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Research Article
Phytochemical and Antioxidant Activity of Avocado Leaf Extract (*Persea americana* Mill.)

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Abstract

Background and Objective: Antioxidants are a necessary nutrient component of the body to prevent and cope with oxidative stress. Avocado leaf allegedly has a natural antioxidant that can meet the needs of antioxidants which are still limited, especially for people with degenerative diseases. This study aimed to identify and analyze the phytochemical content and antioxidant power of avocado leaf extract. **Materials and Methods:** The type of research is pure experiment. The avocado leaf extraction preparation was macerated using 96% ethanol solvent with a ratio of 1:10 (w/v) for 3×24 h. Phytochemical types analyzed were flavonoids, saponins, polyphenols, tannins, alkaloids and steroids. Measurement of antioxidant activity using DPPH (1,1-Diphenyl-2-picrylhydrazyl) method on avocado leaf extract and vitamin C (control group). Data analysis was unpaired t-test, one-way ANOVA test and linear regression test with significant value was $p<0.01$. **Results:** Positive avocado leaf extract contains flavonoids, saponins, tannins and steroids. Antioxidant activity avocado starch extract (absorbance DPPH = 0.797) is strong with IC50 of 72.61 mg L^{-1} and vitamin C as positive control is also very strong with IC_{50} (mg L^{-1}) of 23.03. **Conclusion:** Avocado leaf extract contains phytochemical substances with a strong antioxidant that can be used to prevent and cope with oxidative stress.

Key words: Phytochemicals, antioxidant activity, avocado leaf extract, vitamin C, DPPH

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antioxidants are compounds capable of inhibiting the oxidation of other molecules[1]. The body does not have an excessive antioxidative defense system, so if there is exposure to excessive free radicals, the body needs exogenous antioxidants[2]. Concerns about the side effects of synthetic antioxidants make natural antioxidants the preferred alternative[3]. In recent years there has been an increased interest in getting natural antioxidants[4,5]. Studies show that phenolic compounds such as flavonoids have antioxidant activity of free radical catchers found in plants and fruit leaves which are commonly used as traditional medicine[6,7].

WHO reports that currently traditional medicines coming from plants have been used by society for around 80% as personal healthcare effort[8]. Utilization of medicinal plants include the prevention and treatment of a disease and health care. Certain plant or plant extracts are believed to contain biologically active compounds and are effective in curing diseases[8,9]. Most of the natural antioxidants come from fruits, vegetables, spices, grains and spices such as ginseng, curcuma, ginkgo, rosemary, green tea, grapes, ginger and garlic and avocado (*Persian americana* Mill.)[10-13].

Avocado tree is known only for the fruit that people usually consume. Apparently avocado leaf is one of the natural ingredients that can be used as a traditional medicine[14]. This leaf has been empirically used as a diuretic, analgesic, anti-inflammatory, hypertensive, hypoglycemic, diarrhea, sore throat and hemorrhage cure[14]. Avocado is one of the fruit plant groups which are nutritious as a preservative and antioxidants[15,16]. Avocado flesh can be used as an anti-hyperlipidemia and has antioxidant activity and reduce the risk of metabolic syndrome[17-19].

One part of the avocado plant that has the potential as a natural antioxidant substance is avocado leaf. Previous research has shown that avocado leaf has the potential as a natural antioxidant[20] and positively contains alkaloids, flavonoids, saponins, tannins and steroids using methanol solution to hydrolyze and extract avocado leaf[11]. However, using methanol in the extract is less safer compared to ethanol solution[21]. This research aimed to identify phytochemical content using 96% ethanol solution and antioxidant power of avocado leaf extract using DPPH method (1,1-Diphenyl-2-picrylhydrazyl). Extracting antioxidant compound in the leaf using ethanol will be safer for the future experimental studies on the animal or human settings.

MATERIALS AND METHODS

The study was conducted in the Analytical Chemistry Laboratory of Mathematics and Science Faculty of Tadulako University from June-September, 2017. This type of research is experimental with the sample consisted of 2 groups, namely treatment group and control group. The avocado leaf used both on treatment and control group were butter type. The treatment group was avocado leaf extract consisting of 5 series of concentrations which are 10, 30, 50, 70 and 90 mg L^{-1} while the control group was vitamin C consisting of 5 series of concentration such as 10, 30, 50, 70 and 90 mg L^{-1}.

Materials: About 96% of ethanol, DPPH, aquades, filter paper, concentrated HCl, 5% ferric chloride, Dragendorff reagent, chloroform, acetic acid hydride and concentrated sulfuric acid.

Tools: Blender, 60 mesh sieves, Erlenmeyer 1000 mL, measuring flask, 500 mL measuring cup, analytical balance, glass funnel, chamber, buchner funnel, vacuum pump, rotary evaporator, UV-VIS spectrophotometer.

The data collection were conducted through a series of measurements.

Avocado leaf extraction preparation: Avocado leaf used was fresh green, separated from the stalk. The avocado leaf sample was cleaned, then dried in an oven at 50°C for 24 h. After drying, the sample was blended to obtain dry powder. Samples were macerated using 96% of ethanol solvent with a ratio of 1:10 (w/v) for 3×24 h. The extract solution used was 96% of ethanol. Ethanol is considered in ethanol 20% up, non-toxic, neutral, requires less heat for the concentration process, limited soluble confounding substances and semi polar ethanol to attract polar and non-polar compounds. The maceration results were filtered using filter paper with the help of Buchner's vacuum filter. The filtrate was accommodated in Erlenmeyer. The extract was evaporated using an evaporator to obtain a concentrated extract of avocado leaf. Furthermore, the concentrated extract of avocado leaves was tested for its phytochemical substances (groups of flavonoids, saponins, alkaloids, steroids, tannins) and antioxidant power.

Phytochemical test[22]

Flavonoid test: The flavonoid test is that 1.0 mL of sample solution is fed into the test tube and then a little magnesium powder and a few drops of concentrated HCl (Shinola reagent) were added, so when reacted positively, it will result in an orange, pink or red solution.

Saponin test: A total of 2.0 mL of sample solution is inserted into the test tube and shaken for a few minutes, when reacted positively, it will form a stable foam for 15 min.

Tannins test: Tannins test is that 1.0 mL of the sample solution are fed into the test tube and then a drop of 5% ferric chloride solution were added, when reacted positively it will result in a brown precipitate and a dark blue solution.

Alkaloid test: Alkaloid test is 1.0 mL of sample is fed into the test tube and then 2-3 drops of Dragendorff reagent were added, so when reacted positively, it will result in orange precipitate.

Steroid test: Steroid test is that 1 mL extract is added with 3-5 drops of chloroform, then added again with 3-5 drops of acetic acid hydride and 10 drops of concentrated sulfuric acid. The positive test is marked by a change in the color of the blue or green solution.

Antioxidant activity test using DPPH method (1,1-Diphenyl-2-picrylhydrazyl)[23]: Concentrated extract of the sample whose antioxidant activity was determined, used spectrophotometric method with DPPH reagent. The sample extract was weighed 25 mg then put into a 25 mL measuring flask, then adjusted with an ethanol solvent to obtain a solution concentration of 1000 ppm. A series of dilutions were then performed to obtain a solution of 10, 30, 50, 70 and 90 mg L^{-1}. The solution has been prepared, piped for 0.2 mL and added with 3.8 mL DPPH 50 µM solution. The mixture is homogenized and left for 30 min in the dark. Then, the uptake is measured at a wavelength of 517 nm. Tests were also conducted on DPPH solution.

The obtained absorbance value is used to determine the inhibition (%) using the following Eq.[24]:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance DPPH} - \text{Absorbance sample}}{\text{Absorbance DPPH}} \times 100$$

Subsequently, an inhibition (%) curve was prepared and IC_{50} (inhibition concentration) was determined on the basis of the regression equation obtained. The parameter to interpret the test results by DPPH method is IC_{50}. The smaller the value of IC_{50}, the higher the antioxidant activity. The value of IC_{50} is obtained from several stages, before calculating the IC_{50} value, first calculate the log value of the concentration and the probit value for each percentage of free radical capture activity. Levels of antioxidant power based on IC_{50} values are: very strong (<50), strong (50-100), medium (100-250), weak (250-500) and inactive (>500)[25].

Data analysis: Data analysis was unpaired t-test, one-way ANOVA test and linear regression test with significant value was $p<0.01$.

RESULTS

Qualitative analysis of phytochemical compounds on avocado leaf extract contains flavonoid compounds, saponins, tannins, triterpenoids, steroids and contains no alkaloids (Table 1). Table 2 shows that the absorption of DPPH by the presence of avocado leaf extract and vitamin C decreased along with the increasing of avocado leaf extract and vitamin C concentrations and all concentrations were significantly different with $p<0.01$. DPPH absorption value by avocado leaf extract was significantly different with absorbance of DPPH by vitamin C with $p<0.01$, where the absorption value of DPPH by vitamin C was smaller on average which was 0.288 compared with absorbance of DPPH by avocado leaf extract which was 0.452. The percentage of DPPH inhibition by the presence of avocado leaf extract and vitamin C has increased along with the increasing of avocado leaf extract and vitamin C concentrations and all concentrations were significantly different with the value of $p<0.01$. The percentage of DPPH inhibition by avocado leaf extract was significantly different with absorbance of DPPH by vitamin C with $p<0.01$ value, where DPPH inhibition by vitamin C was greater on average which was 63.83% compared to percentage of DPPH inhibition by avocado leaf extract which was 43.26% (Table 2).

Table 3 shows that the absorbance of DPPH with 70 mg L^{-1} of avocado leaf extract concentration was not

Table 1: Phytochemical screening results of avocado leaf extract

Phytochemical parameters	Reactor	Observation result	Remark
Alkaloid	Dragendorff	Does not produce yellow precipitate	-
Flavonoid	Concentrated HCl	Produce an orange solution	++
Saponin	Aquades	Forming foam	++
Tannin	Ferric chloride 5%	Producing dark blue solution	+++
Steroid	Chloroform, acetic acid hydride, concentrated sulfuric acid	Producing green solution (positive steroid)	+

$^{-}$Negative result, $^{+}$Weak positive result, $^{++}$Strong positive result, $^{+++}$Very strong positive result

Table 2: Absorbance and percent Inhibition of DPPH by the presence of avocado leaf extract and vitamin C with various concentrations (initial DPPH = 0.797)

		Absorbance		Percent inhibition			
Test samples	Concentration	Mean±SD	p-value	Log C	Mean±SD	p-value	p-value
Avocado leaf extract	10	0.559±0.002	0.000*	1.000	29.86±0.35	0.000*	0.002**
	30	0.492±0.008		1.477	38.27±1.06		
	50	0.444±0.004		1.699	44.29±0.53		
	70	0.397±0.127		1.845	50.19±1.59		
	90	0.369±0.011		1.954	53.70±1.42		
	Total	0.452±0.071			43.26±9.01		
Vitamin C	10	0.465±0.004	0.000*	1.000	41.59±0.62	0.00*	0.002**
	30	0.371±0.002		1.477	53.45±0.35		
	50	0.255±0.002		1.699	68.00±0.36		
	70	0.215±0.002		1.845	73.02±0.35		
	90	0.134±0.002		1.954	83.12±0.26		
	Total	0.288±0.123			63.83±15..47		

*Significantly different when p<0.01 in one-way ANOVA test, **Significantly different when p<0.01 in unpaired t-test

Table 3: *Post-hoc* post absorption test of DPPH by avocado leaf extract

	p-value				
Concentration (mg L^{-1})	10	30	50	70	90
10	NA	0.001*	0.000*	0.000*	0.000*
30	0.001*	NA	0.003*	0.000*	0.000*
50	0.000*	0.003*	NA	0.003*	0.000*
70	0.000*	0.000*	0.003*	NA	0.025
90	0.000*	0.000*	0.000*	0.025	NA

*Significantly different when p<0.01 in *post-hoc* test, NA: Not acceptable

significantly different with the 90 mg L^{-1}. This means that the optimum concentration of avocado leaf extract to inhibit DPPH activity is 70 mg L^{-1}. IC_{50} as an indicator in assessing the antioxidant of avocado leaf extract as a strong antioxidant power with IC_{50} value of 72.61 mg L^{-1}, while vitamin C has a very strong antioxidant power with IC_{50} value of 23.03 mg L^{-1}. Levels of antioxidant power based on IC50 values are: very strong (<50), strong (50-100), medium (100-250), weak (250-500) and inactive (>500)[24].

DISCUSSION

The results showed that positive avocado leaf extract contains phytochemical substances such as flavonoids, saponins, tannins and steroids. The extraction process of avocado leaves using 95% of ethanol solution is a universal solvent and capable of dissolving polar compounds[26], so that various polar and nonpolar compounds such as alkaloids, flavonoids, saponins, tannins and steroids contained in avocado leaves can be attracted to the solvent, although there is research showing that ethanol is less effective in screening antioxidants[27]. Methanol is the most efficient solvent for the extraction of antioxidant compounds, followed by water, ethanol and acetone[26]. However, the use of ethanol is safer compared to methanol, because methanol is toxic[28].

Qualitatively, the findings of some phytochemical substances in avocado leaf extract proves that the avocado leaf can be used as a source of food that has antioxidants. Several studies have shown that phytochemicals of phytosterols, saponins, polyphenols, flavonoids and ascorbic acid have the ability to regulate cholesterol metabolism and to improve antioxidant status in hypercholesterolemic rats[29,30]. Flavonoids found in avocado leaf extracts can be used in the treatment of oxidative stress because flavonoids can freeze free radicals by donating hydrogen atoms or by single electron transfer[31,32].

The requirement of flavonoids in daily consumption is still higher than vitamin C (70 mg/day), vitamin E (7-10 mg/day) and carotenoids (otototene, 2-3 mg/day)[33]. Flavonoid intake may range from 50 and 800 mg/day, depending on consumption of vegetables and fruits and certain beverages, such as red wine, tea and unfiltered beer. In particular, red wine and tea contain high levels (about 200 mg per glass of red wine or a cup of tea) of total phenol[33]. But Indonesian people, the consumption of red wine is still limited, so this avocado leaf extract can be a solution in meeting the needs of flavonoids. In addition, the use of avocado leaf as a vegetable is still very limited compared to other food stuffs such as tree nuts and palm fruit containing important nutrients and phytochemicals, including carotenoids, polyphenols and tocopherols that have antioxidant and other bioactivity functions[34,35].

Saponins are common in plants used as medicinal plants. Studies have shown that saponins have antioxidant effects in

the presence of decreased serum lipids in wistar rats[36]. However, saponins can interfere with the absorption of minerals and vitamins in the body that suppress the concentration of the liver Fe through imperfect absorption of Fe by forming the Saponin-Fe complex[37]. Other phytochemical compounds found in avocado leaf extract are tannins. Tannins is a water-soluble polyphenol present in bark and fruit especially bananas, grapes, sorghum, spinach, red wine, coffee, chocolate and tea[38]. The results of this research are in line with several studies showing that avocado leaves contain flavonoid compounds, tannins and saponins[39-41].

The results showed that the higher the concentration of vitamin C and avocado leaf extract, the higher the antioxidant activity shown by the smaller absorbance value ($p = 0.000$). This is because the higher the concentration, the smaller the absorbance of the antioxidant ability to reduce the DPPH free radical. In addition, the inhibition value (%) also experienced a significant increase ($p = 0.000$) along with the increasing of concentrations of vitamin C and avocado leaf extract. The absorbance value and inhibition (%) between vitamin C and avocado leaf extract was found significantly different ($p = 0.002$), where vitamin C as antioxidant is still higher in ability to damp free radical of DPPH compared to avocado leaf extract. Vitamin C has an average value of inhibition (%) for 63.83% while avocado leaf extract is for 43.26%. Factors influencing the stability of antioxidant activity are pH, temperature, rays and oxygen, as well as other factors such as metal ions. The temperature factor influences the level of antioxidant activity in the avocado leaf. Anggorowati *et al.*[42] research showed that the temperature of making avocado leaf tea that is at temperature 60, 70 and 80°C value of IC_{50} is higher due to the number of antioxidants that begin to decompose at this temperature, because the more antioxidants decomposing, the higher the value of IC_{50} and the higher the value IC_{50}, the lower the active ingredient in the avocado leaf.

Then, the comparison of IC_{50} leaf extract of avocado and vitamin C as an indicator of antioxidant power found that vitamin C has a smaller IC_{50} value which was 23.03 mg L^{-1} compared to avocado leaf extract which was 72.61 mg L^{-1}. This means that vitamin C has the power of antioxidants with a very strong category, while the avocado leaf extract is strong category. The small power of antioxidant activity of avocado leaves compared with vitamin C is due to the fact that the avocado leaf extract is still a mixture of several compounds such as flavonoids, saponins, tannins and steroids, while vitamin C is a pure synthetic compound as an antioxidant[13] Vitamin C also has more hydroxyl groups, so vitamin C can donate more hydrogen atoms to react with DPPH free radicals.

This research his in line with Owolabi *et al.*[43], who suggested that avocado leaves have strong antioxidant activity, which may help in preventing or slowing down the progression of various diseases associated with oxidative stress. The extract of avocado's leaves is potential natural antioxidant and anti-inflammatory compounds that prevent the formation of calcium oxalate crystal by interfering the process of epithelial cell damage[44]. The total phenolic content and antioxidant potential of avocado phenolics was influenced by the extracting solvent and avocado variety[45]. In addition to avocado leaf, grape extrane and avocado skin also contain bioactive compounds and high antioxidant activity against DPPH (43 and 35%, respectively) than avocado fruit (only 23%)[19].

This study suggests that avocado leaf is potentially used for averting some diseases associated with oxidative stress. Therefore, avocado leaf as known to have non economic values can contribute to be natural medicine and with a possibility of becoming source of income for farmers and industries. However, avocado leaf assesed in this study were only limited to one variation. Examining other varieties would bring clearly insight on the difference bioactive compounds between avocado leaf variations. Futhermore, phitochemical contents in this study were only examined qualitatively, without providing information on quantity of the compounds. This study also did not measure antioxidant activity for each phitochemical contents separately while each kind of phitochemical substances can have different antioxidant activity level. Further studies on assesing quantitative phitochemical compound, extracting phitochemical contents separately to eximined its antioxidant activity, as well as study in the animals and human settings need to be done to offer more detail understanding in natural antioxidant chemical process of avocado leaf.

CONCLUSION

Phytochemical screening of avocado leaves extracted with 90% of positive ethanol solution contains flavonoid compounds, saponins, tannins, steroids. The absorbance value and DPPH percent inhibition of avocado leaf extract and vitamin C showed a significant difference. The concentration 70% is an avocado leaf extract that gives absorbance value and percent optimum inhibition. Antioxidant activity avocado leaf extract (absorbance DPPH = 0.797) is strong with IC_{50} is 72.61 mg L^{-1} and vitamin C is very strong with IC_{50} that is 23.03 mg L^{-1}. Avocado leaf extract with flavonoid compound and strong antioxidant activity can be utilized in the intervention of disease associated with free radicals.

SIGNIFICANCE STATEMENT

The study discovered that the effects of phytochemical combinations (flavonoids, saponins, tannins and steroids) avocado leaf extracts can be used in the treatment of oxidative stress. This study will help researchers to take advantage of avocado leaf extract as a source of antioxidants is cheap, safe and easily obtained. Thus, a new theory on avocado leaf extract using ethanol solution showed strong antioxidant activity against DPPH (1,1-Diphenyl-2-picrylhydrazyl).

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