ANTIOXIDANT AND ANTI-ELASTASE ACTIVITY OF ETHANOL EXTRACT OF TOMATO (Solanum lycopersicum *L.*)

AKTIVITAS ANTIOKSIDAN DAN ANTI-ELASTASE DARI EKSTRAK ETANOL TOMAT (*Solanum lycopersicum* L.)

Alhoi Hendry Henderson[1*)], I Nyoman Ehrich Lister[2], Edy Fachrial[3], dan Ermi Girsang[4]

Faculty of Medicine, University Prima Indonesia[1]
Department of Physiology, Faculty of Medicine, University Prima Indonesia[2]
Laboratory of Biomolecular, Faculty of Medicine, University Prima Indonesia[3]
Department of Public Health, Faculty of Medicine, University Prima Indonesia[4]
Jalan Belanga, Medan, 20118, Nort Sumatera

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ABSTRACT/ABSTRAK

Skin aging due to the damage caused by ultraviolet radiation and toxic ingredients in cosmetics is still a problem. Tomato has antioxidant and skin protection activities. The study aimed to investigate the potential of tomato as an antioxidant and elastase inhibitor. A 170 g of tomato simplicial powder was extracted using ethanol 70% by the maceration method. Antioxidant activity was measured through 2,2'-Azino-bis 3-ethyl benzothiazoline-6-sulphonic acid (ABTS)-reducing activity. The antiaging activity was measured through anti-elastase activity. Tomato extract (SLE) showed strong ABTS-reducing activity (IC_{50} = 86.66 ± 10.58) and very strong anti-elastase activity (IC_{50} = 19.73 ± 0.44). In conclusion, there was a linear correlation between antioxidant activity and anti-elastase activity. However, the antioxidant activity and anti-elastase activity of tomatoes were still below lycopene (IC_{50} antioxidant = 49.23 ± 2.06 µg.ml^{-1} and IC_{50} anti-elastase = 10.39 ± 0.43 49.23 ± 2.06 µg.ml^{-1}). However, it was worth to be developed as a natural product as an antioxidant and anti-elastase. Further study is required to do fractionation to get the purer lycopene compound from tomato.

Penuaan pada kulit akibat kerusakan yang disebabkan oleh radiasi ultraviolet dan penggunaan kosmetik yang mengandung bahan beracun masih menjadi masalah saat ini. Tomat mengandung senyawa kimia yang memiliki aktivitas antioksidan dan perlindungan kulit. Tujuan penelitian ini adalah untuk mengetahui potensi ekstrak etanol tomat sebagai antioksidan dan antipenuaan (anti-elastase). Ekstrak tomat diperoleh dari serbuk simplisia tomat yang diekstraksi menggunakan etanol 70% dengan metode maserasi. Aktivitas antioksidan diukur melalui aktivitas pengurangan 2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) dan aktivitas antipenuaan diukur melalui aktivitas anti-elastase. Ekstrak tomat menunjukkan aktivitas antioksidan dan anti-elastase yang kuat. Aktivitas IC_{50} penurunan ABTS adalah 86,66 ± 10,58 µg.ml^{-1} dan IC_{50} aktivitas anti-elastase sebesar 19,73 ± 0,44 µg.ml^{-1}. Aktivitas antioksidan dan aktivitas anti-elastase berkorelasi linier, artinya semakin besar aktivitas antioksidan semakin tinggi anti-elastasenya. Walaupun aktivitas antioksidan dan aktivitas anti-elastase tomat masih di bawah likopen (IC_{50} antioksidan = 49,23 ± 2,06 µg.ml^{-1} dan IC_{50} anti-elastase = 10,39 ± 0,43 µg.ml^{-1}), tetapi layak untuk dikembangkan sebagai antioksidan dan anti-elastase alami. Oleh karena itu, perlu dilakukan fraksinasi untuk mendapatkan senyawa likopen dari tomat yang lebih murni.

* Alamat Korespondensi : *hendersonhendry96@gmail.com*

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INTRODUCTION

In the twentieth century, the main extrinsic factor that caused skin aging is ultraviolet from the sun radiation. The overexposure of skin to UV radiation, such as UVB (280-320 nm), would induce skin aging (Kang *et al*. 2020). Skin damage caused by photoaging, including deep wrinkles, hyperpigmentation, chronic inflammation, and abnormal elastin formation, was the main cause of the decrease of collagen, elastin, and hyaluronic acid (Miracle Uwa 2017; Tu and Quan, 2016; Widowati *et al.* 2018). Nowadays, people use cosmetics to prevent UV radiation. Furthermore, dermatologists recommended using sunscreen to protect the skin from incidental exposure such as dehydration, reactive oxygen species, and irradiation (Mohiuddin 2019). Some cosmetic products contain a new ingredient that is not on the licensing list. Their new components can cause allergies and skin damage (Pereira and Pereira, 2018). The toxic chemicals containing in cosmetic and sunscreen formulations, such as benzophenones, hydroquinone, p-phenylenediamine (PPD), and mercury, can cause photoallergies cytotoxic and mutagenic to skin cells (Khan and Alam 2019). The appropriate cosmetic products had high prices unreachable by people with low socio-economic conditions (Donglikar *et al.* 2016). Many big cosmetic companies had found many hypoallergic and non-toxic cosmetic products produced from the number of plant species that had skin care effect (Gonzalez-Minero and Bravo-Diaz, 2019; Septiana and Simanjuntak 2018). Cosmetic products that used various plants as a primary ingredient due to their slight side effects were termed herbal cosmetics (Gediya *et al.* 2011). The natural ingredients can reduce skin disorders and maintain the health, moisturize, and texture of the skin.

Furthermore, natural ingredients are found easily and cheaper than cosmetic products (Mangilal *et al.* 2017). Some roots, flowers, fruits, leaves, seeds, and stems of plants contain bioactive compounds, such as polyphenols (flavonoids, catechins, isoflavones, proanthocyanidins, and anthocyanins) and non-flavonoids (phenolic acids, benzoic acid, stilbene, and resveratrol) (Bosch *et al*. 2015). The natural bioactive in plants, like alkaloids, tannins, flavonoids, phenolic compounds and their family members, such as selenium, polyphenols, vitamin C, vitamin E, β-carotene, lycopene, lutein, and other carotenoids, was being widely used as antiaging and anti-wrinkle (Gulati *et al*. 2017; Volunteer 2017). A study of cosmetic with soy extract confirmed that soy extract had an antiaging effect and maintained skin elasticity. In another study, cosmetics contained curcumin extract also increased the tropoelastin expression in human skin (Weihermann *et al.* 2017).

Sopyan *et al.* (2017) had formulated tomato extract for sunscreen protection. Tomato extract was also used as an anti-wrinkle, related to its antioxidant activities by protecting skin cells from oxidative stress and skin damage induced by heavy metal (Miastkowska and Sikora, 2018; Sharafzadeh 2013). The bioactive compound in tomatoes was lycopene (Simitzis 2018). The lycopene has high antioxidant activity, antiaging ability, skin tanning, skincare, skin protection activity, and inhibition of the stromal fibroblasts' migration. Tomatoes also contained flavonoids with intense antioxidant activity and elastase inhibition (Kashif *et al.* 2017; Mai *et al*. 2018; Simitzis 2018). The antioxidant activity could be investigated using 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. The ABTS had the possibility of determining the capacity of hydrophilic and lipophilic antioxidants (Shah *et al.* 2015). The antioxidant capacity of tomato and its compounds was determined based on the ABTS discoloration by the antioxidant compounds. A strong oxidizing agent, such as potassium persulfate, reacted with the ABTS salt to produce ABTS. The ABTS measurement was based on the loss of blue color due to the reduction of ABTS radical by antioxidant compounds. The ABTS method's advantages were its ability to provide specific absorbance at visible wavelengths and detect the lipophilic or hydrophilic compounds because ABTS can be solved either in water or organic solvents. It had a faster reaction time (Wulansari 2018). Elastase played a role in elastic fiber tissue impairment by UV radiation (Imokawa and Ishida 2015). This study aimed to investigate the potential of ethanol extract of tomato as an antioxidant activity through ABTS-reducing activity and elastase inhibitor.

MATERIALS AND METHODS

Preparation of tomato extract

Tomato fruits var Esculentum were collected from Mangunharjo Village, Lembang Sub-district, Bandung, West Java, Indonesia. Red-rinded fruits were sorted and washed before drying using a food dehydrator to produce dried plant materials and then ground into powder. The tomato powder (170 g) was macerated with distilled ethanol 70% (1,850

ml). Ethanol filtrate was filtered using 0.45 µM filter paper. The filtration results were then thickened using a rotary evaporator to reduce alcohol content and to obtain a paste form. Tomato extract was used as the experiment material, and lycopene was used as a standard compound (Widowati *et al*. 2018).

Antioxidant activity assay

The 2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay was used as an antioxidant assay following Rusmana's method (Rusmana *et al*. 2017). The ABTS solution was produced by reacting 14 mM ABTS and 4.9 mM potassium persulfate (1:1 volume ratio) and incubated for 12-16 hours in the dark at room temperature. The mixture was then diluted with phosphate-buffered saline (PBS) (pH 7.4) until the absorbance of the solution was 0.70 ± 0.02 at wavelengths 745 nm. In brief, 2 µl of samples of tomato extract with 6 concentration levels (1.56; 3.13; 6.25; 12.50; 25.0; and 50.0 µg.ml^{-1}) was added to a 96-well microplate respectively, then put in 198 µl fresh ABTS solution. The absorbance was incubated for 6 minutes at 30°C, then measured at 745 nm. The inhibition percentage of ABTS radical was determined by the ratio of reducing ABTS absorbance in the sample relative to the absorbance in the absence of the sample (negative control). The same procedure was applied to lycopene (Chengdu Biopurify, BP0901) as control. This assay was repeated three times. The median inhibitory concentration (IC_{50}) was also calculated. The IC_{50} is the concentration of the solution test inhibition to find its ability to reduce free radical activity by 50%. The IC_{50} can be used to examine the antioxidant and antiaging activity (Wulansari, 2018). The percentage of reducing activity was measured following Wulansari (2018) formula as follows:

$$\text{Reducing activity (\%)} = 1-\frac{S}{C} \times 100\%$$

C: an absorbance without samples
S: an absorbance with samples

Elastase assay

Elastase inhibitor activity was used as an antiaging assay and measured following Widowati *et al.* (2017). Sample of 10 µl with 6 concentration levels (2.08; 4.17; 8.83; 16.67; 33.33; 66.67) (0.78 – 50 µg.ml^{-1}), 5 µl enzyme elastase from porcine pancreas (0.01 mg.ml^{-1}) and 125 µl buffer tris (100 mM, pH 8) were incubated for 15 minutes at 25°C. A 135 µL buffer tris was added into 5 µl enzyme for control, whereas 130 µl buffer tris was added into 10 µl sample for a blank sample. The mixed solution was then added with 10 µl N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate and incubated for 15 minutes at 25°C. Absorbance was measured at 410 nm wavelength. The same procedure was applied to lycopene as control. This assay was repeated three times. The median inhibitory concentration (IC_{50}) was also calculated following the formula of (Widowati *et al.* 2017):

$$\text{Inhibition (\%)} = \frac{C-S}{C} \text{ x } 100$$

C: an absorbance without samples
S: an absorbance with samples

Statistically data analysis

The tool for analyzing data was the SPSS program with the One-Way ANOVA method, followed by the PostHoc Test Tukey HSD. The value of IC_{50} was determined using linear regression analysis.

RESULTS AND DISCUSSION

Antioxidant activity assay

There was a correlation between the concentration level and ABTS-reducing activity (Table 1). The higher the level of tomato extract and lycopene concentration, the greater the ABTS-reducing activity. However, lycopene had an antioxidant activity higher than tomato extract.

The IC_{50} value of tomato extract was higher than lycopene (Table 2). It indicated lycopene had antioxidant activity, through ABTS-reducing activity, better than tomato extract. Thus, tomato extract had lower antioxidant activity than lycopene.

This study showed a similar result reported by Djermoune *et al*. (2019), where IC_{50} of ABTS-reducing activity from fresh tomato var. Marmande was 6.21 mg.ml^{-1}. Moreover, Widowati *et al.* (2016) also reported IC_{50} of ABTS-reducing activity of *Oryza sativa* extract, which was the highest (145.67 µg.ml^{-1}) among other compounds.

Widowati *et al. (*2017) also revealed the IC_{50} of ABTS-reducing activity of *Hibiscus sabdariffa* extract, which was the highest (74.58 ± 2.97 µg.ml^{-1}) compared to others compound. The bioactive compound of tomato had an antiradical activity which protected the cell from the degeneration process (Treml and Šmejkal 2016).

Lycopene is a nonenzymatic antioxidant that is rich in tomato. It was a potent antioxidant and very useful in removing singlet oxygen, causing oxidative stress (Brar *et al*. 2014). Lycopene also has a crucial role in reducing ROS/RNS production and increasing the body's endogenous antioxidant defense (Kasote *et al.* 2015). The tomato's phenolic compounds scavenged the ABTS, then breaking an electron chain into the free radical and quenching chain to remove the ROS (Brar *et al.* 2014; Goel *et al*. 2012). The flavonoid in tomato reduces oxidative stress and may delay the aging effects by promoting healthy tissue growth, keeping the cellular health, and renewing the cell (Anuj *et al.* 2016).

Elastase assay

A correlation occurred between the level of the concentration and elastase activity (Table 3). The higher the concentration of tomato extract and lycopene, the higher the antiaging activity through elastase inhibitor. Lycopene had better antiaging activity than tomato extract.

The IC_{50} value of lycopene (IC_{50} = 10.39 ± 0.43 µg.ml^{-1}) indicated antiaging activity through elastase inhibitor was better than tomato extract (IC_{50} = 19.73 ± 0.44 µg.ml^{-1}) (Table 4). However, tomato extract still had a strong elastase inhibitor activity.

Table 1. The ABTS-reducing activity of ethanol extract of tomato and lycopene
Tabel 1. Aktivitas penurunan ABTS oleh ekstrak etanol tomat dan likopen

Final concentration of SLE and Lycopene (µg.ml^{-1})	Mean of ABTS-reducing activity (%)	
	Tomato	Lycopene
50.00	27.46 ± 3.57^{d}	49.67 ± 1.87^{e}
25.00	12.78 ± 0.63^{c}	26.45 ± 2.35^{d}
12.50	4.75 ± 0.82^{b}	18.28 ± 3.35^{c}
6.25	2.68 ± 0.18^{a,b}	9.90 ± 0.95^{b}
3.13	0.68 ± 0.01^{a,b}	2.07 ± 0.07^{a}
1.56	0.12 ± 0.02^{a}	0.67 ± 0.09^{a}

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

Table 2. The IC_{50} value of ABTS-reducing activity of tomato extract (SLE) and lycopene
Tabel 2. Nilai IC_{50} dari aktivitas penurunan ABTS oleh ekstrak tomat (SLE) dan likopen

Sample	Equation	R^2	IC_{50} (µg.ml^{-1})	Mean of SLE IC_{50} (µg.ml^{-1})
SLE (1st repetition)	y = 0.5209x - 0.9756	0.99	94.11	86.66 ± 10.58
SLE (2nd repetition)	y = 0.6442x - 1.9739	0.98	74.55	
SLE (3rd repetition)	y = 0.539x - 0.7757	0.99	91.33	
SLE (mean)	y = 0.568x - 1.2418	0.99	85.84	
Sample	**Equation**	**R^2**	**IC_{50} (µg.ml^{-1})**	**Mean of Lycopene IC_{50} (µg.ml^{-1})**
Lycopene (1st repetition)	y = 1.0382x + 0.9735	0.99	47.22	49.23 ± 2.06
Lycopene (2nd repetition)	y = 0.9753x + 2.074	0.95	49.14	
Lycopene (3rd repetition)	y = 0.9323x + 2.1397	0.97	51.34	
Lycopene (mean)	y = 0.982x + 1.7291	0.98	49.16	

SLE: *Solanum lycopersicum L. extract*

Table 3. The anti-elastase activity of tomato extract and lycopene
Tabel 3. Aktivitas anti-elastase dari ekstrak tomat dan likopen

Final cocentration of SLE and Lycopene (µg.ml^{-1})	Mean of elastase inhibitor (%)	
	Tomato	Lycopene
66.67	80.08 ± 3.53^{e}	100.40 ± 0.85^{e}
33.33	63.19 ± 0.60^{d}	81.93 ± 0.61^{d}
16.67	50.80 ± 1.11^{c}	56.34 ± 0.77^{c}
8.83	43.67 ± 2.83^{b}	46.67 ± 0.61^{b}
4.17	37.73 ± 3.73^{ab}	41.84 ± 0.92^{a}
2.08	33.39 ± 0.96^{a}	40.02 ± 0.73^{a}

Table 4. The IC_{50} value of anti-elastase of tomato extract (SLE) and lycopene
Tabel 4. Nilai IC_{50} dari anti-elastase pada ekstrak tomat (SLE) dan likopen

Sample	Equation	R^2	IC_{50} (µg.ml^{-1})	Mean of SLE IC_{50} (µg.ml^{-1})
SLE (1st repetition)	y = 0.6854x + 36.421	0.96	19.81	19.73 ± 0.44
SLE (2nd repetition)	y = 0.6303x + 37.866	0.93	19.25	
SLE (3rd repetition)	y = 0.7733x + 34.438	0.98	20.12	
SLE (mean)	y = 0.6963x + 36.242	0.97	19.76	
Sample	**Equation**	**R^2**	**IC_{50} (µg.ml^{-1})**	**Mean of Lycopene IC_{50} (µg.ml^{-1})**
Lycopene (1st repetition)	y = 0.9645x + 39.935	0.95	10.44	10.39 ± 0.43
Lycopene (2nd repetition)	y = 0.9844x + 40.213	0.96	9.94	
Lycopene (3rd repetition)	y = 0.9764x + 39.457	0.96	10.80	
Lycopene (mean)	y = 0.9751x + 39.868	0.96	10.39	

The IC_{50} of the tomato extract found in the present study (19.73 ± 0.44 µg.ml^{-1}) was similar to that reported by Pientaweeratch *et al.* (2016) for *Manilkara zapota* extract (35.73 ± 0.6 µg.ml^{-1}) and Nema *et al.* (2013) for *Centella asiatica* extract (19.45 ± 0.25 µg.ml^{-1}). Based on the antioxidant and anti-elastase activity results, tomato extract possessed a linear correlation between antioxidant and anti-elastase activity. Djohan *et al*. (2019) stated that tomato extract effectively inhibited hyaluronidase activity to keep the skin moist and smooth.

Elastase was an enzyme on the skin surface, responding to dehydration and wrinkle formation (Syamsudin *et al*. 2017). Elastin significantly reduced aging, injury, and sun exposure (Dayan *et al.* 2014). During the aging process, elastin levels would decrease, and therefore, the skin would lose its strength and flexibility indicated by visible wrinkles (Widowati *et al*. 2016).

Elastin was an interstitial fiber in the skin hydrolyzed by an enzyme called elastase. Elastin decrease in the skin could affect the skin's integrity and elasticity (Pientaweeratch *et al.* 2016). The tomato extract showed an elastase inhibition activity that maintained skin elasticity, increased moisture, and reduced stress. The elastase inhibition activity also could improve texture, firmness, and elasticity of the skin hence preventing age spots and wrinkles (Sahu *et al.* 2013). It was also responsible in the dermal matrix for the degradation of elastin fibrous structure (Abdul Karim *et al.* 2014).

The IC_{50} was used to classify the ABTS-reducing activity and anti-elastase activity. The sample was classified into four categories of antioxidant activity : very strong (IC_{50} less than 50 µg.ml^{-1}), strong (IC_{50} 50 - 100 µg.ml^{-1}), moderate (IC_{50} 101 - 150 µg.ml^{-1}), and weak (IC_{50} greater than 150 µg.ml^{-1}) (Fidrianny *et al*. 2015).

Tomatoes can be used as a cosmetical product because it could trigger collagen metabolism and synthesis; they made skin redder and shinier (Bhowmik *et al.* 2012; Prasetya *et al.* 2015). The ability to inhibit skin fibroblast-derived elastase of tomato extract helped reduce the skin's wrinkle formation (Imokawa and Ishida 2015).

CONCLUSION

Ethanol extract of tomato had an intense antioxidant activity based on ABTS-reducing activity and a very strong antiaging action based on elastase inhibitor. The correlation of antioxidant activity and anti-elastase activity of tomato extract was linear. Tomato extract effectively kept skin health due to its lycopene content, which had very strong antiaging action. Therefore, further study is required to do fractionation to get a purer lycopene compound from tomato.

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CONTRIBUTIONS

AHH was the main contributor in designing, conducting research, and writing scripts. INEL was contributed to conducting research and writing, EF was contributed to conducting research and writing, and EG was contributed to writing the manuscript.

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