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Research Article

Antioxidant Activity Test of Ethyl Acetate Extract of Avocado Seeds (*Persea americana* Mill.) Using the 1,1-Diphenyl-2-Picrylhdrazyl Method

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ABSTRACT: Avocado is a plant that can thrive in tropical areas such as Indonesia and is one of the types of fruit that is popular with the public, however, avocado seeds have not been used optimally so that they are wasted as waste. Based on phytochemical experiments, avocados also contain phenolics, tannins and flavanoids which have antioxidant activity. Antioxidants are compounds that can be used to counteract free radicals. Dietary fiber and antioxidants are two types of components that are very useful in improving health and being able to prevent various diseases. The extraction method used in this study is reflux where the sample is heated for 6 hours with the solvent used to evaporate at high temperatures, but it will be cooled by the condenser so that the solvent which was in the form of vapor will condense in the condenser and fall again into the reaction vessel so that the solvent will still be there during the reaction. This study aims to analyze the antioxidant activity of the ethyl acetate extract of avocado seeds using the DPPH method. Quantitatively, the ethyl acetate extract of avocado seeds (*Persea americana* Mill.) was measured at a maximum wavelength of 516 nm using quercetin as a reference standard. The research results showed that the value was 903.08 µ/mL. Quantitatively, the ethyl acetate extract of avocado seeds (*Persea americana* Mill.) was measured at a maximum wavelength of 516 nm using quercetin as a reference standard. The research results showed that the value was 903.08 µ/mL. Quantitatively, the ethyl acetate extract of avocado seeds (*Persea americana* Mill.) was measured at a maximum wavelength of 516 nm using quercetin as a reference standard. The research results showed that the value was 903.08 µ/mL.

KEYWORDS: Antioxidants; Avocado seeds; DPPH; UV-Vis Spectrophotometry.

1. INTRODUCTION

In Sikka Regency there are several types of soil, one of which is Alluvial soil type found in Maumere. This soil is generally fertile with a fairly thick solum (thought layer). Thick soil is a good medium for root development so that the efficiency of absorption of plant nutrients can be better so that this soil is good enough for plant growth, one of which is avocado. Avocado is a plant that can thrive in tropical areas such as Indonesia and is a type of fruit that is popular with the public because apart from its delicious taste it also has a high antioxidant content (Malangngi et al., 2012).

In general, the part of the avocado that is used is the flesh, while the other parts are discarded and become waste, such as the skin and seeds. Based on phytochemical experiments, avocados contain phenolics, tannins and flavonoids which have antioxidant activity. Antioxidants are compounds that can be used to counteract free radicals. Dietary fiber and antioxidants are two types of components that are very useful in improving health and being able to prevent various diseases (Parinding et al., 2021).

An antioxidant is a compound that has a molecular structure that can provide electrons for unstable free radical molecules without being disturbed at all so that they can break free radical chain reactions (Rusdi et al., 2018; Fawwaz et al., 2023). Several studies have found avocado seeds to be rich in polyphenols with antioxidant properties, examples of compounds found in avocados include fatty alcohols, phenolic compounds with aromatic rings, sugars and sugar alcohols. Therefore, it is considered that avocados are beneficial in supporting heart health, aiding weight loss and preventing premature aging (Ong et al., 2022).

Free radicals are compounds that have one or more unpaired electrons which make these compounds unstable and very creative (Fawwaz et al., 2020). Due to the instability, free radicals tend to grab electrons from other compounds which make these compounds unstable resulting in a free radical chain reaction. If this chain reaction is followed by an unhealthy lifestyle, it will cause various diseases, one of which is degenerative diseases such as cardiovascular disease, cancer, atherosclerosis, osteoporosis and other degenerative diseases. The risk of developing this disease can be reduced by consuming sufficient amounts of antioxidants (Tristantini et al, 2016).

DPPH is a stable free radical compound so that when it is used as a reagent in free radical scavenging tests it is sufficiently dissolved and when stored in a dry state under good and stable storage conditions for years. DPPH absorbance values range from 515-520 nm (Sastrawan et al., 2013).

Measurement of antioxidants using the DPPH (2,2-diphenyl-1-picrylhydrazil) method is a method of measuring antioxidants that is simple, fast and does not require a lot of reagents like other methods. Other methods besides DPPH require a large number of chemical reagents, a long analysis time, are expensive and cannot always be applied to all samples (Sayuti & Yenrina, 2015).

Antioxidant interaction with DPPH either by electron transfer or hydrogen radicals in DPPH, will neutralize the free radical character of DPPH and form reduced DPPH. If all the electrons in the DPPH free radical become paired, the color of the solution changes from dark purple to bright yellow and the absorbance at a wavelength of 517 nm will disappear (Malangngi et al., 2012).

2. EXPERIMENTAL SECTION

2.1. Sample collection and processing

This research began in January to May 2023 at the Pharmaceutical Chemistry Laboratory of the Indonesian Muslim University Makassar. The sample used was avocado seed extract (*Persea americana* Mill.*)* Obtained from Avocado Shake Bumi Tamalanrea Permai, Makassar, which came from Maumere Regency. Sikka, East Nusa Tenggara. The avocado seeds that were collected were then separated from the seed coat, then cleaned with running water. Then dry without exposure to direct sunlight. Furthermore, the dried samples were cut into small pieces and then blended. The refined sample is ready to be extracted by reflux method. The research was carried out experimentally by testing the levels of antioxidants in the ethyl acetate extract of avocado seeds (*Persea americana* Mill.) by UV-Vis spectrophotometry.

2.2. Materials and tools

The materials used are aluminum foil, avocado seeds (*Persea americana* Mill.), DPPH, ethyl acetate, filter paper and quercetin. The tools used were stir bars, blenders, Buchner funnels, rotary evaporators, horn spoons, a set of reflux method extraction tools, a set of glassware (pyrex), UV-VIS spectrophotometer and analytical balance.

2.3. Qualitative Analysis

100 g of avocado (*Persea americana* Mill.) seed powder was refluxed using 200 mL of ethyl acetate. Extraction was carried out by inserting the sample and solvent into a 1000 mL round bottom flask, which was connected to a ball condenser. Then the flask was heated with a heating mantle for 6 hours. After that, the solution contained in the flask was filtered using a Buchner funnel. The filtrate obtained was then concentrated using a rotary evaporator at 50ºC and the samples were stored in a desiccator.

2.4. Quantitative Analysis

2.4.1. Stock solution preparation

A total of 10 mg of sample was dissolved with ethanol in a 25 mL volumetric flask. Stir until homogeneous then a solution with a concentration of 1000 ppm is obtained. Then concentrations were made in a series of 100, 150, 200, 250 and 300 ppm by pipetting each solution as much as 1 mL, 1.5 mL, 2 mL, 2.5 mL and 3 mL.

2.4.2. Maximum wavelength determination

3 mL of DPPH solution (40 ppm) was pipetted into the vial, then 1 mL of 96% ethanol was added and homogenized. The solution was incubated in a dark room for 30 minutes. Then the absorption spectrum was measured using a UV-Vis spectrophotometer at a wavelength of 400-700 nm. The maximum wavelength obtained is 516 nm.

2.4.3. Sample preparation and measurement

Solutions that had been made in concentrations of 100, 150, 200, 250 and 300 ppm were pipetted as much as 1 mL into a vial and then 3 mL of 40 ppm DPPH was added to each. The solution was homogenized by incubation at room temperature for 30 minutes and then the absorbance was measured at a wavelength of 516 nm.

2.5. Data analysis

Values were calculated using the % inhibition regression equation. The value indicates the concentration of the sample solution (avocado seed or quercetin as an antioxidant) that can reduce DPPH free radicals by 50%. From the equation y = a + bx, the value can be calculated using the formula = $IC_{50}\ \frac{50-A}{B}$

3. RESULTS AND DISCUSSION

Antioxidant compounds are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals to normal cells, proteins, and fats. This compound has a molecular structure that can give its electrons to free radical molecules without being disturbed at all by its function and can break the chain reaction of free radicals. (Murray et al., 2009).

In principle, DPPH free radicals will react with sample antioxidant compounds through the mechanism of giving hydrogen atoms and cause a color change from purple to yellow which can be analyzed using UV-Vis spectrophotometry based on the decrease in DPPH absorbance. The results of analysis on UV-Vis spectrophotometry are calculated based on the value (Inhibition concentration 50%), the value can be defined as the amount of concentration that can inhibit DPPH free radical activity by 50%. The smaller the value, the greater the antioxidant activity of the tested

material. Values <50 ppm are classified as very strong antioxidants, 50-100 ppm are classified as strong antioxidants, 100-150 ppm are classified as moderate antioxidants, and 150-200 ppm are classified as weak antioxidants (Tristantini et al, 2016).

Extraction using a solvent consists of a cold method including maceration, percolation and a hot method including reflux, soxhletation, infusion, decoction and digestion. The extraction method used in this study was reflux. (Wijaya et al., 2018). The ethyl acetate extract of avocado seeds (*Persea americana Mill.*) was tested for its antioxidant activity using the DPPH method by preparing test solutions of various concentrations and then mixing them with DPPH, incubating for 30 minutes. Incubation was carried out in the dark without light and then the absorbance was measured using a spectrophotometer with a wavelength of 516 nm. The concentration of the sample is directly proportional to the percentage of inhibition (**Table 1**). This happens because the higher the concentration of the solution, the more antioxidants contained therein.

Table 1. Calculation Results of Antioxidant Activity of Ethyl Acetate Extract of Avocado Seeds (*Persea americana Mill.*)

Sample	Concentration (ppm)	Absorbance DPPH	Sample Absorption	% Inhibition	IC_{50}
Avocado seeds (*Persea americana Mill.*)	100	0.707	0.555	21.499	903.08
	150	0.707	0.543	23.196	
	200	0.707	0.532	24.752	
	250	0.707	0.517	26.874	
	300	0.707	0.505	28.571	

The ethyl acetate extract of avocado seeds (*Persea americana Mill.*) was tested for its antioxidant activity using the DPPH method by preparing test solutions with various concentrations and then mixing them with DPPH, incubating for 30 minutes. Incubation was carried out in the dark without light and then the absorbance was measured using a spectrophotometer with a wavelength of 516 nm. The concentration of the sample is directly proportional to the percentage of inhibition (**Figure 1**). This happens because the higher the concentration of the solution, the more antioxidants contained therein. The graphical results of the linear regression equation for the ethyl acetate extract of avocado seeds are y = 0.0356x + 17.85 where a value = 0.9977 is obtained and a value of r = 0.998.

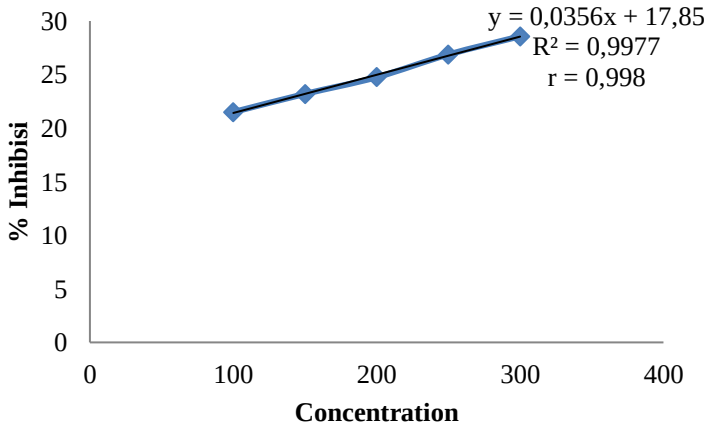

Figure 1. Relationship Between Concentration of Ethyl Acetate Extract (*Persea americana Mill.*) With DPPH inhibition

4. CONCLUSION

Results shows that the higher the concentration of the extract solution, the absorbance of the solution will be smaller. Meanwhile, the greater the concentration of the solution, the percentage of inhibition will be higher. This happens because the higher the concentration of the solution, the more antioxidants contained therein. Samples were extracted using the reflux method where the sample and solvent were heated for 6 hours at 80º C, after the extraction process, the extract was evaporated using a rotary evaporator. Where the function of this tool is to separate a solvent (solvent) from a solution, so that it will produce an extract with a more concentrated content or concentration or as needed. In this study, avocado seed extract (*Persea americana Mill.*) was classified as having no antioxidant activity in terms of the Blois classification (2005) a value of <50 ppm was classified as a very strong antioxidant, 50-100 ppm was classified as a strong antioxidant, 100-150 ppm was classified as moderate antioxidants, 150-200 ppm are classified as weak antioxidants, and >200 ppm have no antioxidant activity.

The solvent evaporation process using heating has the potential to damage and remove antioxidant compounds in samples, especially for compounds that are thermolabile. This is because the longer the extraction time, the longer the contact time between the solvent and the raw material. So that the process of penetration of the solvent into the raw material cell will be better, and cause more and more compounds to diffuse out of the cell.

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Ethical Approval: Not applicable

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