Elastase inhibitory activity of methanol extract and n-hexane extract of *Garcinia xanthochymus* Pericarp

Y Desmiaty¹, N S S Ambarwati²*, B Elya³, D Atmanto² and I Ahmad⁴

¹ Department of Phytochemistry, Faculty of Pharmacy, Universitas Pancasila, Jakarta, 12640, Indonesia
² Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, Jl. Rawamangun Muka, Pulogadung, Jakarta Timur, 13200, Indonesia
³ Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, 16424, Indonesia
⁴ Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia

*neneng_ambarwati@yahoo.co.id

Abstract. *Garcinia xanthochymus* is a plant found in Indonesia and rich in quercetin. Ethanol extract of bark can reduce LDL levels. Xanthon from the *G. xanthochymus* has antidiabetic activity. This study aims to determine the anti-elastase activity of n-hexane extract and methanol extract of *G. xanthochymus* pericarp. Samples were macerated for one time 24 hours using n-hexane and methanol, respectively. The elastase enzyme inhibition test used spectrophotometry methods by reacting extracts with the porcine pancreatic elastase enzyme and using the substrate N-Succ-(Ala)-3-p-nitroanilide (SANA). Absorbance was measured at a wavelength of 405 nm with a microplate reader. The results showed that the concentration of 100 ppm extract of n-hexane extract of pericarp had enzyme inhibiting activity 65.17 ± 6.44%, methanol extract of pericarp 29.81 ± 10.67%. Meanwhile, the inhibitory activity of quercetin elastase enzyme as a positive control was 62.75 ± 1.89%. This research concludes that the n-hexane extract of the pericarp of *G. xanthochymus* has activity as an inhibitor of the enzyme elastase is very high and can potentially be used as a material for making cosmetics.

1. Introduction

*Garcinia xanthochymus* is known as gamboge, yellow mangosteen, and false mangosteen included in the Clusiaceae or Guttiferae family, the *Garcinia* genus is distributed in the Southeast Asian region [1-3]. *G. xanthochymus* leaves and fruit commonly used as traditional medicine for stomach aches, fevers, skin diseases, and sexual disorders [1,2,4]. This plant has activity as an antioxidant, antidiabetic, cytotoxic, potentiating nerve growth factor, antimicrobial, and anti-inflammatory [1-3].

The seeds contained 2.35 g of ash, 6.93 g of protein, 45.22 g of carbohydrate, 12.3 g of crude fiber in 100 g and contained 34.17% saturated fatty acids, 65.79% unsaturated fatty acids [5]. The seed oil of *G. xanthochymus* contains 0.11% myristic acid, palmitic acid 32.96%, stearic acid 96%, palmitoleic acid 17.65%, oleic acid 45.87%, linoleic acid 1.93%, linolenic acid 0, 34%, arachidic acid 0.07%, and behenic acid 0.07% [5]. This oil has activity as an antimicrobial against gram-positive bacteria and as an antioxidant more potent than ascorbic acid and butylated hydroxyanisole [5]. Dichloromethane and
hexane extract of *G. xanthochymus* bark have cytotoxic and anti-inflammatory activity [6]. The leaf powder of these plants contains carbohydrate, glycoside, flavonoid, and tannins [2].

This study aims to determine the inhibitory activity of elastase enzymes from hexane extract and methanol pericarp extract of *Garcinia xanthochymus* fruit.

2. Materials and methods

The pericarp of *G. xanthochymus* was obtained from Bogor Botanical Garden, West Java, Indonesia. The dried sample washed clean, cut into pieces, then dried using a drying cabinet after being ground using a blender [6]. The powder was macerated in stages using hexane and methanol, respectively [7]. Furthermore, the filtrate was evaporated using a rotary evaporator and concentrated using a water bath [8] and stored in an airtight container at 4°C [8]. The extract yield percentage was calculated using the formula [6], as follows:

\[
\text{Extract yield (\%) = \frac{\text{extract weights}}{\text{dried sample weights}} \times 100}
\]

Elastase enzyme inhibitory activity by measuring the enzymatic conversion of N-succ (Ala) 3-Nitroanilide to p-nitroaniline elastase using microplate spectrophotometric method 96 well combination reader (Elx 800) at 405 nm [9]. Each well was containing 0.2 M Tris-HCl buffer (pH 8.0), 1μg / mL porcine pancreatic elastase (PPE), and extract the sample or control sample 100 ppm in pre-incubated for 15 min at 25 °C [9]. The reaction was started by adding substrate 0.8 mM substrate N-Succ-(Ala)-3-p-nitroanilide (SANA), and after 20 minutes, the reaction product (p-nitroaniline) remained at 25 °C absorbance measured at a wavelength of 405 nm. Blank uses all components except PPE enzymes and quercetin as a positive control [9]. Each treatment was performed in triple.

The calculation of the percentage of elastase enzyme inhibitory activity was calculated using the formula [9]:

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

Where A is the enzyme activity without inhibitor, and B is the activity in the presence of inhibitor.

The research data were represented as the mean ± standard deviation (SD) determined from the results of three replications in each test. Comparisons were made using a two-sample T-test analysis. Results differed significantly when the p-value was less than 0.05 (p <0.05).

3. Results and discussion

The maceration process uses the n-hexane solvent first to attract non-polar natural products such as volatile oil and lipids [10]. Furthermore, the dried sample was macerated using methanol as a solvent to attract polar compounds [11]. The results of maceration using non-polar solvent (n-hexane) and polar (methanol) solvent obtained a yield of 2.50% and 41.33%, respectively. Based on the results of this yield shows that the extracted value produced in the maceration process uses a little n-hexane; this shows the least of the extracted compound [12].

The results of the elastase enzyme inhibitory activity test of *G. xanthochymus* pericarp extract with a concentration of 100 ppm extract shows in Table 1.

| The Sample          | Tyrosinase Inhibitory Activity (%) |
|---------------------|------------------------------------|
| the hexane extract  | 65.17 ± 6.44                       |
| the methanol extract| 29.81 ± 10.67                      |
| Quercetin (positive control) | 62.75 ± 1.89                   |
The results of this study indicate that hexane extract of *G. xanthochymus* pericarp has higher elastase enzyme inhibitory activity than the positive control (quercetin). The results of the statistical analysis of two sample T-tests between hexane extract activity and quercetin activity with p-value <0.05 obtained \( t_{\text{test}} (0.62) < t_{\text{table}} (2.92) \), this shows that the inhibitory activity of the elastase enzyme from hexane extract was significantly different compared to quercetin. The high elastase enzyme inhibitory activity of hexane extract due to the presence of flavonoid compounds [9]. Extracts that have high elastase enzyme inhibitory activity can be developed as active ingredients for anti-aging cosmetics [13].

*G. xanthochymus* mainly contain xanthone, benzophenone, depsidone, isocoumarin, glycosides, flavonoids, and tannins [2,3]. Xanthones with cytotoxic activity were isolated from the stem bark of *G. xanthochymus* [14]. Fruits contain high carbohydrates and secondary metabolite compounds: saponins, tannins, alkaloids, terpenoids, and phenolic compounds [4]. The results of other studies also showed that *G. xanthochymus* fruit extracts had antibacterial and antifungal activities [4].

The total phenolic or polyphenols contained in the *G. xanthochymus* are associated with antioxidant activity. Also, it has antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, anti-thrombotic, anti-viral, anti-cancer, and vasodilatation activities [15]. Guttiferone H and gambogenone with cytotoxic activity on colon cancer cells have been isolated from the methanol extract of *G. xanthochymus* [13].

The polyphenol content found in these plants, mainly in the fruit was suspected as a factor that causes an inhibitory activity of the elastase enzyme from the nonpolar extract.

4. Conclusions
The *G. xanthochymus* pericarp hexane extract had elastase inhibitory activity 65.17 ± 6.44 at 100 ppm (parts per million) higher than quercetin used as the positive control (62.75 ± 1.89%) and after statistical analysis using two-sample T-test results of extract activity hexane is significantly different than the positive control activity (quercetin). Whereas methanol extract has lower elastase inhibitory activity than positive control activity.

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