

Antioxidant and Anticollagenase Activity of Tomato (*Solanum lycopersicum* L.) and Lycopene

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Abstract

Antioxidants were any substance that can inhibit oxidation that causes collagen disintegration, lipid breakdown, DNA damage and protein unfolding in the aging process. The ingredients in the cosmetics has a toxin effect in human body. To investigate the bioactive compound, an antioxidant and antiaging activity particularly as collagenase inhibitor of the tomatoes. This study used phytochemical screening for the bioactive compounds, DPPH scavenging activity for antioxidant assay and collagenase inhibition activity for the antiaging property. The phytochemical screening shows that the *Solanum lycopersicum* L. extract (SLE) has flavonoid, phenol, saponin, tannin and alkaloid. The lycopene (79.45 ± 1.01) has an antioxidant activity better than SLE (147.20 ± 16.97) from DPPH scavenging activity. Lycopene ($85.09 \pm 1,81 \mu\text{g/mL}$) also has the higher antiaging activity than SLE ($236,74 \pm 9,74 \mu\text{g/mL}$) particularly as collagenase inhibitor. In conclusion flavonoid was the most common in the SLE. SLE has a higher antioxidant activity through DPPH scavenging activity and antiaging activity particularly as a collagenase inhibitor.

Keywords: *Solanum lycopersicum* L.; Antiaging; Collagenase; Antioxidant.

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1. Introduction

Antioxidants were any substance that can delay or inhibits oxidation of that substrate significantly and can scavenge reactive oxidative stress (ROS) directly and inhibit ROS production [1]. ROS were free radicals produced by mitochondria thought to be oxygen-centered the radicals initially, but also a product of normal cellular metabolism from cellular redox process called reactive nitrogen species (RNS) [2,3]. The physical changes of the skin due to transformation in the connective tissue like the formation of lipid peroxides, cell contents and enzymes caused by the UV exposure [4]. In the aging process, ROS had an important role in collagen disintegration, lipid breakdown, DNA damage, fragmentation and protein unfolding [5]. At least 3.500 years ago, the spices and herbs had been traded existence. Recently, the user of the spices and herbs as natural antioxidants *in the food industries increased rapidly* [6]. The ingredients in the cosmetics such as paraben, coal tar, phthalates, heavy metals and the other compounds have a toxic effect in human body [7]. Tomatoes are the most important vegetables and rich of antioxidants such as lycopene, flavonoid, vitamins C, E, B6, folic acid, niacin, potassium and trace elements which play important role in scavenging free radicals and cofactors of antioxidant enzymes [8,9]. Lycopene is a powerful antioxidant agent and has the ability to prevent skin from aging from the ability to defend the skin against the radiation of UV exposure by blocking the UV light [10,11]. Flavonoid and phenolic compounds have an antioxidant activity generally in scavenging free radicals [12]. By knowing so much the function of the tomatoes, these study objectives are to investigate the bioactive compound, an antioxidant using 1,1-diphenyl 2-picrylhydrazyl (DPPH) method and antiaging activity particularly as collagenase inhibitor of the tomatoes.

2. Experimental Section

Materials

Materials used in this study are tomato (*Solanum lycopersicum* L.), ethanol 70%, DPPH 2,2 Diphenyl-1-picrylhydrazyl (DPPH) (Sigma, D9132), absolute methanol (Merck 1060092500), Dymethylsulfoxide (DMSO) (Merck 1029522500), Lycopene (Chengdu BP0401), N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGPA) (Sigma F5135), Collagenase from *Clostridium histolyticum* (Sigma C8051), Tricine (Sigma SA10377), calcium chloride (Merck 1023821000), Sodium chloride (Merck 106406), distilled water, (Merck 1.02931.1000), Hydrochloric acid solution (Merck 109057),

Instrumentation

Instrument used in this study are Micropipette (1-10 μ L; 50-200 μ L; 100-1000 μ L), Microplate Reader, 96 well, Tube 15 ml, Tube 50 ml, Pipette Tips (1-10 μ l, 50- 200 μ l, 100-1000 μ l), Multichannel Pipette 30-300 μ L, Incubator, analytical balance, vortex, tube eppendorf 1,5 ml, pH meter, beaker glass.

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

The fruits of *Solanum lycopersicum* L. were collected from Bandung, West Java, Indonesia. The plants were identified by the herbarium staff, Department of Biology, School of Life Science and Technology, Bandung

Institute of Technology, Bandung, Indonesia. The *Solanum lycopersicum* L.'s fruits were mashed and extracted using ethanol 70% with maceration method. Every 24 hours the filtrate was filtered and collected until it was colorless. The extract evaporated until the extract become a paste form [13].

2.2. Phytochemical Screening Assay

The phytochemical screening assay on *Solanum lycopersicum* L. was carried out of ethanolic extraction of *Solanum lycopersicum* L. to know the bioactive compounds that were useful in anti-aging and antioxidant [13,14].

Phenols identification

Solanum lycopersicum L. extract (10 mg) was dissolved with 5 ml ddH₂O. Then 500µl of FeCl₃ 1% was added. The presence of green/red/purple/blue/black color indicated the phenol compound [4].

Steroid/triterpenoid identification

Solanum lycopersicum L. extract (10 mg) was placed on dropping plate, then glacial acetate was added until the extract was soaked. After 10-15 minutes, add 1 drop of absolute sulfate acid (H₂SO₄). The presence of green/blue color indicated the steroid compound if the presence of purple/red/orange color indicated the triterpenoid compound [4].

Saponin identification

Solanum lycopersicum L. extract (10 mg) was dissolved with ddH₂O into the test tube and boiled it for 5 minutes, shake vigorously. The presence of the stable foam indicated the saponin compound [4].

Tannin identification

Solanum lycopersicum L. extract (10 mg) was dissolved with 2 ml HCl 2N into the test tube and heated it in the water bath for 30 minutes. Add 500µl amyl alcohol after the mixture was cooled down and filtered. The presence of orange/red color indicated tannin compound [4].

Terpenoid identification

Solanum lycopersicum L. extract (10 mg) into dropping plate. Then vanillin and absolute H₂SO₄ were added. The presence of the purple color indicated terpenoid compound [4].

Flavonoid identification

Solanum lycopersicum L. extract (10 mg) was dissolved with HCl 2N in the test tube, and add Mg/Zn. The mixture sample was heated for 5-10 minutes, add 1 ml amyl alcohol after the mixture cooled down and filtered. The presence of red/orange color indicated flavonoid compound [4].

Alkaloid identification

Solanum lycopersicum L. extract (10 mg) dissolved with 5 ml ddH₂O and evaporated in the water bath. Add 5 ml HCl 2N into the residue and divided into 2 test tube. Add 3 drops of HCl 2N to the first tube for blank. Moved the second tube to drop plate and add 3 drops of Dragendorff solution. The presence of orange sediment indicated alkaloid compound [4].

2.3. Diphenyl-1-picrylhydrazil (DPPH) Assay

DPPH compounds are free radicals that contained hydrogen radical compounds. The reduction of DPPH solution was the antioxidant compounds release the hydrogen atoms into the antioxidant radicals and forms into a nonradical DPPH. Add Solanum lycopersicum L. extract (50 µl) and DPPH 0,077 mmol (200 µl) into the 96-well plate. The mixture was incubated in the dark room for 30 minutes. The absorbance was measured using the microplate reader with 517 nm wavelength [15]. The radical scavenging activity was measured using the following formula:

$$\% \text{ scavenging} = \frac{\text{Sample}}{\text{Control}} \times 100$$

2.4. Collagenase Assay

The mixture of the 10 µl of collagenase from Clostridium histolyticum (0.1 mg/ml), 30 µl of SLE and 60 µl of tricine buffer (50 mM tricine, 10 mM calcium chloride, 400 mM sodium chloride, pH 7.5) were incubated at 37°C for 20 minutes. Add 20 µl FALGPA 1 mM substrate into the mixture. The absorbance was measured using the microplate reader with 335 nm wavelength [16]. The collagenase inhibition activity was measured using the following formula:

$$\% \text{ inhibition} = \frac{C - S}{C} \times 100$$

C: negative control absorbance

S: sample absorbance

3. Result and Discussion

3.1. Phytochemical Screening of SLE

Phytochemical screening of SLE has shown the presence of flavonoid, saponin, phenol, tannin, steroid/triterpenoid, terpenoid and alkaloid.

The result of phytochemical screening of SLE can be seen in table 1.

Table 1: Result of Phytochemical Screening of SLE

Phytochemical content	SLE
Flavonoid	+++
Saponin	++
Phenol	+
Tannin	-
Steroid/Triterpenoid	-/+
Terpenoid	-
Alkaloid	++

++++ : very high content; +++ : high content; ++ : moderate content; +: low content; - : not detected

The aim of this phytochemical screening of the SLE was to investigate the bioactive compounds in the *Solanum lycopersicum* L. such as flavonoid, saponin, phenol, tannin, steroid/triterpenoid, terpenoid and alkaloid. Table 1 shows the presence of the high content (+++) of the flavonoid, the moderate content (++) of the saponin and alkaloid, the low content (+) of the phenol and triterpenoid, while the tannin, steroid, and terpenoid were not detected (-). Flavonoids were the highest bioactive compound in the *Solanum lycopersicum* L. extract, they were contributed to antioxidant in the human health. The bioactive compound can react with the free radical, chelate metals, and inhibit lipid peroxidation [17].

3.2. Diphenyl-1-picrylhydrazil (DPPH) Assay

DPPH was one of the free radical that was occurring at the electron transfer. The DPPH solution will oxidize the plant extract compounds. This process characterized by the purple color was fading away into yellow color [18]. DPPH radical scavenging activity was the most widely method that was used for screening the antioxidant activity of the plant extract [19]. The DPPH scavenging activity of *Solanum lycopersicum* L. in many concentrations can be seen at table 2. The IC₅₀ value of DPPH scavenging activity can be seen at table 3.

Table 2: Diphenyl-1-picrylhydrazil (DPPH) scavenging activity of *Solanum lycopersicum* L. extract and lycopene (mean, the result of the Tukey HSD post hoc test)

Last (µg/mL)	Concentration	Mean of DPPH Scavenging activity (%)	
		<i>Solanum lycopersicum</i> L.	Lycopene
200		54.98 ± 2.61 ^d	90.29 ± 0.46 ^f
100		47.13 ± 1.81 ^c	59.77 ± 0.83 ^e
50		39.95 ± 1.53 ^b	41.60 ± 0.34 ^d
25		35.70 ± 0.94 ^{a,b}	33.72 ± 0.31 ^c
12.5		35.37 ± 1.42 ^{a,b}	29.18 ± 1.19 ^b
6.25		31.82 ± 1.90 ^a	16.34 ± 0.96 ^a

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

Table 2 shows the result of the DPPH scavenging activity, the higher the concentration level, have better potential for scavenging free radical. Based on the result, lycopene has DPPH scavenging activity higher than *Solanum lycopersicum* L. extract.

Table 3: The IC₅₀ value of 2,2-Diphenyl-1-picrylhydrazil (DPPH) scavenging activity of *Solanum lycopersicum* L. extract (SLE) and lycopene

Sample	Equation	R ²	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
SLE (1 st repetition)	$y = 0.0918x + 34,816$	0.95	165.40	
SLE (2 nd repetition)	$y = 0.1250x + 31,953$	0.99	144.38	
SLE (3 rd repetition)	$y = 0.1287x + 33,036$	0.94	131.81	147.20 \pm 16.97
SLE (mean)	$y = 0.1152x + 33,269$	0.96	145.23	
Lycopene (1 st repetition)	$y = 0.3516x + 21.716$	0.97	80.44	
Lycopene (2 nd repetition)	$y = 0.3534x + 22.288$	0.97	78.42	
Lycopene (3 rd repetition)	$y = 0.3481x + 22.327$	0.98	79.50	79.45 \pm 1.01
Lycopene (mean)	$y = 0.3511x + 22.110$	0.97	79.44	

Table 3 shows that the IC₅₀ value of *Solanum lycopersicum* L. was higher (147.20 \pm 16.97) than lycopene (79.45 \pm 1.01). From this result, lycopene has antioxidant activity through 2,2-Diphenyl-1-picrylhydrazil (DPPH) scavenging activity better than *Solanum lycopersicum* L. extract.

The similar result was reported by Widowati and her colleagues [18], which reported that the IC₅₀ of DPPH scavenging activity of *Jasminum sambac* extract was higher (94.13 \pm 10.54 µg/mL) than eugenol (2.28 \pm 0.12 µg/mL). Widowati and her colleagues [4] reported that the IC₅₀ of DPPH scavenging of *Hibiscus sabdariffa* extract was higher (195.73 \pm 18.63 µg/mL) than ascorbic acid (5.91 \pm 0.66). Boulanouar and his colleagues [20] reported that the IC₅₀ of *T. algeriensis* extract (111 \pm 1.12 µg/mL) was higher than ascorbic acid (16 \pm 1.22 µg/mL) Based on the results indicate lycopene more potent as antioxidant particularly as DPPH scavenging activity than *Solanum lycopersicum* L. extract.

The antioxidant activity was influenced with the density of the extract, the high density of the extract presents the higher bioactive compound and the low density of the extract present the lower bioactive compound in the extract [21]. The bioactive compound also has hydrogen donating ability associated with the replacement of hydroxyl groups in the aromatic ring systems of the phenolic compounds [22]. The antioxidant activity from flavonoid was through suppressed the oxidative stress in the cell [23]. DPPH was a stable free radical when the antioxidant reacts with DPPH, the DPPH reduced into hydrazine [24].

4. Hyaluronidase assay

Collagen was a structural protein that was correlated in the skin thickness and strength [25]. Collagenase is a

group of metalloproteinase enzyme that has important role in collagen degradation. One of the aging problems was the increasing the collagen degradation [26]. The collagenase inhibitor activity of *Solanum lycopersicum* L. extract and lycopene in some concentration can be seen at table 4 and the IC₅₀ values can be seen at table 5.

Table 4: The anti-collagenase activity of *Solanum lycopersicum* L. extract and lycopene (mean, the result of the Tukey HSD post hoc test)

Final Cocentration (µg/mL)	Mean of collagenase inhibitor (%)	
	<i>Solanum lycopersicum</i> L.	Lycopene
250.00	52.39 ± 1.89 ^f	84.55 ± 1.10 ^f
125.00	30.88 ± 0.26 ^e	54.99 ± 0.34 ^e
62.50	22.84 ± 0.84 ^d	48.53 ± 1.32 ^d
31.25	19.04 ± 1.03 ^c	43.33 ± 0.46 ^c
15.63	15.74 ± 0.94 ^b	35.95 ± 0.05 ^b
7.81	10.51 ± 1.19 ^a	28.85 ± 0.45 ^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 4 shows that various concentration *Solanum lycopersicum* L. extract and lycopene, the higher of the concentration level, more potential the antiaging activity particularly as collagenase inhibitor. Based on the result, lycopene has an antiaging activity better than *Solanum lycopersicum* L. extract.

Table 5: The IC₅₀ value of anti-collagenase of *Solanum lycopersicum* extract (SLE) and lycopene

Sample	Equation	R ²	IC ₅₀ (µg/mL)	IC ₅₀
				(µg/mL)
SLE (1 st repetition)	y = 0.1667x + 11.794	0,98	229,19	236,74 ± 9,74
SLE (2 nd repetition)	y = 0.1508x + 12.642	0,99	247,73	
SLE (3 rd repetition)	y = 0.1638x + 11.788	0,98	233,28	
SLE (mean)	y = 0.1604x + 12.075	0,99	236,44	
Lycopene (1 st repetition)	y = 0.2057x + 32.844	0,96	83,40	85,09 ± 1,81
Lycopene (2 nd repetition)	y = 0.2123x + 31.981	0,97	84,88	
Lycopene (3 rd repetition)	y = 0.2030x + 32.338	0,97	87,00	
Lycopene (mean)	y = 0.2070x + 32.388	0,96	85,08	

Table 4 shows that the IC₅₀ value in antiaging activity assay, IC₅₀ value of *Solanum lycopersicum* L. has higher value than lycopene (IC₅₀ of lycopene = 85,09 ± 1,81 µg/mL; IC₅₀ of *Solanum lycopersicum* L. extract = 236,74 ± 9,74 µg/mL).

This study has a similar result was reported by Widowati and her colleagues [16]. Which reported that the IC₅₀ value of the collagenase inhibition of *Oryza sativa* extract was 816.78 µg/mL, the result indicated the *Oryza sativa* extract has lower antiaging activity particularly as collagenase inhibition compared with the other compounds. Widowati and her colleagues [18] reported that IC₅₀ of the collagenase inhibition of *Jasminum sambac* extract was 339.30 ± 7.87 µg/mL, the result indicated the *Jasminum sambac* extract has lower antiaging activity particularly as collagenase inhibition compared with the other compounds. Based on this study, *Solanum lycopersicum* L. extract has a potential as antiaging activity particularly as collagenase inhibitor.

The bioactive compound dramatically induced the collagen synthesis as a booster of collagen in the skin and inhibited the collagen activity via reduced the collagen breakdown [27]. The collagenase inhibition activity delays the aging process such as wrinkle formation via delay the forming pre-collagen fibres [28]. The polyphenols such as flavonoid that found in the plant extract have an anti-collagenase activity has been used as a basic material for synthesizing several antiaging molecules [29].

5. Conclusions

From the result, lycopene has potential antioxidant and antiaging activity through collagenase inhibitor higher than *Solanum lycopersicum* L. extract. In conclusion, the bioactive compound that presence in the *Solanum lycopersicum* L. was flavonoid, phenol, saponin, tannin and alkaloid. The compound in the *Solanum lycopersicum* L. made it had antioxidant activity through DPPH scavenging activity and antioxidant activity particularly as collagenase inhibitor, so *Solanum lycopersicum* L. can be used as an antiaging product. For the recommendation, the extract needed to be purified to get the bioactive compound for the antiaging sources.

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