

## In Vitro Inhibition Activity of Elastase and Tyrosinase Enzymes of Parijoto Fruit (*Medinilla speciosa*) Extract and Fractions

Wulandari<sup>1</sup>, Endang Diah Ikasari<sup>2</sup>, Ida Suskawati<sup>3</sup>, Endang Dwi Wulansari<sup>1\*</sup>

<sup>1</sup>Department of Pharmaceutical Biology, College of Pharmaceutical Science (Stifar) Yayasan Pharmasi Semarang, Central Java, Indonesia

<sup>2</sup>Department of Pharmaceutical Technology, College of Pharmaceutical Science (Stifar) Yayasan Pharmasi Semarang, Central Java, Indonesia

<sup>3</sup>Department of Pharmaceutical Chemistry, College of Pharmaceutical Science (Stifar) Yayasan Pharmasi Semarang, Central Java, Indonesia

\*Corresponding Author: [dewi.ung@gmail.com](mailto:dewi.ung@gmail.com)

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#### Corresponding author e-mail

[dewi.ung@gmail.com](mailto:dewi.ung@gmail.com)

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### ABSTRACT

**Background :** Ultraviolet rays and pollution cause premature aging of the skin, darkening, dryness, and decreased elasticity. Parijoto fruit (*Medinilla speciosa*) offers potential for development as an anti-aging agent. This study aims to determine the inhibitory activity of elastase and tyrosinase enzymes from extracts and fractions of parijoto fruit in vitro, and to obtain the active fraction. Extraction was carried out by maceration using 96% ethanol solvent.

**Methods :** Four fractions were obtained through liquid-liquid partition using n-hexane, ethyl acetate, butanol, and water solvents. Elastase and tyrosinase enzyme inhibition tests were carried out in vitro using an ELISA (multi-mode reader). The reference compound in the elastase enzyme inhibition test was gallic acid, and kojic acid was used to inhibit the tyrosinase enzyme. **Results:** The elastase enzyme inhibitory activity of the extract and four fractions and gallic acid with a concentration of 133.33 µg/mL were 13.28; 21.56; 14.88; 15.12; 11.24; and 21.83%. The tyrosinase inhibitory activity of parijoto fruit extract and four fractions and kojic acid with a concentration of 125 µg/mL were 17.01; 19.2; 23.96; 14.76; 18.20; 34.71%, respectively. Parijoto fruit extract and fractions had elastase and tyrosinase inhibitory activity. **Conclusions:** The n-hexane fraction was the active fraction in inhibiting the elastase enzyme by 21.56%, while the active fraction in inhibiting the tyrosinase enzyme was the ethyl acetate fraction which provided an activity of 23.96%.

**Keyword:** *Medinilla speciosa*; Extract; Fraction; Antiaging

### INTRODUCTION:

A tropical climate is a climate that is in an area that is crossed by the equator. This climate

causes the area to receive more sunlight, thus affecting the skin. Skin that is often exposed to the sun can result in adverse effects such as

hyperpigmentation, sunburn, premature aging, and even skin cancer. Tyrosinase inhibitor agents can prevent melanogenesis caused by ultraviolet radiation from sunlight by preventing enzymatic oxidation (Gazali et al., 2014). Tyrosinase inhibitors that are currently widely used to whiten skin are hydroquinone, azelaic acid, mercury and kojic acid. However, some of these compounds have dangerous side effects, namely being carcinogens and mutagens (Normaidah et al., 2024). Kojic acid is a compound that has tyrosinase inhibitory activity in preventing hyperpigmentation. If used in large concentrations it can cause erythema and contact dermatitis (Gazali et al., 2014). Excessive exposure to sunlight, apart from causing an increase in melanin production, can also cause damage to cell components, which can cause the skin to become dry, wrinkled and dull (Dini et al., 2024). Apart from that, UV rays also affect skin elasticity which is degraded by the elastase enzyme in the extracellular matrix which causes wrinkles to appear. This elastase enzyme plays a role in degrading elastin which also has its own role in the aging process (Pittayapruuek et al., 2016). Therefore, research to find effective, safe, and abundant sources of tyrosinase inhibitors is urgently needed.

Prevent the adverse effects of sunlight on the skin, it can be done by using sunscreen or anti-aging cream from natural ingredients such as parijoto fruit (*Medinilla speciosa*). Parijoto fruit contains active substances such as phenols, flavonoids, tannins, saponins, and alkaloids (Sholikhati et al., 2024). These compounds are secondary metabolites resulting from plant metabolism that have various beneficial pharmacological effects, for example as antiaging and inhibition of hyperpigmentation. The mechanism of secondary metabolite compounds as antiaging or hyperpigmentation is by inhibiting the activity of elastase and tyrosinase enzymes. Based on research (Winanta et al., 2024) parijoto fruit extract has elastase enzyme inhibition activity. Methanol extract from parijoto fruit is also reported to

have inhibitory activity against the tyrosinase enzyme (Riantica et al., 2023).

Previous research on parijoto fruit as an inhibitor of elastase and tyrosinase enzymes was limited to its extract. Further studies are needed to determine the potential of parijoto fruit in the preparation of active fractions. Research development to the fractionation stage is necessary to obtain more specific compounds than extracts. Fractionation is the advanced fragmentation stage of compounds from extracts. Fraction compounds are more specific because they have been separated based on polarity. Furthermore, fractionation can increase the concentration of target compounds because unwanted components are separated (Mona et al., 2022). This study aims to determine the inhibitory activity of parijoto fruit extracts and fractions on the elastase and tyrosinase enzymes.

## METHODS:

### Tools and Materials

The equipment used in this study is a maceration vessel, rotary evaporator EV400H, water bath, glassware, KLT silica gel 60 F254 plate, and a microplate reader (BIOTEX Synergi HTX). The materials used in this study are parijoto fruit *Simplicia* powder, ethanol 96%, ethyl acetate, chloroform, FeCl<sub>3</sub>, butanol, acetic acid, aquadest, ammonia, methanol pa, Dragendorff, Lieberman Bouchardat, n-hexane, sulfuric acid, gallic acid, kojic acid, phosphate buffer 0.05 M pH 6.5, Dimethyl Sulfoxide (DMSO), Tyrosinase from mushroom enzyme, L-tyrosine substrate, 1000 ppm acid solution, Tris-HCl buffer 0.1 M pH 8, elastase enzyme from porcine pancreas, N-succinyl-Ala-Ala-Ala-p-nitroanilide elastase substrate.

### Extraction

The extraction method used in this study was maceration using 96% ethanol. 137.7 grams of parijoto fruit powder was added to 1,000 mL of 96% ethanol. The extract was then concentrated using a rotary evaporator and

water bath at 40°C until a thick extract was obtained (Ameliya et al., 2025). The yield of the thick extract was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of simple substance}}{\text{weight of extract}} \times 100$$

### Fractionation

Fractionation was carried out using the liquid-liquid partition method using n-hexane, ethyl acetate, butanol, and air as solvents. 10 grams of thick extract was added with 100 mL of distilled water and 100 mL of n-hexane until dissolution occurred. The n-hexane phase was collected and the distilled water phase was added with 100 mL of ethyl acetate, shaken homogeneously until two immiscible layers were formed. The ethyl acetate phase was collected and the distilled water phase was added with 100 mL of butanol, shaken homogeneously until two layers were formed. Each layer was collected. A quarter of the fraction obtained was thickened on a water bath at 40°C and the yield was calculated using the same calculation as calculating the yield of the extract (Oentari, 2023)

### Identification of Active Compounds

The identification of active compounds in parijoto fruit extract and fraction samples was carried out by the thin-layer chromatography method. Phenolic compounds are dissolved with ethyl acetate: chloroform (3:2), the spray reagent is FeCl<sub>3</sub>, while flavonoids use butanol: acetic acid: water (4:1:5) as mobile phase, ammonia vapor for spotting (Hanani, 2015). Tannin compounds are analyzed using TLC with methanol: water (6:4) as mobile phase and FeCl<sub>3</sub> as spray reagent (Cahyaningsih et al., 2019). Alkaloid compounds are TLC analyzed with ethyl acetate: methanol: water (6:4:2), and Dragendorff as spray reagent (Saepudin et al., 2024). Saponins are expanded by chloroform: methanol : aqueous (13:7:2) of the Liebermann Bouchard patches (Saepudin et al., 2024).

Steroids/triterpenoids are diluted with n-hexane: ethyl acetate (3:1) in the appearance of Liebermann Bouchard patches (Hidayah et al., 2016). Essential oils are diluted with n-Hexane: ethyl acetate 8: 2 and visible spots of sulfuric acid (Handayani, 2014).

### Inhibition of Elastase and Tyrosinase Enzymes

#### Preparation of Extract Samples and Fractions of n-Hexane, Ethyl Acetate, n-butanol and Water Parijoto Fruit

Extracts and fractions were weighed 10 mg dissolved in a flask using 5 ml of DMSO to obtain a sample concentration of 2000 ppm. Then the extract and fraction samples were diluted to 125 ppm (Furi et al., 2022).

#### Elastase Enzyme Inhibitor Activity Test

The elastase enzyme inhibition test was carried out on extract samples, n-hexane fraction, ethyl acetate, n-butanol, and water. The comparative standard for elastase inhibition activity is gallic acid. For elastase enzyme inhibition testing, extract and fraction samples were taken as much as 10 µl then added with 110 µl of Tris-HCl 0.1 M buffer with a pH of 8.0. 10 µl of elastase enzyme that has been dissolved with buffer at a concentration of 1500 units/ml and 20 µl of N-Succinyl-Ala-Ala-Ala-p-nitroanilide mM substrate. For the raw acid of the gallic acid, a concentration of 500 ppm is made and then diluted to 15.625 ppm in Tris-HCl 0.2 M buffer pH 8. Subsequently, each of these concentrations was taken as much as 10 µl then added with 125 µl of N-Succinyl-Ala-Ala-Ala-p-nitroanilide substrate 1.5 mM, pre-incubated for 5 minutes at 25°C. As 15 µl of elastase enzyme that had been dissolved with buffer at a concentration of 1500 units/ml added into the well plate, and incubated for 30 minutes at a temperature of 25°C. The negative control contains 10 µl of Tris-HCl 0.1 M pH 8 buffer, 15 µl of elastase enzyme that has been dissolved with buffer at a concentration of 1500 units/ml, and 125 µl of N-Succinyl-Ala-Ala-Ala-p-

nitroanilide mM substrate. Absorbances were observed at a wavelength of 410 nm, and observations were carried out 3 times (Natanael et al., 2021; Jiratchayamaethasakul et al., 2020).

### Tyrosinase Enzyme Inhibitor Activity Test

The elastase enzyme inhibition test was carried out on extract samples, n-hexane fraction, ethyl acetate, n-butanol, and water. The positive control for tyrosinase inhibition activity is kojic acid. For the testing of tyrosinase enzyme inhibition, 10 µl of extract and fraction samples were taken, then added with 110 µl of phosphate buffer 0.1 M, pH 6.8, 10 µl of tyrosinase enzyme that had been dissolved with phosphate at a concentration of 1500 units/ml and 20 µl of L-tyrosine substrate 1.5 mM. For raw kojic acid, a concentration of 500 ppm will be made and then diluted to 15.625 ppm. Subsequently, each of these concentrations was taken as much as 10 µl then added with 110 µl of phosphate buffer 0.1 M pH 6.8, 10 µl of tyrosinase enzyme that had been dissolved with phosphate with a concentration of 1500 units/ml, and 20 µl of L-tyrosine substrate 1.5 mM. The blanks contain only 110 µl of phosphate buffer of 0.1 M pH 6.5, 10 µl of tyrosinase enzyme that has been dissolved with phosphate paste with a concentration of 1500 units/ml, and 20 µl of L-tyrosine substrate of 1.5 mM. The mixture is then incubated for 30 minutes at 37°C, and then the absorption of the mixture is observed using a microplate reader at a wavelength of 510 nm (Sagala & Ripaldo, 2020; Jiratchayamaethasakul et al., 2020). Testing was carried out in 3 replications.

The formula for solving the inhibitory activity of the enzymes tyrosinase and elastase is as follows:

$$\% \text{ Inhibition} = \frac{(B1-B0) - (S1-S0)}{(B1-B0)} \times 100\%$$

Description:

B1 = Blank absorbance

B0 = Blank control absorbance

S1 = Absorbance of the test solution

S0 = Absorbance of test solution control

(Furi et al., 2022; (Natanael et al., 2021)

### RESULTS:

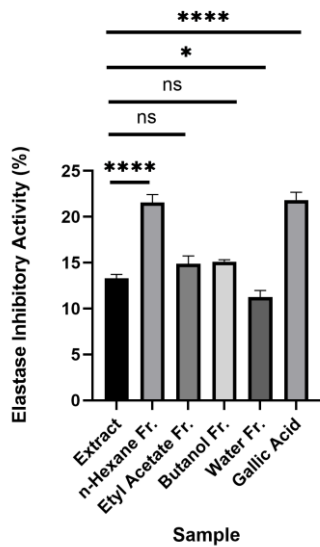
The results of the research on the yield of extracts and fractions of parijoto fruit are presented in Table 1. The identification of secondary metabolite compounds of extracts and fractions of parijoto fruit is presented in Table 2, while the inhibitory activity of elastase and tyrosinase enzymes from extracts and fractions of parijoto fruit is presented in graphs 1 and 2.

**Table 1. Results of the Extract Yield and Parijoto Fruit Fraction**

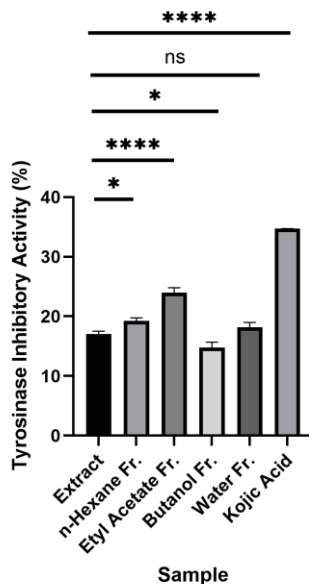
| Sample                 | Weight (g) | Yield (%) |
|------------------------|------------|-----------|
| Extract                | 13.37      | 9.71      |
| n-hexane fraction      | 0.28       | 2.8       |
| Ethyl acetate fraction | 0.78       | 7.8       |
| Butanol fraction       | 0.93       | 9.3       |
| Water fraction         | 0.6        | 6.9       |

**Table 2. Secondary Metabolite Content of Parijoto Fruit**

| Compound/Sample        | Extract | n-hexane fraction | Ethyl acetate | Butanol fraction | Water fraction |
|------------------------|---------|-------------------|---------------|------------------|----------------|
| Phenol                 | +       | +                 | +             | +                | -              |
| Flavonoids             | +       | -                 | +             | -                | -              |
| Tannin                 | +       | +                 | +             | +                | -              |
| Alkaloids              | +       | +                 | +             | -                | -              |
| Steroids/Triterpenoids | +       | +                 | +             | -                | +              |
| Saponin                | +       | +                 | +             | -                | -              |
| Essential oil          | +       | +                 | +             | +                | -              |



**Figure 1. Elastase Inhibitory Activity Test**



**Figure 2. Tyrosinase Inhibitory Activity Test**

## DISCUSSION:

### Extraction and Fractionation

Parijoto fruit extraction was carried out using the maceration method using 96% ethanol with a ratio of 1:4. Extraction is intended to extract secondary metabolite compounds present in parijoto fruit. Ethanol is a universal solvent that can attract compounds with diverse polarities. 96% ethanol solvent is used as a solvent because it is selective, non-toxic, has good absorption and high filtering ability so it can filter non-polar, semi-polar and polar compounds. The

96% ethanol solvent penetrates more easily into the cell walls of the sample than the ethanol solvent with a lower concentration, resulting in a concentrated extract (Wendersteyt et al., 2021).

### Identification of Secondary Metabolite Compounds of Parijoto Fruit

Based on the results of identifying secondary metabolite compounds in the extract, there are phenolic compounds, flavonoids, tannins, alkaloids, steroids/triterpenoids, saponins, and essential oils. Research conducted by (Tinasari et al., 2024) found that parity fruit extract and ethyl acetate fraction contain flavonoids, alkaloids, tannins, and saponins. The compounds contained in the extract have different polarities so it is necessary to carry out fractionation to group the compounds based on their polar properties. In the fractionation process, two immiscible solvents are used, and they have different polarity levels. Multilevel fractionation using different solvents based on their polarity levels produces different natural extracts so that secondary metabolite compounds can be maximally attracted by each solvent (Putri et al., 2023). The fractionation method is generally used as a reference in estimating the polarity of a compound to be separated. Based on this, the fractionation method has advantages compared to other methods, because it can separate bioactive compounds based on the level of polarity because polar compounds dissolve in polar solvents, while semi-polar compounds dissolve in semi-polar solvents and non-polar compounds dissolve in non-polar solvents. The fractionation process in this research uses hexane, ethyl acetate, butanol, and water solvents which have different polarity levels. The identification results of parijoto fruit extracts and fractions are in Table 2. These compounds can provide interactions or activity against the inhibition of elastase and tyrosinase enzymes.

### Elastase Enzyme Inhibitory Activity

Elastin is a protein component in the skin that helps the skin remain elastic. In the body, elastin works with collagen components to produce skin with an elastic and firm texture (Brahmanti & Dyah Puspitasari, 2023). The elastase enzyme causes a decrease in the elasticity of elastin (Ramadhani et al., 2023). The presence of elastase is the result of continuous and excessive exposure to sunlight. As a result of the work of the elastase enzyme, the skin becomes saggy and unhealthy. The inhibitory activity of the elastase enzyme was observed by looking at the reaction between the elastase enzyme and the substrate N-Succinyl-Ala-Ala-Ala-p-nitroanilide to form p-nitroaniline which is yellow in color. The presence of elastase inhibitor compounds causes a decrease in substrate degradation by the enzyme (Ernawati, 2024).

Parijoto fruit contains secondary metabolites that can be used to overcome elastase enzyme activity. Phenols and flavonoids are the most abundant secondary metabolites in plants. Flavonoids are phenolic compounds capable of inhibiting elastase enzymes (Jakimiuk et al., 2021). Tannin is a compound that has an affinity for the elastase enzyme (Hadi et al., 2015). Alkaloids can modulate inflammatory signals that trigger elastase release by neutrophils (Kim et al., 2015). Saponins are steroid glycosides or triterpenoids, these compounds are known as antioxidants through inhibiting elastase and reducing oxidative stress, while essential oils are essential oils that are widely used as ingredients for antiaging products and as antioxidants (Chen et al., 2023). In this study, gallic acid was used as a comparison, this compound is a derivative of a phenol compound that has good stability so that it provides consistent results, besides this compound can interact directly with the elastase enzyme (Eun Lee et al., 2019). The results in Figure 1 show that the n-hexane fraction has almost the same potential as gallic acid. The n-hexane fraction is a nonpolar solvent capable of extracting nonpolar compounds, such

as essential oils, certain alkaloids, and steroids and triterpenoids. The elastase enzyme is a proteolytic enzyme that plays a role in degrading elastic fibers, namely elastin. Elastin is a protein rich in hydrophobic amino acids, so it has a predominantly nonpolar character (Marinaccio et al., 2022). This hydrophobic nature allows the nonpolar compounds in the n-hexane fraction to interact more effectively with the active site of the enzyme, resulting in more optimal elastase inhibitory activity.

### Tyrosinase Enzyme Inhibitory Activity

The presence of the tyrosinase enzyme in the body will trigger the formation of melanin in the skin, especially if the skin is continuously exposed to sunlight. The tyrosinase enzyme inhibition test is based on the principle of inhibiting the occurrence of dopachrome from the reaction of L-DOPA and the tyrosinase enzyme. The intensity of the dopachrome that occurs can be measured at a wavelength of 490 nm (Rauf, 2016). Active secondary metabolite compounds from parijoto fruit are used to inhibit the tyrosine-tyrosinase reaction that occurs so that it can reduce the formation of dopachrome.

From the research results in Figure 2, it can be seen that the ability of active compounds in both extracts and fractions of parijoto fruit is smaller than kojic acid. Kojic acid is a compound that has been proven to inhibit the action of the tyrosinase enzyme and has been widely used in various antiaging products. Kojic acid is able to form a copper chelate on the enzyme so that the enzyme does not bind to the substrate and does not produce melanin (Furi et al., 2022). Based on research on Sinulingga et al., (2022) alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins have tyrosinase enzyme inhibitory activity of 22.45%.

Ethyl acetate is a semipolar solvent that can attract compounds that tend to be semipolar. Phenols, flavonoids, and tannins are compounds that tend to be polar-semipolar and have a high affinity for interacting with the tyrosinase

enzyme through chelation and competition with the substrate (Normaidah et al., 2024). Phenolics are known to have the ability to chelate  $\text{Cu}^{2+}$ .  $\text{Cu}^{2+}$  in the tyrosinase enzyme acts as a cofactor which functions to help the substrate bind to the enzyme. The loss of the cofactor in the enzyme reduces the enzyme's ability to bind its substrate so that melanin is not formed (Furi et al., 2022). Saponin compounds, triterpenoids and essential oils can inhibit the process of melanin formation caused by the activity of the enzyme tyrosinase. Alkaloids are able to interfere with the catalytic reaction of tyrosinase by binding to its active site (Kim et al., 2020)

## CONCLUSIONS:

Parijoto fruit extracts and fractions have inhibitory activity against elastase and tyrosinase enzymes. The n-hexane fraction is the active fraction in inhibiting the elastase enzyme, while the ethyl acetate fraction is the active fraction in inhibiting the tyrosinase enzyme. The n-hexane fraction can be developed into cosmetic preparations that can soften and make the skin supple, such as hand and body lotion preparations. Meanwhile, the ethyl acetate fraction can be developed into cosmetic preparations that can brighten skin pigmentation. In addition, it is necessary to isolate and identify more specific bioactive compounds from the active fraction of parijoto fruit.

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