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Tyrosinase Inhibition, Antiglycation, and Antioxidant Activity of *Xylocarpus granatum*

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Abstract. *Xylocarpus granatum* is mangrove plant that traditionally used as face powder in Central Sulawesi, Indonesia which related to antioxidant, antiglycation and tyrosinase inhibition activities. This study aimed to evaluate the potency of *X. granatum* as a tyrosinase inhibitor, antiglycation, and antioxidant. The leaves, stem, stem bark, fruit flesh, fruit peel, and kernel of *X. granatum* were extracted using ethanol, then their tyrosinase inhibition, antiglycation, and antioxidant were evaluated. Tyrosinase inhibition activity was evaluated using *in vitro* assay with L-tyrosine and L-DOPA as the substrate of monophenolase and diphenolase. Antiglycation activity was studied by measuring the excitation and emission fluorescence from glucose and fructose reaction with Bovine Serum Albumin. Antioxidant activity was measured using the DPPH radical scavenging assay. The result showed that the ethanolic extract of fruit flesh has higher potency as a tyrosinase inhibitor (IC_{50} of 393.8 mg/L and IC_{50} of 448 mg/L, respectively for monophenolase and diphenolase). Antiglycation assay showed that the ethanolic extract of stem bark provides the strongest antiglycation activity with an IC_{50} of 118.1 mg/L. Meanwhile, fruit peel provides the strongest antioxidant activity with an IC_{50} of 5.5 mg/L. Fractionation of ethanolic extracts of each part of *X. granatum* tree yield fractions with lower bioactivity compared to the crude extract. Moreover, stem extract and fractions from two different locations (Tarakan and Kendari) tend to have different bioactivities strengths. The stem part of *X granatum* could be developed as a new raw material of cosmetic products in Indonesia, while ethanol as the solvent for extraction and the different bioactivity of stem extract from a different location can be the consideration for the industry to standardize the extract prior to production of the final product.

Keywords: Antiglycation, Antioxidant, Tyrosinase inhibition, *Xylocarpus granatum*

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INTRODUCTION

Xylocarpus granatum is a mangrove species grows in the upper intertidal zone of mangrove forests and native to Tropical mangrove forests (Allen et al., 2003). This mangrove species is widely used by the local community as traditional medicinal plants, while the wood of this plant is used to make boats and furniture. Previous research reported that bark of *X. granatum* has the potency as anti-diarrhea, anti-bacterial, anti-diabetic, lipase inhibition, and antioxidant (Markers, 1972; De Bruyne et al., 1999; Scalbert, 1991; Veluri et al., 2004; Batubara et al. 2009, Das et al., 2019). Active compounds of *X. granatum* fruit possess a significant antisecretory effect on peptic ulcers (Lakshmi et al., 2010). Another study reported that the extract of *X. granatum* seed kernel has antioxidant and tyrosinase inhibition activity (Zamani et al., 2015).

Besides being used as a medicinal plant, *X. granatum* (especially its fruit) is used by the local community of Togean Island, Central Sulawesi, Indonesia as a facial mask for the bride. Based on that local knowledge, the potency of *X. granatum* as a cosmetic ingredient and personal care is an interesting aspect to be explored. In tropical countries with high intensity of sunshine throughout the year, cosmetics and personal care with lightening activities are very popular. Furthermore, cosmetics that appeal to women are ones with antiaging activity. On the other hand, cosmetics that contain antioxidants are also needed because it can protect the skin from the radical species that cause damage to the skin.

The research purposes was to evaluate the potency of *X, granatum* as a lightening, anti-aging, and antiox-

idant agent using a different part of *X. granatum*, the different solvent of extraction/fractionation, and different growth location. The information about the most effective part of the plant to be used for cosmetics purposes, the best solvent for extraction, and the quality of raw material from a different location, will be scientific evidence to develop the new cosmetic product in Indonesia as well as to standardize the product.

METHODS

X. granatum was collected from Tarakan, North Kalimantan and Kendari, Southeast Sulawesi, Indonesia. Chemical reagents such as 2,2'-diphenylpicryl hydrazyl (DPPH), ethanol, L-tyrosine, L-DOPA, tyrosinase, methanol, ethyl acetate, *n*-hexane, bovine serum albumin (BSA), glucose, fructose, ascorbate acid, dimethyl sulphoxide (DMSO) were purchased from SIGMA Aldrich and Merck and used without any purification. ELISA plate well reader (Merk Biotek Epoc Spektro UV-Vis) was used for antioxidant and tyrosinase inhibition assay, while fluorometer (FluoroStar BMG Labtech) was used for antiglycation assay.

Sample Preparation, Extraction, and Fractionation

The sample was separated into several parts, namely leaves, stem, stem bark, fruit flesh, fruit peel, and kernel. Each part of the sample was washed and dried in the oven at a temperature of 40-60 °C. The dried sample was ground to obtain sample powder with an average size of 20 mesh prior to the extraction process. Ethanol was used as the solvent in the maceration extraction of the samples. The crude ethanolic extracts were then dried using a rotary evaporator prior to use in the bioactivity test. Liquid-liquid fractionation was conducted using *n*-hexane and ethyl acetate to the ethanolic extract to obtain fractions with different polarities.

Tyrosinase Inhibitory Assay

Tyrosinase inhibitory activity was evaluated based on inhibition of the sample to monophenolase and diphenolase activity. The assay was carried out using L-tyrosine and L-DOPA as the substrates, following the method as described by Batubara et al. (2010). Kojic acid was used as a positive control.

Antiglycation Assay

Antiglycation activity was evaluated according to the Povichit et al. (2010) method with modification as mention by Zahra et al. (2016). Aminoguanidine was applied as a positive control.

Antioxidant Assay

Antioxidant activity was evaluated based on DPPH radical scavenging activity, following Batubara et al. (2009) with modification. The amount of 100 µL DPPH solution 125 µM was mixed with 100 µL of the sample in various concentrations. The mixture was then incubated at 37 °C for 30 minutes. The absorbance of the sample was measured at 517 nm. Ethanol was used as solvent and blank, while ascorbic acid was used as positive control.

$$\text{Inhibition (\%)} = \left[1 - \frac{Asample - Acontrol}{Ablank - Acontrol}\right] x\ 100\%$$

Where,
Asample: Absorbance of the sample
Acontrol: Absorbance of ascorbic acid as the positive control
Ablank: Absorbance of ethanol as the blank

Statistical Analysis

The data resulted in this study was performed in triplicate and reported in the average. T-test was used for evaluating the activity data obtained using two ways ANOVA, followed by Duncan multiple comparison tests.

RESULTS AND DISCUSSION

As described before, the fruit of *X. granatum* is empirically used by the local coastal community for facial mask, and it is believed to be able to enhance the woman's beauty. Zamani et al. (2015) reported that fruit peel of *X. granatum* has antioxidant and tyrosinase inhibitory activity, thus make this plant is prospective to be utilized as an active ingredient of cosmetic. Otherwise, the amount of fruit that can be collected from *X. granatum* tree is limited. Therefore, the investigation to evaluate the potency of other parts of *X. granatum* as active ingredients of cosmetics is needed. We selected the aerial part, including leaf that is available every time, stem (including the twig), and fruits. The fruit was also divided into three parts i.e., fruit peel, fruit flesh, and kernel. In this study, the leaves, stem, stem bark, fruit flesh, fruit peel, and kernel of *X. granatum* (Figure 1) were evaluated for their tyrosinase inhibition activity, antiglycation, and antioxidants activity.

(a) (b)

Figure 1. *Xylocarpus granatum* and its parts, stem, leaf, fruit (a) and its fruit with the part: fruit peel, fruit flesh (seed coat), and seed kernel (b)

Table 1. Tyrosinase inhibitory, antiglycation, and antioxidant activity of ethanolic extract of the different part of *X. granatum*

Ethanolic extract of	Tyrosinase inhibitory activity (IC_{50}, mg/L)		Antiglycation (IC_{50}, mg/L)	Antioxidant (IC_{50}, mg/L)
	Monophenolase	Diphenolase		
Leaves	>12500^{d}	>12500^{f}	>500^{e}	253.3^{g}
Stem	885.4^{c}	2150.0^{d}	125.9^{b}	6.8^{c}
Stem Bark	394.4^{b}	1792.3^{c}	118.1^{b}	12.0^{f}
Fruit flesh	393.8^{b}	448.0^{b}	447.9^{d}	8.9^{d}
Fruit peel	417.0^{b}	3340.7^{e}	309.1^{c}	5.5^{b}
Kernel	447.1^{b}	2330.0^{d}	>500^{e}	10.8^{e}
Ascorbic acid	N.A	N.A	N.A	3.5^{a}
Kojic acid	46.1^{a}	78.3^{a}	N.A	N.A
Aminoguanidine	N.A	N.A	20.11^{a}	N.A

N.A: Not applicable; Data followed by the same letter in the same column are not significantly different according to Duncans multiple comparison test (P<0.05).

Tyrosinase inhibitory activity was evaluated to measure the ability of samples as lightening agents. Tyrosinase inhibition is correlated with the decrease of melanogenesis on the skin since this enzyme is responsible for melanogenesis or hyperpigmentation in mammals, including humans (Chang, 2009). Tyrosinase activity was evaluated using two types of substrate, namely L-tyrosine (evaluate the activity of monophenolase) and L-DOPA (evaluate the activity of diphenolase). Investigation of tyrosinase inhibitory activity showed that fruit flesh of *X. granatum* possess higher inhibitory activity against tyrosinase (IC_{50} of 448 and 394 mg/L, respectively for L-tyrosine and L-DOPA) compared to the other parts of the plant, even though its inhibitory activity is lower than kojic acid (positive control). The activity of the fruit flesh is not significantly different compared with stem bark, fruit-peel, and kernel extract on monophenolase reaction but significantly different on diphenolase reaction. On the other word, the other parts of *X. granatum* plant that shows tyrosinase inhibitory activity for the L-tyrosine substrate are stem, stem bark, fruit peel, and kernel (Table 1). Batubara et al. (2010) reported that some Indonesian medicinal plants, including *X. granatum* has the potency as an inhibitor of tyrosinase.

The potency of *X. granatum* extract as an anti-aging agent was evaluated through antiglycation assay. Glycation is a reaction between free reducing sugars with free amino groups of proteins, DNA, and lipids. This spontaneous reaction leads to the formation of advanced glycation end products (AGEs) (Kim et al., 2017) and furthermore lead to a loss of protein function and impaired elasticity of tissues, including skin and associated with promoting aging (Semba et al., 2010; Nguyen & Katta, 2015). Antiglycation activity assay shows that the ethanolic extract of stem and stem bark of *X. granatum* have potency as an antiglycation agent (IC_{50} of 118 and 126 mg/L, respectively), but not as good as aminoguani-

dine as a positive control. This result agrees with the report of Sapitri et al. (2019) that claimed that the stem of *X. granatum* has antiglycation activity. The next prospective extract is fruit peel extract that is also had antiglycation activity (Table 1).

Reactive Oxygen Species (ROS) enhance melanin biosynthesis, induce proliferation of melanocytes (Yasui & Sakurai, 2003), and plays an important role in AGEs formation and accumulation which leads to pathogenesis of skin (Yamakoshi et al., 2003). Therefore, antioxidant activity is an important aspects related to skin health and skin protection. In this study, the antioxidant activity of *X. granatum* extract and fraction were evaluated by DPPH radical scavenging activity assay. The result shows that all of ethanolic extracts from all part of *X. granatum* (stem, stem bark, fruit peel, fruit flesh, and kernel) has big potency as antioxidant agents. Strongest antioxidant activity was performed by fruit peel and stem with IC_{50} of 5.5 and 6.8 mg/L, respectively, while IC_{50} of ascorbic acid as a positive control was 3.5 mg/L (Table 1).

Based on the three *in vitro* assay determination on this study, the most prospective parts are stem and fruit-peel. Stem and fruit-peel have a high tyrosinase inhibition activity on monophenolase, high antiglycation activity, and high antioxidant activity. Fruit-peel can be utilized as cosmetic raw materials because this part is not used for generative purposes, only the seed kernel and seed coat is important for generative purposes. The fruit is not available all year, so it is more reasonable to select the stem part to be developed as a new cosmetic product. These results also suggest not selecting the main stem as cosmetic raw materials, but the small stem such as twig can be used. By using a small stem, it will conserve the plant. By pruning process of *X. granatum*, the farmer could collect the twig or branch and use it as cosmetic raw materials.

In order to select the best solvent to be used to prepare the cosmetic product, the fractionation of ethanolic extract was performed. Fractionation of ethanolic extract of leaves, bark, fruit peel, and stem was carried out using *n*-hexane and ethyl acetate. Evaluation of tyrosinase inhibitory activity, antiglycation activity, and antioxidant activity were also conducted to each fraction. The results showed that bioactivities of *n*-hexane, ethyl acetate, and residual ethanol fraction are lower compared to its crude ethanolic extract (Table 2). The antioxidant activity that gives a higher activity after fractionation was found on the stem-bark extract. The first ethanolic extract of stem bark has IC_{50} of 12.0 mg/L (Table 1), and after fractionation, the activity is higher such as shown on the ethyl acetate fraction with IC_{50} of 4.2 mg/L (Table 2). For the antiglycation activity, the increasing activity after fractionation is found on fruit-peel extract (IC_{50} 309.1 mg/L before fractionation and after fractionation on ethyl acetate and residual ethanol has lower IC_{50} of 103.0 mg/L and 166.0 mg/L, respectively). The results indicated that active constituents in ethanolic extract probably synergistically provide the bioactivities, although this assumption needs to be further proven. These results suggest to use only ethanol as the solvent for extraction of *X. granatum* for cosmetic raw materials, and further separation to find active ingredient is better using ethyl acetate for fractionation.

On the basis of the bioactivities of each extract of *X. granatum* parts and considering the amount of the extract; thus comparison of the bioactivities of the stem extract and fractions collected from two different areas (Tarakan, North Kalimantan and Kendari, Southeast Sulawesi, Indonesia) were carried out. The results indicated that ethanolic extract of *X. granatum* stem from Kendari provide stronger antioxidant compared to the sample from Tarakan, except for its *n*-hexane fraction. Tyrosinase inhibition of *X. granatum* stem from Kendari also tend to be stronger (for L-tyrosinase substrate) compared to that from Tarakan. Meanwhile, tyrosinase inhibition (for L-DOPA substrate) of both samples are comparable (Table 3). From these results, we can state that the origin of the *X. granatum* could influence its bioactivities; this may be due to the difference in environmental conditions.

Based on this research results, it is found that the most prospective parts of *X. granatum* that could be developed as cosmetic raw materials is stem/twig and fruit-peel. As cosmetics raw material, ethanol can be used as a solvent for extraction, and it is not important to separate further. The industry that will use this material as cosmetics raw materials needs to consider the origin of plants and standardize it to make sure the quality, safety, and efficacy of the product.

Table 2. Tyrosinase inhibitory, antiglycation, and antioxidant activity of fractions of ethanolic extract of the different part of *X. granatum*

Sample	Fraction	Tyrosinase inhibitory activity (IC_{50}, mg/L)		Antiglycation (IC_{50}, mg/L)	Antioxidant (IC_{50}, mg/L)
		Monophenolase	Diphenolase		
Leaves	*n*-Hexane	8017.4^{i}	3403.7^{f}	$>500^{g}$	$>500^{i}$
	Ethyl acetate	$>12500^{j}$	$>12500^{k}$	$>500^{g}$	$>500^{i}$
	Residual ethanol	$>12500^{j}$	11992.2^{j}	$>500^{g}$	303.8^{h}
Stem	Hexane	1469.0^{g}	9272.0^{i}	$>500^{g}$	26.9^{f}
	Ethyl acetate	444.6^{b}	665.9^{c}	160.6^{c}	53.5^{g}
	Residual ethanol	2083.4^{h}	578.7^{b}	232.2^{d}	18.6^{e}
Stem Bark	*n*-Hexane	1343.7^{f}	8587.1^{h}	$>500^{g}$	27.9^{f}
	Ethyl acetate	1217.4^{e}	2962.2^{e}	245.6^{e}	4.2^{b}
	Residual ethanol	628.5^{c}	1262.2^{d}	245.7^{e}	12.5^{d}
Fruit peel	*n*-Hexane	2081.2^{h}	4311.2^{g}	398.6^{f}	56.5^{g}
	Ethyl acetate	1149.1^{d}	706.5^{c}	103.0^{b}	8.4^{c}
	Residual ethanol	1454.1^{g}	3395.2^{f}	166.9^{c}	11.3^{d}
Ascorbic acid		N.A	N.A	N.A	3.5^{a}
Kojic acid		46.1^{a}	78.3^{a}	N.A	N.A
Amoniguanidine		N.A	N.A	20.11^{a}	N.A

N.A: Not applicable; Data followed by the same letter in the same column are not significantly different according to Duncans multiple comparison test. P<0.05

Table 3. Tyrosinase inhibition and antioxidant activity of extract and fraction of *X. granatum* stem from Kendari

Origin of Samples	Stem of X. granatum	Antioxidant Activities (IC50, mg/L)	Antityrosinase Activities with L-tyrosine as Substrate (IC50, mg/L)	Antityrosinase Activities with L-Dopa as Substrate (IC50, mg/L)
Kendari	Ethanolic crude extract	3.2a	512.0d	609.3b
	n-Hexane fraction	216.4b	>1000e	>1000e
	Ethyl acetate fraction	3.2a	355.4c	882.7c
	Residual ethanol fraction	3.4a	291.6b	957.4d
Ascorbic acid		2.8a	N.A	N.A
Kojic acid		N.A	39.7a	65.5a

N.A: Not applicable; Data followed by the same letter in the same column are not significantly different according to Duncans multiple comparison test. P<0.05

CONCLUSION

X. granatum has a potency to be utilized as an active ingredient of cosmetics and personal care since it provides tyrosinase inhibitory, antiglycation, and antioxidant activity. Part of *X. granatum* tree that performs higher tyrosinase inhibition activity is fruit flesh. However, the stem of *X. granatum* provides higher antioxidant and antiglycation bioactivities, and this part of *X. granatum* tree has higher availability compared to the other parts of the plant. Fractionation of ethanolic extract of each part of *X. granatum* tree does not provide fraction with higher bioactivities. Meanwhile, stem extract and fractions from different areas tend to have different bioactivity strengths.

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