Anti-elastase activity of methanolic and ethyl acetate extract from *Garcinia latissima* Miq.

N S S Ambarwati¹, B Elya²,* and Y Desmiaty³

¹ Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, Jl. Rawamangun Muka, East Jakarta, 13220, Indonesia  
² Department of Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, UI Depok Campus, Depok, 16424, Indonesia  
³ Department of Pharmacognosy, Faculty of Pharmacy, Universitas Pancasila, Jl. Srengseng Sawah, Jagakarsa, Kota Jakarta Selatan, Daerah Khusus Ibukota Jakarta, 12640, Indonesia.

*berna.elya@gmail.com

**Abstract.** Elastase is a proteinase enzyme that can reduce elastin by dividing specific peptide bonds. Therefore, the inhibition of elastase activity in the dermis layer can be used to maintain skin elasticity. Materials that can inhibit elastase activity can be a cosmetic ingredient in dealing with skin aging. Increased desire to maintain healthy skin without chemicals, encourages the use of materials from natural sources such as plants from Indonesia, a country that has high biodiversity, including *Garcinia latissima* Miq. from the Clusiaceae family. The purpose of this study was to explore and sought an explanation as to why *G. latissima* Miq. inhibits elastase enzyme. Plant extraction was carried out using maceration successively methods. The inhibition activity of elastase enzyme was carried out by measuring the kinetic enzyme N-succ-(Ala)-3-Nitroanilide conversion to p-nitroaniline (substrate) spectrophotometry at 405 nm and using porcine pancreatic elastase (PPE) as an enzyme. The results showed that the *G. latissima* Miq. methanol extract and ethyl acetate extract were active as elastase enzyme inhibitors. *G. latissima* Miq. extract can maintain skin elasticity.

**1. Introduction**

Elastase is a chymotrypsin enzyme from proteases that are found in the dermis and is responsible for breaking down elastin which causes changes in skin elasticity. This causes natural skin aging. Therefore, inhibitors of elastase enzymes can potentially be cosmetic ingredients to prevent skin aging because of their use in preventing loss of elasticity and sagging of the skin [1].

The process of skin aging can also be due to extrinsic factors which are dominated by exposure to solar radiation (photoaging). UV exposure can cause physical changes to the skin due to changes that occur in connective tissue through the formation of lipid peroxides and reactive oxygen species (ROS), which can then cause a reduction in skin elasticity. This can be inhibited by the presence of antioxidant compounds [2].

It has been previously investigated, that the water fraction of *Garcinia indica* bark extract to have an inhibitory effect on elastase enzyme [3]. The inhibitory effect of the elastase enzyme from the ethanolic extract of *Garcinia picrorhiza* Miq. fruit showed IC50 = 152.93 μg / mL, lower than IC50 xanthones
The stem bark and seeds ethanol 70% extracts of *Passiflora edulis* are native plant from Brazil from with IC50 values for inhibition of elastase enzymes, respectively 62.82 ± 1.50 μg/mL and 41.06 ± 0.31 μg/mL. These results indicated that *P. edulis* stem bark and seed extract as an anti-aging cosmetic ingredient [5]. It needs further research on other plants that can be efficacious as inhibitors of the elastase enzyme, especially native plants of Indonesia such as *Garcinia latissima* Miq.

*Garcinia latissima* Miq. is a native plant from Indonesia which has antioxidant activity from fraction G of its leaves ethyl acetate extract by the DPPH method has 9.39% inhibition percentage and IC50 value of 6.4377 g/mL, fractionation method with column chromatography [6]. The leaves methanol extract also has antioxidant activity with the effective percentage (EP) value at 10 μg/mL was 29.47±2.01%. Fraction D (fractionation method with column chromatography) had the highest deterrence activity against DPPH free radicals (37.73±1.44%) when used at 10 μg/mL. The half effective concentration of the extract was 23.40 μg/mL, whereas that of the most active fraction D was 19.38 μg/mL and quercetin as a positive control was 3.72 μg/mL [7-9].

This study aimed to determine the ability of the inhibitory activity of elastase enzyme from plant extract *G. latissima* Miq.

### 2. Material and methods

The *G. latissima* Miq. fruit, leaves, and stem bark extracts were obtained by maceration successively [6]. An anti-elastase assay using the spectrophotometric method used with slight modification was using porcine pancreatic elastase (PPE) with the substrate N-Succ-(Ala)-3-p-nitroanilide (SANA). The product reaction, p-nitroaniline for 20 min at 25°C was monitored by measuring the absorbance at 405 nm with a microplate reader (Elx 800). Reaction mixture were contained 0.2 M Tris-HCl buffer (pH 8.0), 1 μg/mL elastase, 0.8 mM SANA [10,11].

It was pre-incubated for 15 min at 25°C and the reaction was started by adding substrate. Blanks contained all the components except the enzyme. The quercetine was used as a positive control. Each treatment was triplicated. The percentage of inhibition was calculated as:

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\text{Inhibition} \% = (1 - \frac{B}{A}) \times 100
\]

Where A is the enzyme activity without inhibitor and B is the activity in the presence of inhibitor [11]. The qualitative phytochemical tests were carried out to identify different phytoconstituents.

### 3. Results and discussion

We examined the inhibitory activity of porcine pancreatic elastase (PPE) at concentration of extract and control (quercetin) was 100 ppm. The *G. latissima* Miq. stem bark methanol extract showed the highest inhibitory effect with 66.42±5.23% inhibition on elastase activity. The ethyl acetate extract of the *G. latissima* Miq. stem bark showed activity of anti-elastase 64.43±13.39%, higher than the quercetin as control (62.75±1.89%). The percentage inhibitory activity of elastase enzyme at concentration 100 ppm of fruits methanol extract (33.06±3.57%) and leaves methanol extract (23.98±1.52%) were smaller than the control. The results of the elastase enzyme inhibition test are shown in Table 1.

| Sample                        | % inhibition (100 ppm) |
|-------------------------------|------------------------|
| Leaves methanol extract       | 23.98 ± 1.52           |
| Fruits methanol extract       | 33.06 ± 3.57           |
| Stem bark methanol extract    | 66.42 ± 5.23           |
| Stem bark ethyl acetate extract | 64.43 ± 13.39         |
| Quercetine (positive control) | 62.75 ± 1.89           |

Table 1. The result of the elastase enzyme inhibition test (triplicate).
Quercetin, is a flavonoid compound and is products extracted from plants [12]. Quercetin shows a strong antioxidant activity, and inhibit elastase so that elasticity can be formed again after mice treated with elastase once a week for 4 weeks were subsequently administered 0.5 mg of quercetin for 10 days [12]. It has been investigated from the four flavonoids (myricetin, quercetin, kaempferol, galangin), quercetin was the most effective inhibitor from the elastase release [13]. The quercetin was used as a positive control for elastase inhibition at 100 ppm, finally we compared the elastase inhibitory activity of the extracts with the standard used (quercetin).

This study showed that two extracts (stem bark methanol extract and stem bark ethyl acetate extract) were higher potential effect as anti-elastase than the quercetin. The outcome indicated the possible application of compounds from the extracts were found to be potential inhibitors of anti-elastase [14]. The analysis or identify of bioactive compounds present in the plant extract need to do the applications of common phytochemical screening assays [14].

The results of this activity test encourage us to find out the phytochemical content in the extract. The results of the phytochemical screening test can be seen in Table 2.

| Test         | Reagent Used       | Fruits Methanol Extract | Stem Bark Methanol Extract | Stem Bark Ethyl Acetate Extract | Leaves Methanol Extract |
|--------------|--------------------|-------------------------|----------------------------|---------------------------------|-------------------------|
| Tannins      | Acidic FeCl3       | +                       | +                          | -                               | +                       |
|              | Gelatin            | +                       | +                          | -                               | +                       |
| Saponins     | Frothing test      | -                       | +                          | -                               | +                       |
| Flavonoids   | HCl + Mg turnings  | +                       | +                          | +                               | -                       |
| Anthraquinones | Borntragers’s   | -                       | -                          | -                               | -                       |
| Terpenoids   | H2SO4              | -                       | -                          | -                               | -                       |
| Alkaloids    | Dragendorff’s      | -                       | -                          | +                               | -                       |
|              | Mayer’s            | -                       | -                          | +                               | -                       |
|              | Bouchardat’s      | -                       | -                          | +                               | -                       |

The results of the phytochemical screening test showed that the methanol extract of stem bark, the stem bark ethyl acetate extract, and the methanol extract of fruit contained flavonoids. The presence of flavonoids in the extract affects the inhibitory activity of the elastase enzyme [15]. This activity is also influenced by the content of flavonoids in the extract [15]. Therefore, it is possible that the flavonoid content in the methanol extract of stem bark is slightly greater than the content of flavonoid in the stem bark ethyl acetate extract. And the content of flavonoid in the stem bark ethyl acetate extract is twice than the content of flavonoids in the fruit methanol extract. Flavonoids that have inhibitory activity in the elastase enzyme include kaempferol, quercetin, and myricetin [15]. The presence of a catechol group on ring B flavonoids affects the inhibitory activity of the elastase enzyme [16].

The content of flavonoid compounds in the extract influence the potential activity as anti-elastase of the extracts [17]. The theoretical affinity order among flavonoids and amino acid residues (like elastase) interaction has explained the activity of flavonoids using in silico docking studies as an anti-elastase [18]. Flavonoids are a group of natural compounds with variable phenolic strutures and are classified as isoflavones (genistein and daidzein), neoflavonoids, flavones (luteolin, apigenin, and tangeritin), flavonol (kaempferol, quercetin, myricetin and fisetin), flavonone (hesperitin, naringenin and eriodictyol), flavanone, flavanol [18].

The test results of the inhibitory activity of elastase enzymes in vitro from the methanol extract of stem bark and the stem bark ethyl acetate extract at a concentration of 100 ppm showed results greater
than 50%. This shows that the topical use of these two extracts can counteract UV radiation and prevent dry skin from being used in cosmetic preparations [19].

4. Conclusion
The methanol extract of stem bark and the stem bark ethyl acetate extract of *G. latissima* Miq. has the potential as a cosmetic ingredient because of its ability in anti-aging activities.

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Conflict of Interest
The authors declare no conflict of interest.

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