

Correlating Color and Chemical Profiles of *Sterculia quadrifida* Barks for Herbal Raw Materials Quality

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ABSTRACT

The traditional use of faloak (*Sterculia quadrifida* R. Br.) stem bark as an effective treatment for various diseases has led to its widespread cultivation and collection. Therefore, this study aims to determine the correlation between the intensity and color variation of faloak stem bark with its antioxidant activity and phytochemical content using chemometrics analysis. The study procedures were carried out by collecting samples from different locations in East Nusa Tenggara, Indonesia. Stem bark intensity and color variations were then associated with chemical profiles of extract produced. Chemical profiles analyzed were Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity as measured using the DPPH, β -carotene bleaching, and CUPRAC methods. Subsequently, the data were subjected to multivariate statistical analyses utilizing Principal Component Analysis (PCA) and Cluster Analysis (CA). The results showed that color variations on faloak stem bark had no significant impact on cluster formation. CA and PCA showed grouping according to gray value representing color intensity. Meanwhile, PCA revealed significant correlations between gray value of faloak stem bark extract and TPC, antioxidant activity, and extract yield, with TFC having no association. Based on these results, color detection system could be developed to facilitate *S. quadrifida* bark collection as herbal medicine raw materials in the field.

Keywords: CA; Color detection system; Faloak (*Sterculia quadrifida* R.Br.); Multivariate statistical analysis; PCA.

INTRODUCTION

Sterculia quadrifida R.Br. is a local plant belonging to the Sterculiaceae family and is widely distributed in nature in various regions, including Indonesia, Timor Leste, Australia, and India (Akter, 2016). The plant is commonly referred to as faloak in Indonesia and is often cultivated by farmers in Timor Island, East Nusa Tenggara Province (Siswadi et al., 2013). In addition, a previous study revealed that its bark has been traditionally used for treating different health challenges in eastern Indonesia (Hertiani et al., 2017), including malaria, hepatitis, lumbago, anemia, typhus, and rheumatism (Siswadi et al., 2013). The ability of the plant is due to its natural compounds, such as flavonoids, phenolics, terpenoids, and alkaloids (Siswadi et al., 2013; Lulan et al., 2018). Faloak also has various bioactivities, including antioxidants (Saragih and Siswadi, 2019), anti-cancer, and immunomodulating activity (Hertiani et al., 2019; Winanta et al., 2019). Several studies have shown

that its hexadecanoic acid constituent has the potential to reduce serum cholesterol levels (Siswadi and Saragih, 2021). Due to the various benefits of faloak, it is considered an important product in Indonesian herbal medicine. This indicates the need to develop an appropriate and effective standardization system and a broader database to support its development.

In line with previous studies, faloak stem bark has antioxidant activity to fight free radicals and prevent damage. This is primarily due to the constituent beneficial compounds, including phenolics and flavonoids (Hertiani et al. 2017; Lulan et al. 2018). The use of an effective raw materials quality assurance system has been reported to help in improving the antioxidant activity and achieving maximum extraction results. Consequently, various efforts have been made to use digital codes from gray value as well as Red, Green, and Blue value (RGB) for color detection. In this context, a previous study revealed that the quality of raw materials can be determined using the correlation between antioxidant activity, flavonoids, and phenolics with stem bark color.

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The phytochemical content of harvested plant parts depends on harvest time/season and practices (Peschel et al., 2013). According to Siswadi et al. (2021), stem bark typically has higher flavonoids concentration compared with leaves or branches. Bark's diameter indicates a plant's age and has a direct relationship with phytochemical content. Another study revealed that the size of a tree has a positive correction with the tannin content, while plant color is largely dependent on environmental conditions, active compounds, and tree diameter. For example, the presence of some flavonoids has the ability to impact yellow coloration (Zvavamwe et al. 2016).

According to previous studies, standardization of raw materials for faloak stem bark is often carried out with grouping using multivariate statistical analysis. The objective of this method is to evaluate the profiles of flavonoids, phenolics, and antioxidant activity based on stem diameters and various heights at the growing sites. Consequently, analytical chemistry data assessment can be carried out using chemometrics (Widodo et al., 2019). A previous study revealed that the use of the method facilitated the categorization and differentiation of plant fingerprints, leading to rapid outcomes during medicinal plant consistency assessment. Principal Component Analysis (PCA), Discriminant Analysis, K-Nearest Neighbor Method, and Cluster Analysis (CA) have also been reported to have the potential to recognize samples patterns (Miller and Miller, 2005). In a recent report, phenolic-flavonoid content and antioxidant activity were used along with chemometrics to classify medicinal plant samples (Widodo et al., 2019), while PCA and CA were combined for differentiation purposes (Sarbu et al. 2012; Granato et al. 2018). Based on these findings, it is essential to develop an effective harvesting method to prevent the collection of herbal medicine raw materials with poor quality. Therefore, this study aims to determine the correlation between the intensity and color variation of faloak stem bark with its antioxidant activity and phytochemical content using chemometrics analysis. The results are expected to lead to the development of intelligent color identification as a component of a standardizing system for bark harvesting.

MATERIALS AND METHODS

Materials

The study samples comprised faloak trees obtained from Timor Island, East Nusa Tenggara Province of Indonesia. To harvest the samples, the criteria included that no debarking had taken place and the stem was located approximately 1m above

the ground surface. Faloak trees from 3 diameter classes (15cm; 15-30cm and >30cm) were obtained at different altitudes of 300-600-900 and >900 metres asl.

During the process, pulverization of the collected samples was carried out, followed by classification based on altitude strata and diameter class. Subsequently, faloak trees were chopped into pieces of 0.5-1 cm size to facilitate extraction. The stem bark obtained was ground and sieved through a 40-mesh sieve (Siswadi et al., 2021).

Total Flavonoid Content (TFC)

In this study, concentrated extract was obtained by placing ± 50 g pulverized bark in 30 mL of 96% ethanol for 48 hours, followed by filtration and evaporation. Subsequently, AlCl_3 reagent was used to determine flavonoids content of the samples (Ordonez et al., 2006), and quercetin served as a standard.

Total Phenolic Content (TPC)

TPC was assessed using Folin-Ciocalteu reagent, while gallic acid served as the standard, as proposed by Siswadi et al (2021) (Table I).

DPPH Test Method

The ability of various samples to scavenge radicals was tested using DPPH (Diphenyl-1-picrylhydrazyl), and its IC_{50} values were calculated according to Saragih and Siswadi's method (2019). A total of 0.25 mL samples of various concentration series was added into a 5 mL flask; then 1 mL each of DPPH and ethanol was mixed and vortexed together before absorbance measurements at 515 nm were recorded after 30 minutes incubation time - with ethanol serving as blank while 1mL of DPPH as control (control).

$$\% \text{ RSA} = ((\text{absorbance control} - \text{absorbance sample}) / \text{absorbance control}) \times 100\%$$

Note: RSA (Radical scavenging activity)

Antioxidant Activity of Faloak Stem Bark Extract Using The β -carotene Bleaching Method

Sample powder (1 g) was extracted using 10 mL of 96% ethanol for 15 minutes. The β -carotene bleaching test method used was proposed by Karim et al. (2014), and slightly modified. A total of 4 mg of β -carotene was dissolved in 50 μL chloroform and added to 0.5 mL tween 20 and 50 μL linoleic acid. After the chloroform was evaporated, CO_2 -free water was added to 25 mL to form a homogeneous beta-carotene emulsion. Subsequently, 180 μL of beta carotene and linoleic acid emulsion was added to 20 μL of the test solution in a 96-well microtiter plate. In addition,

incubation was conducted for 20 minutes at 50°C, and 200 μ L of the solution was added to 20 μ L tested solution, followed by measurement at 450 nm.

Antioxidant Activity of Faloak Stem Bark Extract Using The CUPRAC Method

A total of 10 mL of 96% ethanol was added to 1g sample powder in a test tube and then placed in the sonicator for 30 minutes. The solution was filtered and placed in a container with a tight lid. The determination of antioxidant capacity using the CUPRAC method was modified following the technique of Apak et al. (2016). Quercetin standard solution was prepared by weighing 10.0 mg of quercetin standard and dissolving in 10 mL of methanol p.a. Determination of the antioxidant capacity (liquid extract of faloak stem bark) was carried out by reacting 0.25 mL of the sample with 0.25 mL of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1.0×10^{-2} M) reagent, 0.25 mL ammonium acetate buffer (pH 7.0), 0.25 mL of neocuproine reagent (7.5×10^{-3} M), and 0.025 mL of distilled water. Subsequently, the solution was allowed to stand according to the operating time, while the absorbance at the maximum wavelength was read with a UV-Vis spectrophotometer. The blanks used were reagents and 96% ethanol, with 2 replications for each sample solution.

Data Collection For Color of The Sample's Powder

Camera settings were set to obtain clear images to be further used by ImageJ. The brightness level of an image was influenced by shutter speed, aperture, and ISO (Figure 1). Capturing images to obtain primary data was carried out at night for 4 consecutive hours from 20.00 WIB to 24.00 WIB in a dark and closed room using only LED lighting, while the primary lighting in the shooting room was turned off. The open gaps of the box were covered with several layers of black cloth to ensure light did not escape. Each image was accompanied by 3 replications, hence, each sample was captured 4 times. Subsequently, the data was analyzed using ImageJ to obtain gray value.

Data Analysis

The data obtained in this study were processed using the PCA and CA methods with Minitab 19 software. In addition, the component variables comprised gray value data of faloak stem bark powder, phenolics content, flavonoids content, antioxidant activity using the DPPH method, bleaching of β -carotene, and CUPRAC, and the extract yield. The analyses aimed to produce score plots, loading plots, biplots, and dendograms from the data. The score plots were

produced to observe the direct correlation between color intensity and each phenolic contents, flavonoids content, antioxidant activity using the DPPH method, β -carotene bleaching, and CUPRAC, and the quantity of yield.

RESULTS

Multivariate Analysis Using PCA and CA

Data from variable components comprised gray value, TPC, total flavonoids content, the antioxidant activity of faloak stem bark extract using various methods (DPPH, β -carotene blanching, CUPRAC), yield data, and grouping using color variations. In addition, stem diameter and growth altitude were analyzed using PCA and CA, as shown in Tables I and II.

The DPPH and CUPRAC data were obtained from Pratiwi et al. (2017). The β -carotene data were sourced from Pamungkas et al. (2017), and the yield data were provided by Siswadi et al. (2015).

Correlation coefficient matrix of all measured variables was presented in Table III. Gray value had a positive correlation with yield, TPC, and CUPRAC, while it negatively correlated with β -carotene and showed no association with TFC.

Figure 2 showed 3 main clusters in the score plot graph based on the gray value, TPC, TFC variables, antioxidant activity (DPPH, β -carotene bleaching, and CUPRAC), and extract yield. In addition, color of stem bark variations was represented by different colors of the numbered sample (orange and brown). Cluster 1 consisted of 7 data, which were samples 5, 14, 13, 35, 4, 15, and 7, while cluster 2 comprised 13 data, including samples 36, 9, 24, 32, 22, 17, 31, 33, 18, 26, 6, 34, 23.

The loading plot (Figure 3) obtained from the PCA analysis formed a vector representing each variable in the data and placed each variable according to its load or contribution to PC1 and PC2 to illustrate their correlation. The angle created between 2 variables could be used to determine their correlation. Angle less than 90° showed that the variables were positively correlated. Meanwhile, variable angle larger or closer to 180° showed that the variables were negatively correlated. Variables were not correlated when the angle formed was close to 90° (Widodo et al., 2019).

Grouping using CA was conducted to group samples based on similarity according to the variables used in the analysis into a cluster (Miller and Miller, 2005). Figure 4 was a dendrogram of grouping using CA based on the Euclidean distance measured according to Complete Linkage. Grouping was carried out at the similarity level and

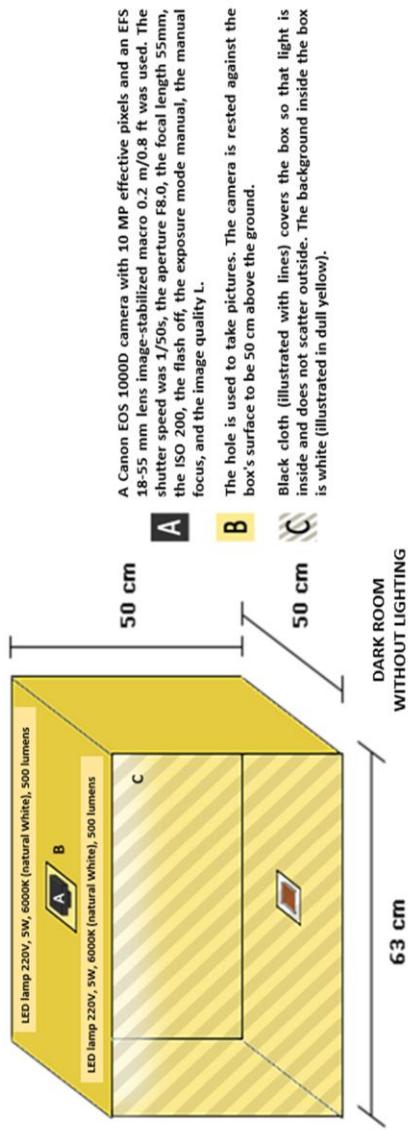
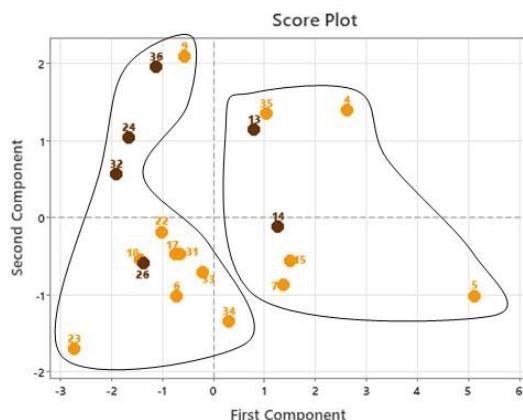


Figure 1. Method for collecting color data for stem bark samples

Table I. Data processed for the multivariate analyses covering Gray value, TPC, DPPH, β -carotene, CUPRAC, and Extracts' Yield

SC	Gray Value	TPC (mg/g)	Antioxidant activity						Antioxidant activity				
			IC ₅₀ DPPH (μ g/ml)	IC ₅₀ β -carotene (μ g/ml)	CUPRAC (μ mol/g)	Yield	SC	Gray Value	TPC (mg/g)	IC ₅₀ DPPH (μ g/ml)	IC ₅₀ β -carotene (μ g/ml)	CUPRAC (μ mol/g)	Yield
4	93240	187.00	137.28	1754.37	3775.21	8.18	22	86110	153.00	160.62	44980.71	1555.16	3.82
5	95950	344.33	84.07	1174.78	6227.76	8.30	23	88210	46.00	415.40	71936.58	957.30	5.96
6	85030	102.00	147.91	35375.82	2779.95	3.30	24	83150	59.00	275.73	16513.55	2029.66	3.08
7	88190	213.00	131.29	27910.34	3579.48	6.32	26	90290	81.50	176.07	26098.77	619.81	1.86
9	88210	105.00	29.29	17151.08	1966.79	5.72	31	80420	137.00	100.42	28599.80	1964.41	4.90
13	93570	220.00	146.63	21094.57	2267.50	3.34	32	85360	63.33	160.76	32988.18	823.25	2.58
14	93440	258.00	127.03	2084.88	2334.52	3.70	33	87830	139.00	137.88	37002.87	2141.76	4.78
15	88110	245.00	143.83	12486.83	3352.72	5.08	34	89860	208.00	138.06	42300.45	2379.00	4.32
17	84570	245.00	115.18	42726.19	912.22	3.43	35	87880	257.00	95.74	14172.67	2690.39	3.74
18	84000	166.00	160.62	52584.78	1211.74	3.92	36	88580	101.67	279.42	14072.23	1839.26	2.34

Note: SC = Sample Code



Note: Samples were color-coded by stem bark variations: brown and orange.

Figure 2. Score Plot Clustering of Gray Value, TPC, TFC, and Antioxidant Activity (Table I)

Table II. Data processed for the multivariate analyses covering diameter and growth altitude as well as RGB, HEX, and color code data based on Encycolorpedia

Sample code	Diameter (cm)	Height (m)	RGB (average)	HEX	Color Shade
4			130,91,58	825B3A	orange (medium dark)
5	15-30		136, 94, 58	885E3A	orange (medium dark)
6		<300	119, 82, 53	775235	orange (medium dark)
7			122, 86, 55	7A5637	orange (medium dark)
9	>30		123, 85, 55	7B5537	orange (medium dark)
13			127, 94, 59	7F5E3B	brown (medium dark)
14	15-30		128, 94, 57	805E39	brown (medium dark)
15		>300-600	122, 87, 54	7A5736	orange (medium dark)
17			116, 83, 53	745335	orange (medium dark)
18	>30		116, 83, 54	745336	orange (medium dark)
22			118, 85, 54	765536	orange (medium dark)
23	15-30		123, 87, 54	7B5736	orange (medium dark)
24		>600-900	114, 83, 51	725333	brown (medium dark)
26	>30		122, 91, 56	7A5B38	brown (medium dark)
31			111, 79, 49	6F4F31	orange (medium dark)
32	15-30		116, 85, 54	745536	brown (medium dark)
33			122, 86, 54	7A5636	orange (medium dark)
34		>900	124, 89, 55	7C5937	orange (medium dark)
35	>30		119, 87, 56	775738	orange (medium dark)
36			122, 89, 54	7A5936	brown (medium dark)

2 main clusters were formed. The first cluster (samples 5, 14, 13, 35, and 4) had a similarity level of 36.50, while the second cluster (samples 36, 9, 24, 32, 22, 17, 31, 33, 18, 26, 6, 34, 7, 15, 23) exhibited a similarity level of 31.42.

Grouping in the dendrogram according to similarities in the gray value, TPC, TFC, antioxidant activity (DPPH, β -carotene bleaching, CUPRAC), and yield did not show any correlation with faloak stem bark color variations in each group. Samples with similar stem bark color variations were not in the same cluster group. This showed that the CA

analysis did not show a relationship between faloak stem bark color variations in the pattern of grouping samples according to gray value, TPC, TFC, and antioxidant activity (DPPH, bleaching β -carotene, CUPRAC), and yield. However, clusters were formed based on the variable gray value, TPC, TFC, and antioxidant activity as measured using the DPPH, CUPRAC, and bleaching β -carotene methods and the yield amount.

Table IV showed that the medium-dark orange faloak stem bark samples tended to have phenolic content, flavonoids, antioxidant activity

Table III. Pearson Correlation matrix (containing correlation coefficient) among the parameters

	TFC	TPC	Yield	DPPH	B-carotene	CUPRAC	Gray Value
TFC	1	0,0983948	0,115466	-0,139939	0,327442	0,167581	0,0592365
TPC		1	0,411527	-0,694167	-0,403376	0,66743	0,515452
Yield			1	-0,0792319	-0,241506	0,720635	0,399274
DPPH				1	0,348702	-0,410912	-0,126914
β -carotene					1	-0,636229	-0,44375
CUPRAC						1	0,542447
Gray Value							1

Note: Moderate correlation: (0,25-0,5), good correlation (0,5-0,75), very good correlation (0,75-1), the same for negative correlation

Table IV. The average value, minimum value, and maximum value of the variables TPC, TFC, antioxidant activity (DPPH, β -carotene bleaching, CUPRAC), and total yield based on color category of the stem bark powder

Average	TPC (mg/g)	TFC (mg/100ml)	IC ₅₀ DPPH (μ g/ml)	IC ₅₀ β -carotene (ppm)	CUPRAC (μ mol/g)	Yield
Brown (6 sample)	130.58	7.97	194.27	21942.03	1652.33	2.82
Orange (6 sample)	181.95	9.08	161.83	30725.52	2535.28	5.13
Brown(min)	59.00	5.05	127.03	14072.23	619.81	1.86
Orange(min)	46.00	3.35	84.07	1174.78	912.22	3.30
Brown(max)	258.00	12.47	279.42	32988.18	2334.52	3.70
Brown(max)	344.33	12.45	415.40	71936.58	6227.76	8.30

(in the DPPH and CUPRAC methods), and a higher yield quantity compared to the medium-dark brown faloak stem bark samples. This result was showed by data on the average variable TPC, TFC, antioxidant activity (DPPH, β -carotene bleaching, CUPRAC), and the yield amount based on bark's color category.

DISCUSSION

PCA technique could be utilized for multivariate analysis to identify groupings of faloak based on similarity among objects and correlation between variables. Score plots were particularly effective at helping interpret complex datasets by grouping variables that shared characteristics together and identifying patterns or clusters. Figure 2 revealed that the distance between sample points and those belonging to the same color variation group was far. This study demonstrated that color variations of bark had no significant influence on TPC, TFC (data obtained from previous publications by Siswadi et al. 2021) or antioxidant activity measured using various methods (DPPH, β -carotene bleaching, and CUPRAC), extract yield or yield per tree. The similarities in properties and chemical composition of samples could be attributable to red, green, and blue primary color mixtures found across samples from various groups of stem bark color variations; or simply due to limited data available.

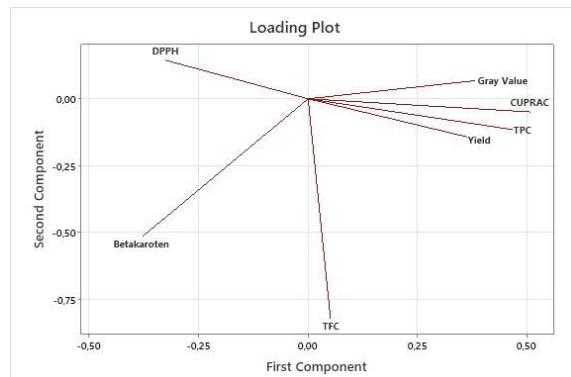
**Figure 3. Loading plot**

Figure 3 showed that gray value had an angle less than 90deg with TPC variable, demonstrating their positive relationship. Furthermore, the gray value formed an angle close to 90deg with respect to TPC variable, showing no correlation. The gray value formed an obtuse angle or more than 90deg with the DPPH variable, demonstrating its robust negative correlation. This revealed that it was directly proportional to antioxidant activity while being directly proportional to its IC₅₀ value. The gray value formed a sharp angle or less than 90deg to the CUPRAC variable, showing they displayed a strong positive correlation and thus directly correlated with antioxidant activity. The variable also had an effective positive correlation with yield variable,

showing a sharp angle (an angle less than 90deg) or strong positive correlation. This indicated that gray value and yield had a strong relationship and indicated the proportionality of faloak stem bark extract yield.

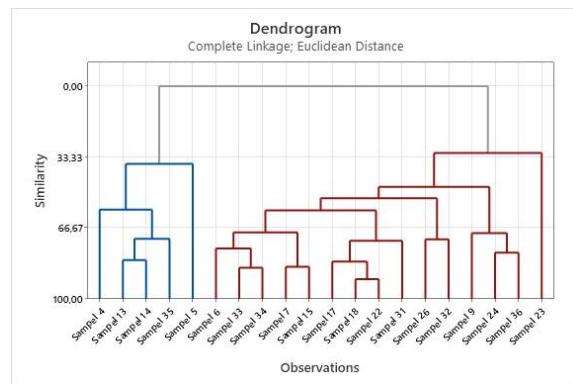


Figure 4. CA based on sample similarity

Based on the correlations shown in Table III, it can be deduced that gray value had a strong relationship with TPC, antioxidant activity as measured by DPPH, β -carotene, and CUPRAC tests, and yield of extract from faloak stem bark extract. The correlation is such that when gray value increased with each category, it increased the phenolic content, antioxidant activity, and yield of faloak stem bark extract. However, TFC did not show correlation, suggesting this did not impact flavonoid levels within faloak stem bark extract.

The results of Siswadi's study (2021) regarding the correlation between flavonoid content and variations in color of faloak stem bark showed that the sample group that had red and yellow bark had the highest flavonoid content, while the group that had orange bark had the lowest flavonoid content. However, results could not be directly compared due to differences in color grading systems and standards. Siswadi et al. (2021) used color classification red, yellowish red, reddish brown, brown, strong brown, dark yellowish brown, and yellowish brown, which referred to the Munsell color chart classification system. Meanwhile, in this study, a simple classification was used according to RGB and hexadecimal data which referred to the Encycolorpedia website color classification system because the "orange" label on the 2 sources defined a different color.

Color data did not have a universal standard and could be subjective. The existence of color variations could be correlated with the content stored in faloak stem bark. For instance, anthraquinones and flavonoids were active compounds that contributed to giving red and

yellow colors (Deveoğlu & Karadağ, 2019), while the presence of tannin compounds contributed to giving a brown color, so trees with brown, red, or yellow stems generally produced many tannin compounds (Malviya and Mahajan, 2013). Based on Siswadi's observations (2021), generally, the intensity of the red color of faloak stem bark was directly proportional to the circumference of the stem diameter. However, faloak with stem diameter class <15 cm living in karst areas also had red stem. The trees found were observed to be old, making environmental factors a growth-inhibiting factor so the tree had a small diameter.

Faloak found in thick solum soil or growing close to waterways had a diameter of >30 cm and exhibited a yellowish bark color. This confirmed that the tree diameter did not correlate with bark color. However, differences in soil conditions at a particular growth altitude location could affect a plant's compound content and color. It was also possible that color did not correlate with TFC because faloak contained colorless flavonoid compounds.

Faloak sampling locations were scattered in several locations with different growth altitudes. The type of soil for each location was also different, these differences could cause significant differences in chemical content of the soil and chemical compounds produced by plants. Siswadi et al. (2021) reported that the East Nusa Tenggara region had a semi-arid climate with a longer dry period and only had a rainy season for 4 months, which made the soil structure capable of binding enough water because the water content in the soil was relatively small. Clay-textured soils with more electrical charge and micro-pores had a higher ability to hold water and nutrients. The small surface area and the many cavities in the sandy soil structure made it difficult for the soil to store water as well as nutrients and easily evaporate. The higher the place, the lower the temperature of the atmosphere and soil. The ability of plants to absorb nutrients and water decreased because the physiological reactions of the root system in absorbing nutrients and water decreased in low-temperature soils.

Chemical content of the soil at faloak stem bark sampling location could be seen in the study of Siswadi et al., 2021. Nutrients absorbed by plants had their roles and different functions. Nitrogen (N) played an important role as a constituent in many critical organic compounds such as proteins, enzymes, and chlorophyll. Phosphorus (P) functioned in transferring energy in plants, aiding metabolized carbohydrates and proteins, and transporting carbohydrates in leaf cells. Carbon (C) was the essential molecular

component of carbohydrates, proteins, lipids, and nucleic acids in plants and played a role in the process of photosynthesis. Potassium (K) was a cofactor and activator in the enzymes of carbohydrate and protein metabolism and helped in regulating osmotic pressure and ion balance in plants. Soils at an altitude of <300 masl had the highest dominance of nutrients compared to other altitude classes and showed a relatively high soil moisture value. The pH level of 6.65 in this stratum also allowed many nutrients. Soils with a pH of 5.8-6.8 were estimated to contain higher levels of manganese, boron, copper, zinc, and nitrogen as well as potassium and sulfur (Kurniawan and Parikesit, 2008). Soil nutrient content influenced the formation of secondary metabolites, so differences in nutrient content could affect the produced metabolites.

Stem Bark Color Detection System Design

The purpose of designing a stem bark color detection system was to create a component detection system based on simplex sample color data. This could simplify raw materials selection process to ensure that raw materials obtained at the supply harvest stage were high quality. According to the PCA and CA analysis results, the samples' color variations did not significantly affect the variables and the extracts' yield. However, when the variable values were averaged as in Table IV, each variable was compared to the gray value based on color grouping. In this study, it could be concluded that the sample data in the medium dark orange color group had TPC, TFC, and antioxidant activity (DPPH values, β -carotene blanching, CUPRAC) and a higher yield compared to the samples in the medium dark brown color group. The results also showed that the gray value correlated with the TPC variable, antioxidant activity, and yield, suggesting that the darker color intensity, the higher the phenolic content, antioxidant activity, and yield of the sample bark.

A more advanced stem skin color detection system design could be obtained using an RGB system that used 3 primary colors simultaneously. As the gray value contained only light and dark color intensities, RGB also contained color intensity data for each primary color, namely red, green, and blue. Correlating color data with the component variables to be seen could be more complex. However, when this system was implemented successfully, grouping samples based on color variations was no longer necessary as the system could automatically analyze color composition.

When using the interpretation method through gray values, RGB, or others, creating an online and offline database that integrated the component variable data was still necessary. Color detection systems depended heavily on lighting, the number of pixels, and the sophistication of the processing technology used. The hope was that a portable detection system could be produced that was conveyable to facilitate the sorting of raw materials in the field with accurate, fast, reliable, and reproducible results.

In conclusion, the gray value as a parameter of intensity or color density of faloak stem bark had a positive correlation with phenolic content, antioxidant activity testing using the CUPRAC method, and yield amount. This negatively correlated with the antioxidant activity test method using the DPPH method and β -carotene bleaching and did not correlate with the flavonoid content of faloak stem bark extract. The lack of correlation between the antioxidant activity measured by the CUPRAC and the DPPH method or β -carotene bleaching assay could be attributed to several factors. Each method assessed antioxidant activity through different mechanisms and targets different types of antioxidants. Consequently, the discrepancies in correlation could arise from variations in the types and concentrations of antioxidants present in the samples, as different antioxidants exhibited varying activities depending on the assay method used. The detection system for stem bark components based on color could be made using intensity as the primary detection system (gray value or RGB) instead of color grouping. In addition, color grouping could be used as a subcategory in a stem bark component detection system that used gray value as the primary detection system to obtain more specific results.

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REFERENCES

- Akter K, Barnes EC, Brophy JJ, Harrington D, Vemulpad SR, Jamie JF. (2016). Phytochemical profile and antibacterial and antioxidant activities of medicinal plants used by aboriginal people of New South Wales, Australia. Evid-Based

- Complementary Altern Med 2016 (1): 1-14.
- Apak R, Ozyurek M, Guclu K, Capanoglu E. (2016). Antioxidant activity/capacity measurement Reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/chemometric assays. J. Agric. Food Chem 64 (5): 1046-1070. DOI: 10.1021/acs.jafc.5b04744
- Deveoğlu O, Karadağ RA. 2019. Review on the flavonoids - A dye source. Int J Adv Eng pureSc 31 (3): 188-200. DOI: 10.7240/jeps.47651.
- Granato D, Santos JS, Escher GB, Ferreira BL, Maggio RM. (2018). Use of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. Trends Food Sci Technol 72: 83-90. DOI: <https://doi.org/10.1016/j.tifs.2017.12.006>.
- Hertiani T, Winanta A, Sasikirana W, Munawaroh R, Setyowati EP, Murwanti R. (2019). *In vitro* immunomodulatory and cytotoxic potentials of faloak (*Sterculia quadrifida* R.Br.) bark. Online J Biol Sci 19: 222-231. DOI: 10.3844/ojbsci.2019.222.231.
- Hertiani T, Permanasari PRISCI, Mashar H, Siswadi S. (2017). Preliminary study on faloak bark potency for prevention of microbial infection. In Proceeding Conf Heal Manag Post Disaster Recover, 22nd May 2017. DOI: 10.5281/zenodo.3568453.
- Hertiani, T., Rumondang, A. (2022). Upaya penjaminan kualitas bahan baku herbal menggunakan variasi warna kulit batang faloak (*Sterculia quadrifida* R. Br.) dan kandungan fitokimianya [Undergraduate thesis, Universitas Gadjah Mada].
- Iannucci L. (2021). Chemometrics for data interpretation: Application of Principal Components Analysis (PCA) to multivariate spectroscopic measurements. IEEE Instrumentation & Measurement Magazin 24(4): 42-8. DOI: 10.1109/MIM.2021.9448250.
- Karim AA, Azlan A, Ismail A, Hashim P, Abd Gani SS, Zainudin BH, Abdullah NA. (2014). Composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activity of cocoa pod extract. BMC Complement Altern Med 14 (1): 381. DOI: 10.1186/1472-6882-14-381.
- Kurniawan A, Parikesit P. (2008). Tree species distribution along the environmental gradients in pananjung pangandaran nature reserve, west java. Biodiversitas 9 (4): 275-279. DOI: 10.13057/biodiv/d090407.
- Lulan TYK, Fatmawati S, Santoso M, Ersam T. (2018). Antioxidant capacity of some selected medicinal plants in East Nusa Tenggara, Indonesia: The potential of *Sterculia quadrifida* R. Br. Free radic antioxid 8 (2): 96-101. DOI: 10.5530/fra.2018.1.15.
- Malviya N, Mahajan S. (2013). Preliminary phytochemical screening of bark of some important trees of college campus with special reference to tannin, glycoside and their medicinal properties. Int res j environ Sci 2 (11): 13-17.
- Miller JC, Miller JN. (2005). Statistics and chemometrics for analytical chemistry, Fifth Edition. Pearson education, England.
- Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. (2006). 'Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts'. Food Chem. 97(3): 452-458. DOI: 10.1016/j.foodchem.2005.05.024.
- Pamungkas, A. W., Hertiani, T., & Murti, Y. B. (2017). Analisis pengaruh ketinggian tempat tumbuh terhadap aktivitas antioksidan ekstrak etanolik kulit batang faloak (*Sterculia quadrifida*) menggunakan metode pemucatan beta karoten [Undergraduate thesis, Universitas Gadjah Mada].
- Peschel W, Prieto JM, Karkour C, Williamson EM. (2013). Effect of provenance, plant part and processing on extract profiles from cultivated European *Rhodiolarosea* L. for medicinal use. Phytochemistry 86: 92-102. DOI: 10.1016/j.phytochem.2012.10.005.
- Pratiwi, A. E., Hertiani, T., Murti, Y. B. (2017). Pengaruh ketinggian tempat tumbuh dan diameter batang faloak (*Sterculia quadrifida*, R.Br.) terhadap kandungan kimia dan aktivitas reduksi kupri-neokuproin ekstrak etanoliknya [Undergraduate thesis, Universitas Gadjah Mada].
- Saragih GS, Siswadi S. (2019). Antioxidant activity of plant parts extracts from *Sterculia quadrifida* R. Br. Asian J Pharm Clin Res 12 (7): 143-148. DOI: 10.22159/ajpcr.2019.v12i7.33261.
- Sârbu C, Nașcu-Briceiu RD, Kot-Wasik A, Gorinstein S, Wasik A, Namieśnik J. (2012). Classification and fingerprinting of kiwi and pomelo fruits by multivariate analysis of chromatographic and spectroscopic data, Food Chem 130 (4), 994-1002. DOI:

- 10.1016/j.foodchem.2011.07.120.
- Siswadi S, Saragih GS, Rianawati H. (2013). Potential distributions and utilization of faloak (*Sterculia quadrifida* R. Br. 1844) on Timor Island, East Nusa Tenggara 5 (6): 165-171 in Langi M et al. (eds.). Proc of the International Conference on Forest and Biodiversity. 5-6h July 2013, Manado, Indonesia.
- Siswadi S, Faridah E, Hertiani T. (2021). Total flavonoid content of faloak (*Sterculia quadrifida*) bark in varieties of bark colour, tree diameter and growth altitude. *J Trop For Sci* 33(3): 298-307. DOI: 10.26525/jtfs2021.33.3.298.
- Siswadi, Faridah, E., & Hertiani, T. (2015). Rendemen ekstrak dan flavonoid total kulit batang pohon faloak (*Sterculia quadrifida* R.Br.) pada beberapa kelas diameter dan strata ketinggian tempat tumbuh [Master's thesis, Universitas Gadjah Mada].
- Widodo H, Sismindari S, Asmara W, Rohman A. (2019). Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *J Appl Pharm Sci* 9(6): 099-105. DOI: 10.7324/JAPS.2019.90614.
- Winanta A, Hertiani T, Purwantiningsih, Siswadi. (2019). *In vivo* immunomodulatory activity of faloak bark extract (*Sterculia quadrifida* R. Br.). *Pak J Biol Sci* 22 (12): 590-596. DOI: 10.3923/pjbs.2019.590.596.
- Zvavamwe C, Mkandhla K, Mpofu C, Phiri V, Bgwoni F, Khonzokuhle B, Sibutha M, Tshuma J. (2016). Yellow dye extraction from *Eucalyptus grandis*bark. *Am J Eng Res* 5 (10): 10-18.