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Potency of Jack Fruit Leaves as Tyrosinase Inhibitor

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Abstract

The aim of this study is to screen whitening agent potency from Jack fruit leaves compared between 96% ethanol extract from young Jack fruit leaves (green to white) and mature Jack fruit leaves (dark green). The phytochemistry examination showed that there was a strong indication of flavonoid in young Jack fruit leaves in 96% ethanol extract. The methods for screening as whitening agent is based on tyrosinase inhibitor potency using mushroom tyrosinase and the result of the young Jack fruit leaves in 96% ethanol extract are the most potent as tyrosinase inhibitor (monophenolase 29,9 µg/mL^{-1} and diphenolase167,3 µg/mL^{-1}) compared with the mature Jack fruit leaves in ethanol extract (monophenolase 214,2 µg/mL^{-1} and diphenolase 358,1 µg/mL^{-1}) and young Jack fruit leaves in Ethyl Acetate Fraction (monophenolase 81,8 µg/mL^{-1} and diphenolase 1557 µg/mL^{-1}). Their IC_{50} values for young Jack fruit leaves 96% ethanol extract monophenolase are not significantly different with kojic acid as positive control.

Key words: Jack fruit leaves; phytochemistry; tyrosinase inhibitor.

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1. Introduction

Many plant extracts form the basis of medical treatments in ancient civilizations and many traditional cultures. Over the past decade, there has been fervent interest in products found in nature because of their perceived safety. Many believe that if a product can be safely ingested, it will also be safe for topical application [1]. In Western countries, skin lighteners are applied for the prevention and treatment of irregular hyperpigmentation, such as melasma, freckles or age spots. In Asia, the use to make the skin whiter is widely extended by traditional beliefs [2]. Plants used in this research are the leaves from the Jack fruit (Artocarpus heterophyllus) which is known in Indonesia as a common fruit which the dried leaves are used in Sumatra for generations as traditional whitening mask. Khan and his colleagues [3] reported this plant has many medicinal properties; the seed and fruit as tonic, the roots for diarrhea and fever, the wood for muscle contraction and skin whitening, the leaves for lactating in human and animals, siphilis, worms, ulcers, wound [4] and also animal feed [5].

Many research have been carried out to find new source of whitening agent from nature to inhibit the biosynthesis of melanin in the human skin. Arung et. al did a screening test in artocarpus species and found 9 flavonoids and a stilbene compound have been isolated. This isolated compound showed to inhibit melanin production in B16 melanoma cells culture [6, 7].

Flavonoids are natural plants polyphenols found in leaves, bark and flowers. This polyphenolic compounds are known to have anti-inflamatory, antiviral, antioxidant and anticarcinogenic properties [2, 8, 9]. Flavonoids may have hypo pigmenting capabilities by directly inhibiting tyrosinase activity at distal portions of the melanogenic pathway [2] Structure-function analysis of flavonoids suggest that flavonoids with an α-keto group show potent tyrosinase inhibition due to the similarity between the dihydroxyphenyl group of DOPA and the α-keto containing flavonoids [9].

Tyrosinase is known to be an key enzyme involved in melanin biosynthesis in plants, microorganisms and mammalian cells. This enzyme catalyses two different reactions: the hydroxylation of monophenols to o-diphenols (monophenolase activity), and the oxidation of o-diphenols to o-quinones (diphenolase activity), which in turn, are polymerized to brown, red or black pigments [10]. Many tyrosinase inhibitors have been tested in cosmetics and pharmaceuticals as a way of preventing overproduction of melanin in epidermal layers [11].

Tyrosinase inhibition is the most common approach to achieve skin hypopigmentation as this enzyme catalyses the rate-limiting step of pigmentation [2]. Tyrosinase inhibitor have been used frequently in cosmetics and depigmenting agents for hyperpigmentation. It catalyzes two different reactions using molecular oxygen; the ortho hydroxylation of tyrosine (mono-phenols) to 3,4-dihydroxyphenilalanine or DOPA (o-diphenols) named monophenolase activity and the oxidation of DOPA to dopaquinone (o-quinones) named diphenolase activity. The oxidation results in polymerized to brown, red, or black pigments by free radical coupling pathway [10].

2. Material and Methods

Plants material: young Jack fruit leaves taken from Bogor Botanical Garden, West Java and the species

determined by The *Herbarium Bogoriensis*. The leaves are divided into 2 categories: young leaves (green to white) and mature leaves (dark green).

3. Preparation of Plants Extract and`Fraction

The extraction of young and mature Jack fruit leaves was done in the laboratory of Pharmacy, Animal Veterinary Faculty, Bogor Agricultural University using simple maceration methods for 3x24 hours with ethanol 96% solvent. All the leaves that have been dried are made into coarse powder 900gr each and extracted with 96% ethanol solvent. The extract was obtained from a rotary evaporator at 40°C and 50 rpm and equivalent to 112,32gr of young Jack fruit leaves and 123,45gr mature Jack fruit leaves.

The concentrated extract of young Jack fruit leaves in 96% ethanol was partitioned with n-hexane, and the n-hexane solution concentrated. The water solution was then partitioned with ethyl acetate and the ethyl acetate and water solution was concentrated. Phytochemistry screening was done in Biopharmaca research Center of Bogor Agricultural University.

4. Phytochemistry Screening

The phytochemistry screening was done to find whether there were secondary metabolite chemical compounds such as: Alkaloid, flavonoid, tannin, steroid, hydroquinone and terpenoid. The procedure of the screening is based on Reji and Rexin [12]. Phytochemistry screening was done in the 96% ethanol extract in the young jackfruit leaves and mature leaves. The sample was filtrated using Whattman no.42 (125mm).

Alkaloid

The sample of plant extract (0.5 g) were diluted in 5ml 1% HCl solution and filtrated. The filtrate was divided into two parts. The first was added Draggendorf (solution of potassium bismuth iodide) reagent and formation of red precipitate. The second added Wagner (iodine in potassium iodide) reagent and formation of brown/reddish precipitate. The sediment formed showed a trace of alkaloid in the sample [13].

Flavonoid

As much as 0,5 g of extract was diluted in a 2 ml NaOH solution. which was then added concentrated H_2SO_4, and formed a yellowish solution which indicates the trace of flavonoids in the sample.

Tannin

As much as 0,5 g of extract was diluted in 10 ml of water. The solution was filtered and the filtrate was added 5% iron chloride and the color green indicates a trace of tannin in the solution.

Anthraquinone

As much as 0,5 g of extract was diluted in a 2 ml chloroform, which was then stirred and filtered. The filtrate

was then stirred with a 100% ammonia solution with the same volume. The red color present in the ammonia layer (bottom layer) indicates the trace of anthraquinone in the sample.

Steroid

As much as 0,5 g of extract was diluted in a 2 ml chloroform. Sulfuric acid was added gently until it formed a layer. The blue ring present between the layers indicates trace of steroid in the sample.

Terpenoid

As much as 0,5 g of extract was diluted in a 2 ml chloroform solution and 3 ml concentrated H_2SO_4. The brown reddish color that appeared indicates that the sample contains terpenoid compounds.

Bioactivity Test

Inhibition of tyrosinase activity (monophenolase and DOPA auto-oxydation (diphenolase). Bioactivity test was performed at Biopharmaca Research Center Bogor Agricultural University, Bogor Indonesia. This assay was performed using methods as described earlier [14, 15] with minor modification. Extract and fraction were dissolve in DMSO (dimethyl sulphoxide) to a final concentration of 20 mg mL^{-1}. This material stock solution was then diluted to 600 µg mL^{-1} in 50 mM potassium phosphate buffer (pH 6,5).

The material were tested at the concentrations ranging from 31,25 to 2000 µg mL^{-1}. Kojic acid, which was used as positive control was also tested at concentrations 7,8125 to 500 µg mL^{-1}. In 96-well plate, 70 µL each extract dilution was combined with 30 µL of tyrosinase (sigma, 333 Units mL^{-1} in phosphate buffer) in triplicate. After incubation at room temperature for 5 min, 110 µL of substrate (2 mM L-tyrosine or 2 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 a multi-well plate reader. The concentration of plants extract and fraction at which half the original tyrosinase activity was inhibited (IC_{50}), was determined for each plant extract and fraction. Kojic acid (sigma, USA) was used as positive control.

5. Results and Discussion

The phytochemistry screening showed that traces of flavonoid was strongly present in the 96% ethanol young leaves extract. (Table 1). Research by Arung *et. al*, 2005 to some Artocarpus species have been results compound of 9 flavonoids and stilbene. This compounds showed to inhibit melanin production in B-16 melanoma cells culture. Chemical compounds with depigmenting activity are used in dermatology and cosmetics for a long time. A huge number of phenolic compounds have been tested as inhibitors of melanin synthesis and as photo protector. Naturally occurring herbal extracts, active compounds such as phenols, flavonoids, coumarins and other derivatives have gained attention as putative hypo pigmenting agents [9, 16].

In some of the research groups, flavonoids were found mostly in polyphenol plants. More than 4000 members have identified it widely distributed in leaves, bark and flowers. All the flavonoids have phenolic and pyran or

oxine rings,which are benzoc-pyran derivatives. Flavonoids are classified into 6 large groups: flavanols, flavones, flavonols, flavanones, isoflavones and anthocyanidins, which differ in the conjugation of rings and the position of hydroxyl, methoxy and glycosidic groups [9]. The material from plant containing flavonoids is mostly used in medication due to its benefits such as seperti anticancer, anti-inflammatory, protection against UV [2].

Table 1: Phytochemistry Screening of Jack Fruit Leaves

Solvent	**Secondary Metabolite**						
	Alkaloid	Flavonoid	Tanin	Terpenoid	Saponin	Steroid	Anthraquinone
Young leaves of 96% ethanol extract	-	++	+	-	+	+	-
Mature leaves of 96% ethanol extract	-	+	+	-	+	+	-
Young leaves n- hexane fraction	-	+	+	-	-	+	-
Young leaves ethyl acetate fraction	-	+	+	-	-	+	-

++ = strong, + = weak, - = not detected

Potency as Tyrosinase Inhibitor

The potential test result of tyrosinase inhibitor in monophenolase and diphenolase in young and mature Jack fruit leaves in 96% ethanol extract (Table 2) indicates that the young leaves extract has more potential in monophenolase (IC_{50}: 29,9 µg mL^{-1}) and diphenolase (IC_{50}: 167,3 µg mL^{-1}) compared to the mature leaves extract in monophenolase (IC_{50}: 214,2 µg mL^{-1}) and diphenolase (IC_{50}: 358,1 µg mL^{-1}). The chemical activity in young Jack fruit leaves was not significantly different with the positive control of kojic acid monophenolase (IC_{50}: 28,2 µg mL^{-1}) and diphenolase (IC_{50}: 85,0 µg mL^{-1}).

Further examination was done through a test for potential tyrosinase inhibitor from the ethyl acetate fraction and the n-hexane fraction of the young leaves extract. The test indicates that the young leaves extract in ethyl acetate fraction in monophenolase (IC_{50}: 81,8 µg mL^{-1}) and diphenolase (IC_{50}: 1557,7 µg mL^{-1}), whereas the n-hexane fraction of the young leaves extract in monophenolase and diphenolase (IC_{50}: >2000 µg mL^{-1}).

The phytochemistry test in young Jack fruit leaves 96% ethanol extract detect strong traces of flavonoids compared to the other samples. The tyrosinase inhibitor potential test indicates the young leaves extract has a result of IC_{50} which is better than the mature leaves extract, ethyl acetate fraction and also n-hexane fraction of

young leaves extract. It is suspected that the more traces of flavonoid compounds then the bigger the potential as tyrosinase inhibitor. The research by Arung *et. al*, used isolated flavonoids from the Jack fruit sapwood, found that an active compound of artocarpanone [7] with a tyrosinase inhibitor of IC_{50} 80,8 µM is good potential compared to the positive control of kojic acid 15.5 µM and the Arung et. al, research in 2008 detected an active substance which is artocarpin that can inhibit the biosynthesis of melanin even though the mouse melanoma B-16 cell was stimulated with α-MSH or forskolin.

Table 2: IC_{50} values of monophenolase and diphenolase activities of tyrosinase

Solvent	Monophenolase	Diphenolase
Young leaves 96% ethanol extract	29,9	167,3
Mature leaves 96% ethanol extract	214,2	358,1
Young leaves n-hexane Fraction	>2000	>2000
Young leaves Ethyl Acetate Fraction	81,8	1557,7
Kojic Acid	28,2	85,0

6. Conclusion

Young Jack fruit leaves are natural substance which has a good potential as a whitening agent. Artocarpin and artocarpanone as an active compound can be isolated from the sapwood of Jack fruit tree which was done by Arung et. al, and found large traces of flavonoids. The phytochemistry screening from 96% ethanol extract of young leaves indicates strong traces of flavonoids, which can be linked to the potential as a whitening agent where artocarpin and artocarpanone is part of the flavonoid compound. Inhibition test of tyrosinase activity (monophenolase) and DOPA auto-oxidation (diphenolase) also showed that the young leaves 96% ethanol extract has the potent activities as tyrosinase inhibitor. The activities are not significantly different with kojic acid as positive control. The limitations of this study is in the tyrosinase bioactivity test which use mushroom tyrosinase as the initial screening, recommendation for further research is to use cell culture methods to reveal the toxicity dosage and activity effect of tyrosinase enzyme as an inhibitor of melanin production.

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