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Tyrosinase Enzymes Activities and Sun Protection Factor of Ethanol Extract, Water Fraction, and n-Butanol Fraction of *Chromolaena* odorata L. Leaves

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Abstract

BACKGROUND: The need for skincare is increasing. One of the indicators of skin health is the brightness of the skin tone. Tyrosinase enzymes can darken the skin color due to their activity against melanin biosynthesis. The skin color will also change when exposed to UV rays, and even at a more severe level, it can cause cancer.

AIM: The purpose of this study was to determine the inhibitory activity of the tyrosinase enzyme and the SPF (Sun Protection Factor) value of ethanol extract, water fraction, and n-butanol fraction from Chromolaena odorata L. leaves.

METHODS: In this study, tests were carried out on ethanol extract, water fraction, and an n-butanol fraction of *C. odorata* leaves to inhibit tyrosinase enzyme activity based on percent inhibition and determination of inhibitory activity against UV light based on the SPF value. Determination of tyrosinase enzyme inhibitory activity using an ELISA reader was carried out by calculating the IC_{50} value with kojic acid as a positive control and measuring the SPF value using UV-Vis spectrophotometry.

RESULT: The results showed that the IC_{50} value of the tyrosinase enzyme inhibitory activity test, kojic acid as a positive control was 24.85 μg/mL (very strong), ethanol extract samples, water fraction, and n-butanol fraction were 191 μg/mL (weak), 65.86 μg/mL (very strong), and 14.59 μg/mL (very strong), respectively. The SPF value, including minimal protection shown by the ethanol extract at a concentration of 60 μg/mL, the water fraction at a 120 μg/mL concentration, and the n-butanol fraction a concentration of 40 μg/mL.

CONCLUSION: The ethanol extract, water fraction, and n-butanol fraction of the *Chromolaena odorata* L. had an inhibitory effect on the tyrosinase enzyme and sun protection capacity used as an ingredient in cosmetic preparations.

Introduction

Tyrosinase is an oxidase enzyme that contains copper and can be found in animals, plants, and microorganisms. Besides, Tyrosinase is a key enzyme that can catalyze the enzyme browning and melanin synthesis. Tyrosinase also exhibits monophenolase and diphenolase activity. It catalyzes L-tyrosine hydroxylation to L-3,4-dihydroxyphenylalanin (L-DOPA) and L-DOPA oxidation dopaquinone, which can undergo non-enzymatic polymerization to produce dark pigments [1], [2].

In humans, excess tyrosinase causes excess melanin production in the skin, which can lead to hyperpigmentation effects such as the appearance of freckles, melismas, age spots, and melanoma. The increase in the number or activity of free radicals in the living system can trigger an increase in melanin production [3], [4].

UV rays come from sunlight. UV rays (ultraviolet) consisting of UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (200–290 nm). Sunlight that reaches the surface of the earth and impacts the skin is UV-A and UV-B rays. UV rays are needed by the skin in skin rejuvenation and other skin health to a certain extent. However, excessive amounts of UV radiation can cause skin problems. Excessive UV radiation exposure is a trigger factor in skin problems such as cracked skin, burns, immune suppression, wrinkles, dermatitis, urticaria, aging, hypopigmentation, hyperpigmentation, and complications of skin cancer [5], [6].

Human skin is constantly exposed to ultraviolet radiation (UVR), an induction factor for reactive oxygen

species (ROS). Excessive ROS levels can cause skin pigmentation or even direct DNA damage and cause injury to the skin. The antioxidant defense system helps improve oxidative stress [7].

Skin pigmentation is another mechanism for preventing UVR damage. Melanin absorbs UVR, thus protecting skin cells from UVR damage. Therefore, normal skin pigmentation is significant for human health. Tyrosinase plays a role in catalyzing skin pigmentation and is directly related to pigmentation disorders in mammals. It is a prime target for discovering and screening new inhibitors because of its central role in melanogenesis [8].

There is an exciting and subtle relationship between the defense system by antioxidants and melanogenesis. This relationship is related to ROS entrapment. The synergistic effect, in this case, is the increased effectiveness of antioxidants in absorbing free radicals when tyrosinase inhibitors work, thereby reducing melanin production. Protection of the skin from the tyrosinase enzyme's excess activity and UV radiation is vital for maintaining healthy skin [9].

The development of plant-based antioxidants is at the center of attention today. Of the various plants that are being developed, one of them is *Chromolaena odorata* L. leaves. Its leaves have an antioxidant profile, as shown by several previous studies. In this research, the benefits of *C. odorata* leaves will be developed as an antioxidant by measuring their ability to inhibit the tyrosinase enzyme and inhibit UV radiation by determining the SPF value.

Methods

Materials

All materials and reagents used in this study were pro-analytic (p.a) specifications and obtained from Sigma-Aldrich (St. Louis, USA). Tyrosinase enzyme from mushrooms in lyophilized powder was obtained from Sigma Chemical Co. (St. Louis, USA).

Sample preparation

Extraction and fractionation

The leaves of *Chromolaena odorata* L. obtained from Takalar District, South Sulawesi, are cleaned, dried, and pollinated. The powder was immersed in 70% ethanol solvent and sonicated using a sonicator (Elma®) for 15 min. Extraction was carried out with 70% ethanol solvent three times until the filtrate became clear. The concentrated filtrate was evaporated at a rotary evaporator and dried using desiccator. The extract was then fractionated with different solvents, which are n-hexane, water, and n-butanol (water-saturated), using the liquid-liquid extraction (LLE) method, respectively. All the fractions were then dried using a freeze dryer Lyophilizer by brand Büchi L200, Germany.

Phytochemical screening of Chromolaena odorata L. leaf extract fraction

Screening using the TLC method with 10% $AlCl_3$ spray reagent for identification of flavonoid compounds and 10% $FeCl_3$ for identification of phenolic compounds. The eluent used was ethyl acetate: methanol with a ratio of 5: 1. The spots were observed using UV 254 nm and 366 nm.

Quantitative test of chemical content of Botto-Botto (Chromolaena odorata L.) leaf extract fraction

Total flavonoid levels were tested using quercetin standards and total polyphenol levels using gallic acid standards.

Tyrosinase enzyme inhibitory activity analysis

Preparation of phosphate buffer solution

A total of 2.7 g KH_2PO_4 was dissolved in distilled water gradually until a volume of 400 mL was sufficient. Then the pH of the solution was measured using a pH meter. A total of 5,6 g KOH was dissolved it in 100 mL of distilled water. The KOH solution was added to the KH_2PO_4 solution until it reached a pH of 6.8. The phosphate buffer solution was stored in the refrigerator.

Preparation of L-tyrosine solution

A total of 18.2 mg of L-tyrosine was put into a 100 mL volumetric flask, added to the phosphate buffer gradually, and homogenized. The volume was sufficient to 100 mL with a phosphate buffer solution.

Preparation of the tyrosinase enzyme solution

A total of 1 mg of tyrosinase enzyme was put into a 10 mL measuring flask dissolved in 10 mL of cooled phosphate buffer solution. The tyrosinase enzyme solution was placed in a container filled with ice to keep the enzyme temperature stable at cold temperatures during processing

Preparation of leaf extract test solution

Preparation of kojic acid as a positive control solution

A total of 5 mg kojic acid was put into an ependorphous tube and dissolved in 5 ml of 50 mM

phosphate buffer solution (pH, 6.5). The kojic acid solution was then diluted to obtain a kojic acid solution with the same concentration as the test solution.

Determination of the inhibitory activity of the tyrosinase enzyme

A total of 50 μl of 1 mM L-Tyrosine, 50 μl of 50 mM of phosphate buffer solution (pH 6.8), 20 μl of tyrosinase enzyme solution, and 100 μl of sample solution were put into the well on the microplate. The mixture was incubated for 5 min at room temperature. Then the absorption was measured using a microplate reader (ELISA) at a wavelength of 490 nm. A blank test was carried out without the addition of enzymes, namely, 170 μl of phosphate buffer solution (pH 6.8), 50 μl L-Tyrosine 1 mM were used. The negative control used the above mixture without additional samples. The positive control used kojic acid as a sample substitute. The steps mentioned above are carried out in triplo.

The determination of the percentage inhibition of tyrosinase activity is based on the formula:

% Tyrosinase inhibition = A−BA × 100%

Note:

A = Absorbance of the sample without inhibitor.

B = Absorbance of the sample with inhibitor.

The test sample's inhibitory activity was determined by IC_{50}, namely the concentration at which the test sample inhibited tyrosinase activity by 50%. IC_{50} was calculated using a linear regression equation. The log sample concentration as the X-axis and the % inhibition probit as the Y-axis, from the equation y = bx±a, and the IC_{50} value could be calculated.

Formula: IC_{50} y = ax + b, x = (50 – a):b

Note:

a = slope

b = intercept

IC_{50} values below 100 μg/mL indicated the strong inhibitory potential of tyrosinase activity, 100–450 μg/mL indicated weak tyrosinase activity inhibitory potential, and 450–700 μg/mL indicated very weak potential inhibition of tyrosinase activity.

Determination of SPF value

SPF value *in vitro* using spectrophotometric method [10], we weighed the sample, and then made several variants of the concentration (μg/mL) by diluting the sample with 70% to 10 ml ethanol. The UV-Vis spectrophotometer was calibrated first using 70% ethanol and 1 ml of 70% ethanol inserted into the cuvette. A test absorption curve was made in a cuvette with a wavelength between 290 and 320 nm, 70% ethanol was used as a blank and then determined the average absorption (Ar) at 5 nm intervals. The absorbance results of each cream concentration were recorded, and then the SPF value was calculated, carried out three times. The SPF value was analyzed using the Mansur method

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Note:

EE: Erythemal effect spectrum

I: Solar intensity spectrum

Abs: Absorbance of sunscreen product

CF: Correction factor (= 10).

Results and Discussion

Table 1 shows that the results of the *Chromolaena odorata* leaf extract are Simplicia weight of 800 g, Extract Weight of 181 gr, and Rendement of 22.625.

Table 1: The results of the extraction of *C. odorata* leaves

Simplicia weight (g)	Extract Weight (g)	Rendement (%)
800	181	22.625

Table 2 shows that the highest value of total polyphenols and total flavonoids is in the n-butanol fraction.

Table 2: Test results for total polyphenols and total flavonoids

Sample	Total polyphenol value (%)	Total flavonoid value (%)
Ethanol extract	10.56	2.70
n-butanol fraction	18.08	7.06
n-hexane fraction	14.30	0.92
Water fraction	11.87	1.00

Identification of *C. odorata* leaves contained Phenolic and Flavonoids in three samples which were checked with positive results (Table 3).

Table 3: Identification results of the *C. odorata* leaf fraction compound components

Compound	Sample		
	Water fraction	n-butanol fraction	n-hexane fraction
Phenolic	+	+	+
Flavonoids	+	+	+

Present (+), Absent (-)

This study has resulted in two categories, tyrosinase enzyme activity in IC_{50} value and SPF value of ethanol extract, n-butanol fraction, and water fraction of *C. odorata* and kojic acid, a comparison standard. Testing tyrosinase enzyme inhibition on the leaf samples was carried out using a microplate reader (ELISA). This study used the tyrosinase enzyme synthesized from fungi and L-tyrosine as the substrate. The maximum activity of the tyrosinase enzyme was at pH 6.8. Hence, it was buffered with a phosphate buffer

of pH 6.8 that had been cooled. The tyrosinase enzyme was also stable at −20°C, so storing and processing it must remain in a cold container.

Testing the tyrosinase enzyme's inhibitory activity was based on the enzyme's ability to catalyze L-Tyrosine's oxidation as a substrate to L-DOPA [11]. A further oxidation process occurred in the tyrosinase enzyme's presence with a catalyst that converts L-DOPA into DOPAquinone. Furthermore, DOPAquinone would spontaneously automerize to become DOPAchrome. The final result in the form of DOPAchrome would give a purple color that absorbed visible light energy at a wavelength of 490 nm. Analyzing the tyrosinase enzyme inhibition activity by ethanol extract, n-butanol fraction, and water fraction from *Chromolaena odorata* L. leaves was carried out using L-tyrosine as substrate, and kojic acid as a positive control. Kojic acid was chosen because it was a tyrosinase inhibitor with the highest inhibition and stability in a skin-lightening cosmetic [4], [12].

The results of the tyrosinase enzyme inhibitory activity test are shown in Table 4. The comparison graph in Figure 1 shows that Kojic acid was used as a positive control.

Table 4: IC_{50} Value of *C. odorata* Leaf

Sample	Concentration (µg/mL)	Percent inhibition (%)	Value IC_{50} (µg/mL)
Ethanol extract	10	24.5	191
	15	30	
	20	29.5	
	25	25.5	
	30	24	
n-Butanol fraction	10	24.9	14.59
	15	28.2	
	20	27.7	
	25	27.2	
	30	5.7	
Water Faction	10	23.7	65.86
	15	22.6	
	20	20.2	
	25	7.5	
	30	22.3	
Kojic acid	10	21.9	24.85
	15	21.7	
	20	18.7	
	25	9.4	
	30	9.2	

The test results were carried out on ethanol extract, n-butanol fraction, and water fraction with five concentration levels, 10, 15, 20, 25, and 30 µg/mL, and carried out in a triplo. The results were then compared with kojic acid made with the same concentration and calculated triplo for each concentration.

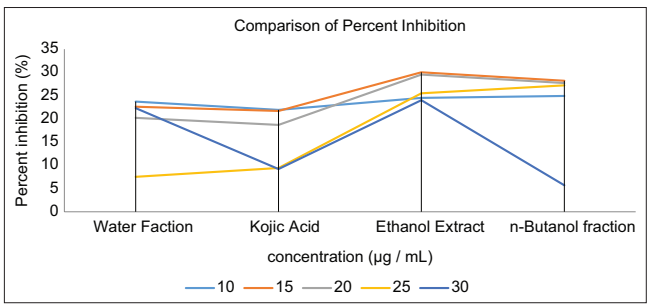

Figure 1: Comparison diagram of botto-botto leaf sample inhibition percentage

The results of the IC_{50} value from the tyrosinase enzyme inhibitory activity test showed a concentration value that could inhibit 50% of the tyrosinase enzyme activity (IC_{50}), the kojic acid was 24.85 µg/mL. Simultaneously, ethanol extract samples, n-butanol fraction, and water fraction were 191 µg/mL, 14.59 µg/mL, and 65.86 µg/mL, respectively (Table 4). The IC_{50} values for inhibition of tyrosinase activity categorized as >100 µg/mL indicated the strong potential, 100–450 µg/mL indicated weak potential, and 450–700 µg/mL indicated the very weak inhibitory potential of tyrosinase activity [13]. The smaller the IC_{50} value, the greater the Tyrosinase inhibitory activity. The ethanol extract was categorized as weak. The water fraction and n-butanol fraction were the strong categories in tyrosinase enzyme inhibition. The IC_{50} value of the n-butanol fraction was smaller than the standard kojic acid as a positive control. These results concluded that the ethanol extract, water fraction, and n-butanol fraction of *C. odorata* leaves have an inhibitory effect on Tyrosinase. Other studies have shown good anti-tyrosinase abilities at IC_{50} (66.28 µg/mL) [14]. Another study demonstrated the many biological effects of the potential tyrosinase inhibitor of structurally related flavonoids (1-9) and found that all tested materials had a tyrosinase inhibitory effect compared to the positive control, and kojic acid showed the strong tyrosinase inhibitory effect with an IC value of 40.94 ± 0.78 µM [15].

The subsequent measurement was to determine the SPF value. Sun Protection Factor (SPF) is a universal indicator that explains a product's effectiveness as UV protective. The higher the SPF value of a product or active sunscreen, the more effective it is to protect the skin from UV rays' destructive effects [16].

The results of the SPF measurement are shown in Table 5. In this study, the water fraction showed the SPF value at a 120 µg/mL concentration, the n-butanol fraction at 40 µg/mL, and the ethanol extract at 60 µg/mL. At this concentration, the SPF value was more than 2, where this value falls within the minimum range (if the SPF range was between 2 and 4). A concentration of fewer than 120 µg/mL for the water fraction, <40 µg/mL for the n-butanol fraction, and <60 µg/mL for ethanol extract has an SPF value of <2. Hence, it could not protect both UV A and UV B rays. At higher concentrations, there was a better protection value (the protection value is higher and still within a range), but further research is still needed to prove the effectiveness and benefits of better protection from this sample. Other studies show that at a concentration of 1% and 2% has a moderate SPF category with SPF values of 17.762 and 28.643. Concentrations of 3% and 4% have a high SPF category with SPF values of 30.592 and 31.325. The higher the concentration used, the better the SPF value [17]. Another study revealed that the

Table 5: SPF Value on *C. odorata* Leaf

No	Sample (µg/mL)	SPF Value	Category
1	Ethanol extract 20	1.24	
	Ethanol extract 40	1.61	
	Ethanol extract 60	2.02	Minimal protection
	Ethanol extract 80	2.59	Minimal protection
	Ethanol extract 100	3.57	Minimal protection
2	n-Butanol fraction 10	1.28	
	n-Butanol fraction 20	1.56	
	n-Butanol fraction 30	1.90	
	n-Butanol fraction 40	2.39	Minimal protection
	n-Butanol fraction 50	2.99	Minimal protection
3	Water faction 40	1.24	
	Water faction 80	1.64	
	Water faction 120	2.20	Minimal protection
	Water faction 60	2.86	Minimal protection
	Water faction 200	3.69	Minimal protection

Asteraceae family's leaf extract showed SPF values ranging from 4.71 to 5.29. Its ability to effectively block UV B was 75%–83%. Thus, the extract is an excellent candidate for medicinal in sunscreen preparations [18].

Conclusion

The ethanol extract, water fraction, and n-butanol fraction of the *Chromolaena odorata* L. leaves had an inhibitory effect on the tyrosinase enzyme and had sun protection capacity to be used as an ingredient in cosmetic preparations. Novelty research, namely, the results of this research are used as the basis for further research development to be used as antiaging material.

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