

## The Leaching of Natural Dyes from Avocado (*Persea americana Mill*) Seeds Using the Ultrasonic-Assisted Extraction Method and Its Application on Cellulose Fibers

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#### Abstract

The development of textiles in Indonesia grows steadily, which increases the use of synthetic dyes. Textile industry uses synthetic dyes because they are cheaper and easier to obtain. Moreover, the color availability is guaranteed and more varied. However, when introduced to the environment, these synthetic dyes have a negative impact on health. The natural dye from avocado (Persea americana Mill) seeds can become an alternative. Polyphenol compounds, such as tannins and flavonoids, are natural color sources found in avocado seeds. The extraction of natural dyes from avocado seeds can be done by using ultrasonic-assisted extraction which offer high efficiency and rapid processing. This study examined the parameters that affect the yield of dye extraction from avocado seeds, namely solvent concentration and extraction time. Qualitative analysis on the pigment content in the yield of extraction using UV-Visible Spectrophotometry and GC-MS were also done. The results indicated that the highest yield obtained from avocado seeds was 16.7% with 90 minutes extraction time using 70% ethanol solvent. Furthermore, if the dye is applied to cellulose fibers, such as the cotton cloth, the color changed depending on the fixator added. Based on the result of the UV-Visible Spectrophotometry test, the avocado seeds contain flavonoids. Meanwhile, the result of the GC-MS suggests the compound with the largest percentage detected in avocado seeds is the 13-Tetradecyno ic acid, methyl ester ( $C_{15}H_{26}O_2$ ). The compound contains a chromophore, such as a carbonyl group (C = O) which is a common feature of flavonoids.

Keywords: Avocado seeds, extraction, natural dyes, ultrasonic-assisted extraction

### 1. Introduction

Indonesia is rich in natural resources. The biodiversity of several types of plants have been widely used as raw materials for traditional medicine, handicraft, industrial products, and natural dyes. This condition allows to exploit and explore natural resources properly. One of the natural resources that can be maximized is natural dyes. The parts of plants, that can be used as coloring agents, are fruit (skin, flesh, and seeds), bark, leaves, and roots.

Textile industry in Indonesia grows steadily, causing the use of synthetic dyes to increase. Color is one of the main attractions and becomes an important criterion for public acceptance towards several products, such as textiles, cosmetics, food, and others (Rymbal et al., 2011). In general, the textile industry uses dyes from chemicals because they are cheaper and easier to obtain. Moreover, the color availability is guaranteed and more varied. However, the excessive use of synthetic dyes may cause health and environmental problems because they are carcinogenic, which can lead to skin cancer in humans and pollute the environment. It even contains heavy metals with very high intensity, which is difficult to color decompose. Therefore, the use of natural dyes for textiles is a viable alternative which is expected to have no negative impact on the environment. More attention is being paid to environmental protection, natural sustainable development lifestvle and (Zhang, et al., 2014).

Natural dyes offer an environmentally safe option for colouring of textiles materials (Rodiah, et al., 2020). Natural colors are present in a wide variety of plant sources, such as roots, seeds, leaves, fruits and flowers (Barrozo, et al., 2013). Avocado (Persea Americana Mill) is a potential plant for dyestuffs. Avocado plant is easily found in Indonesia. In 2015, avocado production in Indonesia was 585,243 tons (BPS, 2016). Furthermore, in 2016, it increased to 841,769 tons. Avocado production has increased year by year. The production of the fruit has reached an average of 243,930 tons per year (BPS, 2016). Most people use avocado only in its fruit, while other parts, such as seeds, are underutilized and often considered waste. Avocado seeds contain several secondary metabolite compounds, including flavonoids, terpenoids, quinones, saponins, tannins, monoterpenoids, and sesquiterpenoids. Cflavonoids, are the main compounds in the process of forming a color (Hakim et al., 2014).

Widarta and Arnata (2017) used leaves avocado to get bioactive components that can be utilized as a source of antioxidants. The result showed that the appropriate solvent used in obtaining the extract of avocado leaves with the highest antioxidant activity was 70% ethanol. The dye extraction from avocado seed was studied with methanol as solvent. The variations of this research were the extraction temperature and ratio of feed to solvent (Arlene et al., 2015).

A potential used of a colored avocado seed extract as natural colorant was studied. The work was to characterize the colour formation, to study the mechanism of colour formation and the colour properties of the extract (Dabas et al., 2011). The isolation and characterization of the most abundant pigment in coloured avocado seed extract, it has named perseorangin, using liquid chromatography-mass spectrometry (LC-MS), and infrared (IR) and nuclear magnetic spectroscopy resonance (NMR) were conducted (Hatzakis et al., 2019). To obtain tannins and flavonoids from plants, extraction is carried out using a solvent according to the solubility level of these compounds. Alcoholic compounds, such as ethanol, methanol, and propanol, can be used as solvents for extracting all flavonoid groups. One of the current unconventional extraction methods is ultrasonic-assisted extraction (UAE). The cell wall of the material is broken down by ultrasonic vibrations so that the contents inside can be (Mason, 1990). easily removed The advantages of using this method are greater efficiency and shorter operating time. By using the UAE method, the samples were irradiated with ultrasonic waves, thereby

accelerating the extraction process. The action of ultrasound is believed to be partially influenced by cavitation phenomena and mass transfer enhancement (Chemat et al., 2011), The extraction process was accelerated using sonication (Ramic et al. 2015). Using ultrasound-assisted extraction (UAE) is an alternative that allows the reduction of the extraction time and the volume of solvents, favouring the reduction of energy consumption during the extraction process (Garcia, et al., 2021).

The ultrasonic extraction process was influenced by several factors such as the position and depth of the vessel in the ultrasonic bath, the diameter and shape of the vessel, the frequency and input power have significant effect on the extraction yield (Kulkarni, 2014). Previous studies using ultrasonic by Both et al. (2014) make the influence of ultrasound technique on physico-chemistry characteristics during the extraction. To obtain phenolic compounds (total phenolic, total anthocyanins and condensed tannins) in a short time, the ultrasonic method can be use (Carrera, et al., 2012). The result show that good color strength is due to the unique mode of action developed by ultrasonic rays which transfer mass into solvent via irradiation of crude powder of walnut bark with solvent (Arifeen, et al., 2021). The ultrasonic bath dveing is also energy efficient and sustainable (Amutha, et al., 2020). In UAE, many factors must be considered, such as amplitude intensity, particle size, type of solvent, pH of the extraction medium, time, and temperature. Time is one of the most important factors because it affects the number of components extracted. In addition, the solvents also have an important role in the extraction process which depends on the degree of solubility.

The previous studies were more focused on the isolation and characterization of the pigment in colored avocado seed extract. The preliminary study of the dye extraction from avocado seed using ultrasonic assisted was conducted by Arlene et al. (2015). Therefore, in this study concerning the extraction of avocado (Persea americana Mill) seeds, effect temperature and ratio of feed to solvent. Based on the variable of analysis the design experiment, it can be concluded that the temperature and the feed to solvent ratio do not affect the yield, the phenolic content and the color intensity. The highest yield of the result is 22,6% obtained on 70°C temperature extraction and 1:12 the feed to solvent ratio.

In this research focuses on extraction avocado (Persea americana Mill) seeds using ultrasonic with the variables of analysis are of solvent, ethanol types solvent concentration and durating the leaching UV-Visible process. In addition, Spectrophotometric test will be carried out to analyze the chromophore and using GC-MS test to validate the presence or absence of a chromophore. Futhermore, the result of natural dye will be applied to cotton fabrics with different fixer solution.

## 2. Methodology

## 2.1. Materials

The materials of avocado seeds were obtained from fruit seller on Z.A Pagar Alam street, Bandar Lampung. For the extraction solvent, Ethanol 96% (w/w), methanol 96% (w/w), and acetone 96% (w/w) and the fixer alum and ferrous sulfate were supplied by Brataco chemicals (Lampung, Indonesia). The aparratus used in this study were 60 mesh sieve, filter paper, rotary evaporator, analytical balance, mortar and pestle, desiccator, petri dish, spatula, 500 mL beaker glass, hotplate, white cotton cloth, and a set of ultrasonicassisted extraction as showed in Figure 1.



(1), inlet water (2), outlet water (3), ultrasonic cleaning batch (4), condensor (5), timer (6), heater (7) and statif (8).

### Figure 1. Ultrasonic-assisted extraction

## 2.2. Preparation of Raw Materials

The avocado seeds were collected and then cut into small pieces. Then the seeds were dried in the sunlight. Also, the seeds were crushed until smooth and then sieved using a 60-mesh sieve. Analysis of the water content of the seed powder showed a value of 12.34% (w/w).

# 2.3. The Leaching Process on Avocado Seeds

The leaching process of avocado seeds to produce natural dyes was carried out using ultrasonic waves from the ultrasonic cleaning batch with a frequency of 80 kHz and a temperature of 70°C. The ratio between the raw material and the solvent in this avocado seed extraction was 0.125 g/mL.

The extract of avocado seed was obtained by mixing 50 grams of avocado seed powder and 400 mL of ethanol solvent in a round-bottom flask. After that, the samples were extracted using a set of equipment for the ultrasonic-assisted extraction. By using the UAE method, the samples were irradiated with ultrasonic waves, thereby accelerating the extraction process. According by Arlene et al. (2015), the influence of the temperature and the feed to solvent ratio towards the yield were obtained and the highest yield of the results was 22.6% obtained from temperature extraction of 70°C (Arlene et al., 2015).

The concentrations of the ethanol solvent used in this study were 50%, 70%, and 96% w/w. Samples were also divided into several time durations, namely 15, 30, 45, 60, 75, and 90 minutes. After conducting sonication, the filtrate and pulp of the avocado seeds were separated using filter paper. Next, the solvent was evaporated using a Buchii Rotavapor R-250 to separate it from the extract of avocado seeds. Then, it was heated until the temperature of 80°C using a hotplate to obtain dye extract in the form of a paste. The dye extract was weighed using an analytical balance, until constant weight then placed in a desiccator. The avocado seed extract was obtained.

## 2.4. The Analysis

Spectrophotometry is a tool used to measure the wavelength of natural dyes by comparing the absorbance relationship with the wavelength (nm). According to Ariviani (2010), the content of the extracted natural the absorbance relationship with the wavelength (nm). According to Ariviani (2010), the content of the extracted natural dye is in line with its absorbance value. The purpose of this absorbance test is to determine the maximum wavelength of the extracted natural dye.

To determine of maximum wavelength using UV-Visible Spectrophotometric (UV-1800 Series). The Gas Chromatograph-Mass Spectroscopy analysis was conducted using Thermo Scientific Trace 1310 Gas Chromatograph and Thermo Scientific ISQ LT Single Quadropole Mass Spectrometer. The separation was performed using HP-5MS UI capillary column with helium UHP as a carrier gas. The injector temperature was set at temperature of 260°C and split flow 50 mL/min.

## 2.5. Application on Cellulose Fibers

White cotton clothes with a size of 10 cm x 10 cm (2 pieces) were prepared. Next, the 2 q of avocado seed extract (the dye) was dissolved using 200 mL of water. Then, the white cloth was dipped in the dye for 15 minutes and then dried and hung it in the open air. This work carried out 3 times. Each cloth that has been dipped in the dye was then dipped in the solution of alum and ferrous sulfate fixation. Alum solution made from 20 g alum was dissolved in 200 mL of water. Ferrous sulphate solution made from 20 g ferrous sulphate was dissolved in 200 mL of water. Therefore, the color change visually was obtained before and after immersion into the fixation solution. The process of dyeing the fabric into the fixator solution was carried out at temperatures above 50°C.

### 3. Results and Discussion

#### 3.1. The Effect of Solvent Types on Yield

Figure 2 shows the result of leaching avocado seeds (*Persea Americana Mill*) for variations solvent with a UAE frequency of 80 Hz and a temperature of  $70^{\circ}$ C and a leaching time of 60 minutes. The solvents used were water, ethanol, methanol and acetone.



Figure 2. The effect of solvents on the yield of natural dyes

Figure 2 shows the best-obtained yield of 9.28% is natural dyes with ethanol solvent. The choice of the solvent for their extraction from plant product depends on the composition of these components (Souissi, et al., 2018). The yield obtained with ethanol solvent is higher than the other three solvents. Aquadest solvent is the most polar compound compared to other solvents, so that components that are polar are extracted and cause the total flavonoids per sample weight to be low (Septiana, 2012). This can be seen in the yield results obtained in the extraction of natural dyes from avocado seeds that the yield with distilled water is lower than some other solvents. In general, phenolic compounds are easier to extract by semi-polar organic solvents (Septiana et al., 2002).

#### 3.2 The Effect of Ethanol Solvent Concentration on Yield

The solvent used in the extraction of avocado seeds was ethanol with concentrations of 50%, 70%, and 96% (w/w). Based on Figure 3, it can be seen that the extraction of natural dyes from avocado seeds which produces the highest yield percentage was achieved by using 70% ethanol solvent.



Figure 3. The effect of ethanol concentration on yield of the natural dyes

The solvent in the UAE extraction process acted as a medium for ultrasonic wave propagation. Ultrasonic waves induced the cavitation phenomenon that played а positive role in the extraction process. The cavitation phenomenon is the formation of small bubbles in an intermediate medium, in which the bubbles will enlarge over time and eventually burst and release a large amount of energy. This energy is used for chemical processes. One of the factors affecting the formation of cavitation is the viscosity of the solvent. The viscosity of this solvent is directly proportional to the concentration of the solvent. The higher the concentration of the solvent is, the more viscous the solvent will be. Meanwhile, this viscosity is inversely proportional to the formation of cavitation, which can cause the extraction yield to decrease (Azmi et al., 2017).

From the results of this study on avocado seed extraction, there was an increase in the yield percentage of the extract from the ethanol 50% and 70%. Meanwhile, the yield percentage decreased in the ethanol 96%. The increase in extraction yield using ethanol 50% to 70% occurs because there is a water content in the solvent which increases the swelling of the avocado seeds. This increases the contact area between the plant matrix and the solvent, thereby increasing the extraction yield. In addition, the change in polarity of the solvent also affects the result of the extraction of the dye produced. For example, distilled water (0% ethanol) solvent has a large degree of polarity with a polarity index of 9.0, while 20% ethanol (80% distilled

water) has a polarity index of 8.2. This

means that distilled water has a higher

degree of polarity than ethanol. The higher

the ethanol concentration is the less polarity (Suhendra et al., 2019). This decrease in polarity can affect the solvent's ability to extract the dye. The higher the solvent concentration, the higher the extract yield obtained.

Natural dyestuff compounds are more easily to be extracted if they are compatible with the solvent used in the extraction process, according to the like-dissolved-like principle. Meanwhile, the extraction yield decreased when using ethanol 96% due to the chemical compounds found in avocado seeds which increased in terms of the solubility in the ethanol 70% and decreased after reaching ethanol 70%. The differences in ethanol concentrations can change the polarity of the solvent, thereby affecting the solubility of bioactive compounds, such as flavonoids. Similar results were also reported by Suhendra et al. (2019) that the use of the ethanol 70% was able to produce the highest total phenolic in the extraction of weeds. In addition, Widarta and Arnata (2017) also reported that the ethanol 70% was able to produce the highest total phenolic content in avocado leaves.

# 3.3. The Effect of the Duration of the Leaching Process on Yield

In this study, time variations were set at 15, 30, 45, 60, 75, and 90 minutes. Based on Figure 4, there is an increase in the extraction yield until it reaches the optimum condition when the extraction time was prolonged. In the extraction of avocado seeds, the most extracted yield was achieved in the extraction time of 90 minutes. This increase was caused by the duration of exposure to ultrasonic waves, resulting in a cavitation event in the solution material that affected the cell walls to break. The break of the cell wall caused the components inside the cell to come out mixing with the solution. This led the solvent to diffuse easily into the avocado seed material matrix. In this condition, the ability of the solvent to extract the dye increases so that the yield of the avocado seed extract is even greater (Maran, 2015).

The extraction yield increases in line with the extraction time. The extraction yield continues to increase until it reaches an equilibrium condition between the dye concentration in the avocado seed and the dye concentration in the solvent. The optimum extraction yields will be obtained if the solvent is saturated so that it is unable to dissolve the solute in the extracted avocado seeds. This condition is called an equilibrium state.



Figure 4. The effect of the duration of the leaching process on yield of natural dyes

## 3.4. The presence of chromophore groups

This UV-Visible Spectrophotometric analysis has been widely used for the determination of organic compounds. Molecular groups that can absorb light are called chromophore groups, for example C = C, C = O, N = N, N= O, etc. If any chromophore or acrosome group attached to the chromophore causes a shift in higher wavelength, the compounds analvzed UV-Visible can he by Spectrophotometry. -OH, -O, -NH<sub>2</sub>, and -OCH3 are acrosome groups that have lone color-bearing pairs and are aroups. Spectrophotometry works based on the absorption of visible ultraviolet light by molecules that absorb electromagnetic light. The maximum wavelength of the dye compounds in the extract can be measured with a wavelength of 200-700nm (Harbone, 1987).

The chromophore groups contained in the compound were characterized based on the peak conditions of dye absorption. Based on the UV-Visible spectrophotometric analysis as shown in Figure 5, two peaks were obtained, in which the peak of the band I was at the wavelength of 557.5 nm and the peak of the band II was at the wavelength of 279 nm. According to Markham (1988), the band I and band II are in the range of anthocyanidin and anthocyanin groups. Furthermore, the chromophore group bonds contained in the avocado seed extract based on its wavelength are categorized in the ketone group (C = O). The presence of the



The GC-MS analysis is an analytical method that combines chromatography and mass spectroscopy methods. Through this GC-MS analysis, the components of chemical compounds contained in natural dye extracts can be produced. Based on the results of the GC-MS analysis, it was found that there were 33 components of organic compounds in the avocado seed extract. From the test results, the area of each peak can be calculated, which is then compared with the total peak, resulting in the data of area percentage. Because the results of this test cannot determine the concentration of the compounds with certainty, the area percentage is used to indicate how much of these compounds are in the avocado seed extract sample. The followings are three compounds that have a dominant area percentage in avocado seed extract. 13-Tetradecynoic acid, and methyl ester  $(C_{15}H_{26}O_2)$  have an area percentage of 21.45% with a molecular weight of 238.37 11-(2-cyclopentane-1-yl) Methyl g/mol. undecanoate  $(C_{17}H_{30}O_2)$  has an area percentage of 15.46% with a molecular g/mol. weight of 266 2-n-Octylfuran (C<sub>12</sub>H<sub>20</sub>O) has an area percentage of 11.35% with a molecular weight of 180.29 g/mol. The compounds from the GC-MS analysis were used to confirm the presence or absence of chromophore groups from the results of the UV-Visible Spectrophotometric test.

From the results of the analysis, it was found that some of the structures of the compounds indicated that the presence of flavonoids in the avocado seed extract. It can be seen from the total of carbon atoms that make up these compounds, namely the 13-Tetradecynoic acid, methyl ester

carbonyl group is a general feature of the flavonoid compounds.

 $(C_{15}H_{26}O_2)$ , with a total of 15 carbon atoms. This is a feature of flavonoid compounds which consist of 15 carbon atoms (Markham, 1988).

In addition, there are also chromophore groups in the form of a carbonyl group (C = O) and a carboxylate group. The presence of the carbonyl group is also a general feature of flavonoid compounds (Azmi, et al., 2017).

### 3.5. Natural dyes on Cellulose Fibers

To produce a good coloring, dye needs to be given a binder or a fixator. The addition of fixation makes the rubbing fastness and washing fastness were also slighty improved due to greater (Rehman, et al., 2021). The use of this type of binder in the process of dyeing fabrics with natural dyes produces different colors. The binders used in this study were alum and ferrous sulfate.



Figure 6. The comparison before and after exposing with color on a cotton cloth



Figure 7. The color cellulose fiber result on a cotton cloth after the fixation process

From the results of experiments that have been carried out using natural dyes from avocado seeds, it was found that avocado seeds can dye cotton cloth with orange color because they contain flavonoid compounds (Fig. 6). The extract could be used in products stored at low temperatures, but a system for stabilizing the color at higher temperatures may be needed (Dabas, et al., 2011). During the dyeing process, the cotton cloth was soaked in the avocado seed extract solution. The dye was adsorbed and settled into the pores or cotton fibers. The deposition of the dye increased when the fabric that has been dyed had passed the fixation process so that the metal salts (alum and ferrous sulfate) that enter the fabric fibers can deposit the dye even more. According to Sulistiawati et al. (2017), metal ions in the fixator can bind natural dyes strongly when the temperature is above 50°C. The cloth that had been dipped in the fixator was then washed using clean water.

Fig. 7 indicates that the natural dye changes in color after the fixation process. The addition of an alum (left side) as the fixators shows that the color of the fabric has not changed significantly. Meanwhile, the addition of the ferrous sulfate (right side) as the fixator results in a darker color change. According to Azizah (2018), the use of different fixators will produce colors in different directions. She added that alum will usually give a color that matches the original color, while the ferrous sulfate will give a darker color. Based on a study conducted by Fakriyah et al. (2015), the results of adding an alum as the fixator to the natural color of turmeric extract indicate that the use of this fixator produces the highest yellow color intensity. This means that the use of an alum as the fixator is able to retain its original color. Alum (KAl(SO<sub>4</sub>)<sub>2</sub>.H<sub>2</sub>O) is a complete salt of aluminum sulfate which has the properties to clear and strengthen the color. The presence of  $AI^{3+}$  ions will react with the polyphenol compounds found in natural dyes to produce colors like the original color.

The results of applying natural dyes to fabrics showed good results, in which natural dye from avocado seed extracts can be used as an alternative textile dye, replacing synthetic dyes that pollute the environment.

### 4. Conclusion

The ethanol solvent with a concentration of 70% at 90 minutes extraction time resulted in the highest yield of 16.67%. In addition, ethanol solvent is the best solvent compared to aquadest, methanol, and acetone. Based on the UV-Visible Spectrophotometric test, two peaks were obtained, in which the peak of the band I was at the wavelength of 557.5 nm and the peak of the band II was at the wavelength of 279 nm. Band I and II are in the range of anthocyanidin and anthocyanin groups. Anthocyanidins and anthocyanins belong to the class of flavonoid compounds. Based on the GC-MS analysis, some compounds have а dominant area percentage. The structure of these

showed the presence compounds of flavonoids which consist of 15 carbon atoms and also a chromophore group, namely a carbonyl group (C = O) which is a general feature of flavonoids. The results of the application of dye from avocado seed extract to cellulose fibers showed that this dye can color cellulose fibers. For example, a cotton cloth produced an orange color. The results of the addition of an alum as the fixator indicated that the color of the fabric did not change significantly, while the addition of the ferrous sulfate as the fixator resulted in a dark gray color. Therefore, avocado seed extract can be applied as a natural dye and an alternative to textile dyes for cellulose fibers.

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