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Extraction and Utilization of Avocado (*Persea americana* Mill.) Seed Tannins as a Biomordant for Natural Dyeing of Timorese Handwoven Fabric

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Abstract: *Natural dyeing of Timorese handwoven fabrics results in less intense and less color fastness of products against washing. Using metal mordants such as alum for color fixation is not sufficiently safe for the environment. In this study, the extracted tannin of avocado seeds was applied as an alternative mordant to increase the color fastness of natural handwoven fabrics. Tannin was extracted by maceration, qualitatively and quantitatively analyzed with UV-Vis, and applied in different concentrations for handwoven fabric dyeing. The color fastness value was determined using the Staining Scale Standard. The results showed that avocado seeds contain tannins with a total tannin content of 22.75 mg/kg dry weight. The FTIR Spectra show several specific functional groups of tannin, such as C-O and -OH, compelling enough to bind dyes and fabric fibers. It was achieved that the higher the tannin concentration, the higher the color fastness of the handwoven fabrics.*

Keywords: *Avocado seeds, Biomordans, Natural dyes, Tannins, Timorese handwoven*

INTRODUCTION

Timorese handwoven fabrics dyeing process, especially in North Central Timor Regency, is carried out using synthetic and natural dyes. Synthetic dyes are more practical, available, and stable against light and pH changes than natural dyes. However, the continuous use of these dyes can lead to environmental pollution, human health problems, and natural eco-balance disturbances (Khan & Malik, 2018; Lellis et al., 2019). Natural dyes extracted from certain parts of plants, such as roots, bark, leaves, and flowers (Affat, 2021), show better biodegradability, higher environmental compatibility, and renewability potential. These dyes produce suitable woven fabrics for people with dermatological disorders and allergic reactions(Li et al., 2022). On the other hand, woven fabrics with natural dyes have less color strength and fastness against washing, sunlight, air, sweat, and moisture (Affat, 2021; Samanta, 2018). Therefore, the natural dyeing process of handwoven fabrics requires a binder compound for dyes and fabric fibers known as mordant.

Mordant forms a link between the dye and the fabric substrate through a specific reaction mechanism, increasing the dye's affinity to the fabric. Mordant consists of three types: metal, oil, and tannin (Prabhu & Bhute, 2012; Saxena & Raja, 2014). Metal mordant is a derivative mordant from metal salts such as Al2(SO4)3, SnCl2, KCrO4, CuSO4, and FeSO4 (Brahma et al., 2019). These metallic mordants are ecologically dangerous, considering the produced effluent will be directly discharged into the environment (Kant, 2012; Khan & Malik, 2018). Even though Al and Fe are relatively safer, their massive long-term use requires consideration of their adverse environmental impact (Alasfar & Isaifan, 2021; Lellis et al., 2019), besides their low availability in nature. Several researchers have developed non-toxic and eco-friendly mordants for mordanting natural dyes from tannins, known as mordants (Chakraborty, 2014; Prabhu & Teli, 2014a; Win et al., 2019). Biomordants are natural substances such as tannic acid or tannins that have affinity for textile fibers and dyes. Thus, they form a link between the fiber and dyestuff. These mordants are used as a primary mordants for cotton and cellulosic fibers as they do not have much affinity for metallic mordants(Saxena & Raja, 2014).

Tannin, a polyphenolic compound, possesses hydroxyl and carbonyl groups, which have potential as a bioburden. Tannins can form stable cross-links with different molecules such as proteins, carbohydrates, and dye molecules (De Hoyos-Martínez, Merle, Labidi, & Charrier – El Bouhtoury, 2019). Tannins have been extracted from various parts of plants and applied as a biomordan on various types of fabrics. In our previous work, using curcumin extract, tannin from tamarind seed husk waste was extracted and applied as a biomordant in natural Timorese woven dyeing. It obtained a higher color fastness (level 4 or good category) than the use of Al2(SO4)3 and FeSO4(Adu et al., 2022). Wangatia (2015) extracted tannins from mango bark to be applied in cotton fabrics dyeing using bitter leaves dyes and obtained a higher fastness value (level 3) than CuSO4 mordant. Singh & Sheikh (2020) achieved a colorfastness level of 4-5 for wool fabrics dyeing with biomordan tannins from myrobalan extract (*Terminalia chebula*) and *Kigelia Africana* dye. In the same year, Sarker, Hosne Asif, Rahman, Islam, and Rahman (2020) reported good fastness values (range 4-5) in dyeing silk fabrics using tamarind seed husk tannin as a biomordant.

In this work, we investigate the possibility of avocado seed (*Persea americana* Mill.) to be used as a biomordant in handwoven fabric dyeing. The phytochemical screening showed that the ethanol extract of avocado seeds contains flavonoids and tannins (Figueroa et al., 2018). The total tannin content of dry avocado seeds ranged from 41-117 mg/kg (Malangngi et al., 2012). The previous researchers reported tannin content in avocado seeds of 6.98, 30.96, and 328.8 mg/100 g (Rosero et al., 2019; Segovia et al., 2018). Until now, avocado seeds have only been discarded into the local environment as agricultural waste; moreover, there is no research examining the potential of avocado seeds as a biomordant. The high tannin content makes avocado seeds a potential source of tannins to be used as a biomordant in the natural dyeing of Timorese handwoven. Using biomass waste as a source of tannins is the best way to preserve the environment and food needs, considering that avocado seeds are environmentally friendly, abundant, easy to gain, and do not compete with food products. In our research, tannins were extracted and characterized from avocado seeds, followed by the determination of tannin content. Furthermore, the different concentrations of tannin were used as biomordants in the natural dyeing of Timorese woven fabrics using curcumin dye, and then the colorfastness was determined.

RESEARCH METHODS

Materials and Tools

Avocado seeds were collected from Kefamenanu local market, woven yarn (Mahkota Putih), fine muslin cloth, Folin-Ciocalteau reagent, Methanol, NaCl (Merck), $FeCl_3$ (Merck), HCl 37% (Merck), $CuSO_4.5H_2O$ (Merck), Na_2CO_3 (Merck) and distilled water.

The equipment used in this study included Genesys 10S Thermo Scientific UV-Vis Spectrophotometer, Bruker Alpha FT-IR, Cosmos CB-282 AP Blender, UN-55 Memmert Drying Oven, water bath, thermometer and a set of glassware.

Procedures

Extraction of Biomordants and Dyes

Biomordant extraction was carried out by maceration. 250 g of avocado seeds powder were weighed and dissolved in 1.25 L of 95% methanol for 24 hours and then filtered to obtain a filtrate. The filtrate was evaporated and cooled in a desiccator before further analysis. The filtrate was heated to a temperature of 100ºC for 30 minutes and allowed to cool, and then it was filtered and precipitated to be used as a biomordant. Using a UV-Vis Spectrophotometer, the precipitate was used for qualitative and quantitative tannin determination.

The dye extraction was done by weighing 6 g of clean turmeric, crushing it with a blender, and then soaking it in 300 mL of water for 30 minutes. The extract was boiled for 60 minutes; then, the solution was cooled and filtered. The filtrate was used as a natural dye

Qualitative and Quantitative Determination of Tannins

Qualitative Test (Win et al., 2019).

A qualitative test was carried out by reacting 1 mL of 0.5% avocado seed solution with various reagents, such as 5% FeCl3, 5% CuSO4.5H2O, and 2 mL of dilute HCl solution (2%). FT-IR further characterized the tannins of avocado seed to determine the tannin functional groups.

Quantitative Test (Blainski, Lopes, & De Mello, 2013)

Determination of tannin content in avocado seeds was carried out using a UV-Vis Spectrophotometer. As much as 0.1 g of tannic acid was dissolved in 50 mL of distilled water, poured into a 100 mL volumetric flask, and added with distilled water up to the mark. A serial dilution of 20, 40, 60, 80, and 100 ppm was made by putting each 1 mL dilution series into a 10 mL volumetric flask containing 7.5 mL aquabidest. 0.5 mL of Folin-Ciocalteau reagent was added to the flask and allowed for 3 minutes, and 1 mL of saturated Na2CO3 solution was added. The solution was stored in the incubator for 15 minutes. The maximum absorption wavelength was determined by measuring one standard absorption in the 400-800 nm wavelength range. Then, a standard curve was plotted by connecting the concentration of the standard solution with the absorption values. The tannin content was determined by weighing 0.5 g of macerate and dissolving in aquabidest 10 mL. A sample of 1.0 mL was poured into a 10 mL flask containing 7.5 mL of aquabidestand, and 0.5 mL of Folin-Ciocalteau reagent was added. The solution was allowed to stand for three minutes, and 1.0 mL of saturated Na2CO3 was added. The solution was incubated for 15 minutes, then the absorbance was read at the maximum wavelength.

Mordanting and Dyeing of Cotton Yarn

The mordanting technique used in this study is the pre-mordanting technique. The white cotton yarn was soaked in avocado seed solution at various concentrations of 0, 5, 15, and 25% at 95oC for 45 minutes with a ratio of biomordant solution and material of 20:1. The biomordant-coated yarn was rinsed and dried to be dyed.

The cotton yarn, fixed with tannin, was put into a 10% dye solution at room temperature, and the temperature was slowly raised to 85oC. The immersion was continued at this temperature for 60 minutes. The dyed yarn is rinsed, dried, and woven. The color characteristics of the resulting woven fabrics were documented. According to ISO 105-A03, the Staining Scale Standard determines the color fastness.

RESULTS AND DISCUSSION

Cotton yarns were dyed with curcumin and fixed with avocado seed tannins as the natural mordant. Avocado seed tannin was prepared by maceration using methanol. Maceration is a simple extraction method because it does not require temperature treatment, which has the potential to degrade the target compounds, in addition to more extended interaction between the solvent and the solute, which can enhance the extraction process. Methanol solvent is used based on its high polarity, as it has been reported as the best solvent that produces the highest yield in tannin extraction (De Hoyos-Martínez et al., 2019). Tannins are polar compounds that are well-dissolved in the polar solvent. Curcumin dye was extracted using hot water, although curcumin is a non-polar compound with a lower solubility in water than in organic solvents such as hexane, acetone, and ethanol (Le-Tan et al., 2022). Water is a common solvent affordable for weavers, and organic-solvent-free products demanded by consumers are in our consideration. The results of tannin extraction from avocado seeds and turmeric extract as natural dyes are shown in Figure 1.

Figure 1. Avocado seed powder and curcumin extract

A qualitative test using various reagents was carried out to determine the tannin type contained in avocado seeds, and the test results can be seen in Table 1. Table 1 clearly shows that avocado seeds contain tannins by producing identical colors and precipitation compared to phlobatannins or condensed tannins, as reported in a previous study (Prabhu & Teli, 2014b). The reaction between tannin and reagent will produce a greenish-black precipitate and a dark green solution color when added with dilute FeCl3, a pale green solution when added with CuSO4 solution, and a dark red solid when added with dilute HCl solution if it contains condensed tannins, and produces a blue-black precipitate if it contains hydrolyzed tannins (Prabhu & Bhute, 2012).

Table 1. Qualitative analysis of avocado seed extract

Reagent	Observations	Picture
$FeCl_3$	black precipitate	
$CuSO_4.5H_2O$	a pale green solution	

HCl	dark red precipitate	

In order to determine the specific functional groups of tannins in avocado seeds which have possibility to crosslink between fibers and dyes, it was carried out an identifications using FT-IR. Measurements were made at wave numbers 4000-500 cm^{-1}. The FT-IR Spectra of avocado seed tannin is given in Figure 2.

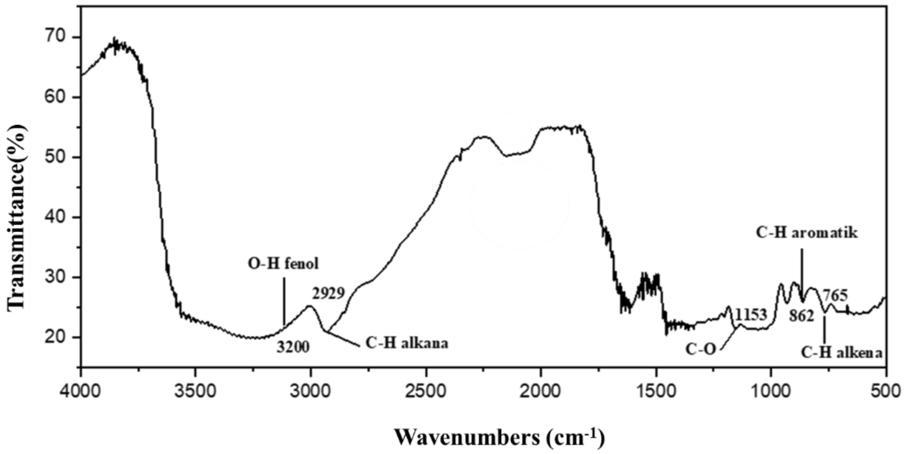

Figure 2. FTIR Spectra of avocado seed tannin

The FTIR spectra of avocado seeds show the presence of specific absorption of tannins functional groups. C-H alkene group absorption was identified in the 675-995 cm^{-1} wave number region. Aromatic C-H absorption occurs at wave numbers 690-900 cm^{-1}. The wave number 1050-1300 cm^{-1} indicates the vibration of the C-O bond from the ether group (Icha Nurfirzatulloh, 2023). At wave numbers 2850-2970 cm^{-1}, there is the absorption of the C-H alkane functional group, as previously reported, the 2950 and 2850 cm^{-1} areas are absorption areas for alkane C-H bonds (Pratini, 2017). Furthermore, there is a specific absorption of O-H phenol at wave number 3200-3600 cm^{-1}.

Table 2. Characteristics of tannin absorption in FTIR spectra

Functional Group	**Range of Absorption Area**	**Absorption Area**	**Reference** (Sahribulan, 2022)
C-H alkene	675-995	765	3010-3095 & 675-995
C-H aromatic	690-900	862	3010-3100 & 690-900
C-O	1050-1300	1153	1050-1300
C-H alkane	2850-2970	2929	2850-2970 & 1340-1470
O-H phenol	3200-3600	3200	3200-3600

Table 2 shows that avocado seeds contain several specific functional groups for tannins. Functional groups such as carbonyl and hydroxyl are reactive groups that will form covalent bonds with cellulose and curcumin dyes through a condensation reaction. Other researchers have stated that the –OH groups in tannins can form hydrogen bonds with cellulose, while the –COOH groups in tannins react with dyes that have hydroxyl groups (Prabhu & Teli, 2014b). The chemical interaction between functional groups of tannin, cotton fibers, and dye can improve the color fastness of handwoven fabrics.

Quantitative Test of Avocado Seed Tannins

A quantitative tannin test was carried out to determine the total tannin content in avocado seeds, which can act as a biomordant. Tannins are polyphenolic compounds, so their concentration in the sample was determined using the Folin-Ciocalteu reagent. The hydroxyl group in the phenolic compound reacts with the Folin-Ciocalteu reagent to form a blue molybdenum-tungsten complex whose absorption can be measured at visible light wavelengths (740 nm). The results of determining the tannin content are shown in Table 3.

Table 3. Tannin content after measured by UV-Vis spectrophotometer

Sample Absorbance	Concentration (mg/kg dry weight)
1.4373	22.75

The total tannin content in this study was 22.75 mg/kg dry weight, which is lower than previous studies (Adu et al., 2022). The low levels of obtained tannins were due to hot extraction. According to Jylhä et al. (2021), thermal treatment can reduce condensed tannin concentrations (16-28%) because tannin compounds are easily degraded at high temperatures. As mentioned in another source, tannin was degraded at a temperature of 37-170ºC (Sebestyén et al., 2019; Wang et al., 2019). Nonetheless, the total tannin in this study still makes it possible to effectively act as a biomordant to form a link between the dye and the fabric substrate in the natural dyeing of woven fabrics.

Characteristics of Timorese Handwoven Colors

Determination of the Timorese handwoven color characteristics was carried out after the mordanting process at various concentrations of avocado seed powder 0, 5, 15, and 25%. The color characteristic determined in this study was the color fastness against washing. The cotton yarns resulting from mordanting and coloring with curcumin are woven without a specific pattern into woven fabrics, as shown in Figure 3.

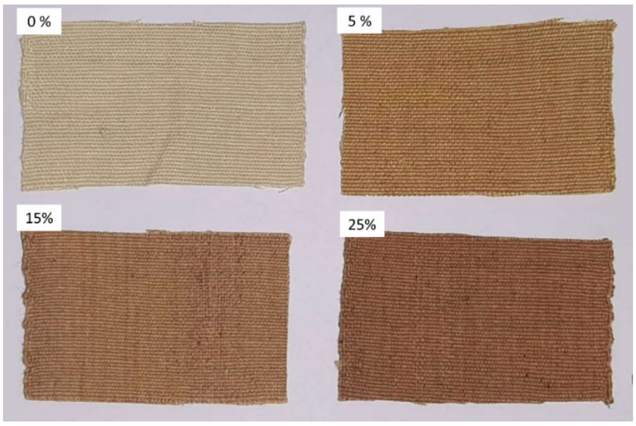

Figure 3. Color characteristics of handwoven fabrics after dyeing and mordanting

The handwoven color resulting from using avocado seed tannins as a mordant differs for each concentration variation. A yellowish-brown color is produced at concentrations of 5 and 15%, while woven with 25% tannin has a more intense reddish-brown color. This shows that the higher the tannin concentration, the more reddish brown the fabric color. The handwoven fabrics with tannin as a natural mordant give a similar color characteristic to the cotton fabrics produced using the same mordant (Sarker et al., 2020; Singh & Sheikh, 2020). The color fastness experiments of handwoven fabrics after natural dyeing are shown in Table 4.

Table 4. Color fastness of the handwoven fabrics

Sample	Color Fastness Grade	Color Fastness Category
Control	2-3	Slightly Fair
Tannin 5% + dye	2-3	Slightly Fair
Tannin 15% + dye	3-4	Good
Tannin 25% + dye	4	Very Good

The color fastness test was carried out to measure the quality of the avocado seed tannin biomordant in binding woven fabric fibers and curcumin dyes. Woven fabrics fixed with 5% tannin have lower colorfastness values compared to woven fabrics treated with 15% and 25% mordant. This result proves that the higher the biomordant concentration, the greater the number of reactive groups that form cross-links with cellulose fibers and curcumin dyes, resulting in higher color fastness. Previous researchers confirmed that the higher the concentration of the fixative compound, the better the color fastness value (Singh & Sheikh, 2020).

The interaction between tannins, cellulose, and curcumin dyes is chemical. According to Chakraborty (2014), the chemical interaction between the dye and cellulose depends on the reactive group number of the mordant. The higher the reactive group number of the mordant, the higher the affinity between the dye and cellulose. The interaction that is suspected to occur between the tannin mordant, cellulose, and curcumin dye is given in Figure 4.

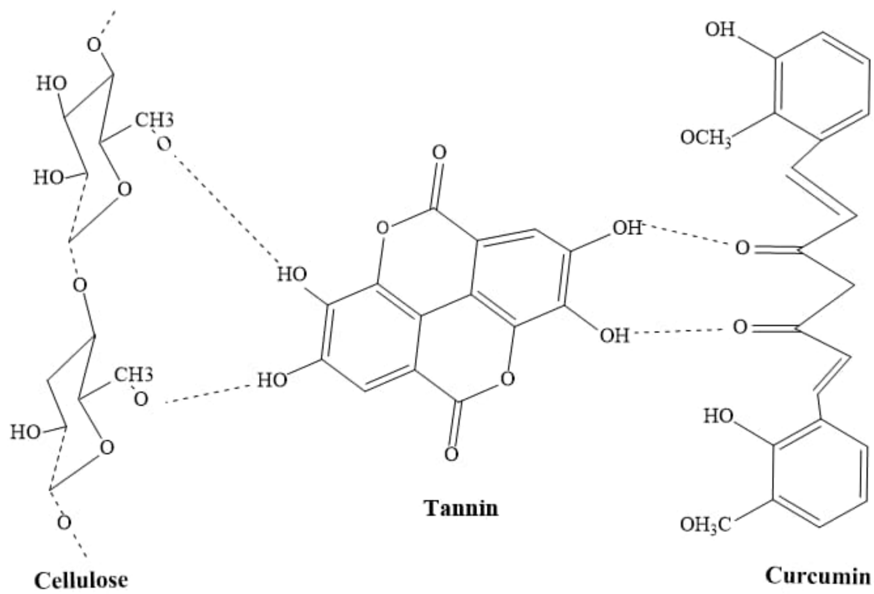

Figure 4. The proposed interaction between cellulose, natural tannin and curcumin dye

CONCLUSIONS

Avocado seed waste extracted using distilled water contains tannin compounds with a total tannin content of 22.75 mg/kg dry weight as a brown powder, which can be used as a biomordant. Identifying functional groups with FTIR spectroscopy involves several functional groups, such as C-O and -OH, compelling enough to bind dyes and fabric fibers. Applying tannins on woven fabrics produces fabrics with several colors at each concentration. It was obtained that the higher the tannin concentration, the higher the color fastness of the handwoven fabrics.

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