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 Henderson¹*, I Nyoman Ehrich Lister², Edy Fachrial³, dan Ermi Girsang⁴

Faculty of Medicine, University Prima Indonesia¹
Department of Physiology, Faculty of Medicine, University Prima Indonesia²
Laboratory of Biomolecular, Faculty of Medicine, University Prima Indonesia³
Department of Public Health, Faculty of Medicine, University Prima Indonesia⁴
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-ethylbenzothiazoline-6-sulphonic acid (ABTS)-reducing activity. The antiaging activity was measured through anti-elastase activity. Tomato extract (SLE) showed strong ABTS-reducing activity (IC₅₀ = 86.66 ± 10.58) and very strong anti-elastase activity (IC₅₀ = 19.73 ± 0.44). In conclusion, there was a linear correlation between antioxidant activity and antielastase activity. However, the antioxidant activity and anti-elastase activity of tomatoes were still below lycopene (IC₅₀ antioxidant = 49.23 ± 2.06 µg.ml⁻¹ and IC₅₀ 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.

Penauan pada kulit akibat kerusakan yang disebabkan oleh radiasi ultraviolet dan penggunaan kosmetik yang mengandung bahan beracun masih menjadi masalah saat ini. Tomat memiliki aktivitas antioksidan dan perlindungan kulit. Tujuan penelitian ini adalah untuk mengetahui potensi kantor tomat dalam menekan peroksidasi (antioksidan) dan antielastase. Ekstrak tomat diperoleh dari serbuk simpilis tomat yang diekstraksi menggunakan etanol 70% by the maceration method. Aktivitas antioksidan dan antielastase tomat masih di bawah likopen (IC₅₀ antioksidan = 49.23 ± 2.06 µg.ml⁻¹ and IC₅₀ anti-elastase = 10.39 ± 0.43 49.23 ± 2.06 µg.ml⁻¹). Namun, tomat dapat dikembangkan sebagai bahan alami antioksidan dan antielastase. Studi lanjutan diperlukan untuk melakukan fraksinasi untuk mendapatkan senyawa likopen dari tomat yang lebih murni.

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* Alamat Korespondensi : hendersonhendy96@gmail.com
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 hypoallergenic and non-toxic cosmetic products produced from the number of plant species that had skin care effect (Gonzalez-Miner 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, stibene, 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 Esulentum 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 (IC₅₀) was also calculated following the formula of (Widowati et al. 2017):

\[
\text{Inhibition}(\%) = \frac{C-S}{C} \times 100
\]

**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 IC₅₀ 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. Thus, tomato extract had lower antioxidant activity than lycopene.

This study showed a similar result reported by Djeromoune et al. (2019), where IC₅₀ of ABTS-reducing activity from fresh tomato var. Marmande was 6.21 mg.ml⁻¹. Moreover, Widowati et al. (2016) also reported IC₅₀ 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 IC50 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 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).

Table 1. The ABTS-reducing activity of ethanol extract of tomato and lycopene

<table>
<thead>
<tr>
<th>Final concentration of SLE and Lycopene (µg.ml−1)</th>
<th>Mean of ABTS-reducing activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tomato</td>
</tr>
<tr>
<td>50.00</td>
<td>27.46 ± 3.57a</td>
</tr>
<tr>
<td>25.00</td>
<td>12.78 ± 0.63c</td>
</tr>
<tr>
<td>12.50</td>
<td>4.75 ± 0.82b</td>
</tr>
<tr>
<td>6.25</td>
<td>2.68 ± 0.18ab</td>
</tr>
<tr>
<td>3.13</td>
<td>0.68 ± 0.01ab</td>
</tr>
<tr>
<td>1.56</td>
<td>0.12 ± 0.02ab</td>
</tr>
</tbody>
</table>

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 IC50 value of ABTS-reducing activity of tomato extract (SLE) and lycopene

<table>
<thead>
<tr>
<th>Sample</th>
<th>Equation</th>
<th>R²</th>
<th>IC50 (µg.ml−1)</th>
<th>Mean of SLE IC50 (µg.ml−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE (1st repetition)</td>
<td>y = 0.5209x - 0.9756</td>
<td>0.99</td>
<td>94.11</td>
<td></td>
</tr>
<tr>
<td>SLE (2nd repetition)</td>
<td>y = 0.6442x - 1.9739</td>
<td>0.98</td>
<td>74.55</td>
<td>86.66 ± 10.58</td>
</tr>
<tr>
<td>SLE (3rd repetition)</td>
<td>y = 0.539x - 0.7757</td>
<td>0.99</td>
<td>91.33</td>
<td></td>
</tr>
<tr>
<td>SLE (mean)</td>
<td>y = 0.568x - 1.2418</td>
<td>0.99</td>
<td>85.84</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Equation</th>
<th>R²</th>
<th>IC50 (µg.ml−1)</th>
<th>Mean of Lycopene IC50 (µg.ml−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene (1st repetition)</td>
<td>y = 1.0382x + 0.9735</td>
<td>0.99</td>
<td>47.22</td>
<td></td>
</tr>
<tr>
<td>Lycopene (2nd repetition)</td>
<td>y = 0.9753x + 2.074</td>
<td>0.95</td>
<td>49.14</td>
<td></td>
</tr>
<tr>
<td>Lycopene (3rd repetition)</td>
<td>y = 0.9323x + 2.1397</td>
<td>0.97</td>
<td>51.34</td>
<td>49.23 ± 2.06</td>
</tr>
<tr>
<td>Lycopene (mean)</td>
<td>y = 0.982x + 1.7291</td>
<td>0.98</td>
<td>49.16</td>
<td></td>
</tr>
</tbody>
</table>

SLE: Solanum lycopersicum L. extract
Elastin 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 IC50 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.

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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.

REFERENCES


