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Potential of Tyrosinase Enzyme Inhibition by Standardized Ethanol Extract and Ethyl Acetate Fraction of Bengkuang Peel (*Pachyrhizus erosus* L.)

(Potensi Penghambatan Enzim Tirosinase dengan Ekstrak Etanol Terstandar dan Fraksi Etil Asetat Kulit Bengkuang (*Pachyrhizus erosus* L.)

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Abstract: This study aimed to examine the tyrosinase inhibitory potential of bengkuang peel standardized ethanolic extract and ethyl acetate fractions. A total of 450 g of bengkuang peel powder (*Pachyrhizus erosus* L.) was macerated with 96% ethanol for 2x24 h, filtered, and concentrated till viscous. Standarized extract was evaluated specific and non-specific parameters. Microplate colorimetric tyrosinase inhibition assays were performed. The result of plant determination identified the bengkuang peel as *Pachyrhizus erosus* (L.) Urb. The organoleptic test revealed a brownish-yellow viscous extract, fresh smell, and bitter taste. Ethanol-soluble and water-soluble extract were 47.60% and 22.42%. Bengkuang peel ethanolic extract contained alkaloids, flavonoids, saponins, tannins, and steroids. The non-specific parameters showed loss on drying (9.71%), water content (7.28%), total ash (9.91%), acid-insoluble ash (3.68%), no residual solvent content, lead content (-0.2324±0.1729 ppm), cadmium content (-0.0841±0.3418 ppm), Total Plate Count (TPC) of $1x10^1$ CFU/g, and a Total Yeast and Mold Count (TYMC) of $2.26x10^2$ CFU/g. These results showed that the ethanol extract met both the specific and non-specific quality standards. The ethanol extract (103.9263 ppm) and ethyl acetate fraction (81.8606 ppm) IC_{50} values of bengkuang peel were lower than those of the control (25.1235 ppm). Bengkuang peel ethyl acetate fraction showed tyrosinase inhibition activity.

Keyword: Bengkuang peel (*Pachyrhizus erosus* L.), extract standardization, tyrosinase enzyme inhibition.

Abstrak: Penelitian ini mengkaji potensi daya hambat tirosinase ekstrak etanol terstandar dan fraksi etil asetat kulit bengkuang. Sebanyak 450 g serbuk kulit bengkuang (*Pachyrhizus erosus* L.) dimaserasi dengan etanol 96% selama 2x24 jam, disaring, dan dipekatkan hingga kental. Standardisasi ekstrak menggunakan parameter spesifik dan non-spesifik. Tes penghambatan tirosinase dilakukan secara kolorimetri dalam lempeng mikro. Hasil determinasi tanaman mengidentifikasi kulit bengkuang sebagai *Pachyrhizus erosus* (L.) Urb. Uji organoleptik menunjukkan ekstrak kental berwarna kuning kecoklatan, bau segar, dan rasa pahit. Kadar sari larut etanol sebesar 47,60% dan larut air 22,42%. Ekstrak etanol kulit bengkuang meliputi alkaloid, flavonoid, saponin, tanin, dan steroid. Parameter non spesifik menunjukkan susut pengeringan (9,71%), kadar air (7,28%), abu total (9,91%), abu tidak larut asam (3,68%), tidak ada kandungan residu pelarut, kandungan timbal (-0,2324±0,1729 bpj), kandungan kadmium (-0,0841±0,3418 bpj), angka lempeng total (ALT) $1x10^1$ CFU/g, dan angka kapang khamir (AKK) $2,26x10^2$ CFU/g. Hasil tersebut menunjukkan bahwa ekstrak etanol memenuhi standar baku mutu spesifik dan nonspesifik. Nilai IC_{50} dari ekstrak etanol (103,9263 bpj) dan fraksi etil asetat (81,8606 bpj) nilai IC_{50} kulit bengkuang menurunkan aktivitas enzim tirosinase dibandingkan kontrol (25,1235 bpj). Fraksi etil asetat kulit bengkuang menunjukkan penghambatan yang cukup terhadap enzim tirosinase.

Kata kunci: Inhibisi enzim tyrosinase, kulit bengkuang (*Pachyrhizus erosus* L.), standarisasi ekstrak.

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INTRODUCTION

SKIN plays an important role in protecting the internal body, including bones, muscles, organ systems, joints, nerves, and blood vessels, from toxic substances, viruses, bacteria, fungi, and parasites[1]. The skin, acts as a barrier against radiation exposure[2], is a characteristic of a person, and becomes an object of beauty. Skin color is determined by the main pigment in the body, melanin, which is produced by melanocyte glands and plays a role in absorbing and distributing energy from UV rays to protect epidermal cells from damage[3]. Thus, exposure to UV rays is one of the factors causing rapid formation of melanin, followed by a rapid premature aging process[4]. Continuous exposure of the skin to sunlight (UV) by both UV-A and UV-B for a long time causes hyperpigmentation, a disorder of the facial skin pigment that commonly occurs because of ultraviolet radiation from the sun[5]. If this persists, it can lead to sunburns and cause malignancy/skin cancer[6]. Some natural product developments in skin protection are intended to protect the skin from UV rays or sunscreens[7]. Some products inhibit tyrosinase activity and prevent excessive melanin production. Tyrosinase plays a crucial role in melanin production, which is essential for melanogenesis. L-DOPA is a precursor of melanin and catecholamines[8]. Tyrosinase acts as a catalyst in two different reactions: conversion of tyrosine hydroxylase to dihydroxyphenylalanine (L-DOPA), and oxidation of L-DOPA to dopaquinone[9].

As alternative remedies, natural compounds such as tyrosinase inhibitors and bengkuang (*Pachyrizus erosus* L., (*Fabaceae*) are still the subject of research[10,11]. Traditionally, bengkuang has been used as a wet powder that functions as a skin freshener and lightener[12]. This plant grows in tropical and subtropical areas, particularly Indonesia[13]. Bengkuang has anti-inflammatory, antibacterial[14], antioxidant, and anti-tyrosinase properties[15]. There were six active compounds found in bengkuang with antioxidant activity and skin whitening activity that have been isolated, namely daidzein, daidzin, genistin, (8,9)-furanyl-pterocarpan-3-ol, 4-(2-(furane-2-yl) ethyl) -2-methyl-2,5-dihydro-furane-3-carbaldehyde, 2-butoxy-2,5-bis (hydroxy methyl)-tetrahydrofurane -3,4-diol[16]. Compounds with tyrosinase inhibitory activity originate from polyphenols and flavonoids[11]. A comparison of anti-tyrosinase activity showed that the ethanol extract of bengkuang peel had higher tyrosinase inhibitory activity than the ethanol extract of the bengkuang tuber[15]. The development of herbal ingredients presents challenges such as the suitability of results and activities that are always standardized, quality, safe, and effective. As expected, standardization is required to ensure the quality of raw materials, processes, and final results. So, this study aimed to standardize the bengkuang peel ethanolic extract and compare the tyrosinase inhibitory activity of the ethanol and ethyl acetate fractions of the standardized bengkuang peel extract.

MATERIAL AND METHODS

MATERIALS. Bengkuang (*Pachyrhizus erosus* L.) gathered in Metro, Lampung, Indonesia; 96% ethanol, n-hexane, ethyl acetate, chloroform, amyl alcohol pro-analysis (Mallinckrodt Chemicals, Ireland); 30% ammonia, Mg powder, concentrated hydrochloric acid, 1% hydrochloric acid, 1% iron (III) chloride, formaldehyde, sodium acetate, NaOH 1N, anhydrous acetic acid, concentrated sulfuric acid, perchloric acid, potassium hydrogen phosphate, sodium hydroxide 0.2 N, potato dextrose agar (PDA) (Merck, USA); Nutrient Agar (NA) (Himedia Laboratories Ltd., Mumbai, India), L-DOPA, Kojic acid, and tyrosinase (Sigma-Aldrich, Saint Louis, USA).

Tools. The instruments were Rotavapor (Buchi R-300, Germany), analytical balance GR-200(AND Company Ltd., Tokyo, Japan), oven (Memmert, Germany), silicate crucible, crucible tong pliers, petri dish, watch glass, evaporation dish, autoclave, desiccator, colony counter, TLC plate 254 (Merck KgaA, Germany), and Whatman filter paper no. 42(GE Healthcare, USA), Atomic Absorption Spectrophotometer AA-6300 (Shimadzu, Kyoto, Japan), microplate reader Elx800 (BioTek Instrument, USA), gas chromatography Shimadzu 17A (Shimadzu, Kyoto Prefecture, Japan).

METHODS. Preparation of Simplicia. A total of 10 kg of bengkuang (*Pachyrhizus erosus* L.) tubers was collected from Metro, Lampung Province, Indonesia. The plants were identified at the Herbarium Depokinensis, Universitas Indonesia (specimen number 147/UN2).F3.11/PDP.02.00/2021. The bengkuang peel was prepared according to established procedures, such as washing, wet sorting, chopping, drying, dry sorting, and storage. The bengkuang peels were dried using direct sunlight for 1-3 days and an oven at 50°C for 10 minutes in order to optimize the drying process. The simplicia were then observed organoleptically, including shape, color, smell, and taste. The dried simplicia were reduced in size to a powder and sifted using sieve no.4/18 to obtain a uniform powder size.

Preparation of Extract. A total of 450 g of bengkuang (*Pachyrhizus erosus* L.) peel powder was

macerated with 96% ethanol for 2×24 h, filtered, and concentrated in a rotary evaporator (Buchi R-300, Germany) at a temperature of 50 °C, pressure of 175 mmHg, and speed of 60 rpm until a viscous extract was obtained, which was then partitioned using n-hexane and ethyl acetate.

Determination of Specific Parameters. Extract Identity Parameter. Extract identity is performed to provide an objective identity based on the plant's common appellation. The nomenclature comprises the name of the extract, the Latin name of the plant, the portion of the plant used, and the Indonesian name of the plant.

Organoleptic Test. The consistency, color, odor, and flavor of the bengkuang peel extract were evaluated during this test.

Evaluation of Water-Soluble and Ethanol-Soluble Compounds Level. Five grams of extract was macerated for 24 hours in 100 mL of water-chloroform while being shaken for the first 6 hours, then left for 18 hours before being filtered. The layers of chloroform and water were then separated. The water filtrate layer (20 mL) was desiccated on average in a shallow dish. The residue was heated to a constant weight at 105 °C. The contents of the ethanol-soluble and water-soluble extracts were calculated as a percentage of the initial extract's weight.

$$\%\text{Water soluble extract} = \frac{Weight\ of\ residu}{Extract\ \ Total} \text{x } 100\%$$

Evaluation of Phytochemical Content[17]. **Alkaloids.** After adding adequate chloroform to a 4 g sample, 10 mL of ammonia and 10 mL of chloroform were added, respectively. After filtering the solution into a test tube, 10 droplets of H_2SO_4 2N were added. The mixture was continuously stirred for a few minutes until two distinct layers formed. The uppermost layer was transferred to three 1 mL test containers. Each vial received a few droplets of the Mayer, Wagner, and Dragendorff reagents. Mayer's reagent formed a white precipitate, Wagner's reagent formed a brown precipitate, and Dragendorff's reagent formed an orange precipitate, indicating the presence of alkaloids in the sample.

Flavonoids. A 200 mg sample was weighed, extracted with 5 mL of ethanol, and heated for five minutes in a test tube. Subsequently, a few drops of concentrated HCl were added to the resulting solution. Then 0.2 g of Mg powder was added. Positive results were indicated by the appearance of a dark red color that persists for three minutes.

Saponin. A sample of 2 g was placed into a test tube, which was then added by water until the entire sample was submerged, boiled for 2-3 minutes, cooled, and shaken vigorously. Positive results were indicated by the formation of stable foam.

Tannin. A 20 mg sample was weighed, and then ethanol was added until it was completely submerged. Then 2-3 drops of a 1% $FeCl_3$ solution were added. Positive results were indicated by the formation of a bluish-black or greenish color.

Steroid/terpenoids. The collection of 50-100 mg samples was followed by the addition of glacial acetic acid until all samples were submerged. After 15 minutes, six drops of the solution were transferred to a test vial, and two to three drops of sulfuric acid were added. The formation of a red, orange, or purple color indicates the presence of triterpenoids, while the appearance of a blue color indicates the presence of steroids.

Determination of Non-specific Parameters. Loss on Drying (LOD). LOD was carried out to determine the water content and the number of volatile compounds in the extract, after the drying process was carried out in an oven at a temperature of 105 °C using the gravimetric method until a constant weight was obtained, the volatile compounds in question were essential oils, thermolabile compounds or water compounds contained.

Water Content. The water content was determined using the Karl Fischer Method. Bengkuang powder (1 g) was added to 10 mL of methanol and allowed to stand for 24 hours at room temperature. Furthermore, pre-titration was carried out on the tool and a leak test was carried out until a drift rate of 10-50 was obtained. Standardization was performed by weighing the syringe containing distilled water, then 1-2 drops of distilled water in a titrator beaker. The final weights of the distilled water and the injection syringe were determined. The weight difference was defined as the weight of the dripped water. Data on the weight of the dripped water were then entered into the tool and titration was performed. The tool calculates the equivalence of the titrant volume to the weight of distilled water. One milliliter of methanol was placed to a titrator beaker. The water content of the methanol blank was determined. A 1 mL sample was placed in a titrator beaker and titrated, and the water content was calculated using the following formula:

$$\%Water\ content(w/w) = \frac{B.R.f.100}{Wt.100}$$

Description:
B.R : Karl Fischer vol. consumed in mL
F : Karl Fischer factor in mg/mL
Wt. : Weight of sample in mg

Evaluation of Total Ash Content. The total ash content was determined using 2 g of extract, which was lined up at a temperature of 450-600 °C. The extract (2 g) was carefully weighed into a crucible that had been tared and ignited slowly. The temperature was then gradually increased to 600 °C until it was carbon-free, cooled in a desiccator, and weighed until a constant ash weight was obtained. The total ash content was calculated as the ratio of ash weight to initial weight.

Acid-insoluble Ash Content. The acid-insoluble ash content was determined by calculating the insoluble ash content in 25 mL of dilute hydrochloric acid boiled for 5 min. It was then filtered using ash-free filter paper that had previously been weighed. The filter paper containing the acid-insoluble ash was then placed back into the furnace at 600 °C until the ash was recovered, cooled in a desiccator, and weighed until a constant ash weight was obtained. The acid-insoluble ash content was calculated as a percentage of the initial weight.

Evaluation of Residual Solvent Contents. The remaining solvent extract was analyzed using a Shimadzu 17A gas chromatography (Shimadzu, Kyoto Prefecture, Japan). Operational conditions used a glass column measuring 30 m x 0.32 mm containing a stationary phase of TR-WAX with a particle size of 100-200 mesh, nitrogen as a carrier gas with a carrier gas flow rate of 20 mL/min, an injector temperature of 200°C and a detector temperature of 160 °C.

Heavy Metal Contamination. The heavy metal contamination levels (Pb and Cd) were determined using a Shimadzu AA-6300 atomic absorption spectrophotometer (Shimadzu, Kyoto Prefecture, Japan). One gram of bengkuang peel extract was weighed, 1 mL of concentrated H_2SO_4 was added, and the mixture was ignited in a furnace until it became ash. Subsequently, 5 mL of 10% nitric acid was added and filtered. The test solution was then added to 10 mL of demineralized water. Then 1 mL of crude lead solution equivalent to 10 ppm was diluted with distilled water up to 100 mL and for the blank solution, nitric acid 10% solution was added to 10 mL of distilled water. The solution was then placed in the instrument for analysis.

Microbial Contaminant. Total Plate Count (TPC) and Total Yeast and Mold Count (TYMC) were used to determine the level of microbial contamination in the extract. In order to determine the Total Plate Number (TPC), 1.0 g of the extract was added to 10 mL of phosphate buffer (pH 7.2), dilution was performed. 1 mL of each dilution was deposited in a sterile Petri dish and replicated three times. At a temperature of 45±10 °C, 15-20 mL of Nutrient Agar medium was added to each Petri dish. TPC was determined based on the number of colonies that grew on the inverted petri dish after 24 hours of incubation at 35-37 °C. Total yeast and mold counts (TYMC) were determined in the same manner as total plate counts (TPC), utilizing Potato Dextrose Agar (PDA) media.

Tyrosinase Inhibition Assays[15]. Tyrosinase enzyme inhibition of 96% ethanol extract and ethyl acetate fraction of bengkuang peel was carried out by inhibiting DOPA quinone produced by reducing the resulting tyrosinase enzyme, and then measuring its absorbance with a Microplate Reader EL × 800 at 490 nm to obtain IC_{50} values and compared with the IC_{50} of kojic acid as a positive control. Tyrosinase enzyme inhibitory activity was calculated as percentage inhibition (% inhibition) using the following formula:

$$\%\ (Inhibition) = \frac{c - s}{c}\ x\ 100\%$$

Description:
C : Absorbance of enzyme activity without inhibition
S : Absorbance of enzyme activity with the addition of the tested sample S1-S0
S1 : Absorbance of the extract as a result of activity with the addition of the enzyme first
S0 : Absorbance control (Absorbance of the extract as a result of activity without the addition of the test extract)

The IC_{50} calculated using linear regression calculation by connecting the concentration data as the (X) axis with % resistance as axis (Y) of the equation y= a+bx can be calculated using the IC_{50} using the following equation:

$$IC_{50} = \frac{50 - a}{b}$$

RESULTS AND DISCUSSION

Preparation of Simplicia. The simplicia preparation yielded 450 g of powder from 10 kg of bengkuang tubers. The simplicia are yellow and crispy peels with thickness of 0.1-0.4 cm. These characteristics are illustrated in Figure 1. The extraction process was carried out using 96% ethanol because this solvent extracts almost all of the simplicia content, both non-polar, semi-polar, and polar[13]. This solvent is selective,

Figure 1. Bengkuang peels simplicia.

non-toxic, universal, and suitable for extracting all classes of secondary metabolites. Cold extraction allows for the extraction of many compounds, although some compounds have limited solubility in extraction solvents at room temperature[18].

Extraction Yields. The extract was obtained using the standard maceration method with ethanol 96% and partitioning was performed using ethyl acetate to obtain a viscous extract of ethyl acetate from bengkuang peel (Table 1).

Result of Specific Parameters. Identity of Extract. The results of plant identification showed that the true plant used was Bengkuang (*Pachyrhizus erosus* (L.)Urb.). The plants were identified at the Herbarium Depokinensis, Universitas Indonesia (specimen number 147/UN2).F3.11/ PDP.02.00/ 2021. Based on the results of plant identification the identity of the extract used was obtained from the name ethanol extract of Bengkuang (*Pachyrhizus erosus* (L.) Urb.) with a part of the plant used as peel. Identity parameters provide an objective identity from the botanical name.

Organoleptic Test. The characteristics of the 96% ethanol extract of bengkuang peel are viscous, brownish-yellow, characteristic of a fresh odor, and bitter taste. These results show similar results from predetermined extract standards[19].

Water-Soluble and Ethanol-Soluble Compounds. The concentrations of the water-soluble and ethanol-soluble extract compounds obtained met the requirements of the Indonesian Materia Medica Monograph (Table 2). The levels of dissolved compounds in the water solvent indicate the number of inorganic compounds present in the extract, whereas the levels of dissolved compounds in the ethanol solvent indicate the number of organic compounds present in the extract. The secondary metabolites extracted in aqueous solvents are saponins and flavonoid glycosides because they are polar. The secondary metabolites extracted in ethanol are thought to be saponins, flavonoids, steroids, and triterpenoids because ethanol is a universal solvent that can attract polar, semipolar, and nonpolar compounds; thus, all compounds can be extracted in the solvent.

Phytochemical Analysis. The objective of this screening was to identify the type of secondary metabolites present in the ethanol extract and ethyl acetate fraction of bengkuang, as shown in Table 3.

Phytochemical screening of the ethanolic extract and ethyl acetate fraction of Bengkuang peel revealed secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and steroids. The phytochemical test is an initial test method used to determine the content of active compounds contained in plants so that they can be used as drugs. It can be seen that the dominant content of both samples is the flavonoid content. We all know that flavonoids have extensive activity as antioxidants and free radical fighters that correlate with their ability to health and skin protection from the sun's UV rays[15,20,21].

Non-specific Parameter Results. Loss on Drying (LOD) and Water Content. The LOD (9.71%) and water content (5.50%) values met the standards for determining the quality of extracts from the Indonesian Food and Drug Authority (BPOM) RI No. 32 of 2019[22], which was 10% (Table 4).

Loss on drying is one of the non-specific parameters that aims to provide a maximum limit (range) on the amount of compounds lost in the drying process while the moisture/water content is related to extract quality. The large amount of water content in the sample causes the potential for the growth of fungal and mold contamination, as well as the active metabolizing enzymes from plants. This test also confirms whether the understanding process has been completed or not[23].

Total Ash and Acid-insoluble Ash Content. The total ash content was determined to determine the amount of physiological and non-physiological ash after burning in the furnace for 1 h at 450 °C, and the levels were determined by the gravimetric method until the weight was constant. Determination of the acid-insoluble ash content shows the amount of ash obtained from external factors originating

Table 1. Result of ethanolic extract & ethyl acetate fraction.

No	Materials	Mass (g)	Yield value (%)
1	Ethanolic extract 96%	45.6230	11.40
2	Ethyl acetate fraction	0.6422	12.84

Table 2. Water and ethanol-soluble compounds.

No	Determination	Result	Standard Requirements
1	Ethanol soluble Compound	47.60%	Not less than 16%
2	Water soluble compound	22.42%	Not less than 4%

*Materia Medica Standard Requirements.

Table 3. Phytochemical screening.

No.	Phytochemical Content	Sample	
		Ethanolic extract	Ethylacetate fraction
1	Alkaloids	+	+
2	Flavonoids	+++	+++
3	Saponins	++	-
4	Tannins	++	+
5	Quinones	-	-
6	Steroids	+++	+
7	Triterpenes	-	-
8	Volatile oils	-	-

Table 4. Loss on drying and water contents.

No	Determination	Result	Requirements
1	Loss of drying	9.71%	<10%
2	Water content	7.28%	

Table 5. Total and acid-insoluble ash content.

No	Determination	Result	Requirements
1.	Total ash content	9.91%	<16%
2.	Acid insoluble ash content	3.68%	<4%

from impurities in sand or soil[19]. The total ash content (9.91%) and acid-insoluble ash content (3.68%) satisfied the MMI requirements and did not exceed 16%[24] (Table 5).

Residual Solvent Contents. The residual solvent shows the remaining solvent content used during the extraction process. The results met the BPOM requirements contained in the Indonesian medicinal plant extract book, which are less than 1%. Based on the determination of the remaining solvent, N/D (Not Detected) cannot be detected by gas chromatography because the residual solvent content is too low. Thus, the interpretation of the results for the residual solvent was that no solvent remained after the extraction process.

Heavy Metal Contamination. The levels of lead (Pb) and cadmium (Cd) were determined heavy metal contamination. Based on the tests conducted, the metal content of Pb was -0.2324 mg/kg, and that of Cd was -0.0841 mg/kg. This value meets the requirements set by BPOM, namely Pb (≤10) and Cd (≤0.30). This shows that the extract meets the safety requirements of the material for heavy metal contamination; therefore, it can be used as a preparation material because it is safe for the body. The results of Pb and Cd contamination determination are presented in Table 6 and 7.

Microbial Contaminant. Determination of microbial contamination obtained a Total Plate Count (TPC) ≤ 10^1 CFU/g and a Total Yeast and Mold Count (TYMC) of 2.26 x 10^2 (Table 7). These results meet the requirements of the BPOM regulation, where TPC (≤ 1 × 10^4) and TYMC (≤ 1 × 10^3)[19]. Extracts were safe for use as preparation materials and can be stored for long periods. Under these conditions, if the extract is used directly in medicinal preparations, its safety can be guaranteed because there is no microbial contamination[25].

Tyrosinase Inhibition Activity[8]. The tyrosinase enzyme inhibitory activity assay is a preliminary test to determine the optimum conditions for the activity test. Optimization was carried out as a preliminary test before testing the tyrosinase inhibitory activity, while the preliminary test was performed to optimize the maximum wavelength at 490 nm, incubation time at 10 min, enzyme concentration at 310 U/mL, and substrate concentration in 5 mM. An optimization test was conducted to determine the optimal conditions for testing tyrosinase enzyme inhibitory activity. The principle of the tyrosinase inhibition test is to oxidize DOPA to yellow DOPA quinone. Enzyme activity was measured based on the absorbance of the yellow color. The absorption measured was the control blank

Table 6. The result of heavy metal Pb contamination.

No	Sample	A	B (g)	Cu (ppm)	Pb (ppm)	Average (ppm) ± SD
1	Standard	0.2050				
2	Sample 1	-0.0002	1.0234	-0.0010	-0.0098	-0.2324±0.1729
3	Sample 2	-0.0007	1.0512	-0.0341	-0.3244	
4	Sample 3	-0.0008	1.0191	-0.0390	-0.3827	

Table 7. The result of heavy metal Cd contamination.

No	Sample	A	B (g)	Cu (ppm)	Cd (ppm)	Average (ppm) ± SD
1	Standard	1.2592				
2	Sample 1	-0.0037	1,0234	-0.0294	-0.2873	-0.0841±0.3418
3	Sample 2	-0.0048	1.0512	-0.0381	-0.3624	
4	Sample 3	0.0051	1.0191	0.0405	0.3974	

Table 8. Microbial contaminant.

No	Type of Contamination	Result (CFU/g)	Requirements (CFU/g)
1	TPC	1 x 10^1	1 x 10^4
2	TYMC	2.26 x 10^2	1 x 10^3

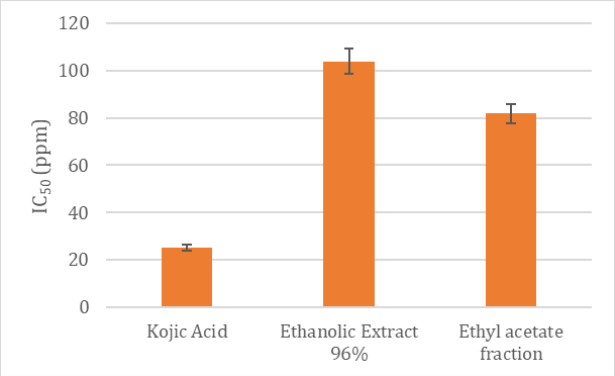

Figure 2. The activity of tyrosinase inhibition.

solution, blank solution, absorption of the test solution with the tyrosinase enzyme, test solution without the tyrosinase enzyme, and control solution.

The optimum enzyme concentration was obtained at a concentration of 310 U/mL and the optimum substrate concentration was obtained at a concentration of 5 mM. The determination of the optimization results is said to be optimal because under these conditions, the absorption obtained is the highest optimum absorption for tyrosinase enzyme inhibitory activity. Optimal absorption was used because the sensitivity of the measurement was optimal even at small concentrations.

The 96% ethanol extract and ethyl acetate of bengkuang peel were tested with kojic acid as the positive control. Each test solution was prepared in five different concentrations: 250, 300, 350, 400, and 450 ppm. This variant was utilized to develop a linear regression equation for calculating the IC50. It was determined the absorption of the test solution, the control solution, the blank solution, and the blank control solution. Kojic acid was utilized as a positive control because it is a circulating anti-tyrosinase compound in Indonesia that inhibits tyrosinase activity.

Tyrosinase plays a role in the formation of skin pigments, and its excessive activity causes hyperpigmentation of the skin. The presence of 96% ethanol extract and ethyl acetate of bengkuang peel inhibited tyrosinase, which was determined from the uptake of DOPA quinone formed and measured by an ELISA reader at a wavelength of 490 nm.

The bengkuang peel extract with the ethyl acetate fraction had greater tyrosinase enzyme inhibitory activity than the 96% ethanol extract. The average IC50 of ethyl acetate extract was 81.86 ppm and the ethanolic extract was 103.93 ppm. This is related to the type of metabolite compounds contained in each extract; therefore, there were differences in the inhibitory activities obtained. The metabolites of terpenoid and flavonoid groups (isoflavonoids) were extracted from ethyl acetate compounds that play a role in tyrosinase inhibition. Isoflavonoids were extracted in a semi-polar solvent, such as ethyl acetate, so that the ethyl acetate extract can extract more isoflavones, and the results of the tyrosinase inhibition test were greater. It related The results of tyrosinase inhibition in the 96% ethanol extract showed IC50 values of 101-250 ppm, where the range was included in the low inhibition criteria[26], and the results of tyrosinase inhibition in the ethyl acetate extract showed IC50 values that were included in the criteria for strong inhibition. This study was also closely related to recent research on the activity of a class of flavonoid compounds, which actively inhibit the DPPIV enzyme by changing the conformation of the structure and making it inactive[27,28].

CONCLUSION

The ethanolic extract of bengkuang peel contains alkaloids, flavonoids, saponins, tannins, and steroids, according to phytochemical analysis. The ethanol extract of bengkuang peel has a viscous brownish-yellow hue, a distinctive aroma, and an acrid flavor. The determination of specific and non-specific parameters of the extract of bengkuang peel in ethanol at a concentration of 96% met the quality standards outlined in the monograph. The test results indicated that the ethyl acetate extract of the bengkuang peel had greater potential than the 96% ethanol extract of the bengkuang peel with an IC_{50} of 81.8606 ppm; therefore, it has greater potential as a tyrosinase inhibitor than the control kojic acid with an IC_{50} of 25.1235 ppm.

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REFERENCES

1. Lautenschlager S, Wulf HC, Pittelkow MR. Photoprotection. Lancet. 2007.
2. Sarkany R. Sun protection strategies. Medicine (United Kingdom). 2017.

3. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci [Internet]. 2013;14(6):12222–48. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3709783/
4. Gilaberte Y, González S. Update on photoprotection. Actas Dermo-Sifiliográficas (English Ed [Internet]. 2010;101(8):659–72. Available from: https://www.sciencedirect.com/science/article/pii/S157821901070696X
5. Schalka S. New data on hyperpigmentation disorders. J Eur Acad Dermatology Venereol. 2017;31(Suppl. 5):18–21.
6. Comprehensive N, Network C. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Melanoma. Journal of the National Comprehensive Cancer Network : JNCCN. 2013.
7. Hakim ZR, Budiman A. The photoprotection of jamblang fruit (*Syzygium cumini*(L.) skeels) ethanolic extract as sunscreen by determining sun protection factor. Int J Pharm Res. 2019;11(4):1180–3.
8. Noor SU, Faridah, Magdalena P. In vitro enzyme tyrosinase inhibitory activity test on liquorice root extract cream (*Glycyrrhiza glabra* L.). J Ilmu Kefarmasian Indones. 2018;16(2):150–8.
9. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem [Internet]. 2019;34(1):279–309. Available from: https://doi.org/10.1080/14756366.2018.1545767
10. Hollinger JC, Angra K, Halder RM. Are natural ingredients effective in the management of hyperpigmentation? A systematic review. J Clin Aesthet Dermatol. 2018;11(2):28–37.
11. Lukitaningsih E, Bahi M, Holzgrabe U. Tyrosinase inhibition type of isolated compounds obtained from *Pachyrhizus erosus*. Aceh Int J Sci Technol. 2013;2(3):98–102.
12. Bashirah D, Putriana NA. Herbal cosmetics with potential as natural skin whitening. Farmasetika.com (Online). 2019;4(4):119–27.
13. Lukitaningsih E. Bioactive compounds in bengkoang (*Pachyrhizus erosus*) as Antioxidant and Tyrosinase Inhibiting Agents. Indones J Pharm. 2014;25(2):68–75.
14. Supari IH, Leman MA, Zuliari K. Antibacterial effectiveness of bengkuang seed extract (*Pachyrhizus erosus*) on the growth of Streptococcus mutans in vitro. Pharmacon. 2016;5(3):33–9.
15. Siregar ID, Kusuma HSW, Widowati W, Marpaung HH, Ferdinand S, Fachrial E, et al. Antioxidant and antityrosinase activities of ethanolic *Pachyrhizus erosus* peel and tuber extract. Maj Kedokt Bandung. 2019;51(2):75–81.
16. Primiani CN. Daidzein compound dynamic of yam bean (*Pachyrhizus erosus*) in blood and its potential to the female white mice. Pros Semin Nas FKIP UNS 2013. 2020;5(3):248–53.
17. Rante TRK, Simbala HEI, Mansauda KLR. Phytochemical Screening and Antioxidant Potential of (*Stachytarpheta jamaicensis* L.) Leaf Extract Using 1.1 Diphenyl-2-Picrylhydracyl (DPPH) Method. J MIPA. 2020;9(2):91–6.
18. Nurhasnawati H, Sukarmi S, Handayani F. Comparison of maceration and soxhletation extraction methods on the antioxidant activity of ethanol extract of malay apple (*Syzygium malaccense* L.). J Ilm Manuntung. 2017;3(1):91–5.
19. Health Department of Republic of Indonesia. Common Standard Parameters of Medicinal Plant Extract. I. Jakarta: Directorate General of Food and Drug Control; 2000. 3–33 p.
20. Saric S, Sivamani RK. Polyphenols and sunburn. Int J Mol Sci. 2016;17(9):1–22.
21. Sies H, Stahl W. Nutritional Protection Against Skin Damage From Sunlight. Annu Rev Nutr. 2004;24(1):173–200.
22. Health Department of Republic of Indonesia. Farmakope Herbal Indonesia. 1st ed. Health Department of Republic of Indonesia; 2008. 111–115 p.
23. Herrera-Arellano A, Flores-Romero S, Chávez-Soto MA, Tortoriello J. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: a controlled and randomized clinical trial. Phytomedicine. 2004 Jul;11(5):375–82.
24. Health Department of Republic of Indonesia. Materia Medika Indonesia. II. Jakarta: Directorate General of Food and Drug Control; 1978.
25. Johnson SS, Oyelola FT, Ari T, Juho H. In vitro. 2013;10:533–40.
26. Charissa M, Djajadisastra J, Elya B. Antioxidant activity assay, inhibition of tyrosinase and efficacy test of gel containing taya cortex (*Nauclea subdita*) extract on human skin. J Kefarmasian Indones. 2016;6(2):98–107.
27. Musoev A, Numonov S, You Z, Gao H. Discovery of novel DPP-IV inhibitors as potential candidates for the treatment of type 2 diabetes mellitus predicted by 3D QSAR pharmacophore models, molecular docking and de novo evolution. Molecules. 2019;24(16):1–13.
28. Pan J, Zhang Q, Zhang C, Yang W, Liu H, Lv Z, et al. Inhibition of dipeptidyl peptidase-4 by flavonoids: structure–activity relationship, kinetics and interaction mechanism. Front Nutr. 2022;9(May):1–17.