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Antioxidant and Antiaging Potential of Salak Fruit Extract (*Salacca zalacca* (Gaert.)Voss))

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ABSTRACT

Natural skin aging is indicated by a loss of skin structure and integrity caused by external factors including UV exposure. This exposure causes oxidative stress on skin cells, initiates aging, and degradation of extracellular matrix (ECM) structure which is composed of many proteins, including collagen and elastin. ECM degradation is caused by the increased activity of proteolytic enzymes, one of which is elastase (closely related to wrinkling). To inhibit oxidative stress during the photoaging process, the skin needs antioxidant compounds. Salak *(Salacca zalacca* (Gaert.)Voss) is a fruit that is rich in antioxidants because it contains flavonoid, phenolic, and polyphenolic compounds, including chlorogenic acid. The purpose of this study is to demonstrate the antioxidant activity and antiaging properties of salak fruit extract (SFE) as a candidate for active ingredients in the prevention of aging. To determine the antioxidant activity of SFE, 2,2-diphenyl-1-picrylhydrazil (DPPH) scavenging and ferric reducing antioxidant power (FRAP) assays were performed, while elastase assays were performed to determine the anti-aging properties of SFE. The IC_{50} values for DPPH scavenging and FRAP activity of SFE were 107.52 µg/mL and 16.82 µg/mL with the highest activity at 68.79% and 97.96%, at concentrations of 200 and 50 µg/mL, respectively. Meanwhile, the anti-aging properties determined through the elastase assay showed an IC_{50} value of 19.71 µg/mL with the highest inhibition 72.50% at a concentration of 66.67 µg/mL. SFE has the potential as an active ingredient in preventing aging through its antioxidant activity and anti-aging properties.
Keywords: DPPH; elastase; FRAP; *Salacca zalaca*; skin aging

INTRODUCTION

Sunlight contains a large amount of ultraviolet (UV) which creates a homeostatic effect in the body, but if exposed to excess, it will cause photoaging and photocarcinogenesis (Lan, 2019). Acceleration of skin aging can occur through several mechanisms, including mtDNA mutations; telomere shortening; hormonal changes; and increased Reactive Oxygen Species (ROS). ROS can be generated endogenously in cells from various metabolic processes and can also occur due to environmental factors (Panich et al., 2016). Excessive ROS production in the body can cause an imbalance and have an impact on increasing oxidative stress, cell damage (including deoxyribonucleic acid (DNA) fragmentation and lipid peroxidation), and apoptosis to cell death (de Jager et al., 2017). The combination of intrinsic and extrinsic factors changes the dermal matrix which contains extracellular matrix (ECM) proteins (elastin, proteoglycans, and collagen which are responsible for providing skin strength and resilience) (Panich et al., 2016) by inducing degradation of the ECM that causing clinical signs such as the presence of wrinkles, irregular pigmentation, fine lines, rough and dry skin, and loss of skin elasticity (Imokawa & Ishida, 2015).

ECM degradation is triggered by an increase in the proteolytic enzymes activity such as elastase and collagenase. The increase in elastase activity involved the physiological elastin degradation in several diseases closely related to wrinkle formation (Younis et al., 2022). In the photoaging process, Matrix Metalloproteinases (MMPs) break down proteins including elastin and collagen in inflamed and aging skin (Deniz et al., 2020). The skin aging process can be inhibited by anti-aging through increasing cell regeneration, induction of autophagy, changes in gene activity, and calorie restriction. Anti-aging treatment maintains a healthy life and longer normal cell function (Gems, 2014). Nutrients such as vitamins; essential amino acids; unsaturated fatty acids and various plant metabolites such as polyphenols; tannins; flavonoids; and quercetin can prevent aging (Bjørklund et al., 2022; Garg, 2017). Antioxidant properties have a role in counteracting the negative effects of free radicals and supporting the maintenance of healthy skin (Rajagopalan et al., 2019).

The application of herbal products as anti-aging treatments for the cosmetics industry is essential for reversing the negative impacts of

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aging caused by excessive exposure to UV radiation. Ingredients in herbal cosmetics possess biological activity and provide nourishment to the skin by enhancing antioxidant enzyme activity that scavenges free radicals. The usage of antioxidants, retinoids, and sunscreen, prevents the UV signaling pathway, elastases, hyaluronidase, and MMP. Plants contain bioactive compounds such as tannins, flavonoids, quercetin, and polyphenols that have the activity to mitigate aging skin and reduce wrinkles, premature aging, loss of pigment, and redness (Garg et al., 2017). Many studies have reported that sources of antioxidant compounds are found in various kinds of plants (Altemimi et al., 2017), one of which is the salak fruit *(Salacca zalacca (Gaert.)* Voss). Salak fruit is a plant species from the palm tree family (Arecaceae) originating in Indonesia (Girsang et al., 2019). Salak fruit is known to contain polyphenol/phenolic compounds. The phenolic compound with the highest concentration is chlorogenic acid, which is also found in high concentrations in the skin of the salak fruit, salak fruits. Salak fruit contains epicatechin, neochlorogenic acid, syringic acid, caffeic acid, ferulic and gallic acid, apigenin or 4,5,7-trihydroxyflavone and isoquercetin, which function as antioxidants (Anitha et al., 2020; Nardini and Garaguso, 2020; Badhani et al., 2015). Salak fruit contains naringenin (Čepková et al., 2021). Therefore, this study evaluates the salak fruit extract (SFE) as an antiaging agent through elastase inhibition and antioxidant activity namely DPPH scavenging, and FRAP activity.

METHODOLOGY

Materials

SFE was produced by the Traditional Medicine Industry (IOT), PT. Fathonah Amanah Shiddiq Tabligh (FAST), Depok, West Java, and was processed according to GMP standards (CoA No. Batch 001.11.23. ERTL.01). Reagents used: 2,2-diphenyl-1-picrylhydrazil (DPPH; Sigma Aldrich, D9132), ferric chloride hexahydrate (Merck, 1.03943.0250), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ; Sigma Aldrich, T1253), Porcine Cell Culture Tested Elastase enzyme (Himedia, TC311), Tris Buffer (Bio-Rad, 1610716), and N-Succinyl-Ala-Ala-Ala-p-nitroanilide, elastase substrate (Sigma Aldrich, 54760), Ethanol (technical).

Methods

Preparation of Salak Fruit Extract (SFE)

The salak fruit extraction process uses the maceration method. The salak fruit was cleaned, dried, and finely ground into powder simplicia. This powder was soaked in 70% ethanol. Filtered and evaporated at 40°C to form a paste then added lactose. The SFE was processed by PT FAST (Depok, Indonesia) following Good Manufacturing Practices (GMP) and CoA No. Batch 001.11.23. ERTL.01. The SFE was dissolved in 100% DMSO as a stock solution for use in further testing (Ginting et al., 2020).

DPPH Scavenging Activity Assay

An amount of 50 µL of SFE in various concentrations (6.25 - 200 µg/mL) was added into a 96-well microplate, then continued by adding 200 µL of DPPH 0.077 mmol/L solution. After that, the mixture was incubated for 30 minutes at 25°C (in a dark room). Then, a microplate reader was used to measure the absorbance at 517 nm (MultiskanTM GO Microplate Spectrophotometer, Thermo Scientific 1510-00778C) (Ginting et al., 2020). The radical scavenging activity was calculated according to formula (1). The regression equation obtained from the percentage of DPPH scavenging activity is used to calculate the IC_{50} value according to equation (2).

$$\% \text{ scavenging activity} = \frac{Ac-As}{Ac} \times 100\ \% \quad (1)$$

As: absorbance of the sample; Ac: absorbance of control without the sample

$$IC_{50} = \frac{50-b}{a} \quad (2)$$

a: constant value; b: regression coefficient value

FRAP Inhibition Activity

Amount of 10 mL of acetate buffer 300 mM (pH 3.6), 1 mL of ferric chloride hexahydrate 20 mM, and 1 mL of 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) 10 mM was added as FRAP reagent. For the assay, 7.5 µL samples with various concentrations (1.56 - 50 µg/mL) were pipetted into 96-well plates and 142.5 µL FRAP reagent was added. Incubation was carried out for 36 minutes at 37°C and then measured at a wavelength of 760 nm (Rusmana et al., 2017).

Elastase Inhibition Activity Assay

An adapted methodology was used to quantify the inhibitory activity of elastase (Thring et al., 2009; Utami et al., 2018; Widowati et al., 2017). Elastase from porcine pancreas (0.5 mU/ml in cold aquadest) and 135 µl Tris buffer (100 mM, pH 8) were pre-incubated with 10 µl samples (2,08 – 66,67 µg/ml) for 15 mins at 25°C. When the mixture had been preincubated, 10 µl of the N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate (2 mg/ml in Tris buffer) was added, and it was then incubated at 25°C for 15 min. At a wavelength of 410 nm, absorbance was measured using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The percentage of inhibition can be measured through

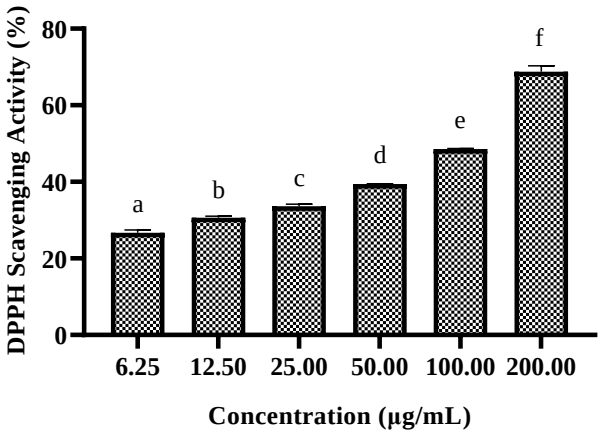

Figure 1. The effect SFE on DPPH free radical scavenging activity.

*The data was presented as mean and standard deviation. A different notation (a, b c, d, e and f) represent a significant difference between various concentration of SFE toward DPPH scavenging activity based on the Tukey ($p<0.05$).

Table I. The IC_{50} of SFE Toward DPPH Scavenging and FRAP Inhibitory Activity

Assays	Equation	R^2	IC_{50} value (µg/mL)
DPPH	y = 0.2081x + 27.635	0.99	107.52 ± 2.61
FRAP	y = 1.4652x + 25.361	0.99	16.82 ± 0.59
Anti-elastase	y = 0.5937x + 38.461	0.99	19.71 ± 3.01

*SFE= Salak Fruit Extract, IC_{50}= Median inhibitory concentration. The data are presented in mean±standard deviation. The assay was done in triplicate. The coefficient of regression (R^2) and the IC_{50} of the sample was calculated by linear regression. IC_{50} of SFE was presented µg/mL

the equation:

$$\% \text{ inhibitory activity} = \frac{Ac-As}{Ac} \times 100\ \% \quad (3)$$

As: absorbance of the sample; Ac: absorbance of control without the sample

Statistical Analysis

The data were examined by applying the SPSS software Version 22.0, and the results were displayed as a histogram (mean ± SD) by applying the GraphPad Prism Version 8.0.1 program.

RESULT AND DISCUSSION

In this study, the DPPH and FRAP assay was carried out to determine the antioxidant activity. The DPPH assay is used to measure the antioxidant ability of bioactive compounds (Yeo & Shahidi, 2019). At room temperature and in alcoholic solvents, such as methanol, DPPH is stable and has a purple color (Widowati et al., 2016; GS et al., 2018). The stability of this radical is due to the resonance of the nitrogen oxide groups in the picryl portion and the electronic balance of the nitrogen atom in the hydrazine group in the molecular structure (Yeo & Shahidi, 2019). In the presence of antioxidants contained in the extract, DPPH is rapidly reduced to 1,1-diphenyl-2-picrylhydrazine and thus loses its color (yellow discoloration). The color change that occurs will be inversely proportional to the concentration of antioxidants in the sample (Gupta et al., 2021). The reaction mechanism that occurs in the DPPH test is a combination of single electron transfer (SET) and hydrogen atom abstraction (HAT) mechanisms, but other studies state that the DPPH test is closer to the HAT mechanism (Rumpf et al., 2023). Similar to the DPPH assay, the FRAP assay is based on the principle of reducing the iron-TPTZ (Fe^{3+}-TPTZ) complex to iron-TPTZ (Fe^{2+}-TPTZ) by antioxidants in samples at low pH. Conditions at low pH are carried out to maintain iron solubility and change the dominant reaction mechanism by increasing the redox potential and decreasing the ionization potential which causes electron transfer. The use of tripyridyltriazine (TPTZ) in FRAP functions as a ligand liaison with iron ions (Munteanu & Apetrei, 2021). The final substance (Fe^{2+}-TPTZ) is colored blue with maximum absorption and changes in absorbance related to antioxidant capacity (Rubio et al., 2016). Even though DPPH and FRAP look

similar, the reaction mechanism in the FRAP assay is non-radical SET-based which has a low relationship with the HAT mechanism. In addition, testing with the SET reaction mechanism has limitations because it only reflects the reduction capacity, so it is less able to identify potential antioxidants that work through the HAT mechanism such as most phenolic compounds (Liang and Kitts, 2014). Therefore, in this study, the FRAP assay was carried out simultaneously with the DPPH test.

In this study, the DPPH scavenging activity of SFE with different concentrations was displayed in Figure 1. The results show a concentration dependence. The highest DPPH scavenging activity was found in the 200 µg/mL concentration with significant differences with the other concentration ($p<0.05$). The strongest DPPH scavenging activity at 200 µg/mL concentration (68.79 %). The IC_{50} value was 107.52 µg/mL with the linear regression y = 0.2081x + 27.635 (Table I). These findings indicate that SFE can scavenge the free radical in the DPPH assay. When compared with the most dominant phenolic compound in salak fruit (chlorogenic acid), the IC_{50} value on the standard is stronger than that of SFE. The IC_{50} value of chlorogenic acid has been reported in other studies, which is 2.56 µg/mL which indicates a very strong antioxidant activity value (Widowati et al., 2021) The DPPH scavenging activity of SFE extract is also reported by Saleh et al. (2018) with IC_{50} value of 49.5 µg/mL (Saleh et al., 2018). Another study demonstrates that the ethanol extract of the SFE showed antioxidant activity through a DPPH scavenging assay (79.48 µg/mL) (Tan et al., 2020). Using ethanol solvents in the extraction process increases the ability of SFE to reduce DPPH free radicals. In the FRAP assay, the results demonstrated that the sample's concentration was closely associated with its inhibiting activity, in other words, the inhibitory activity increased along with the sample concentration used significantly ($p<0.05$) (Figure 2). The strongest FRAP inhibition activity is found in 50 µg/mL with 97.96 % and the lowest FRAP inhibition activity was found in the SFE 1.56 µg/mL with 22.82%. The Inhibitory Concentration (IC_{50}) value of SFE toward FRAP inhibition activity can be seen in Table I (16.82 µg/mL). According to the findings, the SFE expressed high FRAP inhibitory which indicates great antioxidant capability. The FRAP inhibitory activity of SFE extract is also reported by Saleh et al. (2018) with an IC_{50} value of 144 µg AAE/g in higher concentrations (Saleh et al., 2018). Another study demonstrates that the ethanol extract of the SFE showed antioxidant activity through FRAP assay with an inhibitory activity value of 79.48 ± 2.41 µmol /g Fe^{2+} (Tan et al., 2020). The solvent used during extraction can affect the ability of an extract to inhibit free radicals, this is influenced by the total metabolites compounds such as phenols and flavonoids extracted. This was stated in the research by Tan et al. (2020) that the ethanol solvent used for extraction has a higher inhibitory ability than distilled water (Tan et al., 2020). This was also reported by Siregar et al. (2022) which stated that the DPPH scavenging activity in ethanol solvent is higher than in methanol (Siregar et al., 2022). Another study also stated that the content of phenol and flavonoid compounds was higher in samples extracted with ethanol compared to methanol, water, and acetone (Do et al., 2013).

Furthermore, elastase inhibition was performed to determine elastase inhibitory activity. In the current study, the elastase inhibitory effect of SFE at the final concentration (2.08 - 66.67 µg/mL) was determined and explained as shown in Table I. It was noted that the highest elastase inhibitory effect belonged to the extract with the highest concentration, significantly ($p<0.05$, Figure 3) (77.73 %). Furthermore, the IC_{50} value of SFE was 19.71 µg/mL with the linear regression y = 0.5937x + 38.461 (Table I). These results indicate that SFE has the potential to exert a good effect on anti-elastase activity. Based on these results, SFE showed high activity to inhibit elastase. Several studies showed that the phenolic and flavonoid content of SFE possesses anti-elastase activity (Girsang et al., 2019, 2021). The high activity of SFE in the present study might be due to the high content of the phytochemical compound of the extract. Characterization of secondary metabolite content in salak fruit extract will be reported in the future. The DPPH, FRAP, and elastase assays have shown the potential of SFE to inhibit aging through its antioxidant activity. SFE as an antioxidant can inhibit the antioxidant by protecting tissues from oxidative damage. The phytochemicals present in SFE act as anti-aging properties. The phytochemicals present in SFE act as anti-aging properties. Due to their chemical structure, polyphenol compounds have strong antioxidant activity and can scavenge various kinds of ROS such as hydroxyl radicals and superoxide radicals. In addition, polyphenols can inhibit proteolytic enzyme activity in vitro by acting as complexing or precipitating agents. However, the mechanism of action of polyphenols is not fully understood, and studies on the inhibitory effect of natural active ingredients on skin-degrading enzymes are scarce (Wittenaauer et al., 20145). As a candidate for active ingredients in a mixture of cosmetic products, plant extracts with higher antioxidant

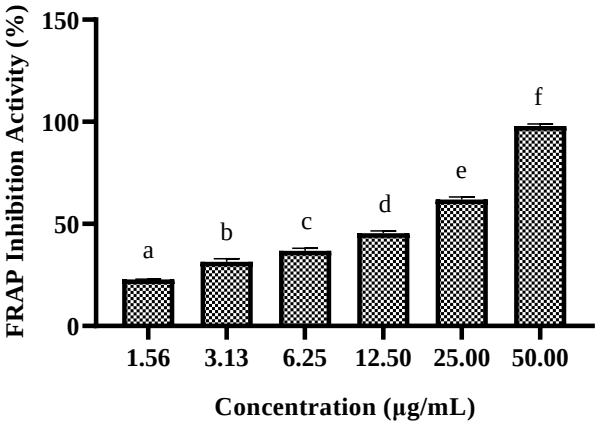

Figure 2. The effect SFE on FRAP Inhibitory Activity.

*The data was presented as mean and standard deviation. A different notation (a, b c, d, e and f) represent a significant difference between various concentration of SFE toward FRAP inhibitory activity based on the Kruskal-Wallis and Mann-Whitney U test ($p<0.05$).

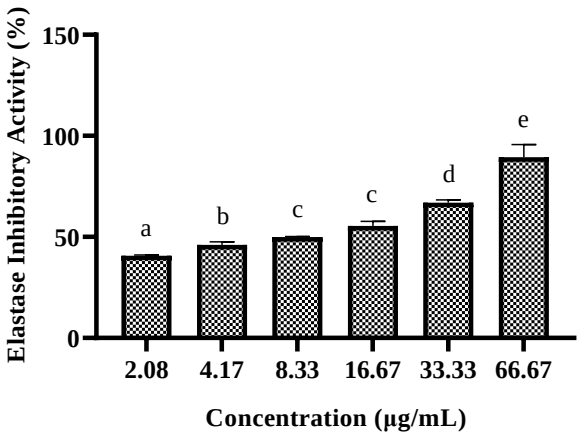

Figure 3. The effect of SFE on Elastase Inhibitory Activity.

*The data was presented as mean and standard deviation. A different notation (a, b c, d and e) represent a significant difference between various concentration of SFE toward elastase inhibitory activity based on the Kruskal-Wallis and Mann-Whitney U test ($p<0.05$).

activity in the healing process are important to use. As a pre-study, this research provides information regarding the potential of salak fruit extract as an inhibitor of aging. Further studies are needed to optimize the salak fruit extract formula with other ingredients as a product.

CONCLUSION

In summary, through the DPPH and FRAP assay, it was shown that Salak Fruit Extract has antioxidant activity and has the potential to inhibit aging through elastase inhibitory activity, thus it can be used as a basis for information that SFE is a potential candidate for active ingredients in a mixture of cosmetic products.

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