

Antioxidant properties of the methanolic extract of avocado fruit peel (*Persea americana* Mill.) from Indonesia

Nurdin Rahman, Sri Mulyani Sabang¹, Rukman Abdullah², Bohari Bohari³

Departments of Nutrition and
¹Chemistry Education, Tadulako University, Palu, Departments of
²Medical Education and ³Nutrition, Sultan Ageng Tirtayasa University, Banten, Indonesia

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ABSTRACT

This study analyzed the antioxidant activity and the phytochemical substances in avocado fruit peel extracted with methanol. In this study, antioxidant activity was determined by IC₅₀ based on the regression value of DPPH free radicals' inhibition. Phytochemical content was measured qualitatively concerning the total content of phenols, flavonoids, tannins, saponins, and alkaloids. Our measurements showed that the methanolic extract of avocado fruit peels from Indonesia had the value of each phytochemical compound as follows: total phenol was 21.833 ± 0.118 mg/100 g extract; total flavonoids were 2.607 ± 0.111 mg/100 g extract; total tannin was 38.357 ± 0.202; saponin content was 8.874% ± 0.031%; and total alkaloid was 9.95 ± 0.035 mg CE/g extract. They then provided the antioxidant activity in IC₅₀, which reached 185.891 ± 1.598 ppm. Avocado fruit peels are identified as a phytochemical source that contributes to antioxidant activities.

Key words: Antioxidant activity, avocado, fruit peels, phytochemicals

INTRODUCTION

Free radical is a compound that cannot be separated from daily human life. In normal amounts, free radicals are beneficial for health, but they can cause oxidative stress in excess. This situation leads to oxidative damage from the cells, tissues, and organ levels, accelerating the aging process and disease emergence.^[1] Free radicals are commonly from excessive sun rays, industrial smokes, cigarettes, or vehicle emissions; a molecule with one or more unpaired electrons causes the free radicals to be reactive against cells by binding

to molecular cell electrons; this reaction is often referred to as oxidation. Undesirable oxidation of DNA, nucleic acids, fats, or proteins accelerates aging and triggers degenerative diseases, including cancers, coronary heart disease, and cognitive disorders. Antioxidants are needed to balance and neutralize those free radicals' effects.^[2,3]

Antioxidant compounds' role is crucial for health, mainly their ability to scavenge free radicals. Commonly, plant-based antioxidants, such as carotene, Vitamin E, Vitamin C, and phenols (polyphenols and flavonoids), potentially reduce the risk of degenerative diseases caused by free radicals.^[4,5] Antioxidant compounds can be obtained from vegetables and fruits that we often consume, such as avocados.

The avocado peel is rich in biochemical properties, potentially useful for humans. Phytochemically tested

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Address for correspondence:

Mr. Bohari Bohari,
Persada Banten Blok A6 No 27, City of Serang, Banten,
Indonesia.
E-mail: bohari@untirta.ac.id

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avocado peel contains secondary metabolites, including flavonoids, tannins, and anthocyanins.^[6] Furthermore, studies on the positive impact of specific phytochemicals show promising results on health, so it is predicted that this avocado waste will also be beneficial.

Avocado (*Persea americana* Mill.) is the *Lauraceae* plant family that grows in subtropical and tropical areas. It is one of the essential pharmaceutical plants used as traditional medicine for thrush, urinary stones, high blood pressure, dry facial skin, toothache, swelling due to inflammation, and diabetes.^[7] The fruit is rich in nutrients and plays as natural antioxidants. The fruit peels provide the largest source of natural antioxidants in avocados. Previous studies show that avocado peels can be developed as a promising natural antioxidant, even though it is not often utilized. The avocado peel contains compounds with hydroxy groups that play antioxidant activity.^[8]

In general, plants, such as avocados, mainly contribute antioxidant activity, containing secondary metabolites or active compounds, including flavonoids, phenolics, tannins, and anthocyanins.^[9] This study aimed to analyze the potential of avocado peel extract as a source of antioxidants, total phenols, total flavonoids, total tannins, saponin levels, and total alkaloids.

MATERIALS AND METHODS

Preparation of avocado peel extract

Sample preparation starts from the sampling stage, separating the peels from the fruit flesh, sorting, cleaning with running water, drying in a place not exposed to direct sunlight, then powdered. Extracts were made by placing a powdered sample in a maceration container and then adding methanol. The maceration container was closed and stored in a place protected from direct sun rays for 24 h, stirring occasionally. It was then filtered to separate the dregs and the filtrate. The dregs were re-extracted with the same amount of methanol as a solvent; re-extraction ended when the filtered liquid was clear. The filtrate was then evaporated with a rotary evaporator and was weighed using an analytical scale.

Antioxidant determination

The concentrate obtained was determined for its antioxidant activity through DPPH methods.^[10] The sample extract was weighed as much as 10 mg and then put into a 10 ml volumetric flask, adjusted with an ethanol solvent to obtain a solution concentration of 1000 ppm. Then, serial dilutions were carried out to obtain 20, 40, 60, 80, and 100 ppm solutions. The solution was pipetted as much as 1 ml, added with 3 ml of 50 M DPPH solution, then homogenized, and placed in the dark for 30 min. The wavelength used in the spectrophotometry in this study was 517 nm. The

absorbance value obtained was used to determine the %inhibition using the following equation:

$$\% \text{Inhibition} = \frac{\text{Abs. DPPH} - \text{Abs. Sample}}{\text{Abs. DPPH}} \times 100\%$$

Next, create a %inhibition curve and determine IC_{50} based on the obtained regression equation.

Determination of total phenols, flavonoids, tannins, saponins, and alkaloids

Total phenol was identified by making a gallic acid calibration curve with the Folin–Ciocalteu reagent and determining the total polyphenol content using the Folin–Ciocalteu method.^[11]

Total flavonoids were determined by the colorimetric method referring to Chang *et al.*'s procedure with several standards with quercetin.^[12] Total tannins were revealed by using the Folin–Ciocalteu method.

The method for determining the level of saponins is based on Adawiyah's research (2017), about 1.25 g of extract was refluxed with 50 ml of petroleum ether at a temperature of 60°C–80°C for 30 minutes.^[13] After cooling, the petroleum ether solution was removed, and the residue left was dissolved in 50 ml of ethyl acetate. The solution was transferred to a banana funnel and then separated from the ethyl acetate solution. The residue left was dissolved with n-butanol 3 times with 50 ml each. The entire n-butanol solution was mixed and evaporated using a rotary evaporator. The remaining evaporation was dissolved with 10 ml of methanol, and then, this solution was dropped into 50 ml of ether while stirring. The precipitate formed in the mixture was poured on filter paper whose weight was known. The precipitate on filter paper was dried and then weighed to a constant weight. The weight difference in the filter paper before and after filtering was the weight of the saponins.

Determination of total alkaloid content was carried out using a sample of 200 g/ml of each extract test solution added with 2 ml of phosphate buffer pH 4.7 and 2 ml of BCG solution (Bromocresol green) then extracted with 3 ml of chloroform for 3 minutes using a vortex. The chloroform phase was taken up to the volume limit and measured the absorbance at a wavelength of 430 nm. Blanks were all reagents without extract solution.^[14]

Data analysis

All experiments were carried out with two replications, and the data obtained from the test results were processed and then analyzed statistically using Statistical Package for the Social Sciences (SPSS). Descriptive analysis was used to analyze the data by describing the data that have been collected as it is.

RESULTS

The antioxidant ability of methanolic extract of avocado peel was determined twice in two replicates using 5 concentrations: 20, 40, 60, 80, and 100 ppm at 517 nm. Table 1 shows that the antioxidant of avocado peel in IC₅₀ was 185.891 ± 1.598 ppm.

Determination of the phytochemical compounds of avocado peel was carried out on the total phenols, total flavonoids, total tannins, saponins, and total alkaloids. Table 2 shows the value of each phytochemical compound; total phenol was 21.833 ± 0.118 mg/100 g extract; total flavonoid was 2.607 ± 0.111 mg/100 g extract; total tannin was 38.357 ± 0.202; saponin content was 8.874% ± 0.031%; and total alkaloid was 9.95 ± 0.035 mg CE/g extract.

DISCUSSION

The antioxidant activity in this study is expressed in IC₅₀ values. This study found that the methanolic extract from avocado peels had antioxidant activity in the weak category. The phytochemical content in this extract revealed that tannins have the most significant proportion, followed by phenols, alkaloids, saponins, and flavonoids, respectively. Thus, it was assumed that the low proportion of phytochemicals affects the antioxidant activity of the observed material.

According to this study, the antioxidant activity of avocado peels from Indonesia had an IC₅₀ value of 185.891±1.598 ppm obtained from methanol extraction. Different things were revealed by Kamaraj M *et al* (2020) research,^[15] which obtained an antioxidant activity of aqueous extract of avocado peels in IC₅₀ reaching 71.96 ppm, where the total content of phenols in their extraction reached 51.58 mg GAE/g extract, which is twice higher than the total phenols in our methanolic extract. Then, the flavonoid values tend to be at the same level, but the tannin content in their aqueous extract was lacking at 4.33 mg TAE/g extract compared to the tannin in our methanol of 38.3 mg/100 g extract. Thus, it can be understood that the low antioxidant activity in our study resulted from the low content of total phenols, which is also affected by the extractant used.

Phytochemicals are beneficial substances in plant parts because of their biological activity on animal metabolism, including humans. Phytochemicals themselves can function as inhibitors and cofactors in enzymatic reactions and scavengers of harmful chemical toxic substances, including free radicals, increase the metabolism of nutrients in the body, and influence taste and color. Various studies have shown a close relationship between phytochemicals and their essential role in inhibiting and repairing inflammation, diabetes, heart disease, cancer, tumors, and diseases related to metabolism in animal cells, even though there are also adverse effects on the body.^[16-18] In anticancerogenic behavior, phytochemical mechanisms include increasing the neutralization and excretion of carcinogens, inhibiting the rate of inflammation and mitosis, and inducing apoptosis.^[19]

The phytochemical substance consists of complex polyphenols, a biocompound contributing to the aromatic rings of hydroxyl substance, which leads to potent antioxidant activities. In our avocado peel extract, specifically the avocado from Indonesia, phenolic phytochemicals, such as flavonoids, tannins, alkaloids, and saponins, were detected. The detection of saponins in our avocado peel methanol extract showed different results from the study by Kamaraj *et al.*^[19] on aqueous extract of avocado peels from Ethiopia, which shows the absence of saponin components. It is presumably due to the influence of the type of extractant used.^[20] Total phenols were strongly associated with antioxidant activity. Several studies have shown that a high total phenol substance contributes to a more potent antioxidant activity.^[21] In addition, Zhang *et al.* showed a positive correlation between total phenols and flavonoids on the antioxidant activity as measured by the inhibition of DPPH free radicals.^[22] The partial least squares projection of latent structures method has also illustrated the critical role of flavonoids on antioxidant activity.^[23] Another phytochemical substance detected in our methanolic extract that was particularly valuable was tannins, consisting of many phenols rings. This substance consists of hydrolyzable tannins (a combination of phenols and ester bonds, which can be hydrolyzed by enzymes, mineral acids, or alkaline) and condensed tannins (containing flavonoids with some degree of condensation).^[24] Then, Dalimunthe *et al.*^[25] proved that the alkaloid components obtained from *Litsea cubeba* Lour. contribute as an antioxidant due to hydroxyl,

Table 1: Antioxidant activities of methanolic extract of avocado peel

Concentration (ppm)	Absorption at 517 nm		Inhibition (%)		IC ₅₀ (ppm)		IC ₅₀ (ppm) (Mean±SD)
	a	b	a	b	a	b	
20	0.387	0.385	10.624	11.085	187.021	184.761	185.891±1.598
40	0.365	0.362	15.704	16.397			
60	0.345	0.340	20.323	21.478			
80	0.327	0.325	24.480	24.942			
100	0.304	0.302	29.792	30.254			

a: Repetition 1, b: Repetition 2. SD: Standard deviation

Table 2: Total phenol, total flavonoid, total tannin, saponin level, total alkaloids of methanolic extract of avocado peels

Parameter	Measurement result		
	a	b	Mean±SD
Total phenol (mg/100 g extract)	21.750	21.917	21.833±0.118
Total flavonoids (mg/100 g extract)	2.529	2.686	2.607±0.111
Total tannins (mg/100 g extract)	38.214	38.500	38.357±0.202
Saponin content (%)	0.852	0.896	8.874±0.031
Total alkaloids (mg CE/g extract)	9.975	9.925	9.95 ± 0.035

a: Repetition 1, b: Repetition 2. SD: Standard deviation

methoxyl, methylene, and methyl groups. In another study, it was shown that in *Lepidium mayenii*, alkaloids were able to contribute more than phenols to their antioxidant effects, as measured by hydroxyl radical scavenging ability, ferric reducing antioxidant potential, and lipid peroxidation inhibition ability.^[26]

The use of avocado peels, a by-product waste, shows a bright potential for further processing. Avocado peels, together with other fruit peels, such as the fruit peels of mango, apples, melons, papayas, and other fruit products in Australia, which are freeze-dried, have been shown to have the potential to contain phenols that work as antioxidants.^[27,28] One of the processing products, a fruit peel tea, shows satisfactory results; tea from drying avocado fruit peel has antioxidant activity with flavonoid and phenols content that is more promising than apple peel tea.^[29] Thus, avocado fruit peel is identified as a source of phytochemicals that contribute to antioxidants.

CONCLUSIONS

The antioxidant power of methanol extract from the avocado peel is about 185.891 ± 1.598 ppm and positively contains total phenol (21.833 ± 0.118 mg/100 g extract), total flavonoids (2.607 ± 0.111 mg/100 g extract), total tannins (38.357 ± 0.202 mg/100 g extract), saponin content ($8.874\% \pm 0.031\%$), and total alkaloids (9.95 ± 0.035 mg CE/g extract). It is a promising waste product to be further used as an antioxidant source.

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Conflicts of interest

There are no conflicts of interest.

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