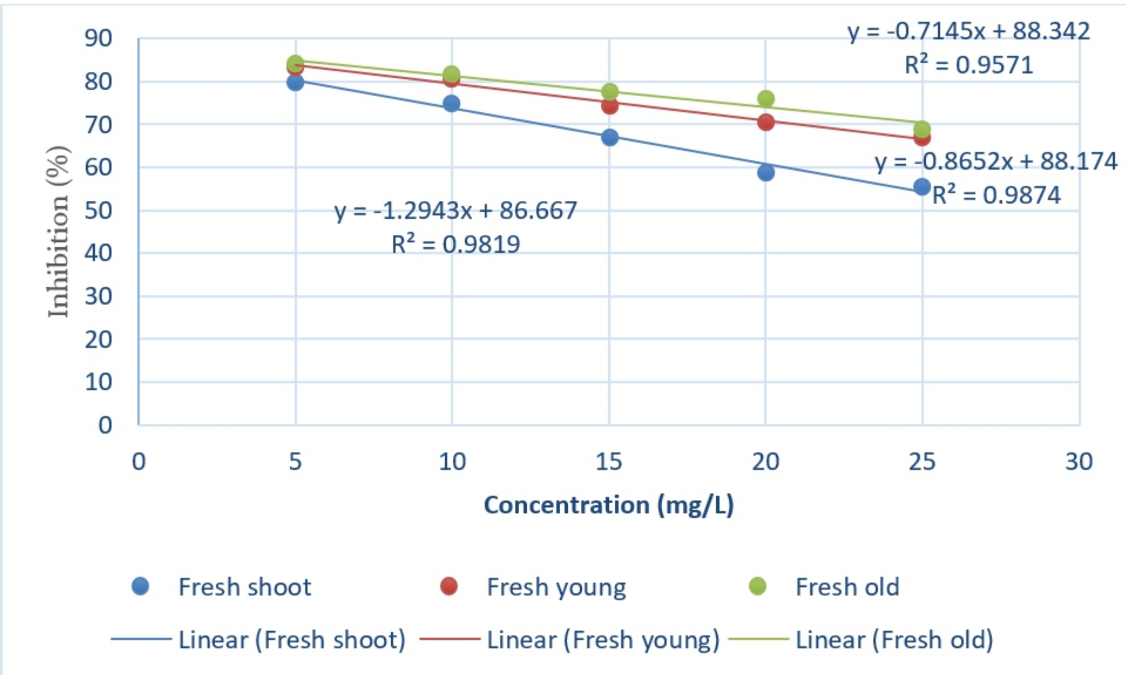


Figure 3. Correlation of concentration and inhibition percentage of fresh sungkai leaf extract

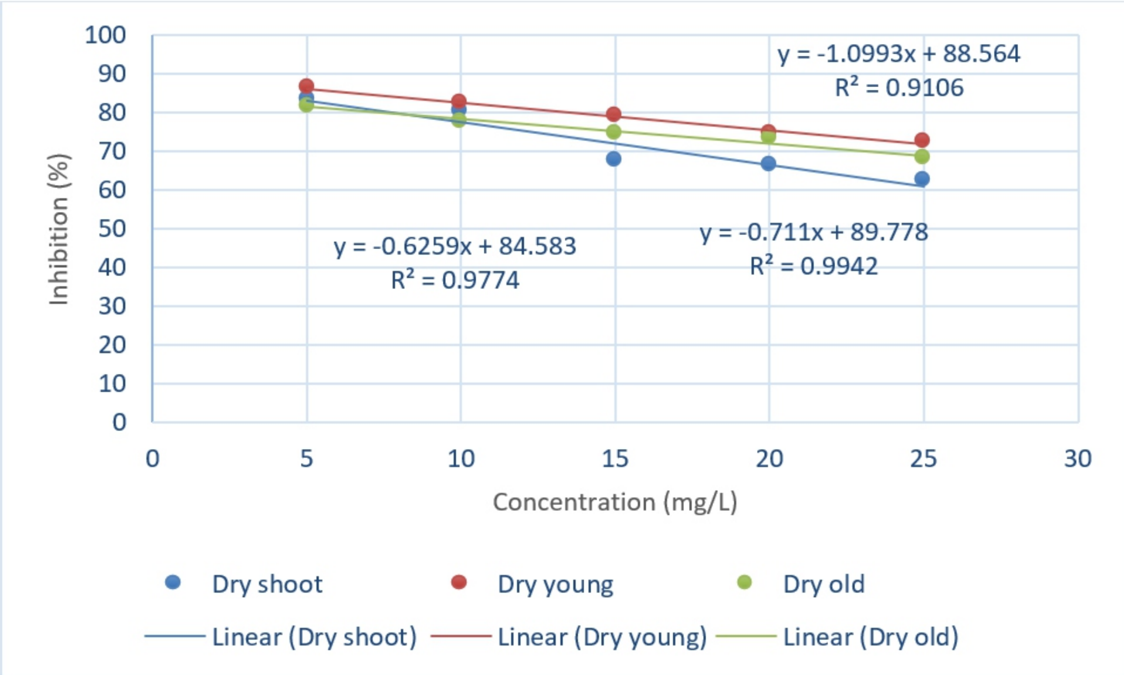


Figure 4. Correlation of concentration and inhibition percentage of dry sungkai leaf extract

Table 6. IC50 of sungkai leves extract

Sample	Linier Equation	IC50	Antioxidant Activity
Fresh shoot	y = -1.2943x + 86.667	28.33	Very strong
Fresh young	y = -0.8652x + 88.174	44.12	Very strong
Fresh old	y = -0.7145x + 88.342	53.66	Strong
Dry shoot	y = -1.0993x + 88.564	34.90	Very strong
Dry young	y = -0.711x + 89.778	55.25	Strong
Dry old	y = -0.6259x + 84.583	59.75	Strong

The IC50 (Inhibitory Concentration) value expresses the free radical scavenging activity. The highest IC50 value was 59.7 µg/ml from boiled dried old leaves, while the lowest, 28.33 µg/ml, was from fresh shoot leaves (Figure 4). The higher IC50 values were inversely proportional to the antioxidant activity. A higher IC50 value was required to produce antioxidant activity and vice versa. A compound with strong antioxidant properties had an IC50 value of less than 50 parts per million (ppm). The categories for antioxidant activity were classified as strong (50-100 ppm), moderate (100-150 ppm), and weak (151-200 ppm), as by Rumagit et al. (2015). In this research, samples of fresh, fresh young, and dried shoots exhibited powerful antioxidant activity, while fresh old, dried young, and dried old leaves showed strong antioxidant activity. This finding aligned with research by Cendrowski et al. (2024), which demonstrated that total vitamin C and polyphenol values were correlated with antioxidant activity. It was observed that the higher the total vitamin C and polyphenol content, the stronger the antioxidant activity of the samples. The amount of these compounds in the extract from the shoot, young, and old leaves decreased, while the IC50 value increased for fresh and dry samples. Additionally, the shoots had the highest vitamin C and polyphenol content and the lowest IC50 value (<50 mg/l), indicating that sungkai leaves extract from sungkai shoot leaves had a powerful antioxidant.

Organoleptic Test

The attributes observed were appearance (color), taste, aroma, and overall assessment. The results of the samples are shown in Table 7.

Table 7. Average value and ranking of the Friedman's test results for organoleptic tests

Boiled sungkai leaves water		Appearance		Aroma		Taste		Overall	
		Average	Rank	Average	Rank	Average	Rank	Average	Rank
Fresh	Shoot	4.29	6	4.57	6	1.89	1	3.06	2
	Young	3.63	5	2.91	1	3.69	3	3.66	4
	Old	2.83	1	3.40	4.5	4.46	6	3.46	3
Dry	Shoot	3.51	3.5	3.34	2	2.91	2	2.66	1
	Young	3.23	2	3.37	3	3.74	4	3.74	5
	Old	3.51	3.5	3.40	4.5	4.31	5	4.43	6

Appearance

Appearance encompasses the display and color that contribute to the product's appeal. The Friedman organoleptic test resulted in the panelists' ratings for appearance ranging from 2.83 to 4.29, indicating significant differences between the samples from each treatment ($p<0.05$). The most favored sample among the panelists was the boiled fresh shoots, which exhibited a bright reddish-purple color and received an average score of 4.29. In contrast, the least favored sample was the fresh old leaves, with an average score of 2.83 and a less attractive yellow color.

Aroma

The Friedman's test for the aroma of the sungkai leaves extract by panelists varied from 2.91 to 4.57, indicating significant differences between the samples of each treatment ($p<0.05$). The most preferred sample was the boiled fresh shoots leaves water, with an average score of 4.57, while the least preferred sample was the fresh young samples, with an average score of 2.91. The aroma was challenging to distinguish due to the high similarity of all the samples.

Taste

The Friedman test for the taste of the boiled sungkai leaves extract ranged from 1.89 to 4.46, indicating significant differences between the samples of each treatment ($p<0.05$). The most favored sample was the fresh old leaves, which had a neutral taste and an average score of 4.46. On the other hand, the fresh shoot leaves sample received the lowest score of 1.89 due to its high bitterness, leaving a sticky or astringent taste after consumption. Julianto (2019) attributed the bitter and astringent tastes to the alkaloid and tannin compounds present in the plants.

Overall

The overall sensory evaluation of boiled sungkai leaves water involved assessing its appearance, aroma, and taste. The Friedman's test resulted in scores ranging from 2.66 to 4.43, indicating significant differences in the levels between the samples of each treatment ($p<0.05$). The extract from dry old leaves had the highest score, characterized by an orange-yellow color, a neutral aroma, and taste. On the other hand, extract from dried shoot leaves had the lowest score. Panelists generally favored the extract from dry old leaves due to its appealing appearance and color and its moderately aromatic and neutral taste, with no flavonoids and negligible tannins and saponins.

Conclusion

In conclusion, the color of the sungkai leaf extract was influenced by the level of maturity and condition of the leaves. Extracts from shoots, young, and old leaves resulted in reddish or purple, orange-brown, and bright yellow colors, respectively. Additionally, the color of fresh sungkai leaves was lighter than that of dried leaves. This research also identified secondary metabolite compounds such as flavonoids, tannins, and saponins. The maturity levels and conditions of the leaves significantly impacted the total vitamin C content. Older leaves exhibited lower total vitamin C, polyphenols, and antioxidant activity in boiled water, whereas fresh leaf extracts showed higher levels of these compounds. The preferences of the panelists varied based on appearance, aroma, taste, and overall assessment. The results indicated that the fresh shoot extract scored the highest in appearance and aroma, while the fresh old leaves extract scored the highest in taste. Ultimately, the dry old leaf extract received the highest score in the overall assessment.

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