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Neneng Siti S. Ambarwati, Berna Elya, Yesi Desmiaty, Dwi Atmanto, and Islamudin Ahmad

Tyrosinase Inhibitory Activity of *Garcinia xanthochymus* Fruit Pericarp Extract

Neneng Siti S. Ambarwati[1, a)], Berna Elya[2, b)], Yesi Desmiaty[3, c)], Dwi Atmanto[1, d)], and Islamudin Ahmad[4, e)]

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

[a)]Corresponding author: neneng_ambarwati@yahoo.co.id
[b)]berna.elya@gmail.com
[c)]yesidesmiaty@gmail.com
[d)]dwiatmanto64@gmail.com
[e)]islamudinahmad@yahoo.com

Abstract. The tyrosinase is the main enzyme involved in melanin biosynthesis. The amount of melanin produced by melanocyte cells will affect a person's skin color. Therefore, the body's skin needs a compound that can inhibit the work of the enzyme tyrosinase. The purpose of this study was to determine the potential of *G. xanthochymus* fruit extracts as a whitening agent. The dried sample was continuously macerated using n-hexane and methanol. Furthermore, each extract's tyrosinase enzyme inhibitory activity was measured using a microplate reader 96 well spectrophotometers at 490 nm and a substrate of 3,4-dihydroxy-L-phenylalanine, L-D OPA. The results showed that the results of the Inhibitory Activity test at a concentration of 100 ppm from each extract were 35.41 ± 11.10% (hexane extract), and 28.83 ± 6.77% (methanol extract). Percentage inhibition of the positive control (kojic acid) was 65.07 ± 0.03 % at 100 ppm. The conclusion of this research is that *G. xanthochymus* pericarp hexane and methanol extract have the potential as skin lightening with hexane extract results better than methanol extract, with inhibitory activity of tyrosinase enzyme half of kojic acid.

INTRODUCTION

Natural products (plants) have an essential role in health, mainly in the utilize and discovery of drugs and supplements to overcome various diseases that arise along with the increasing world population [1-2]. Garcinia is a genus of plants native to Southeast Asia, Polynesia, Africa, Australia, China, India, and Bangladesh [1-3]. The plants of the Garcinia genus is rich in phytochemical sources, namely xanthones, phenolic acids, and flavonoids [1]. *Garcinia xanthochymus* is also called false mangosteen, Himalayan Garcinia, sour mangosteen, Asam Kandis, Mysore gamboge, Egg tree is a traditional medicine commonly used to treat diarrhea, dysentery, nausea, vomiting, worm medicine, eliminating toxins in food, and gallbladder disorders. [1-3]. *G. xanthochymus* is a tropical plant that has a height of up to 15-18 m, has a fruit that is yellow and tastes of acid, edible for making jams, vinegar, curry, drinks, round, with wide about 2–3.5 cm, and leaves with 25–40 cm long by 4–10 cm wide, and rough texture [2-3].

Che Hassan et al. has reported the content of the fruit of this plant include fat, protein, carbohydrates, vitamins (thiamine, riboflavin, ascorbic acid, niacin, vitamin B12), minerals (sodium, potassium, iron, phosphorus, magnesium, calcium) [2]. The isolated polyphenols compound (xanthone and flavonoids) from leaf, fruit, and root extract of *G. xanthochymus* showed antioxidant activity [1-2]. Xanthone from methanolic extract of *G. xanthochymus* wood has

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tested for its activity as a nerve growth factor (NGF) potentiating [2]. Some of the phytochemicals found from this plant include xanthochymol, acetophenone A, alloathyriol, amentoflavone, fukugetin, guttiferone H, 3,8 ″ biapigenin, cycloxanthochymol, guttiferone E, isoxanthochymol, fukugisone, fukugeron, fukugone. [2], 14,5,6-tetrahydroxy-7-prenyl xanthone, 1,4,6-trihydroxy-5-methoxy-7-prenyl xanthone, 1,2,5,6-tetrahydroxy-7-geranylxanthone, 6-prenyl-apigenin, garciniaxanthone E, 1,4,5,6-tetrahydroxy-7,8-diprenylxanthone, and 1,3,5,6-tetrahydroxy-4,7,8-triprenylxanthone and has cytotoxic activity on breast cancer cell lines and pulmonary adenocarcinoma [1-2]. Root and bark extract of *G. xanthochymus* shows anti-inflammatory activity [1]. Garcienone from *G. xanthochymus* has activity as allelopathy [3]. *G. xanthochymus* seed oil has antibacterial activity mainly at *Escherichia coli, Bacillus subtilis,* dan *Staphylococcus aureus* [2]. *G. xanthochymus* fruit extracts have antibacterial activity in some bacteria include *S. aureus, Streptococcus mutans, S. faecalis, pyrogens, Vibrio cholera, E. Coli, Shigella flexnerii, Pseudomonas aeruginosa, Salmonella typhimurium,* and antifungal (*Candida parapsilosis*) [2]. Xanthochymol, angelicoin B, morelloflavone, volkensiflavone, arexanthochymones A, B and C, garcinexanthones A, and 1,4,6-trihydroxy-5-methoxy-7-prenyl xanthone which have been isolated from *G. xanthochymus,* which have antibacterial activity [2].

This study aims to determine the antityrosinase activity of n-hexane extract and methanolic extract from *G. xanthochymus* pericarp. Tyrosinase is an enzyme that catalyzes the hydroxylation reaction of monophenol to o-diphenol and oxidation of o-diphenol to o-quinone in melanin biosynthesis. [4]. Inhibitory activity of a compound from the tyrosinase enzyme can be used as a cosmetic ingredient because it can maintain the whiteness of the skin. [4].

METHOD

The pericarp of *G.* xanthochymus was obtained from Bogor Botanical Garden (Kebun Raya Bogor) West Java. The pericarp sample was cut into pieces and then dried. [5]. Samples were macerated using n-hexane (PT Smart Lab Indonesia) and methanol (PT Smart Lab Indonesia), respectively [6]. The obtained extract solution was concentrated using a rotary evaporator followed by a water bath [7]. The dried extract was stored in a tightly closed container at 4^{o}C [8]. The extract yield was calculated by the following formula [5]:

$$\text{Yield (\%)} = \frac{Extract\ Weight}{sample\ weight}\ X\ 100\% \qquad (1)$$

The anti-tyrosinase activity of the extract was carried out using a spectrophotometric method with a microplate reader at a wavelength of 490 nm (Versamax ELISA Microplate Reader, USA) [9]. A sample of 100 ppm, enzyme tyrosinase from mushroom lyophilized powder (Sigma-Aldrich, USA) 250 U/mL, phosphate buffer solution (Merck, Germany) 120 µL, 0.1 M, pH 6.8, and 18,488 nM 3,4-dihydroxy-L-phenylalanine (L-DOPA) substrate (Sigma-Aldrich, USA) was inserted into 96-well-microtiter plates, then measured the absorbance using kojic acid as a positive control [10-13]. The assay was carried out in triplicate, and data analysis was performed by calculating the percentage of inhibition using the formula [14]:

$$\text{\% Inhibition} = \frac{(A-B)-(C-D)}{(A-B)}\ X\ 100 \qquad (2)$$

Where A is blank absorbance, B is blank control absorbance, C is sample absorbance, and D is sample control absorbance. This research was carried out in triplicate, and data obtained were averaged so that the mean ± standard deviation (SD) was obtained [15]. The mean results were then compared using the t-test to find out whether the data differed significantly or not at the p-value was less than 0.05 ($p < 0.05$) [16].

RESULT AND DISCUSSION

The endocarp of the *G. xanthochymus* fruit is highly acidic fruit flesh with a slightly bitter but tasty taste. In India's northeast region, this endocarp is used as food for pickles, curry spices, seasoned sauces, and traditional medicine [17]. *G. xanthochymus* fruit was analyzed containing 9.06% flour, 11.44% carbohydrates, 1.64% oil, and 28.04% saponins [17]. Several compounds have been isolated such as benzophenone (guttiferone E, guttiferone A, guttiferone H, acetophenone A, gambogenone, cycloxanthochymol, garcinialiptone, garcicowin C, garcinialiptone, garcixanthochymones A, garcixanthochymones B, garcixanthochymones, cycloanthanthymymonics, cycixanthochymones) biflavonoids, saponins, tannins, alkaloids, and lipids [2,17].

The yield of *G. xanthochymus* pericarp extract was 2.50% (n-hexane) and 41.33% (methanol). *G. xanthocymus* methanolic extract has 219.23 ± 0.15 mg GAE/g total phenolic content, and 63.50±2.12 mg RE/g flavonoid content [18]. The results of phytochemical screening showed a methanolic extract of *G. xanthochymus* fruit containing

saponins, tannins, flavonoids, alkaloids, and terpenoids [17]. Guttiferon H and gambogenone isolated from methanolic extract of *G. xanthochymus* fruit have the activity of preventing colon cancer and breast cancer and antioxidants [17-20]. Also, polycyclic prenylated xanthones from *G. xanthochymus* fruit has an inhibitory activity of glioma cancer cells with IC_{50} values between 1.6 to 6.5 μM [21]. The results tyrosinase inhibition assay demonstrated in Table 1:

TABLE 1. The activity of tyrosinase inhibitory with the concentration of the sample 100 ppm in triplicate

The Sample	% inhibition	Average inhibitory activity (%)
	48.22	
G. xanthochymus pericarp n-hexane extract	29.27	35.41 ± 11.10*
	28.73	
	35.85	
G. xanthochymus pericarp methanolic extract	22.35	28.83 ± 6.77*
	28.28	
	65.07	
kojic acid (positive control)	65.10	65.07 ± 0.03*
	65.04	

*Values of average ± standard deviation for triplicate tests

The tyrosinase inhibition assay results showed that n-hexane and methanolic extract of *G. xanthochymus* pericarp had inhibitory activity even though it was lower than the positive control (kojic acid). The ability to inhibit the work of the tyrosinase enzyme means that it can hinder the process of biosynthesis of melanin because the hydroxylation and oxidation reactions involved in melanocytes require the enzyme catalyst tyrosinase [4]. The tyrosinase enzyme known as a polyphenol oxidase is a key enzyme in the formation of melanin [22-23]. Excessive melanin can cause melasma and age spots that depend on one's heredity and can be a severe aesthetic problem [22-24]. The glutathione depletion and cell damage caused by melanin synthesis [23]. Therefore, anti-tyrosinase use can be used as a preservative for skin protection in cosmetic products [24]. From the results of this study, Table 1 shows that at a concentration of 100 ppm, kojic acid can inhibit 65.07%, meaning that the IC_{50} value of kojic acid is below 100 ppm. While the extracted sample with a concentration of 100 ppm has an inhibition percentage below 50%, it means that the IC_{50} value of the extract is above 100 ppm, with the IC_{50} amount of n-hexane extract lower than methanol extract.

The results of this study indicate that hexane extract of *G. xanthochymus* pericarp has higher tyrosinase enzyme inhibitory activity than the methanolic extract. The results of the statistical analysis of two sample t-tests between hexane extract activity and methanolic extract activity with p-value <0.05 obtained t-test (0.88) $<t_{table}$ (2.92), this shows that the inhibitory activity of the tyrosinase enzyme from the hexane extract was significantly different compared to the methanolic extract. It's due to the presence of the non polar compounds contained in the hexane extracts which contribute in tyrosinase inhibition.

The example of non-polar compounds are fatty acids and polar compounds are amino acids, sugars and other polar metabolites [25-26]. This research showed that the percentage of the yield from the extract using polar solvent (methanol) is bigger than the percentage of the yield from the extract using non-polar solvent (hexane) by multiple times. But the result of the tyrosinase inhibitory activity of the extract using non-polar solvent is higher than the tyrosinase inhibitory activity of the extract using polar solvent. The different of the solvents for extraction affect of the yield percentage and the activity of tyrosinase inhibition.

A study on tyrosinase inhibition carried out in molecular simulations shows that the inhibiting compounds enter the cavity of the tyrosinase enzyme's hydrophobic activity, changing the enzyme conformation so that it affects the catalytic activity of the tyrosinase enzyme [24]. Anti-tyrosinase activity of the compound can be influenced by the presence of hydroxyphenyl groups or hydrophobic groups that can bind to the hydrophobic side of the tyrosinase enzyme [24]. Several studies have shown that the tyrosinase inhibitory activity is also related to its flavonoid content [23].

CONCLUSION

The n-hexane extract had a better tyrosinase inhibitory activity than the methanolic extract, although its activity was lower compared to kojic acid as a positive control.

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