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Antioxidant activities of extracts, methanol, and n-hexane partitions of cayenne pepper (*Capsicum frutescens* L.)

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ABSTRACT

Antioxidants are substances that can neutralize and/or stabilize free radicals. Cayenne pepper (*Capsicum frutescens* L.) is a natural source of antioxidants because it contains carotenoids, flavonoids, and vitamin C. This study aims to examine antioxidant activities of n-hexane, methanol, and extract of cayenne pepper (*Capsicum frutescens* L.) using the ferric reducing antioxidant power (FRAP) method. The extraction method was done by maceration, and the fractionation method was done by partitioning using unmixed solvents: methanol and n-hexane. The phytochemical screening test on extracts and partitions has discovered positive content of alkaloids, flavonoids, phenols, and terpenoids. Meanwhile, the TLC test used F254 silica gel as a stationary phase with chloroform:ethyl acetate as a mobile phase (6:4). The test discovered positive results containing flavonoids and phenols. Furthermore, the antioxidant activities were tested using the FRAP method with a UV-Vis spectrophotometer instrument at a maximum value of 598.05 nm and positive control of vitamin C. The results of measurement show that antioxidant activities are found in 505.67 ± 0.56 mol Fe2+/g of partition extract, , 448 ± 16.19 µmol Fe2+/g of methanol fraction, and 353 ± 5.29 mol Fe2+/g of n-hexane fraction.

1. Introduction

Indonesia is rich in plants that are possibly used as a source of natural medicines. There are approximately 30,000 plants that can be used as medicines. Some of them are also commonly used as seasonings by mothers to cook daily meals for families. One of the plants is cayenne pepper, an important horticultural commodity in Indonesia and is inseparable from daily needs. Cayenne pepper contains a lot of vitamins, such as vitamins A, B1, and C, as well as minerals. Cayenne pepper also contains flavonoids (S.M., 2017). Vitamin C in cayenne pepper is an enzymatic antioxidant that works by cleaning compounds and new-free radicals (Firdaus et al., 2013).

Free radicals are molecules or compounds that can independently stand and contain one or more unpaired electrons. The impacts of free radical reactivities can damage cell structures that interfere with the physiology of the cells. Finally, all cells become damaged; inflammation, aging processes, declining immunity, cancer, and atherosclerosis occur (Yuslianto, 2018).

The ferric reducing antioxidant power (FRAP) is a method to measure antioxidants' ability to reduce the Fe3+ ions to Fe2+ so that the antioxidant power of a compound is analogized by increasing absorbance values that indicate the dimensions of antioxidant activities of the tested sample (Pratama et al., 2018).

Cayenne pepper is used by society to cook a dish, and this ingredient has diverse efficacy. However, this diverse efficacy is not widely studied. Therefore, this research aims to investigate

antioxidant activities in the cayenne pepper's extract, n-hexane partition, and methanol by using the ferric reducing antioxidant power (FRAP) method.

2. Materials and Methods

2.1. Preparation of samples

The materials used in this research were cayenne pepper taken from Sokayasa Village of Banjarnegara Regency, vitamin C, n-hexane, gallic acid, quercetin, and folic ciocalteau. Meanwhile, the tool used in this research was a rotary vacuum evaporator and UV-Vis spectrophotometer (Shimadsu, YEAR). The research sample was determined in the Laboratory Science and Technology, Universitas Ahmad Dahlan (UAD). The sample of this research was cayenne pepper (*Capsicum frutescens* L.) from Sokayasa Village, Banjarnegara District. The sample was picked in the morning when it was ripe and red.

The research sample was 3 kg of fresh cayenne pepper. The cayenne pepper was sorted wet and washed with running water. It was chopped and dried under the sun indirectly (covered in black cloth). Then, it was sorted dried, subsequently mashed using a blender, and sieved with a sieve of 40 mesh to get Simplicia powder. The next step was packaging the powder and giving etiquette on the packages as an identity.

2.2. Processes of Extract Making

The Simplicia powder of cayenne pepper (*Capsicum frutescens* L) was weighed as much as 500 g and macerated with ethanol 96% as much as 2.5 L. The maceration process was done in a medium-sized container sealed by the aluminum foil for five days. During the process, the solution was stirred for one hour per day. After that, the solution was filtered to separate the pulp and its filtrate. The pulp was macerated using the same solvent as much as 500 mL. The result of maceration was then refiltered. The filtrate was concentrated by using a rotary evaporator at a temperature of 60°C. The liquid extract was evaporated using an oven at a temperature of 60oC to get a thick extract.

2.3. Extract Partitions

The results of the condensed extract of cayenne pepper were weighed as much as 30 g, put in a separating funnel, added with 100 mL of n-hexane, shaken, added with 100 mL of more solvent methanol, shaken again, and let rest for one day. The partition produced two separate layers because of different weights. The results of the methanol partition and n-hexane were collected and evaporated to obtain a thick partition.

2.4. Phytochemical Screening

The first step was phenol identification. 10 mg of extract, n-hexane partition, and methanol partition were dissolved with 5 mL of distilled water and added with a few drops of FeCl3 5%. Positive results would contain phenol when producing a dark green color (Hanani, 2015).

The next step was flavonoid identification. In this stage, 10 mL of hot water was added to 10 mg of extract, n-hexane partition, and methanol partition. The solutions were boiled for 5 minutes and then filtered. Then, 5 mL of filtrate was added with 0.05 mg of Mg powder and 1 mL of concentrated HCl. The solution was then shaken vigorously. A positive result is indicated by the formation of red, yellow, or orange colors (Hanani, 2015).

The following step was alkaloids identification. 1 mg of extract, n-hexane partition, and methanol partition were dissolved with 5 mL of HCl and then filtered. The filtrate was added with Mayer's, Wagner's, and Dragendorff's reagents for 1-2 drops. If the filtrate positively contains alkaloids, white precipitate would form in Mayer's reagent, red to brown precipitate would form in the Wagner's reagent, and yellow precipitate would form in the Dragendorff's reagent (Hanani, 2015).

The last step was terpenoid-steroid identification. Lieberman-Burchard's reagents were added to the extract of n-hexane and methanol. Then, the solution was gently shaken and let rest for a few minutes. Positive results would contain blue or green steroids; orange, red, or purple colors indicate the presence of triterpenoids (Hanani, 2015).

2.5. Thin-Layer Chromatography

Samples of concentrated extract, n-hexane partition, and methanol were spotted on the TLC plate. The standard comparisons of vitamin C, piperine, and quercetin were spotted. Then, the standard comparisons were eluted by using ethyl acetate eluent:methanol (6:4). After the emulsifier solution had reached the boundary line, the elution stopped. The Rf value of each formed stain was measured. Finally, the stains were examined under UV light at a wavelength of 254 nm and 366 nm.

2.6. Total Phenol Test

The first step was making gallic acid standard curves. A gallic acid solution with a 1 mg/mL concentration was made as a stock solution. Then, this solution was made a gallic acid solution with 25, 50, 75, and 100 µg/mL concentrations. A gallic acid solution was pipetted 0.2 mL, and 1 mL of the Reagent Folin-Ciocalteu was added and whipped. Then, 3 mL of a solution of Na2CO310% was added. Afterward, 10 mL of distilled water was added. The solution was shaken until homogeneous. Finally, the solution was let rest for two hours at room temperature. Meanwhile, the absorption at a maximum wavelength of 768 nm was measured. Then, a calibration curve of the relationship between the concentration of gallic acid (µg/mL) and absorption was made (Marjoni & Afrinaldi, 2015).

The second step was determining total phenolic content. 10 mg/mL of sample solution of extract, results of n-hexane partition, and methanol was diluted so that the 1 mg/mL of extract concentration was obtained. A solution of 1mg/mL was pipetted for 0.2 mL. Then 1 mL of the reagent Folin-Ciocalteu was added and whipped homogeneously. The next step was adding 3 mL of a Na2CO310% solution. Then, distilled water was added up to 10 mL and whipped homogenously. Finally, the solution was incubated for two hours at room temperature. The absorption of the solution was measured at a maximum wavelength of 768 nm, and then a calibration curve of the relationship between the concentration of gallic acid (µg/mL) and absorption was made (Marjoni & Afrinaldi, 2015).

The total phenol levels were calculated using the formula referring to (Yamin et al., 2018), as in Equation

$$Total\ Fenol\ = \frac{Cx\,Vx\,fp}{g} \quad (1)$$

The Description of aforementioned equation 1 as follows. C mean phenolic concentration (value of x), V mean Volume of extract (mL), fp mean Dilution factors and g mean of Weight of sample (g)

2.7. Total Flavonoid Test

The first step was making standard quercetin curves. 0.1 mg/mL stock quercetin solution was diluted to get the concentration of 25, 50, 75, and 100 µg/mL quercetin. 0.5 mL comparison solution (quercetin) was diluted with 1.5 mL methanol. Then, 0.1 mL aluminum (III) chloride 10%, 0.1 mL sodium acetate 1M, and 10 mL of distilled water were added. After the solution was incubated for 30 minutes at a temperature of 25°C, the absorbance of a comparison solution was measured using ultraviolet-visible spectroscopy at the maximum wavelength (Abozed et al., 2014).

The second step was determining total phenolic content. A solution sample with a concentration of 2000 µg/mL in ethanol was made. Then, a 0.5 mL test sample was added with 1.5 mL methanol. Afterward, 0.1 Aluminum (III) chloride 10%, 0.1 mL sodium acetate 1M, and 10 mL of distilled water were added. After the solution was incubated for 30 minutes at a temperature of 25°C, the absorbance of a comparison solution was measured using ultraviolet-visible spectroscopy at the maximum wavelength (Abozed et al., 2014).

The total flavonoid content was calculated using Formula 2.

$$Total\ flavonoids\ = \frac{C\,x\,V}{g} \quad (2)$$

The Description of aforementioned formulas as follows. C mean Quercetin concentration (value of x), V mean Volume of extract (ml) and g mean Weight of sample (g)

2.8. Determination of Antioxidant Activities

The antioxidant activity test employed a procedure referring to (Selawa et al., 2013), who calculated the absorbance from the 0.1 mL sample by adding a 3 mL FRAP reagent.

The determination started with making an acetic buffer solution of 3.6 pH. Sodium acetate trihydrate (CH3COONa.3H2O) 0.155 g was added with 0.8 mL concentrated acetic acid and 50 mL of distilled water. The next step was making the TPTZ solution. 30 mg TPTZ was dissolved in 10 mL 40 mmol/L HCl. Afterward, a solution of FeCl3.6H2O was made by dissolving 0.27 g FeCl3.6H2O with 50 mL distilled water (Selawa et al., 2013).

The next step was making and testing the solution of vitamin C. The 1000 µg/mL solution of Vitamin C in methanol was made and diluted so that 2.5, 5, 7.5, and 10 µg/mL concentrations were obtained. Each concentration of vitamin C was pipetted to 1 mL and put into a test tube. Then, 3 mL FRAP reagent was added and diluted with 2 mL DMSO. The solution was homogenized and then incubated at a temperature of 37°C for 30 minutes. The absorbance of each solution was measured at the maximum wavelength.

The next step was making a blank solution by sequentially pipetting the solution as much as 3 mL DMSO, put into a tube test, added with 1.0 mL FRAP reagent, shaken until becoming homogeneous, and incubated at a temperature of 37°C for 30 minutes. Finally, the absorbance of the solution was measured at the maximum wavelength.

2.9. Antioxidant Extract Test

The first step was determining the absorbance sample. 3 mL FRAP reagent was added to 0.1 ml cayenne pepper of sample solution (extract, results of n-hexane partition, and methanol partition) in a test tube. Furthermore, the absorbance of the solution was read using a UV-Vis spectrophotometer with a maximum wavelength of 596 nm (Syarif & Kosman, 2015).

3. Data Analizes

Data from the absorbance of each sample of extract, n-hexane partition, and methanol partition were used to reveal the antioxidant capacity of the FRAP method. Finally, the quantitative data obtained were compared.

4. Results and Discussion

The sample of cayenne pepper (*Capsicum frutescens* L) was determined in the Laboratory of Science and Technology, Universitas Ahmad Dahlan (UAD). The result of the determination indicated that the plant used was cayenne pepper (*Capsicum frutescens* L). In this research, 3 kg cayenne pepper produced 567 g powder and 39.66 g extract. The results of the sample yield are summarized in Table 1.

Table 1. Extract and Partition Yields

Samples	Extract Yield (%)
Extracts	7.93
Methanol Partition	24.6
N-Hexane Partition	11.8

This study employed 7.93% of extract yield of cayenne pepper (*Capsicum frutescens* L), 24.6% of methanol partition, and 11.8% of n-hexane partition. The yield was used to denote a chemical compound abstracted from the sample. This study has revealed that the methanol partition produces more extracts than the n-Hexane partition because the cayenne pepper contains more chemical compounds, which are more soluble in the methanol solvent as it is polar.

Phytochemical screening is the first step to detect a class of chemical compounds found in a plant. The results of the phytochemical screening for the extract of the cayenne pepper, n-Hexane partition, and methanol partition are summarized in Table 2.

4.1. Results of Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a qualitative test to explore the existence of chemical compounds in an extract sample based on its polarity. The results of spotting stains observed using 254 and 366 nm UV rays were calculated to discover the Rf values based on the stain formed. These results of the calculation are presented in Table 3. The identification of phenolic compounds and flavonoids in the sample extracts, n-hexane partition, and methanol using the gallic acid comparison

and quercetin has shown that the extract contains flavonoids and phenol because this extract has similar Rf to those of 0.29 gallic acid comparison and 0.41 quercetin. Meanwhile, the n-hexane partition contains flavonoids.

Table 2. Results of Phytochemical Screening

Classes of Compounds	Extracts	N-Hexane Partitions	Methanol Partition
Phenol	+	+	+
Flavonoids	+	+	+
Alkaloids			
-Dragendorff	+	+	+
-Mayer	+	+	+
Terpenoids	+	+	+
Steroids	-	-	-
Tannin	-	-	-

Table 3. Rf TLC Values of Test Samples and Their Comparison in Separation Flavonoid and Phenol Compounds

Samples	Spotting	UV 254 nm		UV 366 nm	
		Rf	Colors	Rf	Colors
Extracts	1	0.29	Green	0.29	Blue
	2	0.41	Green	0.41	Green
	3	0.54	Brown	0.54	Yellow
	4	0.58	Brown	0.58	Purple
	5	0.67	Brown	0.67	Red
P n-Hexane	1	0.41	Brown	0.41	Red
P Methanol	1	0.43	Brown	0.43	Purple
	2	0.54		0.54	Yellow
	3	0.63		0.63	Red
Gallic acid	1	0.29		0.29	Purple
Quercetin	1	0.41	Green	0.41	Yellow

4.2. Determination of Total Phenolic Levels

To determine total phenolic content in the extract of cayenne pepper, the absorbance of n-hexane partition and methanol was measured using UV-Vis spectrophotometer with a maximum wavelength of 780.05 nm. The results of determining the phenolic levels are presented in Table 4.

Table 4. Determination of Total Phenol of Cayenne Pepper Extract, n-Hexane Partition, and Methanol

Samples	Total Phenolic Content l (mg GAE/g extract)
Extracts	0.037 ± 0.04
n-Hexane Partition	0.0009 ± 0.013
Methanol Partition	0.023 ± 0.02

The result of examining the total phenolic content shows that the extract has the highest phenolic content compared to the n-hexane partition and methanol. The phenolic compound potentially functions as an antioxidant because it can reduce free radicals by donating its electrons through a hydroxyl group and stabilizing resonantly; moreover, since the phenolic compound is not reactive, it can be used as an effective antioxidant (Wirasti, 2019).

4.3. Determination of Total Flavonoids

To determine total phenolic content in the extract of cayenne pepper, the absorbance of n-hexane fraction and methanol was measured using a UV-Vis spectrophotometer with a maximum wavelength of 439.0 nm.

Table 5 shows that the extract of cayenne pepper has the highest total flavonoids compared to the n-hexane partitions and methanol. The flavonoid compound functions as a free radical scavenger because it can inhibit the oxidation reaction. Moreover, the flavonoid also has a hydroxyl group that serves as an oxidant scavenger because more hydroxyl groups would increase antioxidant activities (Adhayanti & Romantika, 2012).

Table 5. Results of Determination of Total Flavonoid Extract of Cayenne Pepper, n-Hexane Partition, and Methanol Partition

Samples	Total phenolic content (mgQE/g extract)
Extracts	0.124 ± 0.008
n-Hexane partition	0.072 ± 0.007
Methanol Partition	0.97 0.001

4.4. Determination of FRAP Antioxidant Activity

The antioxidant activities on the n-hexane partition, methanol, and extract of cayenne pepper (Capsicum frutescent L.) were determined using the FRAP method. This method aims to test the antioxidants of plants (Tahir et al., 2016). Moreover, its work mechanisms are similar to those of the body. The principle of the FRAP method is based on the reduction reaction; a complex Fe3+ from the colorless Fe (TPTZ)3+ becomes the Fe2+ because the Fe (TPTZ)2+ is intensively blue in the acids (Istiningrum, 2013).

A comparison on the antioxidant activity test was vitamin C because it has the best antioxidant activity and can capture free radicals to create stability. The parameter used to determine antioxidant activities was the effectiveness of the antioxidant. The maximum wavelength of the standard solution of FeSO47H2O was determined by measuring the absorbance at a wavelength of 598 nm using a UV-Vis spectrophotometer as depicted on the following Table 6.

Table 6. Measurement of Standard Curve Absorbance of FeSO47H2O

Standard Curve	Concentration (µg/mL)	Absorbance (Abs)	The equation of standard curves
$FeSO_4 7H_2O$	25	0.157	y = 0.001 x + 0.111
	50	0.202	
	100	0.283	
	150	0.393	
	200	0.464	

The content of antioxidant activities in the n-hexane partition, methanol, and extract of cayenne pepper was determined by measuring the respective absorbance of each sample using the UV-Vis spectrophotometer. The data from the absorbance results were employed to measure the complex form of Fe2+ (TPTZ) and was stated in the form of µmol Fe2+/g extract or partition. This statement indicates that micromol Fe2+ or Fe2+ was formed in every gram of extract or partition (Table 7).

Antioxidant activities of each sample were tested using the FRAP method. The test has revealed that the highest antioxidant activities are found in cayenne pepper extract for 505.67 ± 0.56 µmol Fe2+/g at a concentration of 100 µg/mL. Meanwhile, antioxidant activities in the methanol partition are 447.67 ± of 16.2 µmol Fe2+/g. Finally, the lowest antioxidant activities are found in the n-hexane partition for 353 ± 5.29 µmol Fe2+/g. The results conclude that the higher the concentration of the sample extract and the partition of cayenne pepper, the more complex the Fe2+ is formed.

It is predicted that the antioxidant activities in cayenne pepper are derived from secondary metabolite compounds, such as phenols, flavonoids, alkaloids, and terpenoids. This prediction agrees with the statement of (Gurnani et al., 2016) and (Kusnadi et al., 2019).

Table 7. Antioxidant Activities of Comparison (Vitamin C), Extracts, n-Hexane Partition, and Methanol of Cayenne Pepper Using the FRAP Method

Antioxidant Activities (µmol Fe2+/g)				
Concentration (µg/mL)	**Vitamin C**	**Extracts**	**Partition**	
			n-Hexane	**Methanol**
25	458 ± 4.58	215 ± 3.46	91 ± 1	199.33 ± 5.69
50	531.67± 5.51	295.33 ± 1.15	213.33 ± 0.58	290 ± 1
75	634 ± 5.3	438.67 ± 1.53	282.33 ± 8.14	362.67 ± 2.52
100	726.33 ± 3.37	505.67 ± 0.56	353 ± 5.29	447.67 ± 16.2

5. Conclusion

This study concludes that antioxidant activities in 100 µmol/g ethanol extract of cayenne pepper are found in 506 ± 0.57 µmol Fe2+/g of extract chili pepper, 448 ± 16.19 µmol Fe2+/g of methanol fraction, and 353 ± 5.29 µmol Fe2+/g of n-hexane fraction.

Author Contributions: Slamet Slamet conceived and designed the study. Slamet Slamet performed all data analyses. Slamet Slamet, Urmatul Waznah, Fridiana and Yusrilia interpreted the results and revised the paper. Slamet Slamet wrote the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors disclose no conflict.

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