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Research Article

Analysis of Vitamin C and Antioxidant Activity of *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety)

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Abstract

Vitamin C is a nutrient that the body needs in order to increase its metabolism. This helps the body to create collagen and also act as an antioxidant. This study aims to analyze of vitamin C and the antioxidant activity of *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety), obtained from the Pinrang Regency, South Sulawesi Province. The study was carried out experimentally by UV-Vis spectrophotometry method. The measurement of vitamin C was carried out at a wavelength of 265.667 nm while for the antioxidant activity test the maximum wavelength of DPPH was 515.961 nm. Vitamin C levels obtained in *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety), were 0.926±0.75; 0.344±0.35, and 0.281±0.22±0.22% w/w. For antioxidant activity, the results IC_{50} of *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety), were 2.202; 2,260; and 2.751 ppm while the comparator vitamin C was 12.360 ppm. These results indicate that *Capsicum frutescens L.* has higher levels of vitamin C than *Capsicum annuum L.* (curly and large chili variety), while for the antioxidant activity test, the IC_{50} value of *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety) less IC_{50} value antioxidant activity than a comparison vitamin C, which means that they have strong antioxidant activity.

Keywords: *Capsicum frutescens L., Capsicum annuum L.,* IC_{50}, Vitamin C

Analisis Vitamin C dan Aktivitas Antioksidan pada *Capsicum frutescens L.* dan *Capsicum annuum L.* (Varietas Cabai Keriting dan Besar)

Abstrak

Vitamin C atau asam askorbat adalah salah satu nutrisi yang penting dan diperlukan oleh tubuh dalam meningkatkan metabolisme serta berperan dalam proses pembentukan kolagen dan juga berperan sebagai antioksidan. Penelitian ini bertujuan untuk menganalisis kadar vitamin C dan menguji aktivitas antioksidan pada cabai rawit, cabai keriting dan cabai besar yang diperoleh dari daerah Kabupaten Pinrang Provinsi Sulawesi Selatan. Penelitian dilakukan secara eksperimental dengan metode spektrofotometri UV-Vis. Untuk pengukuran kadar vitamin C dilakukan pada panjang gelombang 265,667 nm sedangkan untuk uji aktivitas antioksidan dilakukan panjang gelombang maksimum DPPH yaitu 515,961 nm. Kadar vitamin C yang diperoleh pada cabai rawit, cabe keriting dan cabai besar berturut-turut sebesar 0,9260±0.75; 0.344±0.35, dan 0,281% ±0.22 b/b. Untuk aktivitas antioksidan diperoleh hasil IC_{50} pada cabai rawit, cabai keriting dan cabai besar berturut-turut sebesar 2,202; 2,260; dan 2,751 ppm, sedangkan vitamin C pembanding sebesar 12,360 ppm. Dari hasil tersebut menunjukkan bahwa cabai rawit memiliki kadar vitamin C lebih tinggi dibandingkan cabai keriting dan cabai besar, sedangkan untuk uji aktivitas antioksidan, nilai IC_{50} cabai rawit, cabai keriting dan cabai besar lebih kecil dibandingkan vitamin C pembanding yang menunjukkan sampel cabai tersebut memiliki aktivitas antioksidan dengan kategori sangat kuat.

Kata Kunci: *Capsicum frutescens L., Capsicum annuum L.,* IC_{50}, Vitamin C

1. Introduction

Vitamin C or commonly known as ascorbic acid is an essential nutrient for the human body which is used as a pharmaceutical ingredient which is widely consumed as an antioxidant. Ascorbic acid or vitamin C in pharmaceutical preparations can be determined by iodometric titration methods and ultraviolet spectrophotometry at a wavelength of 265 nm. Absorption at a wavelength of 265 is capable of absorbing raw vitamin C absorbance. The absorbance will be proportional to the number of particles, so that based on the data obtained, the number of particles that is absorbed the most is at a wavelength of 265 nm[1].

The content of vitamin C is found in fruits and vegetables, one of which is chili. Vitamin C in chilies has a function as a good antioxidant for the body, able to increase the body's resistance to absorption by calcium in the body. Apart from that, vitamin C is also one of the most soluble in water for collagen biosynthesis[2].

Based on previous research by Badriah, et.al (2015)[1], vitamin C levels in large curly red chilies from Kediri were obtained by converting the absorbance data into the form of concentration (ppm) which were obtained respectively 4.478; 4,478; 4.434 ppm and obtained an average yield of 4.463 ppm, which is 0.4463% w/b. Then the next study according to Legowo, BD., et.al (2021)[3] determined the levels of vitamin C in various types of chilies by UV-Vis spectrophotometry at a wavelength of 200 nm, the highest levels of vitamin C were obtained in red curly chilies (50 g/100 g) cayenne pepper content 29 g/100 g, large red chili content 22 g/100 g.

The need for vitamin C for men and women are 60 mg/day, babies are 35 mg/day, pregnant women are 70 mg/day, and nursing mothers are 95 mg/day and for men aged 16-64 years need vitamin C as much as 90 mg, whereas in women aged 16-64 years it takes 75 mg. However, this condition varies for each individual[2].

The content of vitamin C in chilies is an enzymatic antioxidant that works by cleaning up new free radical compounds. Free radicals are molecules or compounds that can stand alone containing one or more unpaired electrons. The impact of the reactivity of free radical compounds can cause damage to cell structures that interfere with cell physiology, and eventually all cells become damaged resulting in inflammation, the aging process, decreased immunity, cancer and atherosclerosis[4].

Antioxidant compounds are found in many vegetable groups, one of which is chili. Chili is a source of vitamin C because this compound is resistant to reduction and acts as an antioxidant in exchanges that occur due to the action of hydroxylation elements. Chili contains phenolic compounds, flavonoids and carotenoids, in addition to being a source of vitamin C. Flavonoids are phytochemicals found in plants. Known activities, including antioxidant activity, antibiotic synergism, and antiviral[5].

Based on other studies regarding chilies which function as antioxidants according to Antasoniasti, I., et.al (2022)[4] it shows that cayenne pepper extracts from Kawangkoan and Remboken from Manado, North Sulawesi, have strong antioxidant activity compared to cayenne pepper extracts from the Tomohon area (IC_{50} is at range of 100-150 µg/mL which indicates that the antioxidant activity given is moderate). Although it has moderate antioxidant activity based on the DPPH test, Tomohon bird's eye chili extract has greater activity compared to the toluene ethyl acetate fraction of the ethanol extract of cayenne pepper fruit (IC_{50} 347.998 ± 19.359) reported by Angelista RHE (2016)[6]. The same thing was shown by the study of[7], Java chilies from the Karangasem and Pamekasan areas had relatively weak antioxidant activity with IC_{50} values of 285.61 ppm and 288.037 ppm respectively.

The results of research conducted by Ananta, I. G. B. T., & Anjasmara DGA (2022)[8], the antioxidant activity of curly red chilies in Denpasar City obtained an IC_{50} value of 505.35 ppm. IC_{50} is a number indicating the extract concentration (ppm) which is able to inhibit the oxidation process by 50%. The smaller the IC_{50} value means the higher the

antioxidant activity. Specifically, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 ppm, strong for IC_{50} is 50-100 ppm, moderate if it is 100-150 ppm, and weak if the IC_{50} value is 151-200 ppm. Based on these criteria, curly red chili fruit extract has potential in its activity as an antioxidant in the weak category[8].

In this study, an analysis of vitamin C levels and antioxidant activity tests was carried out on 2 types f chilies, originating from Pinrang, South Sulawesi. My reason for researching chili plants is because chilies are a horticultural commodity that has high economic value. People use chilies as a spice in everyday cooking. In addition to the main function of chili, which is to meet daily needs, chili is also used as raw material for the food and pharmaceutical industries. Chilies contain carbohydrates, fat, protein, calcium, vitamins A, B1, and vitamin C which are needed by the body and contain lasparaginase as an anti-cancer agent[9].

2. Method

2.1. Tools

Stirring rod, beaker, blender, funnel, erlenmeyer (pyrex), measuring cup, watch glass, filter paper, volumetric flask (pyrex), spectrophotometer UV -Vis (Thermo Scientific), spatula and analytical balance (Kern).

2.2. Materials

Materials were pure ascorbic acid ($C_6H_8O_6$) (Merck), CO_2 free distilled water, *Capsicum frutescens L.* and *Capsicum annuum L.* (curly and large chili variety), DPPH (Merck), methanol pa (Merck).

2.3. Procedure

2.3.1. Analysis of Vitamin C levels .

Sample Processing: Samples of 3 types of chilies originating from Pinrang City (South Sulawesi) were picked and then sorted, then separated from the fruit and stems, after that the shape was changed by cutting it into small pieces, then drying it by airing it[10].

Analysis of vit C levels: The dried chilies are then blended into chili powder. Wavelength was determined using standard vitamin C and calibration curves at concentrations of 4, 6, 8, 10, 12, and 14 ppm with CO_2-free aquadest as a blank. Chili powder that has been mashed each is taken and weighed as much as 50 mg and then put into a glass beaker and added with CO_2-free distilled water and then filtered and taken the filtrate. The filtrate obtained was diluted with CO_2-free distilled water up to the mark of 25 mL. Then it is measured at a predetermined wavelength and tested using UV-Vis Spectophotometry[2].

2.3.2. Antioxidant activity test[10]

Preparation of DPPH stock solution: Weighed as much as 2 mg of DPPH and put into a 100 mL volumetric flask, then dissolved using methanol solvent and adjusted the volume up to the mark. Then measure the maximum wavelength of the DPPH starting from 450 nm – 550 nm.

Preparation of Vitamin C Solution: Weighed as much as 2 mg of ascorbic acid and put into a 25 mL volumetric flask, then dissolved using methanol and adjusted the volume up to the mark mark. Next, take each of these solutions as much as 1.0, 1.5, 2.0, 2.5, and 3.0 mL, then dilute again with methanol in a 50 mL volumetric flask to obtain an extract solution with a concentration of 2, 3, 4, 5 and 6 ppm.

Antioxidant Activity Test: Each chili was mashed and weighed as much as 50 mg and added with 25 mL of methanol, vortexed to help dissolve vitamin C into methanol. To determine antioxidant activity, each sample with various concentrations was pipetted as much as 0.2 mL with a micro pipette and put into a vial, then added 3.8 mL of 50 µM DPPH solution. Shake the mixture until it is homogeneous and leave it for 30 minutes in a dark place, measure the absorption by UV–Vis spectrophotometry at the maximum wavelength of DPPH.

2.3.3. Data analysis[11]

The research data was in the form of measuring vitamin C levels and testing the antioxidant activity of *Capsicum frutescens L.* and *Capsicum annuum L.* quantitatively

Table 1. Results of standard vitamin C absorbance measurements of 5 ppm

Wavelength (nm)	Absorbance of standard vitamin C 2 ppm (A)
265,667	0.320

using UV-Vis spectrophotometry to compare vitamin C levels and their antioxidant activity from the samples, then the data were analyzed using the formula :

Analysis of Vitamin C levels

Regression line equation:

$$Y = a + bX.$$

Formula % Content :

$$\frac{Concentration\left(\frac{mg}{ml}\right) x\ Vol\ sample(ml) x Fp}{Weight\ Sample}$$

Information :

Y : Absorption (A)

X : Concentration

a : intercepts

b : slope slope

Antioxidant activity test

Free radical binding formula= $\frac{Abs\ Standar - Abs\ Sampel}{Abs\ Standar}$ 100%

According to[12] IC_{50} value is calculated using the linear regression equation, sample concentration as the x-axis and % inhibition as the y-axis.

From the equation y = a + bx, the IC_{50} value can be calculated using the formula IC_{50} =(50-b)/a.

Description:

y = 50 (50% oxidation inhibitor)

x = $IC5_0$ (a number indicating the concentration of the extract capable of inhibiting the oxidation process by 50%)

a = slope

b = intercepts

3. Result and Discussion

Table 2. Absorption results of ascorbic acid standard solution by UV-Vis spectrophotometry at a wavelength of 265, 667 nm

Concentration (ppm)	Absorbance (nm)
4	0.407
6	0.550
8	0.657
10	0.736
12	0.887

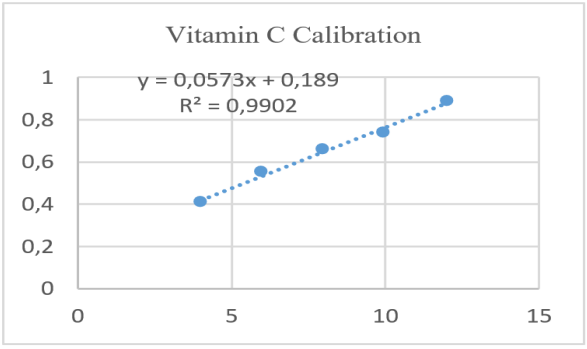

Figure 1. Vitamin C calibration curve

Analysis of Vitamin C Levels

In this study, quantitative analysis was used to determine vitamin C levels from cayenne pepper, curly chilies, and large chilies using UV-Vis spectrophotometry. Standard solution and sample measurements were carried out at the maximum wavelength with the aim of knowing the maximum absorption area that can produce the highest absorbance value from the standard vitamin C solution. Vitamin C is dissolved using CO_2-free distilled water because it is a water-soluble vitamin, besides this solvent used with the aim of reducing the risk of the presence of an impurity[13]. Raw ascorbic acid is prepared a concentration of 5 ppm was then measured for its absorption at a wavelength of 200-400 nm using a UV-Vis spectrophotometer.

Wavelength determination maximum is done by measuring value absorbance of 2 ppm ascorbic acid solution wavelength range 200 - 400 nm. The highest absorbance value is obtained at a wavelength of 265.667 nm with a value of absorbance of 0.320 (can be seen in table 1). After that, a calibration curve was made by making five concentration series of solutions, namely 4, 6, 8, 10, and 12 ppm and then measuring at the maximum wavelength because at that wavelength the sensitivity is also maximum, the absorption power is relatively constant and the shape of the absorbance curve is straight or linear. under these conditions so that the Lambert-Beer law will be fulfilled. Furthermore, for samples of cayenne pepper, curly chili and large chili,

Table 3. Measurement results (λ Max) DPPH 30 ppm

Sample	(Max)	absorbance (nm)
DPPH 30 ppm	515.961	0.921

Table 4. Absorbance of the chili and sample solution at a wavelength of 265.667 nm.

Sample	Absorbance	Average (%)
	0.599 A	
Vitamin C in Cayenne pepper (Capsicum frutescens L.)	0.596 A	
	0.602 A	0.926±0.75
	0.925	
Vitamin C Levels (%)	0.926	
	0.928	
	0.347 A	
Vitamin C in curly chili (Capsicum annum L.)	0.375 A	
	0.376 A	0.344±0.35
	0.200	
Vitamin C Levels (%)	0.416	
	0.418	
	0.325 A	
Vitamin C in chili (Capsicum annum L.)	0.308 A	
	0.309 A	0.281±0.22±0.22
	0.304	
Vitamin C Levels (%)	0.269	
	0.270	

measurements were also carried out at these wavelengths.

Based on the results of measurements of vitamin C (can be seen in table 2), a linear curve is obtained as shown in Figure 1 with the regression equation y = 0.573x + 0.189, r = 0.995. Where the value of r (correlation coefficient) is close to +1 which means that the relationship between concentration and absorbance is very strong. So it can be concluded that there is a linear relationship between concentration and absorbance or absorption that fulfilled the Lambert-Beer law. The regression value obtained can be used to determine vitamin C levels in samples of cayenne pepper, curly chili and large chili.

Vitamin C levels were obtained by calculating the percent content of each absorbance obtained from three replications and then entering the percent content formula. The levels obtained for cayenne pepper were 0.926%±0.75 w/w, curly chilies 0.344±0.35% w/w, and large chilies as much as 0.281±0.22% w/w. The highest vitamin C content was obtained from the cayenne pepper sample, namely 0.926±0.75 %w/w (can be seen in Tables 4).

Antioxidant Activity Test: Antioxidants are compounds that are useful in helping oxidative damage caused by free radicals or reactive oxygen compounds, free radicals are molecules that do not have one or more unpaired electrons and can cause damage to biomolecules. The human body system is exposed to free radicals at any time, both those produced from normal metabolic processes and from the environment, such as cigarette smoke and pollution. Excessive free radical exposure to the body can result in cell damage triggering the pathogenesis of various diseases such as cardiovascular disease, hypertension, hyperlipidemia, Alzheimer's and Parkinson's[14].

Chili contains phenolic compounds, flavonoids and carotenoids, in addition to being a source of vitamin C. Flavonoids are phytochemicals found in plants. Known activities, including antioxidant activity, antibiotic synergism, and antiviral. Flavonoids found in most hot peppers are the glycosides and aglycones myricetin, quercetin, luteolin, apigenin, and kaempferol[5]. The antioxidant activity of cayenne pepper, curly chili and large chili as well as vitamin C as a comparison was tested using the DPPH free radical scavenging method using a UV-Vis spectrophotometer. For sample preparation, cayenne pepper, curly chili and large chili IC_{50} value for each dissolved with methanol, the choice of methanol as a solvent is based

Table 5. Calculation of % inhibition

Sample	Concentration (ppm)	absorbance	% inhibition	IC_{50} (µg/mL)
vitamin C	2	0.643	26.26	
	4	0.556	36,23	
	6	0.462	47.01	12,360
	8	0.368	57,79	
	10	0.264	69.72	
	DPPH	0.872		
Cayenne Pepper	50	0.574	30.25	
	75	0.555	32.56	
	100	0.531	35.48	2.202
	125	0.494	39.97	
	150	0.463	43.74	
	DPPH	0.823		
Curly chili	50	0.593	28.55	
	75	0.570	31.32	
	100	0.545	34,33	2.260
	1 25	0.518	37,59	
	1 50	0.490	40.96	
	DPPH	0.830		
Large Chili	50	0.578	29.33	
	75	0.529	35,33	
	100	0.475	41.93	2.571
	1 25	0.438	46.45	
	1 50	0.388	52,56	
	DPPH	0.818		

on semi-polar properties, having nonpolar (-CH_3) and polar groups (-OH), can attract components non-polar and polar bioactive[15]. After that, the sample was filtered using filter paper and then the filtrate obtained was sufficiently diluted with methanol to the mark limit and then variations in concentration were made to determine its antioxidant activity[16].

Determination of the maximum wavelength of DPPH is carried out by observing the absorption of wavelengths in the range of 490-534 nm. Based on table 3, the maximum DPPH wavelength is 515.961 nm. Furthermore, the maximum wavelength of DPPH is used in determining antioxidant activity. The amount of antioxidant activity is indicated by the IC_{50} value , which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals[17].

Antioxidant activity can be divided into very strong, strong, moderate, weak and very weak categories. An antioxidant is said to be very strong if it has an IC_{50} value of less than 50 µg/mL, a strong antioxidant has an IC_{50} value in the range of 50 to 100 µg/mL, a moderate antioxidant has an IC_{50} value ranging from 100 to 150 µg/mL , a weak antioxidant has the range of 150 to 200 µg/mL and an IC_{50} value of more than 200 µg/mL is a very weak antioxidant[18,19,20].

IC_{50} calculation of samples of cayenne pepper, curly chili and large chili as well as vitamin C as a comparison obtained IC_{50} values respectively 2.202, 2.260, 2.751, and 12.360 µg/mL. Based on the data obtained by the antioxidant activity test, the samples and comparators were all in the very strong category. This stated that the samples of cayenne pepper, curly chili and large chili had high antioxidant activity compared to the vitamin C comparator.

4. Conclusion

Based on the results of the research that has been done, it can be concluded that Vitamin C levels in cayenne chilies, curly and large were 0.926%±0.75, 0.344±0.35% and 0.281±0.22% w/w respectively . The highest

levels of vitamin C were obtained from cayenne pepper samples.

IC_{50} values for cayenne chilies, curly and large chilies were 2.202, 2.260 µg/mL, and 2.751 µg/mL respectively , while the IC_{50} value for vitamin C for the comparator was 12.360 µg/mL . Based on the data obtained by the antioxidant activity test, the samples and comparators were all in the very strong category. This stated that the samples of cayenne chilies, curly chili and large chili had high antioxidant activity compared to the vitamin C comparator.

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