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Antioxidant Activity of Combination Simplicia and Extract of Avocado Peel (*Persea americana* Mill.) and Lime Peel (*Citrus aurantifolia*)

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Abstract

The avocado peel (*Persea americana* Mill.) and lime peel (*Citrus aurantifolia*) are biodegradable waste materials that contain antioxidant activities. This study aims to determine the antioxidant activities of avocado peel and lime peel extracts on their single and combined form. Samples were extracted with the maceration method using 96% ethanol solvent, then an antioxidant activity test towards DPPH (*2,2-diphenyl-1-picrylhydrazyl*) radical inhibition measured using the UV-Vis spectrophotometer. This research showed that the antioxidant activities obtained with the value of IC_{50} (inhibition concentration) are at 24.82 ppm from the avocado peel extract, 131.12 ppm from lime peel extract, and also from the simplicia combination with the ratio of 1:1; 1:2; and 2:1 are respectively at 45.01 ppm, 87.41 ppm; and 38.49 ppm, meanwhile on the combined extracts with the ratio of 1:1; 1:2; and 2:1 are respectively at 31.16 ppm; 39.28 ppm; and 11.69 ppm and followed by ascorbic acid at 4.35 ppm.

Keywords: Antioxidant, Avocado peel, Lime peel, IC_{50}, DPPH

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1 Introduction

Indonesia is a country that has high biodiversity, among which there are various kinds of nutritious plants that are scattered. Utilization of bioavailability especially plants as a source of traditional medicine is still the main choice for most people. In everyday life, the disease can be caused by various sources, one of which is free radicals. Free radicals, if in excessive amounts in the body, will cause oxidative stress. This condition can cause oxidative damage from the cellular, tissue, to organ levels, which accelerates the aging process and the emergence of degenerative diseases, and cancers that are harmful to health [1].

Antioxidants are compounds that donate electrons (electron donors) to free radicals, thereby stopping free radical chain reactions and turning them into stable forms. The benefits of antioxidants for the body include protecting body cells from damage caused by free radicals [2, 3]. Based on the source, antioxidants are divided into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants that come from within our bodies such as superoxide dismutase (SOD), glutathione, and catalase. While non-enzymatic antioxidants consist of antioxidants from natural ingredients and synthetic antioxidants that come from outside the body. Antioxidants from natural ingredients such as ascorbic acid, flavonoids, vitamin E, β-carotene, and xanthophylls. Synthetic antioxidants such as BHA (*butyl hydroxyl anisol*), BHT (*butylhydroxytoluene*), propyl gallate, and TBHQ (*tert-butyl hydroxy quinone*). Currently, the use of synthetic antioxidants such as propyl gallate and TBHQ is getting attention because it has detrimental effects, such as impaired liver, lung function, and poisoning with long-term and continuous use [2, 4]. Therefore, it is necessary to have natural sources of antioxidants that are easily obtained and are available in nature in abundant quantities, and have low side effects compared to synthetic antioxidants.

Natural ingredients known to have antioxidant activity are avocado peel and lime peel. So far, the utilization of avocado peel and lime peel is still not optimal because most people only think of them as biodegradable waste materials. According to previous research, it was shown that the ethanolic extract of avocado peel (*Persea americana* Mill) has antioxidant activity with an IC_{50} value of 137.34 ppm showing the medium antioxidant activity and contains secondary metabolites, namely alkaloids, flavonoids, saponins, tannins, and polyphenols [5, 6]. The content of flavonoid compounds in the avocado peel can act as an antioxidant and role in reducing free radical damage [7]. Meanwhile, the ethanolic extract of lime peel (*Citrus aurantifolia*) has been reported to contain secondary metabolites, namely alkaloids, flavonoids, and saponins, and is known to have antioxidant activity with an IC_{50} value of 54,458 ppm [4, 8].

Combined research was carried out with the aim of obtaining a synergistic effect on the active ingredients. The combination of synergistic effects is a goal pursued in the development of medicinal plants [9]. Therefore, this study aims to determine the antioxidant activity of reducing DPPH radicals from the combination of simplicia and extracts of the two samples based on the absorbance value using a UV-Vis spectrophotometer.

2 Materials and Methods

2.1 Tools and Materials

The tools used are Buchner funnel, beaker, hot plate, cuvette, volumetric flask, oven, rotary evaporator, double beam UV-Vis spectrophotometer (Dynamica Halo DB-20S), test tube, analytical balance (Precisa®).

The materials used are avocado peel, lime peel, pro-analysis ethanol, 96% ethanol, DPPH, ascorbic acid, distilled water, filter paper, aluminum foil, plastic wrap, magnesium powder, concentrated HCl, *Mayer* reagent, *Dragendorff* reagent, *Wagner* reagent, $FeCl_3$ 1%, NaCl 10%, gelatin salt, HCl 2N, *Liebermann-Burchard* reagent.

2.2 Plant Determination

The samples used were avocado peel and lime peel. A determination was made in advance to obtain a clear identity truth that the plants used were actually the peel of the avocado and lime fruit. The determination was carried out at the Laboratory of Ecology and Conservation of Tropical Forest Biodiversity, Faculty of Forestry, Mulawarman University.

2.3 Sample Preparation and Making Simplicia

Peel the avocado and lime and separate the peel from the flesh and seeds. Then, wet sorting is done to separate the parts that are not needed, then washed clean and then sorted dry and then cut into small pieces and dried using an oven. So that the simplicia peel of avocado and lime is obtained, then mashed using a blender to become simplicia powder.

2.4 Extraction

2.4.1 Single Sample Extraction

Fifty grams of samples of avocado peel and lime peel, each put into a maceration jar. Then, 200 mL of 96% ethanol solvent was added until the entire sample was submerged, and the jar was closed, stirred, and allowed to stand for 24 hours. The macerate was filtered using filter paper and accommodated in a container, then concentrated with a rotary evaporator until a thick extract was obtained.

2.4.2 Combination Sample Extraction

Samples of avocado peel and lime peel were made in the ratio of the amount of simplicia 1:1; 1:2; and 2:1. Then 96% ethanol solvent was added until the entire sample was submerged, and the jar was closed, stirred, and allowed to stand for 24 hours. The macerate was filtered using filter paper and accommodated in a container, then concentrated with a rotary evaporator until a thick extract was obtained.

2.5 Calculation of the Extraction Yield

The thick extract of a single sample and the combination of avocado peel simplicia and dried lime peel was calculated as Extraction Yield (%) with the formula in equation 1.

$$\text{Yield}(\%) = \frac{\text{Weigh of the dry extract}}{\text{Weigh of the simplicia before extraction}} \times 100\% \quad \text{(Equation 1)}$$

2.6 Phytochemical Screening Test

2.6.1 Flavonoid Test

2 mL of the extract solution was put in a test tube, added with magnesium powder, and four drops of concentrated hydrochloric acid (HCl), the reaction was positive if the solution turned red, orange, or dark.

2.6.2 Alkaloid Test

2 mL of the extract solution was added with 2 mL of 2% hydrochloric acid (HCl) divided into 3 test tubes. Tube 1 was added with Mayer reagent (positive reaction if a yellowish-white precipitate was formed), tube 2 was added with Dragendorff reagent (positive reaction if it feculent or an orange precipitate formed), tube 3 was added with

Wagner reagent (positive reaction if brown precipitate formed).

2.6.3 Phenolic Test

2 mL of the extract solution was put into a test tube and added with 1% ferric chloride ($FeCl_3$) reagent, if a black color was formed, it indicated the presence of phenolic compounds.

2.6.4 Tannins Test

3 mL of the extract solution was put in a test tube, added with 10 mL of hot water and then cooled. Then five drops of 10% sodium chloride (NaCl) were added and filtered, then the gelatin salt was added. The reaction is positive if a precipitate is formed.

2.6.5 Saponin Test

2-3 mL of the extract solution was put into a test tube, added 10 mL of hot water and cooled. Then shake vigorously for 10 seconds and add one drop of 2N hydrochloric acids (HCl). The reaction is positive if a stable foam of 1-10 cm is formed for not less than 10 minutes.

2.6.6 Steroids and Triterpenoids Test

2 mL of the extract solution was added ten drops of anhydrous acetic acid reagent and two drops of concentrated sulfuric acid. If a blue or green color is formed, it indicates the presence of steroids, while the formation of red or purple color indicates the presence of triterpenoids.

2.7 Antioxidant Activity Test

2.7.1 Preparation of DPPH

DPPH stock solution was made by weighing 4 mg of DPPH, then dissolved with ethanol, and then put into a 100 mL dark volumetric flask, added ethanol to the mark, and homogenized. Obtained DPPH solution with a concentration of 40 ppm.

2.7.2 Determine the Maximum Wavelength

The maximum wavelength of DPPH was measured using a UV-Vis spectrophotometer in the wavelength range of 510-520 nm. Then, the maximum wavelength that has the greatest absorbance value is determined. Furthermore, all measurements were made at the maximum wavelength.

2.7.3 Measurement of Antioxidant Activity of Extract in Single Form and Combination Simplicia of Avocado Peel (AP) and Lime Peel (LP)

A stock solution of a single extract and a combination of simplicia was made in a ratio of 1:1; 1:2; and 2:1 avocado peel and lime peel with a concentration of 1000 ppm and then a series of concentrations of 10 ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm each made three replications. Each series solution with a concentration of 2 mL of extract was pipetted into a test tube lined with aluminum foil. Then 2 mL of 40 ppm DPPH solution was added, homogenized and incubated for 30 minutes at room temperature in a dark place to further measure the absorbance at the maximum wavelength obtained at 514 nm using a UV-Vis spectrophotometer.

2.7.4 Measurement of Antioxidant Activity of Combination Extract of Avocado Peel (AP) and Lime Peel (LP)

Preparation of stock solution of a combination of extracts from a ratio of 1:1; 1:2; and 2:1 were obtained by weighing the avocado peel extract and lime peel extract as presented in Table 1. So that obtained stock solution with a concentration of 1000 ppm and made a series of concentrations of 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm each made three replications. Each series solution with a concentration of 2 mL of extract was pipetted into a test tube lined with aluminum foil. Then 2 mL of 40 ppm DPPH solution was added, homogenized and incubated for 30 minutes at room temperature in a dark place to further measure the absorbance at the maximum wavelength obtained at 514 nm using a UV-Vis spectrophotometer.

Table 1. The ratio of Total Extracts for Each Combination

Combination	Avocado Peel	Lime Peel	Volume	Concentration
1:1	12,5 mg	12,5 mg	25 mL	1000 ppm
1:2	8,33 mg	16,67 mg	25 mL	1000 ppm
2:1	16,67 mg	8,33 mg	25 mL	1000 ppm

2.7.5 Measurement of Antioxidant Activity of Positive Control (Ascorbic Acid)

A stock solution of vitamin C was made with a concentration of 40 ppm, and a series of concentrations of 2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm were made with three replications each. Each series solution with a concentration of 2 mL of vitamin C was pipetted into a test tube lined with aluminum foil. Then 2 mL of 40 ppm DPPH solution was added, homogenized and incubated for 30 minutes at room temperature in a dark place to further measure the absorbance at the maximum wavelength obtained at 514 nm using a UV-Vis spectrophotometer.

2.7.6 Analysis of Antioxidant Activity

Analysis of antioxidant activity data using the DPPH method, the parameter used to show antioxidant activity is the IC_{50} (Inhibition Concentration) value, which is a concentration of antioxidants that can inhibit 50% of DPPH free radicals. The value of the percentage of inhibition with the following formula

$$\%\text{Inhibition} = \frac{(A0 - A1)}{A0} \times 100\% \quad \text{(Equation 2)}$$

Where,
A0 = The absorbance of blank
A1 = The absorbance of the sample

Then the data was processed using probit analysis, and a linear regression curve was made between the log concentration of the test solution (x) and the probit value (y) to obtain the IC_{50} value of the extract in single or combination form. Furthermore, the value of the antioxidant activity is categorized based on the level of antioxidant activity.

3 Results and Discussion

3.1 Data of the Extraction Yield

The initial weight of the avocado peel and lime peel simplicia samples before extraction at 50 grams each. Then the extraction yield (%) was calculated by dividing the weight of the dry extract of avocado peel obtained by as much as 7.91 grams and lime peel by as much as 5.37 grams by the initial weight and then multiplied by 100%. Thus, the yield of avocado peel extract at 15.82%, and lime peel extract at 10.74%.In the sample combination of avocado peel and lime peel simplicia with a ratio of 1:1; 1:2; and 2:1, respectively, the initial total weight before extractionat 120 grams and divided by the weight of the dry extract of the simplicia combination in a ratio of 1:1; 1:2; and 2:1, respectively 13.44 grams; 14.59 grams; and 15.32 grams. Thus, the % yield of the simplicia combination extract in a ratio of 1:1; 1:2; and 2:1 each at 11.2%; 12.15%; and 12.76%

3.2 Phytochemical Screening

Phytochemical screening is an early stage and an approach method that can be used to qualitatively determine the presence of secondary metabolite compounds in avocado peel and lime peel which have potential as antioxidants. The results of the secondary metabolite test are shown in Table 2. The results of phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, and tannins in both samples, with the addition of saponins in avocado peel extract.

Table 2. Result of Phytochemical Screening of Avocado Peel Extract (APE) and Lime Peel Extract (LPE)

Phytochemical Screening		Result		Description	Positive Result
		APE	LPE		
Alkaloid	(Mayer Reagent)	+	+	A white precipitate is formed.	White precipitate
	(Wagner Reagent)	+	+	A brown precipitate is formed.	Brown precipitate
	(Dragendorff Reagent)	+	+	An orange precipitate is formed.	Orange precipitate
Flavonoid		+	+	Formation of red color	Red, yellow, and orange
Phenolics		+	+	Formation of black color	Black color
Tannins		+	+	A precipitate is formed.	Form a precipitate
Saponin		+	-	Foam for 10 minutes and not formed	Forming foam
Triterpenoids		-	-	Not formed	Forming a red or purple color
Steroids		-	-	Not formed	Forming a blue or green color

Description: (+) Positive result : contains compounds, (-) Negative result : no compound content

Table 3. Data of Antioxidant Activity Test of Extract of an Avocado Peel (APE) and a Lime Peel (LPE), Combination Simplicia and Extract of Avocado Peel (AP) and Lime Peel (LP)

Sample	Concentration (ppm)	Log Concentration	% Antioxidant Activity	Probit	IC_{50} (ppm)
APE	10	1	22,04 %	4,23	24,82
	20	1,301	41,89 %	4,79	
	40	1,602	69,55 %	5,51	
	80	1,903	83,07 %	5,95	
	160	2,204	92,53 %	6,44	
LPE	10	1	25,48 %	4,34	131,12
	20	1,301	27,02 %	4,39	
	40	1,602	31,32 %	4,50	
	80	1,903	42,47 %	4,80	
	160	2,204	54,65 %	5,18	
CS AP:LP (1:1)	10	1	11,61 %	3,80	45,01
	20	1,301	24,91 %	4,32	
	40	1,602	35,01 %	4,61	
	80	1,903	68,66 %	5,48	
	160	2,204	89,90 %	6,27	
CS AP:LP (1:2)	10	1	9,69 %	3,70	87,41
	20	1,301	14,10 %	3,92	
	40	1,602	24,19 %	4,29	
	80	1,903	38,86 %	4,71	
	160	2,204	75,56 %	5,69	
CS AP:LP (2:1)	10	1	13,30 %	3,88	38,49
	20	1,301	24,59 %	4,31	
	40	1,602	46,23 %	4,90	
	80	1,903	83,33 %	5,96	
	160	2,204	86,93 %	6,12	
CE AP:LP (1:1)	10	1	25,27 %	4,33	31,16
	20	1,301	25,68 %	4,35	
	40	1,602	48,96 %	4,96	
	80	1,903	82,87 %	5,94	
	160	2,204	93,01 %	6,48	
CE AP:LP (1:2)	10	1	19,20 %	4,27	39,28
	20	1,301	28,84 %	4,90	
	40	1,602	41,56 %	5,92	
	80	1,903	65,00 %	6,02	
	160	2,204	91,10 %	6,19	
CE AP:LP (2:1)	10	1	40,43 %	4,75	11,69
	20	1,301	64,86 %	5,38	
	40	1,602	80,86 %	5,87	
	80	1,903	89,00 %	6,23	
	160	2,204	91,56%	6,37	

Description: CS: Combination of Simplicia; CE: Combination of Extract

3.3 Antioxidant Activity

The antioxidant activity test was carried out using the DPPH method. The antioxidant activity produced is the IC_{50} value, which is the concentration of the test solution to inhibit 50% of DPPH free radicals [10].The DPPH method is a simple, fast, and easy method to do, besides that this method is also accurate, effective, and practical [11].Antioxidant activity can be categorized based on the IC_{50} value. If the IC_{50} value of a sample is <50 ppm, the antioxidant activity is very strong. When the IC_{50} value is in the 100-150 ppm range that the antioxidant activity is moderate. When the IC_{50} value is in the 150-200 ppm range that the antioxidant activity is weak, and if the IC_{50} value is >200 ppm that the antioxidant activity is very weak [5].

Measurement of antioxidant activity was carried out at a maximum wavelength of 514 nm. The results of testing the antioxidant activity of an extractof avocado peel and lime peel using various concentrations of 10, 20, 40, 80, and 160 ppm are shown in table 3.

The results of the antioxidant activity test of an APE, LPE, combination of simplicia and extract ratio 1:1; 1:2; and 2:1 indicates that the higher the concentration of the extract, the higher the value of % antioxidant activity. This can be explained by the color changes that occur in the DPPH compound, which was originally purple but turned yellow when the extract was added. The higher the concentration of the extract, the higher the content of compounds that act as antioxidants, so the higher the inhibition of DPPH, which causes the absorbance value to be lower. This color change occurs when the DPPH radical is captured by antioxidants which release hydrogen atoms to form stable DPPH-H [11].

The results of measuring the antioxidant activity showed that the IC_{50} value of APE at 24.82 ppm means a very strong antioxidant activity, and the LPE showed that the IC_{50} value at 131.12 ppm means moderate antioxidant activity. In the sample combination of Simplicia of AP and LP the ratio is 1:1; 1:2; and 2:1, respectively showing the results at 45.01 ppm (very strong); 87.41 ppm (strong); and 38.49 ppm (very strong). Meanwhile, in the combination of extract of AP and LP the ratio is 1:1; 1:2; and 2:1 respectively showing the results at 31.16 ppm (very strong); 39.28 ppm (very strong); and 11.69 ppm (very strong). The strongest antioxidant activity was shown in the sample combination extract in a ratio of 2:1. This could be due to the synergistic effect resulting from the combination of both samples. A synergistic effect can be produced if the two ingredients can provide a more optimal effect when combined compared to the single form [12].

The measurement results of the positive control antioxidant activity (ascorbic acid/Vitamin C) using various concentrations of 2, 4, 8, 16, and 32 ppm are shown in table 4.

Table 4. Data of Antioxidant Activity of Positive Control (Ascorbic Acid/Vitamin C)

Sample	Concentration (ppm)	Log Concentration	% Antioxidant Activity	Probit	IC_{50} (ppm)
	2	0,301	35,62 %	4,62	
	4	0,602	37,88 %	4,68	
Vitamin C	8	0,903	66,73 %	5,43	4,35
	16	1,204	83,31 %	5,96	
	32	1,505	92,44 %	6,44	

Ascorbic acid or vitamin C is one of the natural antioxidants, it is often used in the comparison of antioxidants and has become a general standard because it is easy to obtain. In addition, vitamin C is also a natural antioxidant recommended by BPOM to be consumed by the

general public [11]. Therefore, in this study, vitamin C was used as a positive control or comparison. In vitamin C, the IC_{50} value at 4.35 ppm means a very strong antioxidant activity

Table 5. Comparison of IC_{50} Value of an Avocado Peel Extract (APE), a Lime Peel Extract (LPE), Combination of Simplicia, Combination of Extracts, and Positive Control (Vitamin C)

Sample	IC_{50} (ppm)	Category
APE	24,82	Very strong
LPE	131,12	Moderate
CS APE:LPE (1:1)	45,01	Very Strong
CS APE:LPE (1:2)	87,41	Strong
CS APE:LPE (2:1)	38,49	Very strong
CE APE:LPE (1:1)	31,16	Very strong
CE APE:LPE (1:2)	39,28	Very strong
CE APE:LPE (2:1)	11,69	Very strong
Vitamin C	4,35	Very strong

Description: CS: Combination of Simplicia; CE: Combination of Extracts

4 Conclusions

Based on the results of the research that has been obtained, it can be concluded that the :

1. The value of yield extraction (%) of avocado peel extract at 15.82%, lime peel extract at10.74%, and the combination of simplicia at 1:1; 1:2; and 2:1 respectively, which is 11.2%; 12.15%; and 12.76%.
2. Phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, and tannins in both samples, with the addition of saponins in avocado peel extract.
3. The antioxidant activity of an avocado peel extract is based on the IC_{50} value at 24.82 ppm (very strong), and lime peel extract at 131.12 ppm (moderate).
4. The antioxidant activity of the combination of simplicia ratio of 1:1; 1:2; and 2:1, respectively at 45.01 ppm (very strong); 87.41 ppm (strong); and 38.49 ppm (very strong). While the combination of extracts ratio of 1:1; 1:2; and 2:1, respectively at 31.16 ppm (very strong); 39.28 ppm (very strong); and11.69 ppm (very strong). The strongest antioxidant activity was shown in the sample combination of extract in a ratio of 2:1.

5 Declarations

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5.2 Author contribution

Elsa Dwi Rahayuningrum contributed to designing methods, conducting research, collecting data, and preparing draft manuscripts. Fajar Prasetya and Nur Masyithah Zamruddin contributed to the direction, adviser, and final synchronization of the manuscript.

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5.4 Conflict of Interest

There is no conflict of interest in this research.

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