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Tyrosinase inhibitory activity of *Garcinia daedalanthera* Pierre

N S S Ambarwati^{1,*}, B Elya², Y Desmiaty³ and I Ahmad⁴

¹ Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, Jl. Rawamangun Muka, Pulogadung, Jakarta Timur, DKI Jakarta, Indonesia

² Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, Indonesia

³ Department of Phytochemistry, Faculty of Pharmacy, Universitas Pancasila, Jakarta, Indonesia.

⁴ Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia

*neneng_ambarwati@yahoo.co.id

Abstract. The tyrosinase enzyme catalyzes the first stage in two reactions in the synthesis of melanin (the hydroxylation of L-tyrosinase becomes 3,4-dihydroxyphenylalanine (L-DOPA) and oxidation from L-dopa to dopaquinone). Hyperpigmentation in human skin is a general phenomenon that is not desirable. Researchers were encouraged to identify potential new tyrosinase inhibitors for cosmetics, especially anti-hyperpigmentation. This study purposed to determine the inhibitory activity of the tyrosinase enzyme from *Garcinia daedalanthera* Pierre leaves. The extract was obtained by maceration successively method. The anti-tyrosinase assay used the spectrophotometric method at 490 nm. The enzyme used tyrosinase from mushrooms lyophilized powder (Sigma), and the substrate used 3,4-dihydroxy-L-phenylalanine, L-DOPA (Sigma). The tyrosinase inhibitory assay results of 100 ppm (triplicate) showed the leaves extract, including ethyl acetate extract of 33.42 ± 5.98 %, hexane extract was 50.67 ± 0.47 %, and methanol extract of 50.68 ± 1.87 %, respectively. Moreover, the stem bark has activity as follows methanol extract of 43.76 ± 1.41 % and the ethyl acetate extract of 55.71 ± 2.80 %. Percentage inhibition of the positive control (kojic acid) was 65.07 ± 0.03 % at 100 ppm.

1. Introduction

The enzyme of tyrosinase has a role in catalyzing two melanin biosynthesis reactions, namely hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to dopaquinone [1]. Tyrosinase enzyme is a copper-containing enzyme that functions as a cofactor [2]. The tyrosinase overexpression causes excessive production of melanin in the skin and triggers the appearance of hyperpigmentation, resulting in melasma, age spots, and melanoma [2].

Hyperpigmentation in human skin is a general phenomenon that is not desirable [3]. Researchers were encouraged to look for a natural product that can inhibit the work of the enzyme tyrosinase so that it can be used as a variety of needs. This matter of improved screening techniques is also developed in the cosmetics industries, especially for skin-whitening agents. Moreover, the need for materials that can inhibit the work of the tyrosinase enzyme is getting higher, so a lot of screening is done to get the natural products that are increasingly needed in the industrial world as skin whitening agents [3].



Garcinia daedalanthera Pierre is a plant of the Clusiaceae family [4]. *G. daedalanthera* plant commonly found in the Sulawesi forest and has been scientifically proven to provide antidiabetic and antioxidant activity [4]. This research aims to determine how much activity inhibits tyrosinase enzyme from this plant.

2. Materials and methods

The leaves and the stem bark of *Garcinia daedalanthera* Pierre were collected from Bogor Botanical Garden, West Java, Indonesia. Each dry leaves and dry stem bark was extracted with maceration successively using three solvents (methanol, ethyl acetate, and n-hexane) [5]. The extract then went through an anti-tyrosinase assay, which tested with a spectrophotometric method. The enzyme used tyrosinase from mushrooms lyophilized powder (Sigma), and the substrate used L-DOPA, 3,4-dihydroxy-L-phenylalanine (Sigma) [6]. Phosphate buffer solution (pH 6.8, 0.1 M, 120 μ L), L-DOPA solution as substrate (40 μ L, 18.488 mM), the sample (100 ppm), and tyrosinase enzyme solution (40 μ L, 250 U/mL) put in 96 well-microtiter plates. The substrate concentration and the enzyme concentration were obtained from the early optimization test. The blank was made without adding extract samples (only dimethylsulfoxide). The mixed solutions were incubated for ten minutes at 37°C. The measurement of absorbance was measured at 490 nm. The assay was done triplicate [7]. Kojic acid (Thornhill, Canada) as the positive control [7].

The tyrosinase inhibitory activity (%) counted as [8]:

$$\text{Inhibition (\%)} = (1-B/A) \times 100 \quad (1)$$

A is the absorbance of the blank, and B is the absorbance of the sample. The qualitative phytochemical assay was done for the highest tyrosinase inhibitory activity result [9].

3. Results and discussion

The previous study showed that the 80% ethanol extract of the *G. daedalanthera* leaves contained flavonoids, saponins, tannins, phenolic groups, and steroids with the content of total phenolic is 55.13 mg GAE/g, the content of total flavonoid is 15.49 mg QE/g, and total tannin content 7.21 mg TAE/g [4]. The previous study also showed that *G. daedalanthera* leaves 80% ethanol extract to have a lowering effect for LDL-C, HDL-C, triglycerides, and total cholesterol of serum lipids in high-fat-diets rats [10]. The phenolic compounds, especially the factors that are responsible for the activities in plant extracts [3].

Tyrosinase, as a polyphenol oxidase enzyme, is widely spread in plants, animals, and microorganisms include mushrooms. Tyrosinase in fungi is already available and useful at many applications, so this research used this enzyme [11]. Tyrosinase prohibitory activity from plant extracts was executed to uncovering anti-tyrosinase new sources [3].

Tyrosinase is a key enzyme in the melanogenesis process for melanin biosynthesis. Melanin affects the occurrence of skin pigmentation. So competitive tyrosinase inhibitors can be used as a whitening agent [12]. Kojic acid is a famous whitening agent and was used as a positive control [12].

Some researchers investigated some active compounds as tyrosinase inhibitory like chalcones and flavanone as competitive inhibitors. Chalcone derivatives is a potent inhibitor to tyrosinase compare to standard arbutin compound. The resorcinol can be a reason for potent tyrosinase inhibition [12]. Pyridyl azachalcones have also been previously detected to inhibit the tyrosinase enzyme more strongly than kojic acid. This is because nitrogen atoms in the pyridine framework can form complexes with copper ions that are on the active site of the tyrosinase enzyme, so that enzyme activity is inhibited. Another chalcone series exhibited potent inhibits the activity of the tyrosinase enzyme higher than kojic acid. In terms of the structure–activity relationship (SAR) analysis, inhibition of tyrosinase enzyme activity occurs due to the presence of para-nitro substituents in the B (ortho-methoxy) ring. The other inhibitory activity is shown by compounds containing the para-dimethyl amino ring on ring B as electron donors. 2,3-dihydro-1H-indent-1-one derivatives such as chalcone are inhibitors of tyrosinase diphenolase activity, were found to be reversible and competitive [12].

Dihydrochalcones and flavanones inhibit the monophenolase and diphenolase actions of tyrosinase. Dihydrochalcone inhibits the activity of monophenolase and diphenolase from tyrosinase effectively. Flavanone compounds containing resorcinol can significantly inhibit monophenolase and diphenolase of tyrosinase competitively. Isoflavone inhibited monophenolase, and diphenolase is an inhibitor that is competitive and reversible. Resveratrol (3,5,40-trihydroxytrans-stilbene, 9) can inhibit the activity of the tyrosinase enzyme from fungi through a mechanism of K cat (suicide substrate) type inhibition. Resveratrol can inhibit the process of cellular melanin production by suppressing proteins in the process of melanogenesis, such as tyrosinase [12].

This research showed that the highest activity of tyrosinase inhibitory was the *G. daedalanthera* stem bark ethyl acetate extract, although smaller than the activity of positive control (kojic acid). The value of the tyrosinase inhibitory activity from the extract was 55.71 ± 2.80 % at the concentration of 100 ppm, was followed by the leaves methanol extract (50.68 ± 1.87 %) and the leaves hexane extract (50.67 ± 0.47 %). The result ultimately can be seen in Table 1.

Table 1. The activity of tyrosinase inhibitory with the concentration of the sample 100 ppm.

The Sample	Tyrosinase Inhibitory Activity (%)
the leaves methanol extract	50.68 ± 1.87
the leaves ethyl acetate extract	33.42 ± 5.98
the leaves hexane extract	50.67 ± 0.47
the stem bark methanol extract	43.76 ± 1.41
the stem bark ethyl acetate extract	55.71 ± 2.80
kojic acid	65.07 ± 0.03

The phytochemical test of the active extract showed the secondary metabolite, including flavonoids, alkaloids, tannins, glycoside, and anthraquinone.

The tyrosinase inhibitory ability of the extract shows that the extract can be used as a skin whitening ingredient so that it can be used as a cosmetic ingredient. Using plant sources as cosmetics, which is in high demand, is the development of safe and effective tyrosinase enzyme inhibitors. Tyrosinase plays an essential role in the process of melanogenesis. The melanogenesis process plays a role essentially in protect skin from negative effects due to sun exposure, especially the occurrence of skin discoloration [2]. But tyrosinase inhibitors safe and effective from natural sources need to find.

The results of the study exhibited that extracts of *G. daedalanthera* can inhibit the activity of the tyrosinase enzyme. The highest inhibitory activity is the stem bark ethyl acetate extract. The publication describes lest the compounds contained responsible primarily for inhibiting the action of the tyrosinase enzyme [8]. The ability of kojic acid to inhibit the work of the tyrosinase enzyme was higher than the assay samples in this study. The methanol and hexane extract also have tyrosinase inhibitory activity more significant than 50% at 100 ppm concentration.

The content of compounds in the extract affects the inhibitory activity of the tyrosinase enzyme [13]. Kojic acid was reported to have tyrosinase inhibitory activity five times of quercetin [13]. This result showed that the *G. daedalanthera* Pierre plant could be used for skincare. The extract had the ability as a whitening agent.

4. Conclusions

The *G. daedalanthera* Pierre stem bark ethyl acetate extract had tyrosinase inhibitory ability 55.71 % at 100 ppm (part per million) followed by the leaves methanol extract (50.68 %) and the leaves hexane extract (50.67 %). In this research ability to inhibit the action of the tyrosinase enzyme from kojic acid at a concentration of 100 ppm was 665.07 %. The compounds containing *G. daedalanthera* Pierre stem bark ethyl acetate extract were flavonoids, alkaloids, tannins, glycoside, and anthraquinone.

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