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Antioxidant Activity Evaluation from Tomatoes' N-Hexane, Ethyl Asetate, and Water Fraction with DPPH

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Abstract

Antioxidants are compounds that can stabilize free radicals in the body. Free radicals are highly reactive molecules as they have unpaired electrons to interact with body cell molecules. Tomatoes contain flavonoids, saponins, solanine tannins, folic acid, malic acid, citric acid, protein, fat, vitamins, minerals, and histamine, which can be used as antioxidants. This study aims to evaluate the antioxidant activity of n-hexane, ethyl acetate, water fraction, and ethanol extracts of Tomatoes and to determine the greatest antioxidant activity between *n*-hexane, ethyl acetate, water and vitamin C. Tomatoes (*Lycopersicum esculentum* Mill.) was extracted using the maceration method with ethanol followed by fractionation using n-hexane and ethyl acetate solvents. The test of antioxidant activity to DPPH radical was conducted on n-hexane, ethyl acetate, water, and vitamin C. The antioxidant activity results, expressed by IC_{50} value to the n-hexane, ethyl acetate, water fraction of Tomatoes fruit, were 4.4603 ppm; 4.0868 ppm; and 4.0527 ppm, respectively. Thus, the greatest antioxidant activity was the water fraction.

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INTRODUCTION

Tomato is one type of fruit with polyphenolic compounds, carotenoids, and vitamin C, which can act as antioxidants.[1] Tomatoes contain a carotenoid compound called lycopene.[2] Atherosclerosis is strongly related to Low-Density Lipoprotein (LDL)-cholesterol. Unsaturated lipid acid components in the LDL-Cholesterol are easy to bind with free radicals and transform into oxidative LDL. It triggers sponge cells, releases reactive oxygen species (ROS), and induces oxidative stress. ROS can form atherosclerosis, plague, lipid oxidation, and endothelial disorder. Simultaneously oxidative stress will decrease the immune response to keep the body healthy. It can be avoided through high antioxidant food consumption.

The antioxidant is easily obtained from various foods containing vitamin E, vitamin C, polyphenol, and carotenoid, especially lycopene and β-carotene. Tomato is one of the abundant and affordable fruit or vegetables. Tomato (*Lycopersicum esculentum* Mill.) is a species from the Solanaceae family. It contains a

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lot of natural antioxidants, such as vitamin E, vitamin C, and carotenoid. Based on the chemical composition, carotenoid is divided into two chemical compound groups, namely, carotene and xanthophyll. The most abundant carotene in tomatoes is lycopene and β-carotene.[3] Carotene is an antioxidant that can reduce malignant cells of cancer. Of all these carotenoid compounds, it turns out that lycopene is relatively more efficient as a singlet oxygen scavenger than other carotenoids (higher than beta-carotene and alpha-tocopherol).[4] Singlet oxygen is a non-radical electrophilic ROS.[5] Lycopene is a special carotenoid that is potential to prevent prostate cancer and degenerative cardiovascular disease and anti-aging.[6] Lycopene is the main carotenoid in tomatoes which is a powerful antioxidant and has received much attention as it is associated with a lycopene-rich diet and reduces the risk of heart disease, cancer and disease in old age.[7] Aging is a biological process that occurs naturally and affects all living things, including all body organs such as the heart, lungs, brain, kidneys, and skin.[8]

The community prepares various techniques of tomato processing. Some people eat fresh tomatoes directly without any treatment. Meanwhile, most Indonesian people always cook tomatoes in various processing, such as steaming, boiling and frying. The cooking process techniques are predicted to impact antioxidant amounts inside tomatoes. Understanding the antioxidant capacity found in tomato fruits requires studying the antioxidant activity using the DPPH method to identify the antioxidant compounds in the tomato sample.

The research on samples of fruit tomato leaves (*Lycopersicon esculentum* Mill, var. Pyriforme Alef.) and vegetable tomato leaves (*Lycopersicon esculentum* Mill, var. Commune Bailey.) showed that it has antioxidant activity with IC50 values of 279.482 µg each. / mL and 280.190 µg / mL. It indicated the ethanol extract of fruit tomato leaves (*Lycopersicon esculentum* Mill, var. Pyriforme Alef.) and vegetable tomato leaves (*Lycopersicon esculentum* Mill, var. Commune Bailey.) have very weak activity as antioxidants.[9] Lycopene can hinder endometrial cancer growth, breast cancer, and lung cancer on cell culture with a higher activity than α and beta-carotene.[10] Generally, tomatoes have high antioxidants. However, high temperatures can reduce the antioxidant amount.[11] The vitamin C was measured using ascorbic acid colorimetric assay kit, and the number of α-tocopherol analyses was performed using an ELISA kit for α-tocopherol. The antioxidant activities were measured by using 1-1-diphenyl-2-pic-1-1-diphenyl-2-pic-rylhydrazyl (DPPH) and stoichiometry method.[12] DPPH provided maximum absorption on 516 nm wavelength and resulted in purple color.[13] This method was utilized due to its simple, easy, rapid, sensitive ability and only involved a minimum sample.[14]

METHOD

This research used 14 kg tomatoes (Lycopersicum esculentum Mill.) obtained from Desa Sepanjang, Tawangmangu, Karanganyar. Centra Java, Indonesia and were collected through purposive random sampling. The tomatoes' criteria included ellipse form, red color, and approximately weighted 1,5-2 ons, fresh and peel. Tomatoes processed in 6 different ways were then extracted by the same technique. This experimental study was conducted at the Laboratory of Biochemistry, Department of Biology, Universitas Ahmad Dahlan. This research used a complete random design with a post-test Only Randomized Controlled Group Design.

RESULTS AND DISCUSSION

The results of data analysis showed that all data were normally distributed and homogeneous. The data were then tested with IC_{50} (Inhibition Concentration 50).

Phytochemicals

The below compounds were identified to determine the chemical compounds in tomatoes. Phytochemical screening was carried out to determine the secondary metabolites in the extract and powder on tomatoes. Secondary metabolites tested qualitatively included alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids.

Table 1. Identification of Tomato Extract Chemical Compounds

No	Chemical Content	Simplicia	Extract	Observation
1	Alkaloids	+	+	Yellow-orange, no sediment with Dragendrof Dark orange with Mayer reagent
2	Flavonoids	+	+	Yellowish orange and Brown orange with $FeCl_3$ reagent
3	Tannins	+	+	Brownish orange, white sediment with Gelatin
4	Saponins	+	+	The foam was formed with a foam test
5	Steroids/ Triterpenoids	-	-	Yellow with acetic acid anhydrous Yellow-brown precipitated with acid

Fractionation

The results of the extraction of tomato fruit samples (*Lycopersicum esculentum* Mill.) were then fractionated. Fractionation was carried out to separate a class of compounds from other groups based on the difference in polarity of the solvent. N-hexane, ethyl acetate and water were the solvents used in fractionation in this study.

Table 2. Tomato Extract of Fraction Yield

Extract Weight (gram)	Fraction	Fraction weight (gram)	Yield (%) b/b
1.6	*n-hexane*	0.48	30
1.6	Ethyl acetate	0.65	40.625
1.6	water	1.53	95.625

Antioxidants activity

Several methods can determine antioxidant activity. In this study, the method used was antioxidant testing with DPPH free radical scavenger. Antioxidant activity was characterized by trapping electrons by free radicals, which caused electrons in free radicals to become paired electrons, resulting in a color change from purple to yellow.

Table 3. The Absorbance Average Value of the Test Solution

Test solution	Concentration (ppm)	Average Absorbance	% Inhibition
Fractio n-hexane	1.000	0.705	11.209
	1.250	0.624	21.410
	1.500	0.546	33.111
	1.750	0.542	45.661
	2.000	0.351	55.793
Ethyl acetate fraction	250	0.676	14.861
	500	0.597	24.811
	750	0.504	36.523
	1.000	0.415	47.695
	1.250	0.300	62.216
Water fraction	5.000	0.676	14.861
	10.000	0.590	25.693
	15.000	0.493	37.909
	20.000	0.382	51.889
	25.000	0.324	59.194

Inhibition Concentration 50

The results of the measuring absorbance were used to obtain the percentage of inhibition value. The antioxidant activity of the n-hexane fraction, ethyl acetate fraction, and the water fraction of the ethanol extract of tomato fruit can be expressed by IC_{50} (*Inhibition Concentration* 50). IC_{50} is the sample concentration needed to capture 50% of DPPH free radicals during operating time. The data from n-hexane, ethyl acetate, and water fractions were then calculated for the IC_{50} value using a linear regression equation based on the formula Y = a + bx.

Table 4. Inhibition Concentration 50

No	Test solution	IC_{50} (ppm) Value
1	n-hexane fraction	4.4603
2	Ethyl acetate fraction	4.0868
3	Water fraction	4.0527

The IC_{50} values were grouped into several groups, namely: IC_{50} values of less than 50 ppm were included in the very strong group, IC_{50} values of 50 ppm to 100 ppm were in the strong group, IC_{50} values of 101 ppm to 150 ppm were in the moderate class, IC_{50} values of more than 150 ppm were in the weak group while the IC_{50} values of more than 500 ppm were in the inactive group

The N-hexane fraction had an IC_{50} value of 4.4603 ppm, indicating that the n-hexane fraction had the weakest antioxidant activity compared to the antioxidant activity of the ethyl acetate fraction, water fraction, and the vitamin C. Meanwhile, hexane had strong antioxidant activity (IC value <50 ppm).

Ethyl acetate fraction had the greatest antioxidant activity compared to the antioxidant activity of the n-hexane fraction and water fraction with an IC_{50} value of 4.0868 ppm. It indicated that the ethyl acetate fraction had strong antioxidant activity as it contained flavonoids. It was stated that flavonoid compounds could inhibit oxidation reactions through a radical scavenger mechanism.[15]

Furthermore, the water fraction had an IC_{50} value of 4.0527 ppm, indicating that the antioxidant activity of the water fraction was greater than the antioxidant activity of the n-hexane fraction due to its triterpenoid compounds, tannins, saponins, and flavonoids that might capture radicals.

CONCLUSION

Based on the results of this research, it can be concluded that the n-hexane fraction had an antioxidant activity with an IC_{50} value of 4.4603 ppm; the ethyl acetate fraction had an antioxidant activity with an IC_{50} value of 4.0868, and the water fraction from the tomato extract had an antioxidant activity with an IC_{50} of 4.0527 ppm. Therefore, the water fraction of the tomato fruit extract had the best antioxidant activity with an IC_{50} value of 4.0527 ppm.

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