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Analysis of Lycopene Content and Antioxidant Activity Test of Katokkon Chili Extract (*Capsicum chinense Jacq*)

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Abstract

This study aimed to determine lycopene contents and the antioxidant activity of katokkon chili extract. The extraction process of katokkon chili used the maceration method with the mixture of n-hexane: acetone: ethanol (2:1:1). The concentrated extract of katokkon chili was obtained through the evaporator process at a temperature of 40 ˚C. The analysis process used a UV-Vis spectrophotometer in which the absorbance reading was at λ = 472 nm. The average lycopene content in katokkon chili extract was 1.457 mg/100g. Testing the antioxidant activity of katokkon chili extract used the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with positive control the vitamin C. The absorbance readings for the antioxidant activity test were at λ = 517 nm. The test results showed that the antioxidant power of katokkon and vitamin C extracts in reducing DPPH free radicals had a similar effect which was equally strong. The IC_{50} value for chili katokkon extract was 13.84 mg/L, and the IC_{50} value for vitamin C was 5.78 mg/L, so it can be concluded that chili katokkon can be an alternative source of natural antioxidants to replace vitamin C.

Keywords: Lycopene, antioxidants, chili katokkon, maceration, UV-Vis spectrophotometer, DPPH

Introduction

Indonesia has a tropical climate, so that it supports making Indonesia a cultivator and exporter of vegetables such as chilies (Prajnata, 2007). Chili in Indonesia can be classified into three species, namely, large chili (*Capsicum annuum L.*), cayenne pepper (*Capsicum frustescens L.*), and sweet chili (*Capsicum logum L.*). Warisno & Dahana (2010) said that one type of chili in Indonesia that has high economic potential but has not been explored much is the katokkon chili variety (Capsicum chinense Jacq), which is the most popular chili and is widely cultivated in the highlands of Tana Toraja district and Enrekang, so it is also known as Toraja chili. Katokkon chili is included in the type of large chili (Capsicum annnuum L.), which can generally be planted in the lowlands to the highlands (mountains) ± 2000 meters above sea level (Flowrenzhy & Harijati, 2017; Mutmainnah & Masluki, 2017).

Katokkon chili is often used as a cooking spice, chili powder, and sauce industry. Apart from the strong aroma and spicy taste, this chili also has a striking color, which is red. The red color in chilies is an indicator of the presence of natural pigments, the red color in chilies is a carotenoid type pigment (Puspita et al., 2018). According to Wall et al. (2001), Capsicum chinense contains 446 ± 41 µg/g per wet weight, and this carotenoid content is beneficial to the body if you consume it. In general, chilies have a lot of nutritional content or chemical elements, including calories, protein, fat, carbohydrates, calcium, vitamin A, vitamin B1, and vitamin C (Winarno, 1989), so that chili is also widely used in the health sector because it functions as an antioxidant and anti-inflammatory derived from carotenoids chili. Chili peppers are also used to treat arthritis, rheumatism, sore skin rashes, and snake bites. This therapeutic application is related to capsaicin, phenolic compounds, and carotenoids of chili plants (Ortega et al., 2012).

Carotenoids are a group of pigments: yellow, orange, red, and oil-soluble (lipids). Carotenoids are usually found in papaya, banana peels, tomatoes, red chilies, mangoes, carrots, sweet potatoes, and yellow and red flowers. The rubbernoid group, which is well-known and has become the most popular research object, is lycopene (Winarno, 1989).

Lycopene is an open-chain unsaturated hydrocarbon compound with 13 double chains in which there are 11 conjugated double chains. The unique properties of lycopene make it a very potent antioxidant (Rao & Rao, 2007). According to Shi et al. (1999), lycopene is an essential compound

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because its use as a natural dye and because lycopene has been recognized for its health benefits. Lycopene does not have the activity of provitamin A, but lycopene functions as an antioxidant and traps singlet oxygen in vitro. Lycopene is the most potent antioxidant globally, which can help prevent the development of various forms of cancer; the effect varies according to sex and type of cancer. Its ability to control free radicals is 100 times more efficient than vitamin E or 12500 times than glutathione (Desmiaty et al., 2008).

This article presents descriptions of lycopene levels and antioxidant activity in katokkon chili extract.

Methods

The tools used in this study were a UV-Vis spectrophotometer (Genesys 10 UV), shaker, rotary evaporator, blender, vacuum filter, analytical balance, measuring cup, measuring flask, Erlenmeyer, beaker, test tube, tube rack, volume pipette, suction ball, spatula, stirring rod, spray bottle.

The materials used in this study were katokkon chilies, n-hexane, 96% ethanol (*Merck*), acetone, vitamin C (*Merck*), DPPH (2,2-diphenyl-1-pichrylhydrazyl) (*Merck*), distilled water, filter paper, and aluminum foil.

Sample preparation

The katokkon chilies that will be analyzed are sorted first by selecting red chilies that are not rotten, then washing them with running water to keep them clean from dirt, then removing the chili seeds and cutting them into small pieces, then mashed in a blender.

Katokkon chili extraction

Katokkon chilies that have been mashed are weighed 10 grams and extracted by maceration method using 100 mL of n-hexane: acetone: ethanol (2:1:1) (v/v) solvent in a closed container and coated with aluminum foil and shaker at the speed of 160 rpm for 24 hours, then filtered with a vacuum filter. The filtered filtrate is put into a separating funnel, and 10 mL of distilled water is added, then shaken for 15 minutes then allowed to stand for a while until two phases are formed, then separated from the polar and non-polar layers, all the upper layers (non-polar layers) are taken and stored in a closed and dark container. This extraction process is carried out three times, and the three extracts are combined and concentrated using a rotary evaporator at a temperature of 40 °C. The concentrated extract was put into a glass bottle covered with aluminum foil.

Lycopene analysis

One gram of concentrated extract was added with 25 mL of n-hexane, the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 472 nm and 502 nm, with n-hexane as a blank.

Antioxidant activity test

Preparation of DPPH solution (2,2-diphenyl-1-picrylhydrazyl)

2.5 mg of DPPH dissolved with 96% ethanol in a flask up to 25 mL to obtain a DPPH solution with a concentration of 100 ppm.

Blank solution absorbance

2.5 mL of DPPH 100 ppm solution dissolved with 96 % to 25 mL ethanol, homogenized so that a concentration of 10 ppm was obtained, the solution was left to stand for 30 minutes in a dark place. The absorbance was measured using a UV-Vis spectrophotometer at λ = 517 nm.

Antioxidant activity

25 mg of concentrated chili extract dissolved with 96 % ethanol in a flask up to 25 mL to obtain a concentration of 1000 ppm, then diluted with 96 % to 25 mL ethanol so that the test solution was obtained with various concentrations (20, 40, 60, 80 ppm). The antioxidant activity was determined by pipetting 1 mL of each concentration and put into the vial, and 2.5 mL of 10 ppm blank solution was added. The mixture was homogenized and allowed to stand in a dark room for 30 minutes, and then the absorption was measured using a UV-Vis spectrophotometer at λ = 517 nm. As a comparison, a solution of vitamin C (20, 40, 60, and 80 ppm) was used with the same treatment as the test solution.

Results and Discussion

The extraction method used is the maceration method. The maceration method is one of the simplest extraction methods. The procedure is carried out by immersing the sample with a suitable solvent in a closed container at room temperature (Erawati, 2012). The separation method by extraction works based on the solubility principle like dissolve like, that is, polar solvents will dissolve polar substances, and vice versa (Khopkar, 2003).

The extraction process was carried out for one × 24 hours using a mixed solvent, n-hexane: acetone: ethanol, with a ratio of 2:1:1. This is based on research (Barba et al., 2006) which shows that a mixture of hexane: acetone: ethanol with a ratio of 2:1:1 (v/v/v) is the best solvent that can extract almost all carotenoids. Remaceration is carried out 3 times with the aim that the solvent can attract and bind all active compounds contained in the sample properly (Syaifuddin, 2015).

The extraction results have separated the non-polar layer from the polar layer. Then, the sample extract is concentrated using a rotary evaporator at a temperature of 40 °C to obtain a concentrated extract of katokkon chili brick red. This concentrated katokkon chili extract is stored in a bottle covered with aluminum foil. It is free from exposure to light, and the sample is not easily damaged until the analysis process.

Lycopene Analysis

Analysis of lycopene content in this study used 1 gram of concentrated sample extract added with 25 mL of n-hexane because the compound to be analyzed was soluble in n-hexane. UV-Vis spectrophotometer was used to measure the absorbance value of the sample at a wavelength to identify lycopene compounds, namely 472 nm and 502 nm; these two wavelengths were used to see which wavelength had the highest absorbance value, which would be used to calculate the lycopene content in the sample. (Alda et al., 2009; Ratu et al., 2016). The results of measuring the absorbance value can be seen in **Table 1**.

Table 1. Absorbance of katokkon chili extract at λ = 472 and λ = 502

Replication	Absorbance (λ 472 nm)	Absorbance (λ 502 nm)
1	1.933	0.732
2	2.054	0.678
3	2.042	0.730
λ_{maks} lycopene = 472 nm		

The absorbance reading was carried out three times to reduce the measurement error so that the data obtained was accurate, from the measurement data the absorbance value could be seen that λ = 472 nm had a better absorbance value, so the absorbance value was used (1,933; 2,054; and 2,042) is to calculate the levels of lycopene in the sample. Lycopene levels were calculated using the standard value of A (1%, 1 cm) = 3450 with the results in mg/100g units (Alda et al., 2009; Ratu et al., 2016).

$$lycopene\ levels = \frac{A_{sample}}{A(1\ \%, 1\ cm)} \times \frac{Vp}{weight\ (g)}$$

The results of the calculation of lycopene levels can be seen in **Table 2**.

Table 2. Lycopene levels in katokkon chili extract samples

Replication	Lycopene Levels (mg/100g)
1	1.400
2	1.490
3	1.480
Average	1.457

Antioxidant activity test

Antioxidants are compounds that can delay, slow down and prevent the process of lipid oxidation by delaying or preventing the occurrence of autoxidation reactions of free radicals in lipid oxidation (Shui et al., 2004).

The method used to test the antioxidant activity in this study was the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. This method is the simplest, easy, fast, and sensitive testing method for antioxidant activity. It only requires a small number of samples for testing the antioxidant activity of natural compounds (Molyneux, 2004).

The antioxidant activity test in this study used a vitamin C comparison solution; the reason for using a vitamin C comparison is because, in addition to being cheap and easy to obtain, vitamin C is a group of flavonoids, lycopene, beta carotene, bilirubin, and albumin, which are included in the secondary antioxidant class (Juniarti et al., 2009).

The concentrations of the test solution and the comparison solution were varied (20, 40, 60, and 80 ppm) to determine the degree of color attenuation due to the presence of antioxidant compounds that can reduce the purple color intensity DPPH. The mixed solution can stand at room temperature for 30 minutes with conditions protected from sunlight (Simajuntak et al., 2004).

The absorbance measurement used a UV-Vis spectrophotometer at the maximum wavelength of DPPH, namely 517 nm so that the absorbance data were obtained as in **Table 3**.

Based on the absorbance data in **Table 3**, a comparison chart of the percentage of katokkon chili extract inhibitor with vitamin C is obtained as in **Figure 1**. The graph in **Figure 1** explains that the free radical inhibitor activity by katokkon chili extract is quite close to the ability of vitamin C as a positive control. This can prove that the katokkon chili extract with the same concentration as vitamin C can strongly inhibit free radical compounds.

Table 3. DPPH absorbance measurement results

Concentration	DPPH in Katokkon chili extract	DPPH in Vitamin C Solution	Blanko
20	0.376	0.286	0.800
40	0.351	0.252	
60	0.307	0.195	
80	0.303	0.186	

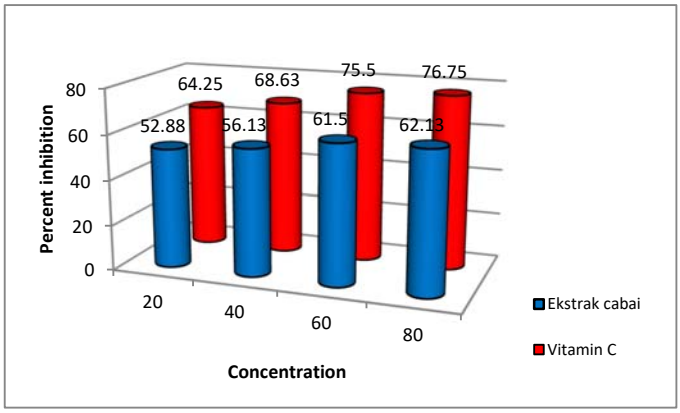

Figure 1. Comparison of the inhibitor percentage of katokkon chili extract with vitamin C

This katokkon chili extract sample has this ability because the sample contains several active antioxidant compounds, including carotenoids (β-carotene & lycopene), anthocyanins, chlorophyll, capsaicin, and vitamin C (Octaviani et al., 2014; Puspita et al., 2018), but the amount of antioxidant content is not necessarily a trigger for katokkon chili extract to be equivalent to the ability of pure vitamin C as a positive control, the cause can be influenced by the length of the sample extraction process and storage of the extraction results until the antioxidant activity test is carried out, the cause can also be caused by the lamp UV-Vis spectrophotometer tools are not good and errors that can appear when making concentration series (Masrifah et al., 2017).

Figure 1 can be further interpreted using the IC_{50} (inhibition concentration) parameter. IC_{50} is the substrate or sample concentration that will cause a reduction in DPPH (2,2, -diphenyl-1-picrylhydrazyl) activity by 50%. The smaller the IC_{50} value means, the higher the antioxidant activity (Molyneux, 2004).

The IC_{50} value in this study was obtained by calculating the x value obtained from the linear regression equation from the graphs in **Figures 2** and **3**. The graph was made by plotting the log value of the concentration with the probit value of each percentage of free radical inhibiting activity (DPPH) (Nur et al., 2016).

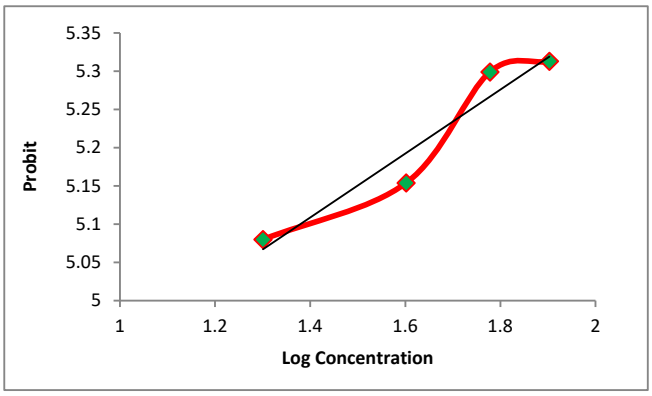

Figure 2. Relationship between log concentration and probit value of katokkon chili extract

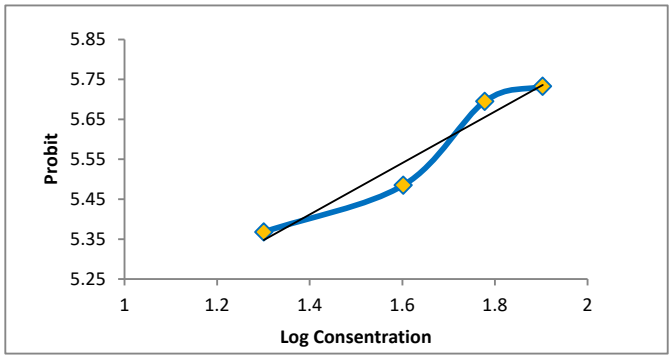

Figure 3. Correlation log concentration with vitamin C probit value

Based on the graphs in **Figures 2** and **3**, it is obtained a linear regression equation for katokkon chili extract Y = 0.41184x + 4.5228 and a linear regression equation for positive control of vitamin CY = 0.6448x + 4.5089, with a correlation coefficient value (R^2) for katokkon chili extract 0.9281 and for vitamin C 0.9421. R2 value close to 1 indicates that the data obtained is excellent. The R2 value was obtained compared with the literature, and it can be said that this result is quite good (Hastono & Sabri, 2011).

The X value in the linear regression equation from the two graphs above is used to obtain the IC_{50} value after replacing the Y = 5 value (probit of 50%). The log value of the concentration obtained is converted to the antilog form so that the IC_{50} value is obtained in ppm concentration (Pratiwi et al., 2014). The calculation of the IC_{50} value for katokkon chili extract results is 13.84 mg/L (ppm), and the IC_{50} calculation for vitamin C as positive control is 5.78 mg/L (ppm).The IC_{50} value obtained in the previous calculation is then used to determine the antioxidant power. The category of antioxidant capacity was adapted from Molyneux (2004) as in **Table 4**.

Table 4. Category of antioxidant power

Value of IC_{50} (mg/L)	Antioxidant Power
<50	Very strong
50-100	Strong
101-150	Moderate
151-200	Weak
>200	Very weak

Based on the IC_{50} value data set in **Table 4**, it can be concluded that the katokkon chili extract and vitamin C are potent antioxidants because they have an IC_{50} value of less than 50 mg/L, and based on the IC_{50} value obtained, it can be said that the antioxidant power of katokkon chili extract which is the main objective of this study has less antioxidant power than vitamin C as a positive control.

Conclusions

The data from the analysis using the UV-Vis spectrophotometer showed that the average level of lycopene in the sample of katokkon chili (*Capsicum Chinense Jacq*) was 1.457 mg/100g. Katokkon chili extract has extreme antioxidant power in reducing free radicals with a value of IC_{50} = 13.84 mg/L. In comparison, vitamin C as a positive control also has extreme antioxidant power in decreasing free radicals with IC_{50} = 5.78 mg/L. Katokkon chilies can be an alternative source of antioxidants to replace vitamin C.

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