The antioxidant and anti-elastase activities of tamarillo fruit extract (*Solanum betaceum*)

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**ABSTRACT**

Skin aging is a complex process characterized by structural and functional loss of skin tissue. Aging is triggered by reactive oxidative stress (ROS), which activates enzymes involved in the aging process, such as elastase. Tamarillo is one of the natural plants that have various chemical compounds that have the potential to be antioxidants and anti-aging. The objective was to examine the antioxidant and antiaging activity of Tamarillo Fruit Extract (TFE). The H₂O₂ scavenging assay and anti-elastase assay were used. The result shows that TFE has a high antioxidant activity, as evaluated by H₂O₂ scavenging activity. The highest scavenging activity was found in a TFE concentration of 500 µg/mL (72.96%). Meanwhile, TFE shows its highest anti-elastase activity at a concentration of 66.67 µg/mL (32.33%). TFE's activity in both antioxidant and antiaging assays was concentration-dependent. The IC₅₀ of the H₂O₂ assay was 278.20 µg/mL, and the IC₅₀ of the anti-elastase assay was 110.67 µg/mL. Therefore, TFE shows its antioxidant and antiaging activities and has the potential to be used in further studies, both in vivo and clinical studies.

**Keywords:** Antioxidant, Antiaging, Elastase, ROS, Tamarillo

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INTRODUCTION

The skin is a complex biological system that comprises various crucial activities such as blood circulation, nerve supply, muscular support, immune function, ultraviolet radiation detection, and endocrine processes (Tobin, 2017). The overexpression and hyperactivity of tyrosinase, elastase, and collagenase enzymes characterize skin aging. These enzymes break down the skin dermal Extracellular Matrix (ECM) constituent, leading to clinical manifestations such as dry skin, over-pigmentation, wrinkles, and loss of elasticity (Orqueda et al., 2022).

In healthy skin, the ECM comprises proteins and carbohydrates, with the main component consisting of collagen, elastin, fibronectin, laminin, and glycoproteins (Lee et al., 2016). These components include a role in flexibility, elasticity, moisture, smooth skin texture, and youthfulness (Ramatelhe et al., 2018). Several types of acute and chronic responses in human skin are mainly caused by exposure to UV rays from the sun. The external form of skin aging is predominantly shaped by environmental factors, notably exposure to ultraviolet (UV) radiation, contributing to approximately 80% of this process (Farage et al., 2008). UV light, as a primary external factor, causes the formation of free radicals through a homolytic process, which then initiates DNA damage, lipid peroxidation, and inflammatory responses (Lan et al., 2019).

Reactive Oxygen Species (ROS) are the free radicals generated internally and externally associated with aging. ROS produced by mitochondria plays an integral part in the aging phase; mitochondrial impairment is recognized as contributing to aging (Maldonado et al., 2023). In addition, apart from mitochondria, other crucial cellular organelles, including peroxisome and endoplasmic reticulum, also generate ROS (Warraich et al., 2020). One of the free radicals generated by the human body metabolized by Superoxide Dismutase (SOD) when it is exposed to UV damage is hydrogen peroxide (H$_2$O$_2$) (Masaki, 2010). Nevertheless, nature has bestowed free radical scavengers known as antioxidants, which safeguard against the detrimental impacts of ROS. However, the efficacy of antioxidant defense decreases as individuals age, along with the enhanced formation of ROS, contributing to the progression of oxidative stress (OS) conditions (Maldonado et al., 2023).

Prolonged UV radiation exposure causes collagen and elastase denaturation and triggers the creation of Matrix Metalloproteinases (MMPs) (Laga & Murphy, 2009; Qun et al., 2009). MMPs play a crucial role in the restructuring of the ECM, including collagen, enzymes, and glycoproteins (Cancemi et al., 2020). Proteins within ECM, like elastin, have the potential as dependable markers of aging due to their slow turnover rate and capacity to accumulate damage gradually (Halper et al., 2014). Elastase enzymes are the protease family that degrades elastin, collagen, fibronectin, and other extracellular matrix proteins (Azmi et al., 2014).

Skin aging will affect self-confidence in an individual’s social life because the skin is the first visible part of an individual when interacting with other people (Ahmad et al., 2018). Antioxidants and antiaging compounds can solve this problem by preventing skin aging from ROS damage and degradation of the ECM. The use of natural ingredients derived from plants has become a significant concern. The advantage of natural ingredients is that they have high antioxidant and antiaging activities to slow the aging process and have low side effects (Naniek, 2022).

One natural plant with high medicinal properties is Tamarillo (Solanum betaceum). The fruit is often widely used as a functional ingredient in drinks, food, and dairy products or consumed directly (Gannasin et al., 2015). This plant has various applications in traditional medicine, such as serving as a natural antioxidant, treating fever, combating bacterial infections, relieving constipation, and aiding in avoiding heart disease and cancer (Rahmawati et al., 2021). Studies have reported that aqueous tamarillo extract has a high content of phenols, triterpenoids, and polysaccharides analyzed using FITR assay (Rito et al., 2023). The phytochemical composition of tamarillo fruit contains vitamin C, A (β-carotene), E (γ-tocopherol), phenolics hydroxycinnamic acid derivatives, anthocyanins, squalene, phytosterols, alkaloids (pyrophilidine, tropane, calystegins), organic acid (glucose, fructose, and sucrose) (Wang & Zhu, 2019). It is reported that the utilization of g-Tocopherol is beneficial in the inhibition of melanogenesis and the modulation of mRNA expression of tyrosinase and suppress the inducible nitric oxide synthase in B16 melanoma cells (Masaki, 2010; Kamei et al., 2009). Tamarillo has been extensively studied with biological activities such as anti-inflammatory, antioxidative, antiproliferative, antinoceptive, and anti-obese properties. Based on the phytochemical contained in tamarillo fruit, it has excellent potential for further use. Therefore, this study aims to evaluate tamarillo fruit extract's antioxidant and anti-elastase activities (TFE) through H$_2$O$_2$ scavenging and anti-elastase activities assays.

MATERIAL AND METHODS

Sample Extraction

Fresh Tamarillo fruit obtained from Berastagi fruit market, North Sumatra. The plant was determined in Laboratorium Bandungense, Institut Teknologi Bandung. The fruit is dried and then ground into simple powder. The Tamarillo fruit powder was extracted using a maceration, utilizing 70% ethanol as a solvent. The liquid is filtered and collected every day. Then, the 70% tamarillo liquid evaporates until a Tamarillo Fruit Extract (TFE) is obtained as a paste (Siregar et al., 2019; Mahadi et al., 2019).
Measurement of \( H_2O_2 \) Scavenging Activity

The samples of TFE were placed in a 96-well plate. Then, 12 \( \mu L \) of Ferrous Ammonium Sulfate 1 mM into the control healthy plate and the one containing the sample (sample well). Add 90 \( \mu L \) of sample solvent (DMSO) to the blank well. Add 63 \( \mu L \) of sample solvent (DMSO) to the control well. Add five mM \( H_2O_2 \) to the healthy plate containing the sample (well sample), and incubate the plate for 5 minutes in a dark place at room temperature. Add 1,10-phenanthroline one mM to the healthy plate containing the sample (well sample) and blank, incubate the plate for 10 minutes in a dark place at room temperature. Absorbance was measured using a microplate reader at \( \lambda = 510 \) nm (Girsang et al., 2020; Jusri et al., 2019; Dewi et al., 2020). The scavenging activity is determined using the equation:

\[
\text{% scavenging activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

Measurement of Anti-Elastase Activity

In 96 healthy plates, TFE was placed in 96 wells following the mapping. Then, the elastase enzyme solution was added. Tris HCL Buffer solution pH eight was added to the control well, sample, and blank. Incubate the plate for 15 minutes at 25\(^\circ\)C. Then, the SucAla3-pNa substrate solution was added to each blank sample and controlled well. The plate was incubated for 15 minutes at a temperature of 25\(^\circ\)C using an incubator. Absorbance was measured using a microplate reader at \( \lambda = 410 \) nm (Sujiatmo et al., 2020; Mawani et al., 2020). The inhibition activity is determined using the equation:

\[
\text{% inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

Statistical Analysis

The data were analyzed with SPSS Statistics software 25 version. The data interpretation was based on One-way ANOVA followed by Tukey Post Hoc (p<0.05), which was interpreted as statistical significance. The results were then shown as mean ± standard deviation (SD) (Dewi et al., 2020).

RESULTS

Sample Extraction

Based on the results of plant determination in Laboratorium Bandungense, Institut Teknologi Bandung, the samples used were Dutch eggplant (Indonesia), Tree tomato (England), and Menen eggplant (Indonesia) with the species name Solanum betaceum Cav. The extraction process took seven days. The calculation yield of TFE is shown in Tables 1 and 2.

Table 1. Wet and Dry Weight of Tamarillo Fruit

<table>
<thead>
<tr>
<th>No</th>
<th>Local Name</th>
<th>Scientific Name</th>
<th>Source of Sample</th>
<th>Wet (g)</th>
<th>Dry (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terong Belanda</td>
<td>Solanum betaceum</td>
<td>Berastagi fruit market, Berastagi, Karo Regency, North Sumatra</td>
<td>2000</td>
<td>250</td>
</tr>
</tbody>
</table>

Table 2. Yield of Tamarillo Fruit Extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>Simplicia (g)</th>
<th>Ethanol volume mL</th>
<th>Period of maceration (Day)</th>
<th>Filtrate Volume (mL)</th>
<th>Yield extract (g)</th>
<th>Yield extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamarillo Fruit</td>
<td>100</td>
<td>1200</td>
<td>7</td>
<td>996</td>
<td>22.38</td>
<td>22.38</td>
</tr>
</tbody>
</table>

The Effect of Tamarillo Fruit Extract towards \( H_2O_2 \) Scavenging Activity

The rate of \( H_2O_2 \) scavenging activity of TFE is provided in Figure 1. According to the findings, the \( H_2O_2 \) scavenging activity of TFE is increased significantly (P<0.05) along with the increasing sample concentration. It can be seen at the
highest concentration of the samples, concentrations of VI (500 µg/mL) showing the highest H$_2$O$_2$ free radical scavenging activity with a scavenging value of 72.96%, and the lowest scavenging activity was found in concentrations I (15.63 µg/mL) with value at 18.86%. The Inhibitory Concentration (IC$_{50}$) value of TFE toward H$_2$O$_2$ scavenging activity can be seen in Table 1.

![Graph showing H$_2$O$_2$ scavenging activity](image)

**Figure 1. The effect of various concentrations of TFE on H$_2$O$_2$ scavenging activity**

*The data is analyzed in three repetitions and presented as mean ± standard deviations. Different symbols present statistical differences among treatments (p<0.05). Number I-VI represents the final concentration of TFE. I = 15.63 µg/mL; II = 31.25 µg/mL; III = 62.5 µg/mL; IV = 125 µg/mL; V = 250 µg/mL; VI = 500 µg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Equation</th>
<th>$R^2$</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Average IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFE Rep 1</td>
<td>$Y = 0.1093x + 19.241$</td>
<td>0.99</td>
<td>281.42</td>
<td></td>
</tr>
<tr>
<td>TFE Rep 2</td>
<td>$Y = 0.179x + 20.384$</td>
<td>0.99</td>
<td>274.48</td>
<td></td>
</tr>
<tr>
<td>TFE Rep 3</td>
<td>$Y = 0.111x + 19.063$</td>
<td>0.99</td>
<td>278.71</td>
<td></td>
</tr>
<tr>
<td>TFE Average</td>
<td>$Y = 0.1094x + 19.563$</td>
<td>0.99</td>
<td>278.22</td>
<td></td>
</tr>
</tbody>
</table>

*Rep = Repetitions

**Table 1. The IC$_{50}$ value of TFE towards H$_2$O$_2$ scavenging activity**

The Effect of TFE on Anti-Elastase Activity

All samples with the final concentrations showed high anti-elastase activities in a concentration-dependent manner (Figure 2). The sample of TFE at a concentration of 66.67 µg/mL showed the highest anti-elastase capability; meanwhile, the sample of TFE at a concentration of 2.08 µg/mL had the lowest inhibitory activity (32.22% and 5.80%, respectively). The Inhibitory Concentration (IC$_{50}$) value of TFE toward anti-elastase activity can be seen in Table 2.

![Graph showing anti-elastase activity](image)

**Figure 1. The effect of various concentration of TFE on anti-elastase activity**

*The data is analyzed in three repetitions and presented as mean ± standard deviations. Different symbols present statistical differences (P<0.05). Number I-VI represents the final concentration of TFE. I = 2.08 µg/mL; II = 4.17 µg/mL; III = 8.33 µg/mL; IV = 16.67 µg/mL; V = 33.33 µg/mL; VI = 66.67 µg/mL.
The Inhibitory Concentration (IC₅₀) value of TFE toward anti-elastase activity can be seen in Table 2.

**DISCUSSIONS**

Skin aging is a complex process in the body characterized by structural and functional loss of skin tissue caused by many factors. The skin will be susceptible to damage, wrinkles, and sagging due to intrinsic (natural) and extrinsic (environmental) factors (Farage et al., 2008; Polisak et al., 2012). Ultraviolet (UV) radiation is one of the extrinsic factors that will induce ROS production through an inflammatory response, causing skin aging. Long-term exposure to UV can accelerate the premature aging process of the skin by up to 80% (Huertas et al., 2016; Zhang et al., 2022). Tamarillo is one of the natural plants that have high medicinal value. This plant possesses many biological activities, including antioxidants contributing to ROS development (Diep et al., 2020).

In our current study, TFE has antioxidant activity in a concentration-specific way, as shown in Figure 1 and IC₅₀ in Table 1. A previous study conducted by Diep et al., 2020 reported that Tamarillo fruit and peel have high antioxidant activities when evaluated using Cupric Ion-Reducing Antioxidant Capacity, Ferric Reducing Ability of Plasma (FRAP), and Folin–Ciocalteu tests. Another study reported that Tamarillo fruit extract showed a high antioxidant activity of up to 22.92%, scavenging activity of up to 47.38 μg/mL Ferric reducing activity of up to 12.17 μM Fe (II)/g (Atiqa et al., 2014). The antioxidant activity of a compound can be expressed in terms of the IC₅₀ value (Safirli et al., 2020). The IC₅₀ value exhibits an inverse relationship with the antioxidant capacity of a compound. A compound's antioxidant activity is more potent when its IC₅₀ value is smaller. A compound is categorized as a powerful antioxidant if the IC₅₀ < 50 μg/mL, strong 50-100 μg/mL, moderate 100-500 μg/mL, and weak > 500 μg/mL (Irwan, 2020; Widowati et al., 2017; Jusri et al., 2019).

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Based on the study shows that TFE has an average IC50 of 278.20 μg/mL. These results correspond with a previous study, which documented that Tamarillo has high antioxidant activities in a concentration-dependent manner compared with ascorbic acid with IC₅₀ 54 μg/mL (Ralte et al., 2021). The antioxidant capability of TFE can be linked to the presence of phytochemicals that serve as primary antioxidants through various pathways in the body. Superoxide formed in the body is converted into hydrogen peroxide (H₂O₂) (Masaki, 2010). This hydrogen is transformed into hydroxyl radicals (•OH). These hydroxyl radicals induce lipid peroxidation in cellular membranes, resulting in cellular damage. In order to prevent cell damage, the antioxidant termination reaction typically works by snagging hydroxyl radicals (•OH) during the peroxidation reaction stage of lipids, proteins, or other molecules in healthy cell membranes (Parwata, 2015).

We also examined the antiaging properties of TFE by using anti-elastase activity assays. Recent findings showed that TFE exhibits an anti-elastase activity with IC₅₀ 110.67 μg/mL, categorized as a moderate anti-elastase activity (Table 2). The anti-elastase inhibitory activity is shown in Figure 2. It is demonstrated that TFE could inhibit the elastase enzyme activities in the assays. This aligns with the previous study that reported that Tamarillo (S. betaceum) peel extract has elastase, tyrosinase, and collagenase inhibition activities. At the same time, S. betaceum seeds could inhibit hyaluronidase (Orqueda et al., 2022).

The phytochemical compounds present in Tamarillo, such as vitamin C and vitamin A, are high in fiber, minerals, and flavonoids and are believed to have biological activities, including antioxidant and anti-aging properties. The anti-elastase activity is correlated with the antioxidant activity of TFE, as evaluated in this research. Studies have demonstrated that aging is attributed to residual free radicals present in the body (Aversa et al., 2017). TFE's antioxidant compounds can scavenge free radicals and prevent cell damage in aging. During aging, the activity of the enzymes elastase, collagenase, hyaluronidase, and tyrosinase increases, resulting in the skin losing its strength and flexibility, which causes wrinkles to appear (Widowati et al., 2016; 2018). The activity of these enzymes can also affect skin moisture, hyperpigmentation of the skin, and the thickness of the epidermis (Favas et al., 2021).

However, further clinical and molecular research is required to identify the precise mechanisms underlying TFE's impact on antiaging processes. Plant extracts with more potent antioxidant and antiaging activity are essential for improving human life. This research suggests that TFE might be one solution to improving problems, especially in aging processes for human health. TFE can be used as a material for further study to be tested in vivo or in vitro.
CONCLUSION

TFE shows its antioxidant activity evaluated by H$_2$O$_2$ scavenging assay and antiaging properties evaluated by anti-elastase assay in a concentration-dependent manner. TFE has excellent potential to be used in further studies for human health.

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REFERENCES


