

The Study of Tyrosinase and Antioxidant Activity of *Xylocarpus Granatum* Koenig Seed Kernel Extract toward Evidence Based Indigenous Knowledge from Togeian Archipelago, Indonesia

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Abstract

The tyrosinase inhibitory activity and antioxidant activity of three (3) extracts (methanol, chloroform, and n-hexane) from seed kernel of *Xylocarpus granatum* Koenig was tested. The tyrosinase inhibitory activity and antioxidant activity was estimated as IC_{50} values and the content of total phenolics in extracts was calculated as gallic acid equivalent (GAE). The best result of tyrosinase inhibitory activity was obtained in methanol extract in monophenolase activity ($IC_{50} = 323.11 \pm 9.0 \mu\text{g/mL}$) that correlated significantly with the total of phenolic content ($R^2 = 0.98$, $Y = -12.27x + 471.73$) and the antioxidant activity have $IC_{50} = 10.61 \pm 0.6 \mu\text{g/mL}$. We indicate that this methanolic extract of *X. granatum* seed kernel possesses potency as tyrosinase inhibitor and antioxidant agent. This result has already shown that the indigenous knowledge in utilization of *X. granatum* seed kernel for lightening skin has been evident.

Keywords: Antioxidant agent; Tyrosinase inhibitor; *Xylocarpus granatum* koenig

Introduction

Indonesian traditional medicine known as Jamu is an all natural medicine involving the use of natural natural resources including plants, animal and minerals. Originally, Jamu was a term used for Javanese traditional medicine, but how it is used as general term pertaining to Indonesian Traditional medicine. Indonesia is consist of 17.508 islands [1] and each area has own specific traditional medicine. The use of plants and other natural resources for medicinal purposes in Indonesia dates back to pre-historic times. Knowledge of Indonesian traditional medicine has been passed down from generations to generations. About 300 distinct native ethnicities live in Indonesia and each ethnic group has used indigenous knowledge to develop folklore medicine based on availability of material in their own area. There have been a few studies of the traditional medicine of the indigenous tribal people of Togeian (Sulawesi). Many local communities in Togeian archipelago have already been utilized coastal plant for curing disease, health care, beauty care (cosmetic), tonic and sunscreen. Unfortunately, the use of medicinal plants by coastal community has not yet been documented well. Moreover, mangrove's finding for lightening product already becomes a national patent from searching of indigenous knowledge in coastal area.

Coastal area of Indonesia is famous for its richness and biodiversity. One of the coastal ecosystem that provide natural resources for human life is mangrove. Mangrove in Indonesia possess the highest biodiversity in the world with a total of 89 species, consisting of 35 species of plants, 9 species shrubs, 9 species of lianas, 29 species of epiphytes, and two species of parasitic. Mangroves biodiversity is a high valuable asset due to the ecological benefits as a source of food and traditional medicine. The extensive use of natural plants, such as mangrove, as health remedies due to their pharmacological properties is quite common. The investigation of efficacy of plant-based drugs has been paid great attention because of their side effects, cheap and easy availability. According World Health Organization (WHO) around 80% of the world people still rely mainly on plant drugs [2,3]. Mangrove plant has already been used as bioactive compound resources such as

alkaloid, phenolic and steroid [4,5].

Many species of mangrove that uses traditional medicine, for instance, *Xylocarpus granatum* Koenig, local name in Indonesia is Nyirih have many activities such as antibacterial [6], antidiarrheal [7], antifeedant [8], cytotoxicity [9], tyrosinase and antioxidant activities [10]. *Xylocarpus granatum* is one species of Meliaceae family have been traditionally used for medicine by South East Asia shoreline inhabitants. Bioactive compounds of *X. granatum* have been known such as triterpenoid, alkaloids, phenolic acids, flavanol, steroids, and monoterpenoids [11].

Finding skin lightening agent from natural sources is one of our research focuses. To decrease hyperpigmentation and oxidative stress on skin, we need to reduce the formation of melanin in the human body. The formation of melanin in human body is influenced or reduced by several mechanisms, including antioxidant, direct tyrosinase inhibition, melanin inhibition of migration from cell to cell and hormonal activities, etc., [12,13].

Tyrosinase (EC 1.14.18.1), a copper-containing monooxygenase, is a key enzyme that catalyzes melanin synthesis in melanocytes [14,15]. It catalyzes two major reactions, including hydroxylation of L-tyrosine and oxidation of the o-diphenol product, L-DOPA (3,4-dihydroxyphenylalanine). DOPA oxidation produces a highly reactive intermediate that is further oxidized to form melanin by

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a free radical-coupling pathway. If free radicals are inappropriately processes in melanin synthesis, hydrogen peroxide (H_2O_2) is generated, leading to the production of hydroxyl radical ($HO\cdot$) and other reactive oxygen species (ROS) [16]. Oxidative stress may be caused by increasing generation of (ROS) and other free radicals. These ROS enhance melanin biosynthesis, damage DNA and may induce proliferation of melanocytes [17]. Yamakoshi et al. [18] also found evidence that oxidative stress plays a role in the pathogenesis of skin disorders. Besides, ROS scavenger such as antioxidants may reduce hyperpigmentation [19].

The utilization of *X. granatum* seed kernel as tyrosinase inhibitor and antioxidant substances have not been explored which that this part have already been used by Togeian's women for skin care passed down through their ancestor. The objectives of this study were to examine the tyrosinase inhibition and antioxidant activity of *X. granatum* seed kernel extracts. This result will be toward evidence of indigenous knowledge from Togeian Archipelago, Sulawesi.

Material and Methods

Plant material

X. granatum seed kernel was collected from Bangkagi Coast Archipelagic of Togeian, Central Sulawesi in July 2013. Specimen sample (voucher number 1918291) has been identified and deposited in Herbarium Bogoriense Bogor, Indonesian Institute of Sciences (LIPI) Cibinong, West Java, Indonesia.

Chemical and instruments

Mushroom tyrosinase (Sigma Aldirch), L-Tyrosine (Merck), L-DOPA. Kojic acid was obtained from Sigma ($\mu\text{g/mL}$), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbate acid were purchased from Sigma (Czech Republic). Folin-Ciocalteu's phenol reagent, gallic acid, ELISA plate well reader and UV-VIS Spectrophotometer (USA).

Preparation of plant extracts

X. granatum seed kernel was dried and grounded before extraction process. Extraction was performed by using maceration method with 3 different solvents namely n-hexane, chloroform and methanol (25 g sample: 75 mL solvent) for 24 h in triplicate. The extracts were filtered using Whatman filter paper (No. 2) and concentrated in vacuo at 30°C using a rotary. The yields of extracts were calculated.

Phytochemical screening

The preliminary phytochemical screening of various active compound from *X. granatum* seed kernel were accomplished.

Detection of tannins [20]: 10 ml of the ethanolic extract (70% of seed kernel of this plant were filtered by filter paper. Many drops of ferric chloride reagent ($FeCl_3$) (1%) have been added to the different filtered extracts. The presence of tannins is indicated by a blue color.

Detection of saponins [21]: The test tube containing each the previous filtered extracts were shaken for 5 minutes using a vortex. The appearance of big foamy indicates the presence of saponins.

Detection of alkaloids [21]: 0.2 g of the powder of two parts of this plant was dissolved in 10 ml of HCl 1%. Then, they were transferred to a water bath for few minutes. After, 1 ml of the filtrated extract was treated with 2-4 drops of dragendorff's reagent. The presence of alkaloid is indicated by the appearance of an orange reddish precipitation.

Detection of terpenoids [20]: 1 ml of acetic anhydride and 2ml of concentrated sulphuric acid were added to beaker containing 1ml

of previous extracts. The presence of reddish brown on the interface indicated the presence of terpenoids.

Detection of flavonoids [22]: Two solution A and B from both part of the plant extract were prepared. The solution A contains 5 ml of ethanolic extract previously prepared. The solution B is consist of 5 ml of ethanolic solvent added to 5ml of KOH (50%). Then the two solution A and B were mixed together. The presence of flavonoid is indicated by appearance of yellow color.

Tyrosinase activity test

This assay was performed using methods as described earlier [10,23,24]. Our extracts were dissolved in DMSO (dimethyl sulphoxide) to a final concentration of 20 mg/ml. This extract stock solution was then diluted to 600 $\mu\text{g/mL}$ in 50 mM potassium phosphate buffer (pH 6.5). The extracts were tested at the concentrations ranging from 7.812 to 500 $\mu\text{g/mL}$ in a 96-well plate, 70 μL of each extract dilution was combined with 30 μL of tyrosinase (Sigma, 333 Units/mL in phosphate buffer) in triplicate. After incubation at room temperature for 5 min, 11 μL of substrate (2 mM L-tyrosinase or 12 mM L-DOPA) was added to each well. Incubation commenced for 30 min at room temperature. Optical densities of wells were then determined at 492 nm with multiwell plate reader. The tyrosinase activities (monophenolase and diphenolase activities) were analyzed in the present extract of *X. granatum* seed kernel compared to control. Kojic acid (Sigma, Czech Republic) was used as positive control.

Antioxidant assay

The antioxidant activity test was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described in [25].

Total phenolic content (TPC)

Total phenolic content of the extracts obtained from solvents having different dielectric constant values was measured at 725 nm using Folin-Ciocalteu spectrophotometric method with slightly modification [26]. The extract were mixed with Folin Ciocalteu reagent for 5 min, 5% Na_2CO_3 was then added and final volume made up to 25 mL with distilled water. After 60 min the absorbance was measured at 725 nm. Total phenol content was expressed as mg gallic acid equivalents (GAE)/100 g tissue.

Statistical analysis

Data of tyrosinase inhibitory and antioxidant activity were calculated as follow in Equation 1

$$\% \text{ inhibition} = [(A-B)/A] \times 100$$

Where :

A is the absorbance of blank

B is the absorbance of sample

Its correlation between total flavonoid content and tyrosinase inhibition as well as antioxidant activity were analyzed with correlation test, $r=+1$ (positive correlation), $r=0$ (no correlation) and $r=-1$ (negative correlation). All data were calculated using the microsoft excell computer program.

Result and Discussion

Xylocarpus granatum seed kernel extract

The extraction of *X. granatum* seed kernel resulted rendement of

methanolic extract is 21.48% whereas the rendement of chloroform extract is 1.87% and n-hexane extract is 1.97%. Three extracts of *X. granatum* seed kernel, namely methanol, chloroform, n-hexane extracts contain different secondary metabolite content (Table 1). Methanolic extract contained polar type compound such as phenolic compound groups (flavonoid and tannin) and saponin while chloroform and n-hexane extracts which contain semi-polar and non polar type compound were not consisted of flavonoid, tannin and saponin. According to Chang [27] that *X. granatum* species contains flavonoid compound group which are potent as tyrosinase inhibitor and antioxidant.

Based on the different constituents including n-hexane, chloroform and methanol extracts. We screened their tyrosinase inhibitory activity (monophenolase and diphenolase) and also antioxidant activity. This result on Table 2 shown that tyrosinase inhibitory activity of *X. granatum* seed kernel methanolic extract is the best extracts which can inhibit monophenolase ($IC_{50}=323.11 \pm 9.0 \mu\text{g/mL}$) and diphenolase activity ($IC_{50}=1926.03 \pm 81 \mu\text{g/mL}$) with kojic acid as positive control. Furthermore, the methanolic extract also has antioxidant activity ($IC_{50}=10.61 \pm 2.1 \mu\text{g/mL}$). Moreover, n-hexane and chloroform extracts have no IC_{50} value until concentration of 2000 $\mu\text{g/mL}$ for tyrosinase inhibition and 100 $\mu\text{g/mL}$ for antioxidant. These result shown that non-polar and semi-polar compound in *X. granatum* seed kernel has not possess ability in inhibition of tyrosinase and antioxidant activities.

Previous study stated that *X. granatum* wood have already been tyrosinase inhibitory and antioxidant activity [10]. The part of *X. granatum* stem and bark also possess potency as tyrosinase inhibitor [28] and *X. granatum* fruit peel also have tyrosinase inhibitory and antioxidant activities [29]. Thus, *X. granatum* seed kernel extract that tested their bioactivity in tyrosinase inhibition and antioxidant activities was new finding based indigenous knowledge in Togeian Archipelago, Sulawesi (Figure 1).

Phytochemical Properties	Extracts		
	n-hexane	Chloroform	Methanol
Flavonoid	-	-	+
Saponin	-	-	+
Tannin	-	-	+

Table 1: Phytochemical components of *X. granatum* seed kernel.

Extract	IC_{50} ($\mu\text{g/mL}$) Monophenolase	IC_{50} ($\mu\text{g/mL}$) Diphenolase	IC_{50} ($\mu\text{g/mL}$) Antioxidant
Methanol	323.11 ± 9.0	1926.03 ± 81	10.61 ± 2.1
Chloroform	-	-	-
n-hexane	-	-	-
Aqueous	-	-	-
Kojic acid	46 ± 0.9	204.08 ± 0.9	
Ascorbate Acid			2.11 ± 0.6

Note: IC_{50} = Concentration of extract can inhibit tyrosinase activity as much as 50%; - : it didn't achieve 50% inhibition at maximum concentration of 2000 $\mu\text{g/mL}$. For antioxidant activity can achieve 50% inhibition at maximum concentration of 100 $\mu\text{g/mL}$.

Table 2: Tyrosinase IC_{50} value of three extracts from *X. granatum* seed kernel.

These result addressed the truth of indigenous knowledge from Togeian Archipelago in utilizing of *X. granatum* seed kernel by Togeian women as lightening product. According to interview with Togeian women that mangrove *X. granatum* in Togeian Archipelago was abundant especially in Bangkagi Coast. The extent of *X. granatum* species estimated about ± 17 hectares. Flowering and fruiting throughout year. In India, the seed kernel of *X. granatum* are used as tonic [7]. Unfortunately, *X. granatum* species have decreased because utilization of wood for making of boat, bridge, charcoal and others. Thus, we conducted socialization of mangrove conservation to local community to keep sustainability of *X. granatum* species.

Therefore, new finding of tyrosinase inhibitor and antioxidant agent from *X. granatum* seed kernel give fruitful information for cosmetic industry. All *X. granatum* parts could be complementary each other in providing the raw material of cosmetic product. Great concern in searching natural compound particularly *X. granatum* that explored by scientist to tackle problem concerning aesthetic and dermatology. Thus, *X. granatum* species constitutes prospective marine plant for cosmetic interest. This utilization still traditionally used by coastal community for skin care.

Total phenolic content

Total phenolic of three extracts (methanol, ethanol and aqueous) and its extract were expressed as gram gallic acid equivalent/100 g dry material. The amount of total phenolic content varied in 3 extracts ranged from 7.55 to 24.12 gram gallic acid equivalent/100 gram dry material. The highest total phenolic contents were detected in "methanolic extract" and the lowest in "water extract". (Figure 2).

Its correlation coefficient of $R^2=0.98$, ($Y= -12.27x + 471.73$) whereas for IC_{50} of diphenolase (Figure 3) had a correlation coefficient of $R^2=0.52$, ($Y= -0.28x + 25.67$) (Figure 4). These results suggested

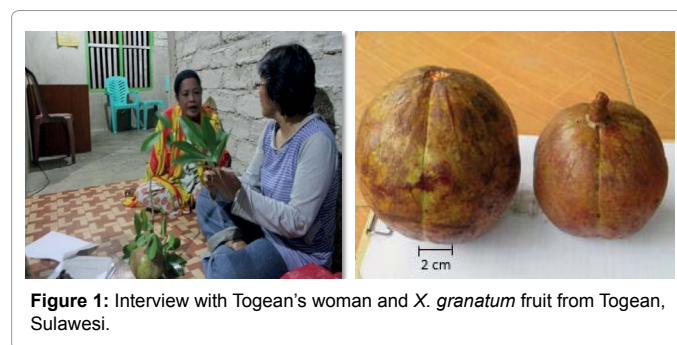


Figure 1: Interview with Togeian's woman and *X. granatum* fruit from Togeian, Sulawesi.

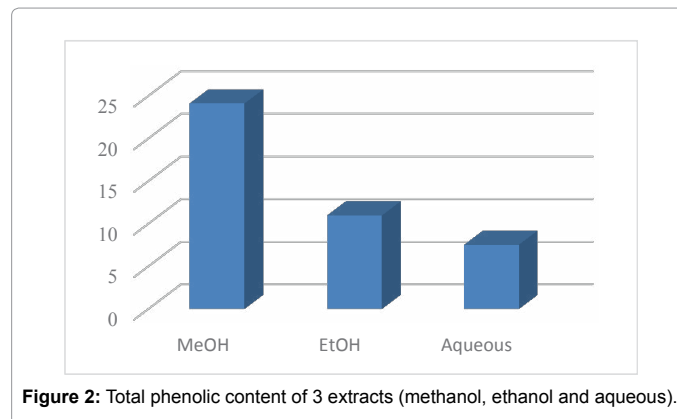
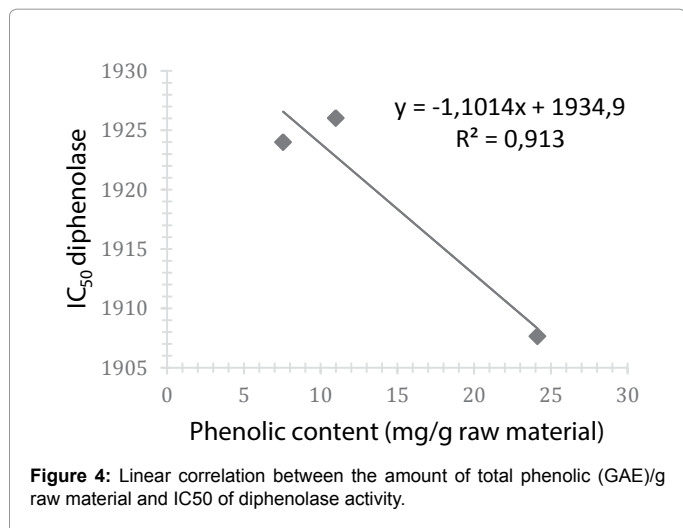
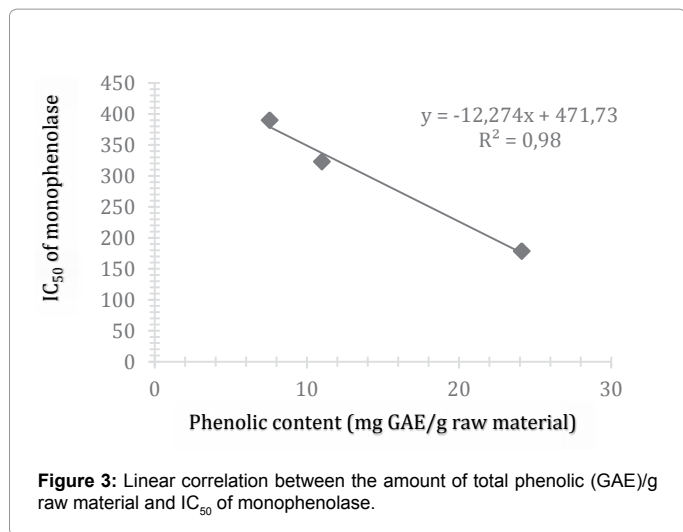


Figure 2: Total phenolic content of 3 extracts (methanol, ethanol and aqueous).



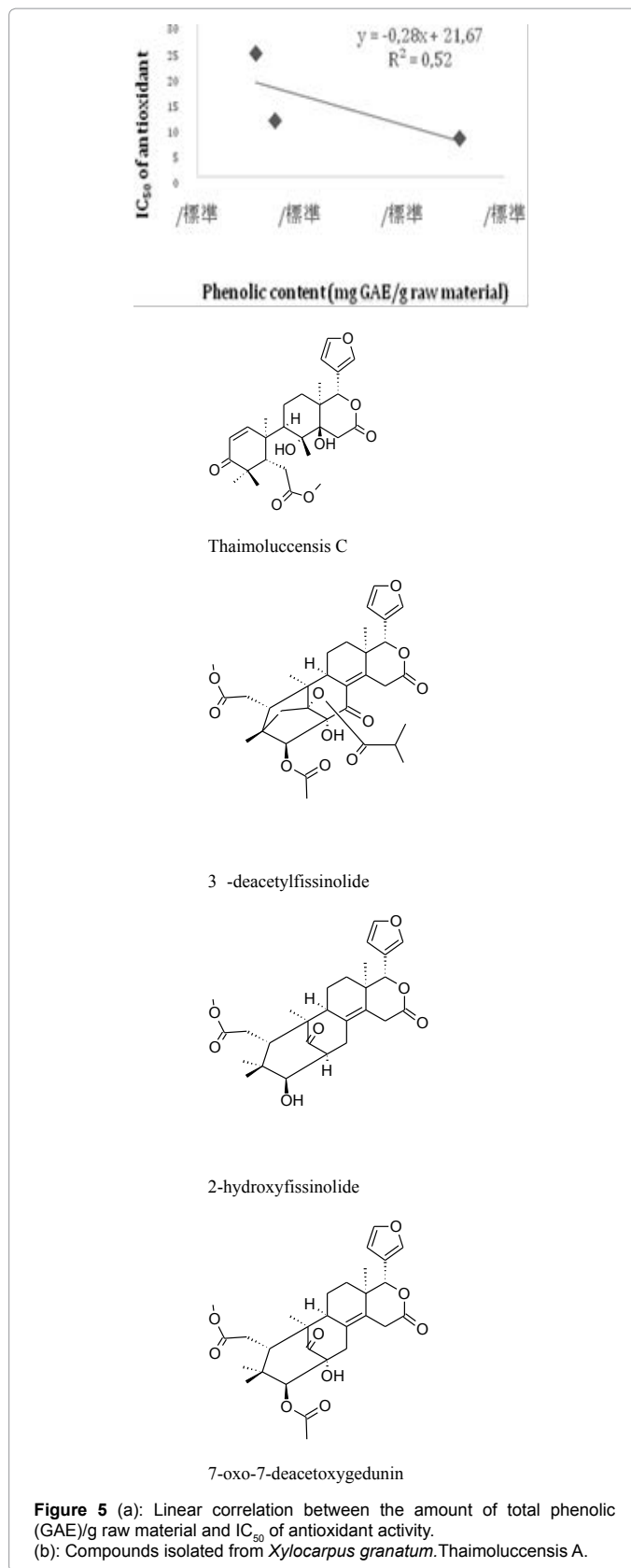
positive correlation of monophenolase and diphenolase activity with tyrosinase activity, the antioxidant activity of three extracts was not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites like e limonoid, alkaloid and hidroquinone. Some compounds such as thaimoluccensis A, thaimoluccensis C, 3 β -deacetylfissinolide, 7-oxo-7-deacetoxygedunin, could be involve to the skin care since these compounds had been isolated and reported as anti-inflammatory activities [30]. Structure the two compounds is shown in Figure 5.

Conclusion

Part of *X. granatum* seed kernel have potential as tyrosinase inhibitor and antioxidant agent. It shown the truth of indigenous knowlegde in utilizing *X. granatum* seed kernel by Togeang's women as skin lightening have been scientifically proved by this research.

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