

# Tyrosinase Inhibitory Activity of *Garcinia latissima* Miq. Extracts

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## ABSTRACT

**Background:** Tyrosinase is an enzyme that plays an essential part in the process of melanin synthesis. High exposure to ultraviolet (UV) radiation or high stimulation of melanocytes could cause excessive melanin pigments to lead to hyperpigmentation. **Objective:** This study aimed to find potential natural skin lightening ingredients from *Garcinia latissima* Miq. **Methods:** Stem bark, fruits, and leaves of *Garcinia latissima* Miq. were extracted with successive maceration. The tyrosinase inhibitory activity test was measured spectrophotometrically at 490 nm using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as substrate and kojic acid as a positive control. **Results:** The tyrosinase inhibitory activity test at a concentration of 100 ppm showed that the bark ethyl acetate extract 15.94% ± 7.70, bark methanol extract of 28.94% ± 5.73, fruit n-hexane extract 25.16% ± 10.22, fruit methanol extract 23.26% ± 9.10; and leaf methanol extract 30.59% ± 0.63 with kojic acid inhibition 65.07%. **Conclusion:** Methanol extract of leaf from *Garcinia latissima* Miq was the most active extract as a tyrosinase inhibitor.

**Key words:** Successive maceration, Extract, Tyrosinase, *Garcinia latissima* Miq.

## INTRODUCTION

The plant of *Garcinia* family hasn't been much tested for its tyrosinase. There are some plants from *Garcinia* family that have been tested for their tyrosinase. They are : *G. daedalanthera* Pierre, *G. atroviridis* Griff., *G. xanthochymus*, *G. livingstonei*, and *G. kola*<sup>1-4</sup>.

Tyrosinase is enzyme that has a role to develop skin pigmen. The inhibition of this enzyme can help to lighten the skin<sup>5</sup>. The inhibition of the tyrosinase enzyme can be tested in vitro by using spectrophotometric<sup>6</sup>. Tyrosinase test of this plant has not been published yet.

Tyrosinase is an enzyme that catalyzes the hydroxylation of tyrosine into 3,4-dihydroxy-L-phenylalanine (L-DOPA), oxidized L-DOPA into hydroxy indole, oxidized into dopaquinone and after several transformations in melanin biosynthesis<sup>7</sup>. Melanin has a significant role in skin protection from ultraviolet (UV), but excessive melanin synthesis can cause hyperpigmentation<sup>8</sup>. Tyrosinase inhibition is the most used way in the skin depigmentation process<sup>5</sup>.

*Garcinia latissima* Miq. as an indigenous plant from Seram Island, Maluku, and Papua, is known as Dolo magota, but already cultivated in Bogor Botanical Garden, Jawa Barat<sup>9</sup>. It had been proven qualitatively in the previous research that ethyl acetate extract of bark, methanol extract of bark, ethyl acetate extract of the fruit, and methanol extract of fruit from *Garcinia latissima* Miq. contained flavonoid<sup>10,11</sup>. Anti-oxidant test and anti-elastase test of this *G. latissima* Miq. have been conducted<sup>12-14</sup>. By conducting anti-tyrosinase test of *G. latissima* Miq., it can be utilized for cosmetics substance.

Flavonoid containing natural resources can inhibit tyrosinase activity directly in melanogenesis.

Flavonoid is a secondary metabolite that has a polyphenol structure and important component in drugs and cosmetics. Flavonoid is also known as a strong inhibitor for several enzymes<sup>15</sup>. Flavonoid has an important role in inhibiting tyrosinase in melanogenesis. Therefore, able to be a depigmentation agent through bonding with copper and antioxidant mechanism<sup>16</sup>. This research aimed to examine and prove the tyrosinase inhibitor scientifically from *Garcinia latissima* Miq. extracts.

## MATERIALS AND METHODS

### Materials and Equipment

*Garcinia latissima* Miq. was cultivated from Bogor Botanical Garden, and part of bark, leaves, and fruit was used. The solvent used for extraction was n-hexane, ethyl acetate, and methanol (Duta Pratama Chemica, Bogor, Indonesia), lyophilized mushroom tyrosinase ≥ 1000 unit/mg (Sigma Aldrich, Singapore), 3,4-dihydroxyphenylalanine (Sigma Aldrich, Singapore), and kojic acid (Thornhill, Kanada). Rotary evaporator (Buchi R-205, Germany), ELISA Microplate Reader (VersaMax™, USA) in Phytochemistry Laboratory in Faculty of Pharmacy, Universitas Indonesia.

### Extraction Process

Extraction process was conducted by multiple maceration method using three different solvents with a different polarity such as n-hexane, ethyl acetate, and methanol. Each of the materials including fruit, leaf and bark was dried, powdered, and soaked by using solvent for 24 hours. After that each of the extract was dried and tested.

### Tyrosinase Inhibitory Activity Assay

The tyrosinase inhibitory activity assay was using mushroom tyrosinase lyophilized powder, 3,4-dihydroxyphenylalanine (L-DOPA) as substrate,

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**Table 1: Result of tyrosinase inhibitory activity test at sample concentration 100 ppm.**

No.	Sample of <i>G. latissima</i>	Absorbance 1	Absorbance 2	Absorbance 3	Tyrosinase inhibitory activity (%)*
1.	ethyl acetate extract of bark	0.64	0.73	0.69	15.61 ± 5.48
2.	methanol extract of bark	0.63	0.56	0.54	28.94 ± 5.73
3.	n-hexane extract of fruit	0.62	0.52	0.68	25.16 ± 10.22
4.	methanol extract of fruit	0.64	0.69	0.54	23.26 ± 9.10
5.	methanol extract of leaf	0.56	0.57	0.56	30.59 ± 0.63
6.	Kojic acid	0.29	0.28	0.28	65.07 ± 0.86

\*Result in triplicate

and kojic acid as the standard<sup>17,18</sup>. Phosphate buffer (120 µL, 0.1 M, pH 6.8), L-DOPA solution (18.488 mM), sample solution (100 ppm), and tyrosinase (250 U/mL) was added in 96-well-plate based on method optimization<sup>19</sup>. Each sample was used a control without tyrosinase<sup>20</sup>. Blank was using DMSO and blank control without enzyme<sup>21</sup>. After 10 min incubation at 37°C, then absorbance was measured using a microplate reader at 490 nm, triplicate<sup>22</sup>.

Inhibition percentage was calculated using formula below<sup>23</sup>:

$$\% \text{ inhibition} = \frac{(A-B) - (C-D)}{(A-B)} \times 100 \quad (1)$$

where A is blank absorption, B is control blank, C is sample, and D sample control.

## RESULTS

Results of the tyrosinase inhibitory activity test can be seen in Table 1.

## DISCUSSION AND CONCLUSION

The result showed *Garcinia latissima* Miq. extract either from leaves, bark, and fruit had an inhibitory activity but lowered from kojic acid. Kojic acid is a synthetic tyrosinase inhibitor that has been reported and used as a skin-lightening agent in cosmetics<sup>24</sup>. Unfortunately, there are several side effects while using kojic acid, such as erythema and contact dermatitis; therefore, natural products are needed to replace this<sup>24</sup>.

In this research, the tyrosinase enzyme was isolated and purified from *Agaricus biosporus* mushroom. It is the primary source of tyrosinase enzyme, affordable, and possess high similarity and homology with human tyrosinase, dan found by Bourquelot and Bertrand since 1895. Mushroom tyrosinase has two copper, interacts with oxygen in the active site, and is bonded with six histidines<sup>25</sup>.

The substrate used in this research was L-DOPA that is o-diphenol and can be oxidized by tyrosinase into o-quinone (dopaquinone); in contrast, the tyrosinase itself becomes deoxy-tyrosinase<sup>25</sup>. The two molecules of dopaquinone will change into dopachrome that can be measured with UV-spectrophotometry at 490 nm<sup>26</sup>.

The highest tyrosinase inhibitory in *Garcinia latissima* Miq. was leaf methanol extract (30.59 ± 0.63 %), but still lower than kojic acid (65.07 %). Further research was needed to isolate the bioactive compound from the leaf methanol extract in *Garcinia latissima* Miq. Another research from the similar genus *Garcinia subelliptica* was reported to find an isolate of dimer flavon-flavon as a tyrosinase inhibitor and higher than kojic acid<sup>27</sup>. The activity of methanol extract *G. subelliptica* in 500 ppm as a tyrosinase inhibitor was reported 57.2 ± 2.2 % showed a strong tyrosinase inhibitory<sup>28</sup>.

Natural ingredients, especially plants and plant extracts, which have activity as an inhibitor of the activity of the tyrosinase enzyme and inhibit melanin production can be used as an ingredient to maintain skin brightness or a whitening agent, which is usually found in cosmetic preparations<sup>29</sup>. The antioxidant activity of skin lightening

is also related to the inactivation of the melanogenesis process. UV radiation produces reactive oxygen species (ROS) which can activate the tyrosinase enzyme in the skin and cause melanogenesis because the enzymes prefer superoxide anion radicals<sup>29</sup>. The leaf methanol extract *G. latissima* Miq. is most active and most potential as tyrosinase inhibitory activity, so it can be further investigated by fractionating or isolating the extract.

Biflavonoid compound of *Garcinia* has been reported to have a competitive mechanism naturally and unique to tyrosinase based on an in-vitro test<sup>30</sup>. Based on molecular docking, the mechanism of the biflavonoid compound is ligand interaction with copper in the active site<sup>30</sup>. This research showed hydrogen bond interaction between Glu322, His85, and His263 residue with a hydroxyl group in monoflavonoid subunit and formed a complex of stable biflavonoid-tyrosinase. Biflavonoid compound is a promising agent for dermatological disturbance related to hyperpigmentation<sup>30</sup>.

The conclusion of the research is the leaf methanol extract of *Garcinia latissima* Miq. showed a strong tyrosinase inhibitory, probably due to biflavonoid compound.

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## CONFLICTS OF INTEREST

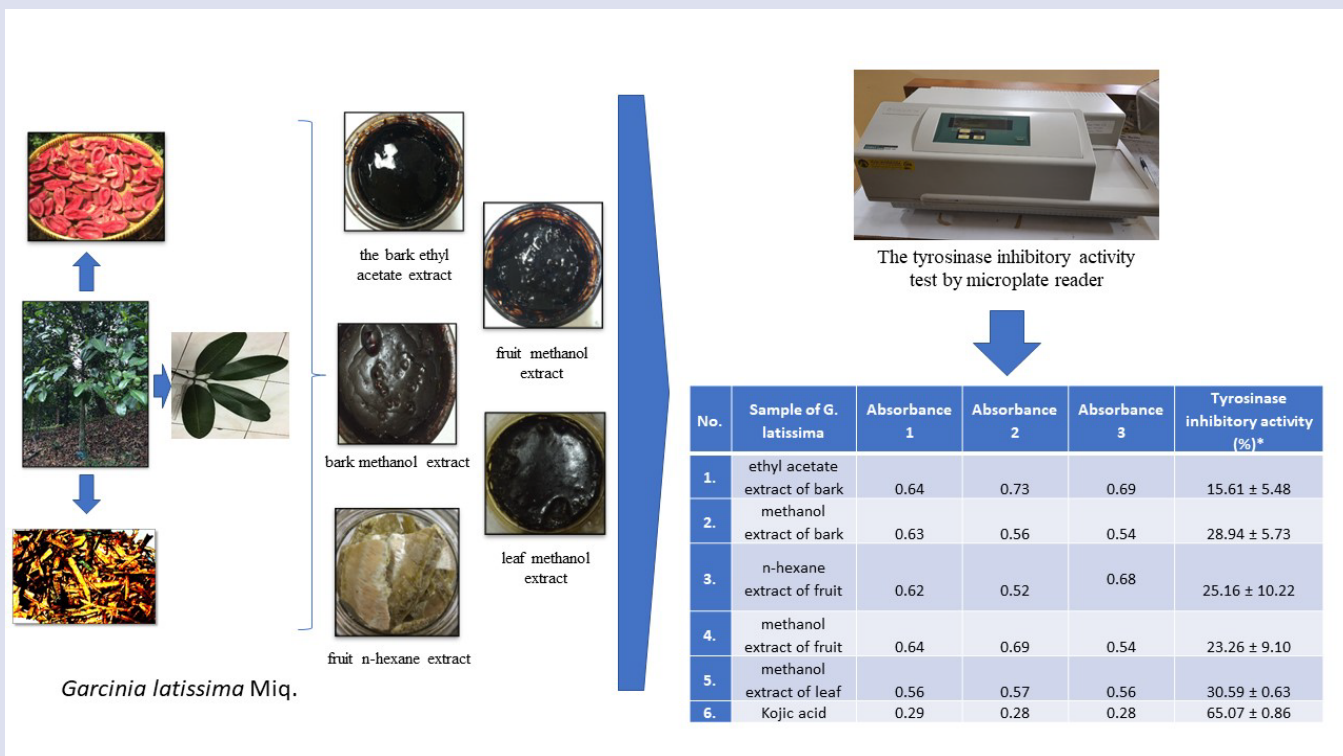
The authors declared no conflicts of interest.

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## GRAPHICAL ABSTRACT



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