



**The Potential of Tamarillo (*Solanum betaceum*) Peel as an Antioxidant by Radical Scavenging DPPH and Antiaging by Collagenase Inhibition**

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**ABSTRACT**

**Background:** The skin aging process, which is impacted by various variables, including genetics, advancing age, environmental conditions, and lifestyle choices, has emerged as a significant concern in skincare. Signs of skin aging, such as decreased firmness and elasticity and the appearance of fine lines and discoloration, have raised significant concerns. The link between free radicals and skin aging has been the subject of in-depth research, while collagenase activity has also been found to influence skin aging. The natural antiaging potential of herbal plants has been widely studied to overcome this problem, one of which is tamarillo (*Solanum betaceum*). **Objectives:** This study aimed to examine the antioxidant and antiaging properties of *S. betaceum* Peel Extract (SBPE). **Method:** Tamarillo peel was extracted using the maceration method. The antioxidant test was analyzed by trapping 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the antiaging test was analyzed by collagenase inhibitory activity. **Results:** SBPE showed vigorous DPPH scavenging activity ( $IC_{50}$ :  $60.91 \pm 0.36$   $\mu\text{g/mL}$ ), while SBPE also showed moderate collagenase inhibitory activity ( $IC_{50}$ :  $121.47 \pm 2.32$   $\mu\text{g/mL}$ ). **Conclusion:** SBPE has high antioxidant activity and moderate antiaging activity.

**Keywords:** Antioxidant, Antiaging, Collagenase, DPPH, *Solanum betaceum*

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## INTRODUCTION

Skin aging is an inevitable biological phenomenon and occurs with increasing age. Several internal and external factors can accelerate this process. Internal factors include genetics and hormonal changes that occur with aging. Additionally, extrinsic influences encompass prolonged exposure to sunlight, smoking, pollution, and unhealthy habits like excessive alcohol intake. (Kang et al., 2021). These factors can cause skin damage that manifests as indicators of the aging process, such as wrinkles, decreased skin flexibility, and uneven pigmentation (Huang & Chien, 2020).

Skin aging is closely related to molecular mechanisms in various skin components, particularly the dermis. In this area, fibroblast cells facilitate the development of the extracellular matrix (ECM), collagen production, elastin synthesis, and tissue regeneration (Huang et al., 2022). Collagen, as the primary protein constituent of the ECM, consists of three polypeptide chains arranged in a triple helix structure that makes it very strong and stable, thus providing strength and elasticity to the skin (Reilly & Lozano, 2021). However, collagen production may decrease with age. Extrinsic factors can also increase the degradation of collagen in the skin. One mechanism associated with collagen degradation is the increase in collagenase enzyme activity. This enzyme is responsible for breaking down or cleaving the molecular bonds of collagen. Therefore, collagenase enzyme activity needs to be inhibited to prevent collagen damage in the skin.

Exposure to UV radiation and environmental pollution are major external factors influencing skin aging (Chen et al., 2021). This exposure will result in Reactive Oxygen Species (ROS), which can react with sulfhydryl (-SH) groups on proteins, disrupting protein structure and altering their activity. This can cause protein dysfunction and disrupt various biochemical pathways within cells, including skin aging (Zhang et al., 2016). ROS can also damage the structure of DNA and RNA, leading to mutations and damage to genes that contribute to aging and cancer risk (Robert & Wagner, 2020). To counteract ROS, the body has antioxidant mechanisms that can provide cellular protection against ROS damage. However, the availability of antioxidants in the body is limited, hence the need for exogenous antioxidants that can help detoxify ROS.

Various skincare products and cosmetic procedures have been developed to slow down or prevent skin aging. These include anti-aging lotions containing vitamin C, hyaluronic acid, and retinol. Cosmetic procedures such as chemical peels, laser treatments, and filler injections can also help reduce signs of skin aging. These procedures are considered expensive and may have potential side effects on the body. Many studies have explored the antioxidant and anti-aging activities delivered by safer natural herbal ingredients, including pineapple, mangosteen peel, black tea, turmeric, moringa leaves, and so on (Ajagun-Ogunleye et al., 2020; Widowati et al., 2020, 2022; Wargasetia et al., 2023).

Another natural herbal ingredient suspected to possess biological activity is the Tamarillo (*Solanum betaceum*). This fruit has high levels of antioxidant compounds, including polyphenols and flavonoids. (Viera et al., 2022). Research has shown that extracts from *S. betaceum* have more potent antioxidant activity compared to kiwi and grapes based on 2'-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), DPPH, and ferric reducing ability of plasma (FRAP assay) tests (Espin et al., 2016). In addition to antioxidants, *S. betaceum* has various benefits such as anticancer, antimicrobial, anti-inflammatory, and many more (Diep et al., 2022; Syarafina & Sudiono, 2023). In addition to its fruit, *S. betaceum* peel also contains several phenolic compounds identified by LC-MS, including chlorogenic acid, ellagic acid, caffeic acid, epicatechin, gallic acid, kaempferol, p-coumaric acid, rutin, and ferulic acid (Espin et al., 2016; Mutalib et al., 2016; Diep et al., 2020). Recently, *S. betaceum* Peel Extract (SBPE) has been extensively studied for its antioxidant and anti-inflammatory potential (Li & Li, 2021; Syarafina, A., & Sudiono, J., 2023; Silitonga et al., 2024;), but there has been no research on its potential as an anti-aging agent. This study evaluates the potential of SBPE as an antioxidant agent through DPPH scavenging activity and anti-aging through the inhibition of collagenase enzyme activity. The findings of this study have the potential to offer new insights into the use of herbal ingredients in the development of more effective and natural skin care products to combat signs of skin aging.

## MATERIAL AND METHODS

### *Preparation of S. betaceum Peel Extract*

Tamarillo fruits (*S. betaceum* Cav.) were collected from Berastagi, Karo, North Sumatra, Indonesia. The plants were identified by the herbarium staff, Department of Biology, School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia. 100 g of dehydrated Tamarillo fruit peels were extracted with 1200 mL of 70% ethanol using the maceration procedure. The ethanol filtrate was sieved, and the residue was exposed to two more cycles of maceration. The macerates were concentrated and evaporated using a rotavapor at 50°C to obtain a paste form. The yield of *S. betaceum* peel extract (SBPE) was 5.45 g, stored at -20°C (Girsang et al., 2019; Priyandoko et al., 2022).

### *DPPH Scavenging Activity*

The DPPH scavenging activity method was performed following the prescribed protocol established by Vrianty et al. (2019) and Widowati et al. (2022b), with specific changes. 200 µL of a solution containing 0.077 mmol of DPPH (Sigma Aldrich D9132) in methanol was combined with 50 µL of an SBPE sample (6.25-200 µg/mL) in a 96-well plate. The solution was kept at room temperature for 30 min, and then the amount of light absorbed was determined using a

microplate reader (517 nm). The negative control was prepared by combining 50 µL of DMSO with 200 µL of DPPH. On the other hand, the blank was made by mixing 50 µL of the sample with 200 µL of 10% DMSO. The percentage of DPPH antioxidant activity was determined using the following formula:

$$\% \text{ Inhibition} = \frac{\text{absorbance negative control} - \text{absorbance sample}}{\text{absorbance negative control}} \times 100$$

### Collagenase Inhibition Activity

The collagenase inhibition method followed the protocol of Widodo et al. (2019) and Sutjiatmo et al. (2020) with modifications. The solution mixture contained 30 µL of sample (with a concentration range of 0.78 – 250 µg/mL), 10 µL of Collagenase enzyme (Sigma C8051), and 60 µL of tricine buffer. The mixture was then incubated at 37°C for 20 min. In addition, a control was prepared consisting of 10 µL of enzyme and 90 µL of phosphate buffer. A blank consisted of 10 µL of enzyme, 80 µL of phosphate buffer, and 30 µL of sample. Afterward, FALGPA substrate (20 µL) (Sigma F5135) was added to the mixture, excluding the blank. The measurement of absorbance was conducted at 335 nm. The inhibitory activity percentage is determined using the following formula:

$$\% \text{ Inhibition} = \frac{\text{absorbance negative control} - \text{absorbance sample}}{\text{absorbance negative control}} \times 100$$

## RESULTS

### DPPH Radical Scavenging Activity

The DPPH scavenging activity can be employed as an assessment tool to determine the antioxidant activity of a test sample. This experiment utilizes the reduction process of the DPPH solution in an alcohol solvent by the action of hydrogenation antioxidants. This reaction leads to the creation of a non-radical molecule known as DPPH. The research findings indicate that SBPE exhibits antioxidant activity by scavenging DPPH, which is enhanced as the concentration of SBPE increases (Figure 1). The concentration that yields the best results is 200 µg/mL, which can effectively scavenge 93.3% of DPPH. The IC<sub>50</sub> value of SBPE, determined using the DPPH scavenging assay, is 60.91 ± 0.36 µg/mL (Table 1).

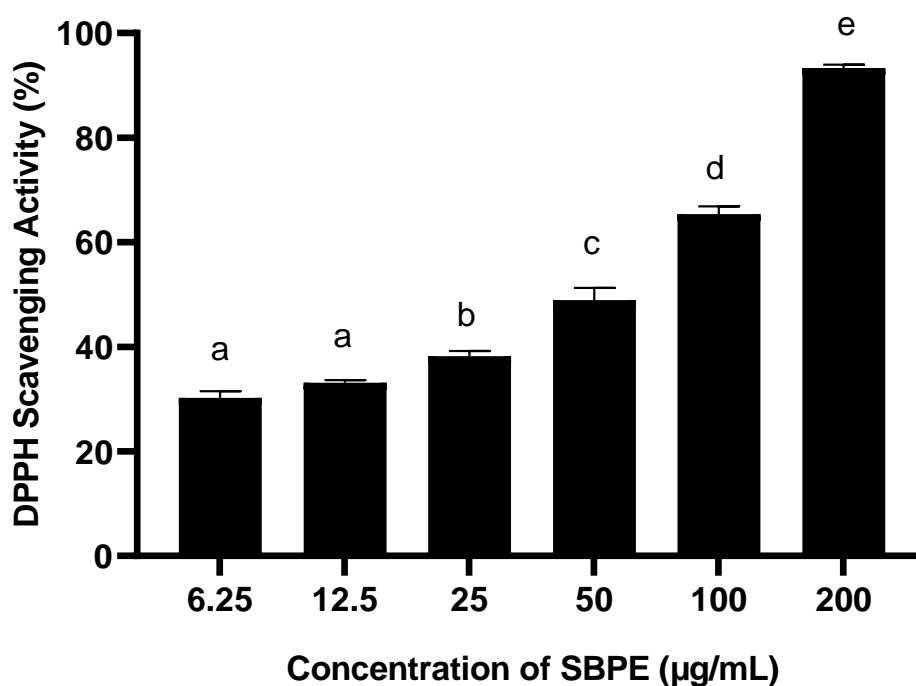


Figure 1. Effect of various concentrations of SBPE on DPPH scavenging activity

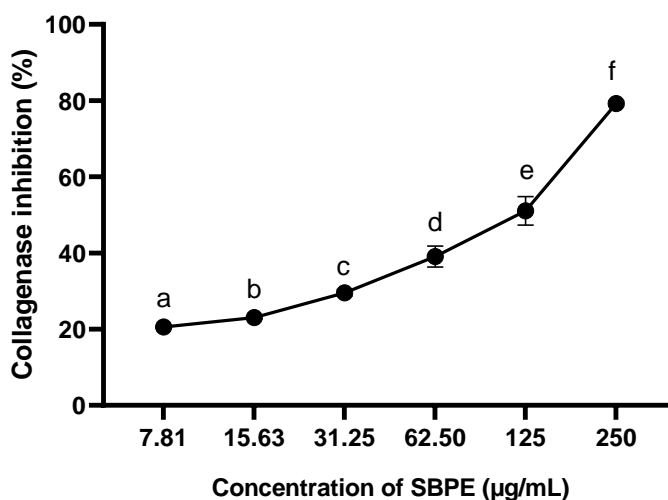
\*The data presented are mean values ± standard deviation from 3 replications. The graph displays several letters (a, a, b, c, d, e) to represent statistical significance at a level of P < 0.05, as determined by the ANOVA and Tukey HSD tests.

**Table 1. IC<sub>50</sub> DPPH scavenging activity of SBPE**

Sample	Equation	R <sub>2</sub>	IC <sub>50</sub> (µg/mL)	IC <sub>50</sub> (µg/mL)
SBPE (replication 1)	y = 0.3303x + 29.784	0.99	61.20	
SBPE (replication 2)	y = 0.3218x + 30.365	0.99	61.02	
SBPE (replication 3)	y = 0.3085x + 19.039	0.99	60.51	60.91 ± 0.36
SBPE (average)	y = 0.3243x + 30.245	0.99	60.92	

**Collagenase Inhibition Activity**

Collagenase is an enzyme that breaks down collagen, the main protein in connective tissue. Inhibiting collagenase is necessary to prevent collagen damage, which causes loss of skin elasticity and slows down the aging process. Research results indicate that SBPE exhibits collagenase inhibition activity that increases with concentration. The highest concentration (250 µg/mL) can result in 79.22% inhibition of collagenase (Figure 2). The IC<sub>50</sub> value for collagenase inhibition by SBPE is 121.47 ± 2.32 (µg/mL).



**Figure 2. Effect of various concentrations of SBPE towards collagenase inhibition**

\*The data presented are mean values ± standard deviation from three replications. The graph displays several letters (a, b, c, d, e, f) to represent statistical significance at a level of P < 0.05, as determined by the ANOVA and Independent T-tests.

**Table 2. IC<sub>50</sub> collagenase inhibition of SBPE**

Sample	Equation	R <sub>2</sub>	IC <sub>50</sub> (µg/mL)	IC <sub>50</sub> (µg/mL)
SBPE (replication 1)	y = 0.2368x + 20.603	0.99	124.14	
SBPE (replication 2)	y = 0.2415x + 20.989	0.99	120.13	
SBPE (replication 3)	y = 0.2332x + 21.293	0.99	120.13	121.47 ± 2.32
SBPE (average)	y = 0.2372x + 20.961	0.99	123.10	

**DISCUSSION**

Skin aging is a process of declining skin function due to various factors such as genetics, aging, environment, and lifestyle. The relationship between ROS and skin aging has been the focus of in-depth research, while collagenase activity also plays a role in this process (Gu et al., 2020; Son et al., 2021). Herbal plants, such as Tamarillo (*S. betaceum*), have become exciting research subjects as potential natural anti-aging agents to address these skin issues. Tamarillo is known to contain flavonoid and polyphenol compounds that are antioxidant-active. Some phenolic compounds found in this fruit are Caffeic acid, Caffeoylquinic acid, Caffeoyl glucoside, Dicaffeoylquinic acid, Dehydrodiferulic acid, Ferulic acid, Feruloyl glucoside, Gallic acid, Kaempferol, Rosmarinic acid, and p-coumaric acid, (Espin et al., 2016; Garcia et al., 2016; Diep et al., 2020).

The involvement of antioxidants in combating free radicals has been proven to play a crucial role in slowing down skin aging. By reducing oxidative damage, antioxidants can help maintain healthy skin and delay signs of aging. Research results show that Tamarillo extract has antioxidant activity based on DPPH scavenging, which increases with its

concentration (**Figure 1**). The IC<sub>50</sub> result (60.91 ± 0.36 µg/mL) (**Table 1**) indicates that SBPE is an antioxidant with a strong category because IC<sub>50</sub> < 100 µg/mL (Sukweenadhi et al., 2020). This is consistent with further research showing that SBPE has antioxidant activity through FRAP, ABTS, and ORAC assays (Espin et al., 2016). The compound content influences the antioxidant ability in Tamarillo. Based on LCMS, Tamarillo contains catechin, epicatechin, ellagic acid, kaempferol-3-rutinoside, isorhamnetin-3-rutinoside, and rutin (Diep et al., 2020). Several studies have revealed the antioxidant activity of these compounds. For example, kaempferol is known to prevent the formation of nitrate oxide and ROS in LPS-induced RAW 264.7 macrophage cells (Wang et al., 2018).

Collagenase activity significantly affects the presence of collagen fibers in the dermis. This enzyme activity must be inhibited to prevent collagen degradation in the dermis. Research results show that SBPE has anti-aging activity through collagenase inhibition, which increases with its concentration (**Figure 2**). The IC<sub>50</sub> result (121.47 ± 2.32 µg/mL) indicates that SBPE has moderate anti-aging properties. This could be influenced by the bioactive compounds in SBPE, including gallic acid and ferulic acid. Other studies have shown that gallic acid coated with gold nanoparticles has a superior ability to inhibit the degradation of type I collagen mediated by high glucose in dermal fibroblast cells (Wu et al., 2021). Another study illustrated that the collagenase inhibition activity of ferulic acid is 52.85 µg/mL, which falls into the strong category (Girsang et al., 2020). With collagenase inhibition activity and DPPH scavenging, SBPE shows promising potential as a good antioxidant and anti-aging agent. Further in vitro and in vivo research on its antioxidant and anti-aging potential is necessary before it can be used as a component in anti-aging skincare products.

## CONCLUSION

SBPE exhibits high antioxidant activity, particularly in DPPH scavenging. Additionally, based on collagenase inhibition tests, it demonstrates moderate anti-aging activity.

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