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Comparison of Antioxidant and Anti-hyaluronidase Activity of Tomato (*Solanum lycopersicum* L.) Extract and Lycopene

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Abstract

Aging process contributed with poor of lifestyle and photodamage can induced wrinkles formation because of extracellular matrix destruction. To investigate the potential of tomato as antiaging sources particularly as the hyaluronidase inhibitor. This study used H_2O_2 scavenging activity for antioxidant assay and hyaluronidase inhibition activity for the antiaging property. The *Solanum lycopersicum* L. extract (SLE) has lower antioxidant activity (221.30 ± 1.94) compared with lycopene (208.37 ± 4.87) through H_2O_2 scavenging activity. The *Solanum lycopersicum* L. (119.81 ± 14.23) has lower antiaging activity particularly as anti-hyaluronidase activity compared with lycopene (81.65 ± 5.95). Our findings suggest that the *Solanum lycopersicum* L. has weak antioxidant activity and moderate antiaging activity, particularly as a hyaluronidase inhibitor. *Solanum lycopersicum* L. can be used as an antiaging source, particularly as hyaluronidase inhibitor.

Keywords: Antioxidant; Hyaluronidase; *Solanum lycopersicum* L.; Lycopene; Antiaging.

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1. Introduction

Skin is the largest organ has an important role in protecting the body and internal organs from the environmental exposure [1]. Poor of lifestyle and photodamage were a better combination to accelerate depression of the skin that caused benchmarks called wrinkles [2]. The exogenous factors were the major factors contributed to the aging process by increasing reactive oxidative stress in mitochondrial dysfunction and oxidative stress [3]. Prevent skin against UV damage (use clothes can protect skin from the sun, avoid UV radiation between 10 am and 4 pm) and use medications (topical retinoids, 5-fluorouracil cream, cosmeceuticals, and antioxidant) to reverse any skin damage [4]. There is one source of natural antioxidant was the tomato. Tomato was a fruit rich of polyphenol and antioxidant content such as vitamin C was water soluble, vitamin E, β-carotene and lycopene were fat soluble and hydrophobic compounds like flavonoid, quercetin, glycosides, naringenin, chalcone, and chlorogenic acid, potassium and folate which are essential for human health [5,6]. The compounds can reduce oxidative stress, mutations of DNA, malignancy and the other parameters of cell damage [6]. Hyaluronidases are enzymes that used to increased uptake, dispersion and delivery of drugs, increasing the tissue permeability and reduce the hyaluronic acid's viscosity [7]. Hyaluronic acid has the moisture effect in the skin by bind and retains the water molecules [8]. Hyaluronic acid also has a contribution in extracellular matrix destruction associated with wrinkle formation [9]. Flavonoid involved in the aging process because the compound can be scavenging reactive oxygen species (ROS) and inhibit enzymes that were associated with aging [10]. The aim of this study is for investigating the potential of tomato as antiaging sources particularly as the hyaluronidase inhibitor.

2. Experimental Section

Materials

Materials used in this study are tomato (*Solanum lycopersicum* L.), ethanol 70%, hydrogen peroxide solution, Ferrous Ammonium Sulfate, sulfuric acid, 1,10-phenanthroline, distilled water, Hyaluronidase from bovine testes type I-S (Sigma Aldrich H3506, USA), sodium phosphate monobasic, hyaluronic acid, sodium chloride, bovine serum albumin, sodium acetate, acetic acid, hydrochloride acid solution, sodium hydroxide, Dymethilsufoxide (DMSO), lycopene.

Instrumentation

Instrument used in this study are Micropipette (1-10µL; 5-50 µL, 100-200 µL, 1000µL), Multichannel pipette 3-400 µL, multi-scan GO reader, vortex, 96-well plate, falcon tube 15 ml, falcon tube 50 ml, tube eppendorf 1,5 ml, yellow tips, blue tips, pH meter, Erlenmeyer, incubator, analytical balance, water bath.

2.1. Preparation of tomato (Solanum lycopersicum L.*) extract*

The experimental fruit tomato (Solanum lycopersicum L.) was collected from a garden in Lembang, Bandung, West Java, Indonesia. The fruits were identified at Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia by the herbarium staff. The fruit

of Solanum lycopersicum L. (6000 g) was mashed into simplicial powder. The simplicial powder of fruit of Solanum lycopersicum L. (170 g) was extracted with distilled ethanol 70% (1850 mL) by maceration method. Ethanol filtrate was filtered every 24 hours. Macerates were filtered and condensed using 50^{o}C rotavapor to obtain Solanum lycopersicum L. extract in a gel form. Solanum lycopersicum L. extract was used as the experiment and lycopene was used as control [11].

2.2. Hydrogen Peroxide (H_2O_2) Scavenging Activity Assay

The reaction of ferrous ammonium sulfate with phenanthroline would form the Fe^{2+}-tri-phenanthroline complex with the orange color, but if there was had H_2O_2 in the reaction will not form the complex, so if there was antioxidant that scavenging H_2O_2, then would form the orange color of Fe^{2+}-tri-phenanthroline complex again. Mix the 60 µl sample of Solanum lycopersicum L. extract, 12 µl ferrous ammonium sulfate 1 mM and 3 µl H_2O_2 5 mM into a well plate, then incubated the plate for 5 minutes in dark room temperature. Add 75 µl 1,10-phenanthroline 1 mM into the mixture then incubated for 10 minutes in dark room temperature. Absorbance was measured used a microplate reader at wavelength λ = 510 nm [12]. The same procedure was also applied with lycopene as a control.

$$\%\ scavenging = \frac{Sample}{Control}\ x\ 100$$

2.3. Hyaluronidase Assay

The mixture of the 25 µl sample, 3 µl hyaluronidase enzyme from bovine testes type I-S and 12 µl phosphate buffer were incubated at 37°C for 10 minutes. Then, added 10 µL hyaluronic acid substrate and incubated again at 37°C for 45 minutes. The reaction was stopped using 100 µl acidic albumin into the mixture and left it in room temperature for 10 minutes. The absorbance was measured at 600 nm wavelength [13].

$$\%\ inhibition = \frac{C - S}{C} x100$$

C: negative control absorbance

S: sample absorbance

3. Result and Discussion

3.1. Hydrogen Peroxide (H_2O_2) Scavenging activity

Hydrogen peroxide (H_2O_2) was one of the reactive oxygen species can cause oxidative damage to cellular, but if the H_2O_2 combine with free transition metal ions can cause biomolecules damaged such as lipids and nucleic acid associated to age-related disorders [14]. H_2O_2 when reacts with Fe^{2+} and Cu^{2+} ions inside the cell can from a hydroxyl radical and produced the toxic effects [15]. H_2O_2 scavenging activity used to determine the antioxidant activity of *Solanum lycopersicum* L. and its compounds. The H_2O_2 scavenging activity of *Solanum lycopersicum* L. and lycopene in many concentrations can be seen in table 1 and the IC_{50} values can be seen in

table 2.

Table 1: Hydrogen Peroxide (H_2O_2) scavenging activity of *Solanum lycopersicum* L. extract and lycopene (mean, the result of the Tukey HSD post hoc test)

Last Concentration (µg/mL)	Mean of H_2O_2 Scavenging activity (%)	
	***Solanum lycopersicum* L.**	**Lycopene**
500.00	72.09 ± 0.41^{f}	75.14 ± 0.82^{e}
250.00	56.40 ± 0.49^{e}	56.20 ± 1.15^{d}
125.00	44.28 ± 1.47^{d}	40.76 ± 0.46^{c}
62.50	39.36 ± 0.27^{c}	40.24 ± 1.36^{c}
31.25	30.58 ± 0.27^{b}	36.86 ± 0.70^{b}
15.63	26.65 ± 0.37^{a}	26.78 ± 0.14^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test).

Table 1 shows the result of the H_2O_2 scavenging activity, the higher the concentration level, the greater the H_2O_2 scavenging activity. That lycopene has an antioxidant activity higher than *Solanum lycopersicum* L. extract.

Table 2: The IC_{50} value of Hydrogen Peroxide (H_2O_2) scavenging activity of *Solanum lycopersicum* L. extract (SLE) and lycopene

Sample	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
SLE (1st repetition)	y = 0.0889x + 30.152	0.95	223.26	221.30 ± 1.94
SLE (2nd repetition)	y = 0.09x + 30.086	0.95	221.27	
SLE (3rd repetition)	y = 0.0887x + 30.541	0.94	219.38	
SLE (mean)	y = 0.0892x + 30.259	0.95	221.31	
Lycopene (1st repetition)	y = 0.0869x + 31.618	0.96	211.53	208.37 ± 4.87
Lycopene (2nd repetition)	y = 0.0898x + 31.792	0.94	202.76	
Lycopene (3rd repetition)	y = 0.0944x + 30.099	0.97	210.82	
Lycopene (mean)	y = 0.0904x + 31.17	0.96	208.30	

Table 2 shows that the IC_{50} value of *Solanum lycopersicum* L was higher (221.30 ± 1.94) than lycopene (208.37 ± 4.87). From this result, lycopene has antioxidant activity through Hydrogen Peroxide (H_2O_2) scavenging activity higher than *Solanum lycopersicum* L. extract.

The similar result was reported by Soundararajan and his colleagues [16], which reported that the IC_{50} of H_2O_2 scavenging activity of *Elaeis guineensis* leaf extract was higher (1052.02 µg/mL) than the other compound. Sundararajan and his collegues [17] reported that the IC_{50} of H_2O_2 scavenging of *Buddleja asiatica* extract was higher (35.05 µg/mL) than the other compound. Based on the results indicate *Solanum lycopersicum* L. extract has low antioxidant activity particularly as H_2O_2 Scavenging activity compared to lycopene.

The IC_{50} was used to classified antioxidant activity of sample compare to standard. If the sample IC_{50} less than 50 µg/mL is a very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a moderate antioxidant, over than 150 µg/mL is a weak antioxidant [18]. Antioxidants have direct scavenging or quenching action of the OH scavengers as well as the compound of the counteract oxidizing precursors, chelate metal ions and boost the antioxidant enzyme activity and production [19].

4. Hyaluronidase assay

Hyaluronic acid has the moisture effect in the skin by bind and retains the water molecules [8]. Hyaluronic acid also has a contribution in extracellular matrix destruction associated with wrinkle formation [9]. The hyaluronidase inhibitor activity of *Solanum lycopersicum* L. extract and lycopene can be seen at table 3 and the IC_{50} values can be seen at table 4.

Table 3: The anti-hyaluronidase activity of *Solanum lycopersicum* L. extract and lycopene (mean, the result of the Tukey HSD post hoc test)

Final Cocentration (µg/mL)	**Mean of hyaluronidase inhibitor (%)**	
	***Solanum lycopersicum* L.**	**Lycopene**
166.67	62.11 ± 6.07^{d}	78.20 ± 2.55^{e}
83.33	44.38 ± 4.44^{c}	53.29 ± 5.69^{d}
41.67	25.55 ± 1.82^{b}	35.84 ± 1.92^{c}
20.83	21.89 ± 1.36^{ab}	32.27 ± 4.56^{bc}
10.42	18.02 ± 0.13^{ab}	24.81 ± 2.37^{ab}
5.21	13.52 ± 1.15^{a}	19.41 ± 3.87^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$ (Tukey HSD post hoc test).

Table 3 shows that the higher concentration of *Solanum lycopersicum* L. extract and lycopene would result the higher anti-hyaluronidase activity. From the result, lycopene has an antiaging activity higher than *Solanum lycopersicum* L. extract.

This study has a similar result was reported by Widowati and her colleagues [13]. Which reported that the IC_{50} of the hyaluronidase inhibition of *Jasminum sambac* extract was 249.94 ± 16.51 µg/mL, the result revealed that *Jasminum sambac* extract has a lower hyaluronidase inhibitor compared to other compounds. Widowati and her colleagues [11] reported that IC_{50} of the hyaluronidase inhibition of *Oryza sativa* extract was 2013.13 µg/mL, the result revealed that *Oryza sativa* extract has a lower hyaluronidase inhibitor compared to other compounds. Based on the results indicate *Solanum lycopersicum* L. extract has low antiaging activity particularly as hyaluronidase inhibitor compared to lycopene.

The IC_{50} was used to classified anti-hyaluronidase activity of sample compare to standard. If the sample IC_{50}

less than 50 µg/mL is a very strong antioxidant, 50-100 µg/mL is a strong antioxidant, 101-150 µg/mL is a moderate antioxidant, over than 150 µg/mL is a weak antioxidant [18]. The bioactive compounds in *Solanum lycopersicum* L. inhibit the hyaluronidase activity. In the keratinocytes and fibroblast the bioactive compounds increasing the mRNA expressions hyaluronan synthase-2 and hyaluronan synthase-2, so the content of hyaluronic acid will increase too [20]. The hyaluronidase inhibitor effective on balancing the body from anabolism and catabolism of hyaluronic acid, and kept the skin moist and smooth [21].

Table 4: The IC_{50} value of anti-hyaluronidase of *Solanum lycopersicum extract* (SLE) and lycopene

Sample	**Equation**	**R^2**	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
SLE (1st repetition)	y = 0.2859x + 14.788	0.94	123.16	119.81 ± 14.23
SLE (2nd repetition)	y = 0.3358x + 15.012	0.99	104.19	
SLE (3rd repetition)	y = 0.2722x + 14.053	0.99	132.06	
SLE (mean)	y = 0.2979x + 14.618	0.98	118.77	
Lycopene (1st repetition)	y = 0.3310x + 21.144	0.95	87.18	81.65 ± 5.95
Lycopene (2nd repetition)	y = 0.3605x + 22.838	0.98	75.35	
Lycopene (3rd repetition)	y = 0.3565x + 20.616	0.98	82.42	
Lycopene (mean)	y = 0.3493x + 21.533	0.98	81.50	

Table 4 shows that the IC_{50} value in antiaging activity assay, IC_{50} value of lycopene has antiaging activity particularly as hyaluronidase inhibitor higher than *Solanum lycopersicum* L. extract (IC_{50} of lycopene = 81.65 ± 5.95 µg/mL; IC_{50} of *Solanum lycopersicum* L. extract = 119.81 ± 14.23 µg/mL).

5. Conclusions

Lycopene has a higher antioxidant and antiaging activity particularly as hyaluronidase inhibitor than *Solanum lycopersicum* L. extract. In conclusion, *Solanum lycopersicum* L. has weak antioxidant as free radical scavenging activity and moderate antiaging activity particularly as hyaluronidase inhibitor. For the recommendation, it needed to purify the active compounds to obtain a better result and *Solanum lycopersicum* L can be used as antiaging sources.

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