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Phytochemical Screening And Tyrosinase Inhibition Activity Of Leaves *Cassia siamea* L.

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ABSTRACT

Cassia siamea L. has been used traditionally as medicine because it has chemical constituents such as saponins, anthraquinones, alkaloids, flavonoids, tannins, terpenoids and steroids. Indonesia, which is located in a tropical region with high temperatures and ultraviolet radiation, can cause skin disorders such as hyperpigmentation due to excess melanin synthesis. Tyrosinase enzymes can prevent or inhibit melanin formation. The purpose of this study was to determine the chemical content of *Cassia siamea* L. leaves extract with various solvents and the inhibitory activity of tyrosinase enzymes. In this study, the leaves of *Cassia siamea* L. were macerated with methanol as a solvent. Dry methanol extract was fractionated using the liquid-liquid method using aquadest, butanol and chloroform as solvents. The four dried extracts were then phytochemical screening to determine their chemical content. Tyrosinase inhibition test was carried out in vitro with L-Dopa as a substrate using ELISA plate well reader at concentrations of 100, 1000 and 10000 ppm with three repetitions (triplo). Methanol extract had percent inhibition of 19.993±1.125%, 28.984±0.624%, 57.164±0.623%, respectively; butanol fraction 25.914±0.541%, 32.566±0.767%, 52.120±1.616%; chloroform fraction 19.920±0.730%, 26.425±0.937%, 62.865±0.167% and aquadest fraction 23.830±0.879%, 30.885±0.778%, 67.471±0.352%. Kojic acid had tyrosinase activity and acts more active than the other extract and fraction with 91.155±0.228% at 500 ppm. The aquadest fraction had the highest percent inhibition (67.471 ± 0.352%) at 10000 ppm which was thought to be due to the presence of alkaloids, tannins, flavonoids, steroids and terpenoids from the results of the phytochemical screening test.

Keywords *: Cassia siamea* L; phytochemical screening; tyrosinase

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INTRODUCTION

Indonesia is a country located in a tropical region which is famous for its high temperatures and ultraviolet (UV) radiation at the highest level. Exposure to UV rays for a long time with frequent frequency can cause skin irritation such as darkening of the skin color. The brown color of the skin is the result of excessive melanin formation[1]. Hyperpigmentation is the most common pigment disorder of the skin. An increase in melanin synthesis or an uneven distribution of melanin can cause hyperpigmentation or patches of skin. The process of forming melanin compounds (melanogenesis) occurs with the help of biocatalysts, especially the enzyme tyrosinase[2].

In the melanogenesis process, tyrosinase acts as a catalyst in two different reactions, namely the hydroxylation of tyrosine to dihydroxy-phenylalanine (L-DOPA) and the oxidation of L-DOPA to dopakuinone. Dopakuinone compounds have very high reactivity so they can polymerize spontaneously to form dopaque which then becomes melanin[3]. Tyrosinase in skin tissue is activated by solar UV radiation, thereby accelerating the process of melanin production. Tyrosinase enzymes can prevent or inhibit melanin formation[1].

One way to lighten skin color is by inhibiting melanin formation through inhibition of the tyrosinase enzyme. Bleach acts as an inhibitor of melanin production and is known as a competitive tyrosinase inhibitor. Various tyrosinase inhibitors are found in cosmetic ingredients including hyaluronic, arbutin, kojic acid, mercury, and hydroquinone. This compound has a very large bleaching power, but is dangerous because it is carcinogenic[4]. The discovery of natural ingredients that are safe for human health, one of which is looking for tyrosinase inhibitors found in nature.

Cassia siamea L. has been used traditionally as medicine and in previous studies had an IC_{50} antioxidant activity of 144.12 µg/mL[5]. *Cassia siamea* L. potential as a medicinal substance is thought to have chemical constituents such as saponins, anthraquinones, alkaloids, flavonoids, tannins, terpenoids and steroids[6]. The total phenolic content (TPC) that has been carried out in previous studies was 88.5 mgGAE/g, while the total flavonoids content (TPC) obtained were 35.6 mgRE/g[7]. Flavonoids with their antioxidant effects play a role in inhibiting the tyrosinase enzyme. The purpose of this study was to determine the chemical content of *Cassia siamea* L. leaves extract with various solvents and the inhibitory activity of tyrosinase enzymes.

METHODS

Cassia siamea L.dry leaves was collected from Balai Penelitian Tanaman Rempah dan Obat (Balitro) which has been determined at the Botanical Garden Plant Conservation Center, Lembaga Ilmu Pengetahuan Indonesia (LIPI). Chemical reagents such as methanol 75%, L-DOPA (Sigma), tyrosinase enzyme (Sigma), kojic acid (Sigma), chloroform, butanol, aquadest, HCl 2 N, Dragendorff reagent, Mayer reagent, Wagner reagent, Bouchardat reagent, HCl p, $NaNO_2$ 5%, $AlCl_3$ 10%, NaOH 1N, $FeCl_3$ 1%, sodium hydroxide NaOH 2N, ether, H_2SO_4, potassium dihydrogen phosphate, dimethyl sulfoxide

(DMSO) (Merck), phosphate buffer (pH 6.5). ELISA plate well reader was used for tyrosinase inhibition assay.

Sample Preparation, Extraction, and Fractionation

Cassia siamea L.dry leaves was ground to obtain 500 gram sample powder to the extraction process. Methanol 75% 5 L was used as the solvent in the maceration extraction of the samples for 3x24 hours. The crude methanol extracts were then dried using a rotary evaporator. Liquid-liquid fractionation was conducted using aquadest, buthanol, chlroform to the methanol extract to obtain fractions with different polarities then dried using a rotary evaporator. The four dried extracts were then phytochemical screening to determine their chemical content and tyrosinase inhibition assay.

Phytochemical Screening

Alkaloids

0.25 grams of sample was added with 0.5 mL of 2N hydrochloric acid and 4.5 mL of distilled water, heated over a water bath for 2 minutes, cooled and then filtered. The filtrate as much as 3 drops was transferred into three test tubes. To tube 1 are added 2 drops of Wagner solution. If there is a brown to black sediment, the powder contains alkaloids. Tube 2 is added with 2 drops of Mayer reagent. If a white precipitate is formed, the powder contains alkaloids. Tube 3 is added 2 drops of Dragendorff reagent. If a brown sediment is formed, the attack contains alkaloids.

Flavonoids

0.5 gram of sample was extracted with 50 mL of hot water, then filtered with filter paper until the filtrate was obtained. 2.5 mL of the filtrate is poured into the test tube. The filtrate was added with 0.5 mL of 5% $NaNO_2$ solution and 0.5 mL of 10% $AlCl_3$ and then shaken. 1 mL of 1N NaOH was added slowly into the filtrate through the test tube wall. The presence of flavonoids indicates a red or orange color

Saponins

0.5 gram of sample was extracted with 100 mL of hot water, then filtered with filter paper until the filtrate was obtained. 5 mL of the filtrate is poured into a test tube then shaken vigorously for 10 seconds. If a foam is formed that is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N hydrochloric acid, it indicates the presence of saponins

Tannins

0.5 gram of sample was extracted with 50 mL of hot water, then filtered with filter paper until the filtrate was obtained. 2.5 mL of the filtrate is poured into the test tube. Then add 2-3 drops of 1% iron (III) chloride solution into the test tube containing the filtrate. The presence of the phenol group is indicated by the formation of a green-blue-black color

Steroids & Terpenoids

0.5 gram of sample was macerated using 10 mL ether for 2 hours then filtered with filter paper. 2.5 mL of the filtrate is poured into the test tube. 0.5 mL H_2SO_4 is poured into a test tube containing the

filtrate. Then drop the liebermann-burchard reagent slowly through the wall. The formation of green violet or blue color indicates the presence of steroids / triterpenoids

Tyrosinase Inhibitory Assay

Tyrosinase inhibitory activity was evaluated based on inhibition of the sample (dilute DMSO) to diphenolase activity. The assay was carried out using ELISA plate well reader with tyrosinase enzyme, L-DOPA as the substrates, phosfat buffer pH 6.5, with three repetitions (triplo)[17]. Kojic acid was used as a positive control. The percentage of tyrosinase inhibitory activity can be calculated by the following formula:

$$\text{Inhibition (\%)} = [1 - \frac{A-C}{B-D}] \text{ x } 100\%$$

Where,

A: Absorbance of the sample

B: Absorbance of blank

C: Absorbance of sampel control

D: Absorbance of blank control

RESULTS

The extraction process using methanol as a solvent is based on previous research that the leaves of *Cassia siamea* L. had the lowest IC_{50} value in the antioxidant activity test[5]. The low IC_{50} value of antioxidants shows the effectiveness in counteracting free radicals from UV rays which indirectly affect the inhibition of the tyrosinase enzyme. The maceration method is chosen because it has advantages, namely in the fast processing process, the method of working and the equipment used is simple, relatively easy and inexpensive[8]. Below is the yield of the extract and fraction of the leaves of *Cassia siamea* L.

Table 1. the yield of the extract and fraction of leaves of *Cassia siamea* L.

Simplicia	Fraction	Powder Weight (g)	Extract Weight (g)	Yield %
Cassia siamea L. Leaves	Methanol	500	70.5	14.1
	Chloroform	40	9.1	22.75
	Aquadest	40	12.2	30.5
	Butanol	40	5	12.5

Phytochemical screening was carried out using the previous method[9]. The most important types of phytochemicals found in extract and fraction of leaves of *Cassia siamea* L. are alkaloids, flavonoids, tannins, steroids, terpenoids positively detected during phytochemical confirmation as shown in Table 2.

Table 2. Phytochemical screening test results of leaves of *Cassia siamea* L.

Sample	Phytochemical Tested		Test Result
Methanol Extract	Saponin		-
	Alkaloids	Mayer	-
		Wagner	-
		Dragendorff	+
	Tannins		-
	Flavonoids		+
	Steroids & terpenoids		-
Butanol Fraction	Saponin		-
	Alkaloids	Mayer	+
		Wagner	-
		Dragendorff	+
	Tannins		+
	Flavonoids		+
	Steroids & terpenoids		+
Chloroform Fraction	Saponin		-
	Alkaloids	Mayer	-
		Wagner	-
		Dragendorff	-
	Tannins		-
	Flavonoids		+
	Steroids & terpenoids		-
Aquadest Fraction	Saponin		-
	Alkaloids	Mayer	+
		Wagner	-
		Dragendorff	+
	Tannins		+
	Flavonoids		+
	Steroids & terpenoids		+

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 hyperpigmentation in humans[10]. In this study, using the L-DOPA substrate because this substrate with the tyrosinase enzyme will produce dopaque which can then form melanin to prevent it by tyrosinase inhibitors. The formation of dopaid products is characterized by the appearance of a brown color which can cause inhibition of the substrate-tyrosinase reaction, resulting in reduced dopaque production which is indicated by a decrease in the intensity of the brown color. This dopaque formation uses a microplate reader using 96 wells microplates at a wavelength of 492 nm[11].

Table 3. Tyrosinase inhibitory of leaves of *Cassia siamea* L.

Sample	Concentration (ppm)	Tyrosinase Inhibition (%)
Methanol Extract	100	19.993 ± 1.125
	1000	28.984 ± 0.624
	10000	57.164 ± 0.623
Butanol Fraction	100	25.914 ± 0.541
	1000	32.566 ± 0.767
	10000	52.120 ± 1.616
Chloroform Fraction	100	19.920 ± 0.730
	1000	26.425 ± 0.937
	10000	62.865 ± 0.167
Aquadest Fraction	100	23.830 ± 0.879
	1000	30.885 ± 0.778
	10000	67.471 ± 0.352
Kojic Acid	500	91.155±0.228

DISCUSSION

Each phytochemical showed potency towards some biological action; for example, flavonoids with their antioxidant effects play a role in inhibiting the tyrosinase enzyme[12]. Tannins also have the ability to act as anti-tyrosinase because they inhibit the biosynthesis process of melanin so that an increase in melanin production does not occur after exposure to UVB rays[13]. Inhibitors of other classes, such as terpenes, steroids, alkaloids, have also been reported in the tyrosinase inhibitory activity[14].

Investigation of tyrosinase inhibitory activity showed that aquadest fraction had the highest percent inhibition (67.471 ± 0.352%) at 10000 ppm compared to the other extract and fraction. The presence of alkaloids, tannins, flavonoids, steroids and terpenoids from the results of the phytochemical screening test of aquadest fraction play a role in inhibiting the tyrosinase enzyme. Kojic acid has tyrosinase activity and acts more active than the other extract and fraction with 91.155±0.228% at 500 ppm. This was affected by the extract conditions, which was a crude extract and fraction which had not been purified, so it was suspected there were other compounds that did not act as tyrosinase inhibitors Kojic acid has an inhibitory effect of tyrosinase enzyme and the greatest stability in cosmetic products[15], but it is carcinogenic and its use in high concentration can damage the skin[16].

CONCLUCIONS

The highest yield of aquadest fraction amounted to 30.5%. Concentration 100, 1000, 10000 ppm shows that methanol extract had percent inhibition of 19.993±1.125%, 28.984±0.624%, 57.164 ±0.623%, respectively; butanol fraction 25.914±0.541%, 32.566±0.767%, 52.120±1.616%; chloroform fraction 19.920±0.730%, 26.425±0.937%, 62.865±0.167% and aquadest fraction 23.830±0.879%, 30.885±0.778%, 67.471±0.352%. Kojic acid had tyrosinase activity and acts more active than the other extract and fraction with 91.155±0.228% at 500 ppm. The aquadest fraction had the highest percent inhibition (67.471 ± 0.352%) at 10000 ppm which was thought to be due to the presence of alkaloids, tannins, flavonoids, steroids and terpenoids from the results of the phytochemical screening test.

Aquadest fraction of leaves *Cassia siamea* L. can be used as active skin lightening ingredients and formulated into cosmetic because have highest yield and inhibitory effect of tyrosinase enzyme.

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