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Research Article

Comparison of Antioxidant Activity of Methanol Extract of Young and Old Leaves of Avocado (*Persea americana* Mill.)

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Abstract

Avocado plants (*Persea americana* Mill.) are widely grown in tropical and subtropical regions. Avocado leaves contain natural antioxidants for traditional medicine. This study aims to determine the total phenolic content, total flavonoids, and antioxidant activity values of young and old avocado leaves, and analyze the relationship between total phenol content and total flavonoids to the antioxidant activity value of avocado leaf methanol extract (*Persea americana* Mill.). The macerated avocado leaf extract was quantitatively tested for antioxidant activity using the DPPH method and the total phenolic and flavonoid contents were calculated using the Follin-Ciocalteu and Follin-Ciocalteu methods, respectively.The results showed that the highest total phenolic content of old avocado leaves was 1.682,27mg GAE/100g dry weight. The highest total flavonoid content of avocado old leaves was 3.858,96mg QE/100g dry weight. Antioxidant activity of methanol extract of young avocado leaves has a value of IC50 321.32 ± 80.43 ppm and avocado old leaves amounted to 288.54 ± 33.06 ppm. Total flavonoid content was more influential on antioxidant activity, with a correlation coefficient of 0.856.

Keywords: *Antioxidant Activity, extract, avocado leaves*

1. Introduction

Compounds that have the ability to prevent oxidation reactions are known as antioxidants. Antioxidant activity serves to counteract free radicals produced from the body, namely reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and peroxide radicals. Sources of free radicals can also be generated from external influences (exogenous) such as exposure to excess ultraviolet light, gamma radiation, environmental pollution, and cigarette smoke. High concentrations of free radicals will oxidize the molecules that make up cells, thus initiating the onset of diseases including cancer, hypertension, conditional disorders, and other degenerative diseases. Antioxidants work to stabilize compound reactions to prevent free radical damage (Anwar, 2014). Consumption of foods rich in antioxidant substances is one of the efforts in reducing radical exposure to the body. There are many sources of antioxidants in plants such as vegetables and fruits, including avocados. Apart from fruit, avocado leaves are also thought to have high antioxidant activity. Avocado plants (*Persea americana* Mill.), a member of the Lauraceae family, generally live in tropical to subtropical environments. Avocado production in Indonesia, according to data from the Directorate General of Horticulture and the Central Statistics Agency (2021), reached 669,260 tons. This number

has increased by 9.89% compared to last year, which was 609,049 tons. Central Java Province was recorded to produce 75,707 tons of avocado trees.

Avocado is a horticultural product with high economic value. Avocado pulp is used for consumption, while the seeds and leaves are used by the Indonesian people as alternative herbal medicines (Paramawati and Dumilah, 2016). Avocado leaves, which are seen as waste or organic waste, potentially contain antioxidants that can be used as traditional medicine such as antihypertension, rheumatism, antidiabetes, and kidney stones (Paramawati and Dumilah, 2016). The antioxidant ability of avocado leaves helps prevent oxidative stress related to various diseases. Avocado leaf extract produces flavonoid, saponin, tannin, and alkaloid compounds that can help prevent the onset of herpes simplex disease. Extraction with methanol solvent is able to extract compounds of both polar, semi-pattern, and non-polar characters. Methanol compounds can filter flavonoids, essential oils, saponins, tannins, and triterpenoids (Verdiana et al., 2018).

Widarta and Arnata's research (2017), shows the extraction process of avocado leaves with various solvents. The results showed that there were secondary metabolite compounds in avocado leaves including total phenols (23.28 mg/g) and total flavonoids (93.97 mg/g). The study also showed the antioxidant results of avocado leaves with the DPPH method (18703 mg/l). Phenolic is an organic material consisting of one or more bonds of hydroxyl substrate (OH). Phenol production is abundant in plants and is useful as a source of antioxidants (Puspitasari and Prayogo, 2017). Flavonoids are phenolic derivatives commonly found in leaves, fruits, stems, and flowers. Other antioxidant substances in leaves are flavonoids that can function to prevent cancer due to free radicals that can damage the cell structure (Waji and Andis, 2009).

One of the factors that affect the production of bioactive compounds produced in avocado leaves is the level of leaf age. Various studies show different flavonoid content in young leaves and old leaves of plants. Izzreen and Fadzelly (2013) reported that leaves (Camellia sinensis) have the lowest flavonoid compounds in old leaves compared to young leaves, while Mu'nisa et al. (2011), conveyed that old seagrass leaves produce higher flavonoids than young leaves. The results of another study stated that in tin leaves, the older the leaves, the lower the water content, tannins, and antioxidant activity of tin leaves and significantly affect the level of taste preference and overall acceptance (Amanto et al., 2020). The results of research by Felicia et al. (2016), explained that old avocado leaves with steaming techniques have a higher average value of total flavonoids than young avocado leaves.

The difference in the content of secondary metabolite compounds in young leaves and old leaves in plants is not yet known for certain. Thus it is necessary to conduct research to determine the comparison of total phenolic yield, total flavonoids, and antioxidant activity of avocado leaf extracts seen from the level of leaf aging.

2. Material and Method

2.1 Tools

The tools used in this research are analytical scales, blender, oven, 3 maceration jars, micropipette, measuring pipette 1 mL, 2 mL, 5 mL, and 10 mL, dropper pipette, 250 mL measuring cup, test tube and tube rack, 25 mL volumetric flask, 50 mL, and 100 mL, vortex, watch glass, spatula, stirrer, washing basket, knife, ruler, funnel, marker, waterbath, rotary evaporator, cuvette, UV-Vis spectrophotometer, incubator, and camera.

2.2 Materials

The materials used in this study are young avocado leaves with the criteria of light green color, smooth texture, taken 4-6 leaflets under the shoots, and old avocado leaves with the criteria of dark green color, smooth and firm leaf surface, taken 5-7 leaflets under young leaves originating from the Plikon area, Temanggung, distilled water, methanol, DPPH (1,1-diphenyl-2-picrylhydrazyl), Follin-Ciocalteu reagent, aluminum chloride reagent ($AlCl_3$), gallic acid, quercetin, ascorbic acid, Tris-HCl buffer (pH 7.5), Na_2CO_3, KCH_3COO 1M, aluminum foil, filter paper, tissue, and labels.

2.3 Methods

Young avocado leaves and old avocado leaves from the Plikon area, Temanggung were taken as much as 3000 g and 5000 g respectively. Samples of young avocado leaves and old avocado leaves were rinsed with running water and drained. The samples were placed in an oven for 24 hours at 40°C to dry (Widarta and Wiadnyani, 2019). The dried symplisia was pulverized into powder with a bleander, then sifted using a 60mesh sieve (Widarta and Wiadnyani, 2019). Young avocado leaf simplisia powder and old avocado leaves were taken as much as 100g and put in a dark maceration jar, added methanol solvent in a ratio of 1: 5 and covered with aluminum foil (Hayati et al., 2012). Then soaked for 2 x 24 hours at room temperature and protected from light, then the solution was filtered on filter paper with the help of a funnel to obtain filtrate (Talapessy et al., 2013). Avocado leaf extract is placed in a glass jar, then covered with aluminum foil, and can be tested for quantitative analysis.

The method to determine total phenolic content refers to the technique of Chun et al. (2003) in Malik and Ahmad (2015), with gallic acid for standard solution. The absorption wavelength was measured at 765 nm. Repetition5 was done 2 times (duplo) until the phenolic content obtained was obtained as mg gallic acid equivalent/g (mg GAE/g).

The method of determining total flavonoid content refers to the technique of Chang et al. (2002) in Ahmad (2014), with quercetin (QE) for the standard solution. Absorbance value was measured by UV-Vis spectrophotometry at a wavelength of 415 nm. The sample solution was repeated twice (duplo) until the flavonoid content was obtained as mg quersetin equivalent/g (mg QE/g)

Antioxidant Activity Testing DPPH method refers to the technique of Suyatmi et al. (2019), with ascorbic acid for standard solution. DPPH crystals in powder weighed 5 mg were dissolved in a 50 mL flask with methanol to obtain a 100ppm concentration DPPH solution. Sample concentrations of 0 ppm, 25 ppm, 50 ppm, 100 ppm, 200 ppm and 250 ppm and each made 2 repetitions (duplo). The absorbance value of the solution was measured using a visible spectrophotometer at an optimum wavelength of 517 nm, methanol was used as a blank, then the inhibition ability of DPPH radicals was calculated in percentages. Calculation of Inhibitory Concentration (IC50) to determine antioxidant activity was calculated by the equation formula (1).

$$\% \text{ Inhibition} = (\text{Abs. Control} - \text{Abs. Sample}) / (\text{Abs. Control}) \times 100\% \quad (1)$$

Data processing using normality test and analysis for correlation between total phenolic content, total flavonoids, and antioxidant activity of each methanol extract of avocado leaves using Pearson correlation. The results of the P value <0.05 state that there

is a significant relationship to determine whether or not there is a relationship between the total phenolic content and total flavonoids to the antioxidant activity of avocado leaf extracts.

3. Results and Discussion

3.1. Results

Avocado leaf simplisia is made by oven drying technique at 40°C. The temperature was chosen to avoid degradation of the structure of the content in the leaves such as flavonoid compounds with the nature of the substance easily oxidized and can't stand the heat if exposed to high temperatures. the highest average yield value is found in old avocado leaves of 22.5%, while young avocado leaves are 17.5%. This is because the greater the yield value indicates the value of the extract produced more and more. The requirement for the yield value of thick extracts is said to be good if the value is not less than 10% (Indonesia Herbal Pharmacopoeia, 2017). This means that the yield of young leaves and old leaves meets the requirements, because it is more than 10%. The use of methanol solvent for avocado leaf extract produces a high yield value, this indicates that methanol can extract compounds well in the leaves. Based on the nature of the solubility of a solvent can affect the acquisition of compounds in the extract (Badriyah and Farihah, 2022).

The total phenolic content of avocado leaf extract was carried out with gallic acid modification as a standard solution (Table 1).

Table 1. Test results of total phenolic content of methanol extracts of young and old leaves of avocado

Avocado leaf extract	Total phenolic content (mg GAE/100g dry weight)
Young leaves	1.454,55
Old leaves	1.682,27

The highest measurement for total phenolic content in old avocado leaves with a value of 1.682,27mg GAE/100g dry weight, while young avocado leaves have the lowest total phenolic content value of 1.454,55 mg GAE/100g dry weight. The above is in line with the research of Aziz and Jack (2015) on older *Nypa fruticans leaves that* have large phenol levels compared to young leaves. During plant growth, it is able to synthesize bioactive components and secondary metabolites in different amounts due to the influence of the level of leaf age and morphology (Farhoosh et al., 2007). Old leaf parts found trichomes as epidermal derivatives more than young leaves. This is because mature cell tissue (old leaves) has reached maximum growth, so that photosynthate is transferred to young leaves as a source of growth material. Biosynthesis of old leaves produces carbon atoms as a source of phenol secondary metabolites that function as a defense in plants from pests or other threats (Kuntorini et al., 2013).

Table 2. Test results of total flavonoids content of methanol extracts of young and old leaves of avocado

Avocado leaf extract	Total flavonoid content (mg QE/100g dry weight)
Young leaves	2.198,87
Old leaves	3.858,96

In Table 2. can be seen the content of the total flavonoid value of methanol extracts of young and old avocado leaves. Based on the data above, it is known that the highest total flavonoid compound measurement results are found in old avocado leaves with a value of 3.858,96 mg QE/100g dry weight, while young avocado leaves have the lowest total flavonoid content value of 2.198,87 mg QE/100g dry weight. The flavonoid content in this study shows that higher levels of compounds are found in old avocado leaves. The same thing was stated by Tehubijuluw et al. (2018), old seagrass tea leaves have a high flavonoid content of 0.1623%, while young seagrass tea leaves have a lower flavonoid content of 0.0888%. Factors that influence the yield of total flavonoids are the same as total phenols in that the increase in leaf age and morphology affects the content of bioactive compounds and the production of secondary metabolites (Farhoosh et al., 2007). Old leaves have better maturity of constituent components, because they contain sufficient nutrients and chlorophyll as a catcher of sunlight, resulting in a high rate of photosynthesis. The high rate of photosynthesis goes hand in hand with the production of secondary metabolites, one of which is flavonoids, which are formed in old leaves more than young leaves. Carbon compounds from photosynthesis are useful as a source of secondary metabolite formation (Tehubijuluw et al., 2018).

Antioxidant activity test research was conducted using the DPPH method which is a stable radical compound. The use of DPPH to measure the activity of antioxidant substances is based on their ability to capture free radicals. According to Suyatmi et al. (2019), the advantages of the DPPH method are a simple, fast, simple and sensitive way of analysis for low concentration samples, but testing with DPPH is limited because DPPH can only dissolve in organic solvents, making it difficult to analyze hydrophilic compounds.

Increasing concentration of the sample solution, the percentage of inhibition against DPPH is getting higher. The percentage of inhibitory activity or inhibition of young leaves and old avocado leaves increases mutually. At a concentration of 250 ppm, the highest inhibition of old avocado leaves was 45,98%, while young avocado leaves were 44,90%. The higher the concentration, the higher the percentage of inhibition, so that the reaction of the sample with DPPH is stronger and more stable. This is because more DPPH binds to hydrogen particles in the extract tested to reduce the absorption of DPPH. The results of this measurement are relevant to Molyneux (2004), that the percent inhibition of DPPH can affect the determination of antioxidant activity, if the inhibition of a sample is high, the higher the activity of antioxidant substances in the sample. The antioxidant activity test was conducted using the DPPH method to determine the activity level of the sample in inhibiting the stable DPPH radical through hydrogen atom donation. Samples with antioxidant activity reduce DPPH to DPPH-H (Molyneux, 2004).

Table 3. Measurement IC50 antioxidant activity of methanol extract of young and old leaves of avocado

Avocado leaf extract	Values IC50 (ppm)
Young leaves	321,32±80,43
Old leaves	288,54±33,06

Table 3. shows the value of IC50 antioxidant activity of methanol extract of young avocado leaves amounted to 321.32 ± 80.43ppm and old avocado leaves amounted to 288.54 ± 33.06ppm. Based on the calculation of the value of IC50 antioxidant activity shows that the sample of methanol extract of old avocado leaves has higher antioxidant activity than young avocado leaves. The antioxidant activity of old avocado leaves is categorized as very weak because the value is more than 200 ppm. IC50 more than 200 ppm, but still has potential as an antioxidant compound (Tristantini et al., 2016). The extract sample produced a weak antioxidant presumably due to the presence of impure compounds that have not been separated from some other component substances, so it is necessary to carry out a fractionation process for the separation of golonga. value IC50 is used as a good determination of the antioxidant efficiency of pure compounds or extracts. According to Molyneux (2004), the lower the value, the higher the antioxidant activity of the compound. IC50 the higher the antioxidant activity of the compound. The result of the value of IC50 The value of old avocado leaves shows smaller than young leaves, meaning that the antioxidant content is greater in old avocado leaves. This is due to the level of leaf age can affect the production of secondary metabolites in a plant. The increasing age of the leaves, the levels of bioactive compounds that act as antioxidants such as flavonoids are increasing.

Antioxidant properties in avocado leaves can be influenced by the biosynthesis mechanism of secondary metabolites such as flavonoids or phenol compounds. These compounds include types of chemical compound components of antioxidant substances that are abundant in plants. According to research by Achakzai et al. (2009) in Felicia (2016), phenolic and flavonoid content compounds are higher in old leaves than young leaves. The age of the old leaves, the antioxidant activity is high, because the concentration of phenols and flavonoids including secondary metabolites that act as antioxidants is higher.

Pearson correlation test based on the results of the table above shows that the total flavonoid content has a p-value <0.01, while for the total phenolic content has a p-value> 0.01 can be seen from the large correlation coefficient. The relationship between the total flavonoid content of avocado leaf methanol extract and DPPH free radical inhibition has a positive correlation with a coefficient value of 0.856. This result states that the total flavonoid content has a strong relationship with the antioxidant activity of a sample.

Conclusion

The conclusion of this study is that the highest total phenolic content of avocado leaf methanol extract (*Persea americana* Mill.) is found in old leaves amounting to 1.682,27mgGAE/100g dry weight. The highest total flavonoid content of avocado leaf methanol extract (*Persea americana* Mill.) was found in old leaves at 3.858,96mgQE/100g

dry weight. The highest antioxidant activity value of avocado leaf methanol extract (*Persea americana* Mill.) is found in old leaves amounting to 288.54 ± 33.06 ppm. The total flavonoid content in the methanol extract of avocado leaves (*Persea americana* Mill.) has more influence on antioxidant activity, with a correlation coefficient of 0.856.

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