

Antioxidant and Antityrosinase Activities of Ethanolic *Pachyrhizuserosus* Peel and Tuber Extract

Irma Dolsyeria Siregar,¹ Hanna Sari W. Kusuma,² Wahyu Widowati,³ Hans Hotma Marpaung,⁴
Sahna Ferdinand,⁴ Edy Fachrial,⁴ I Nyoman Ehrich Lister⁴

¹Master in Biomedical Science, Faculty of Medicine Universitas Prima Indonesia, Medan, Indonesia,

²Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia,

³Faculty of Medicine Maranatha Christian University, Bandung, Indonesia, ⁴Faculty of Medicine Universitas Prima Indonesia, Medan, Indonesia

Abstract

Aging process is a physiological process in living organisms caused by, among others, free radicals. One of the free-radical-related aging problems is skin hyperpigmentation (excessive melanin) due to increasing tyrosinase enzyme activities. Natural compounds are widely used as antioxidant and antiaging agents. *Bengkuang* (*Pachyrhizuserosus*) is known as a source of various active compounds which can be used against free radicals to reduce the risk of skin aging through tyrosinase enzyme inhibition. This study was performed in September 2018 in Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia to examine the antioxidant and antityrosinase properties of *Pachyrhizuserosus* peel extract (PPE) and *Pachyrhizuserosus* tuber extract (PTE). The extraction of PPE and PTE was performed using 70% ethanol by maceration method, followed by phytochemical analysis using modified Farnsworth method. Antioxidant activities were measured through 2,2-Diphenyl-1-picrylhydrazil (DPPH) scavenging activities while antiaging assay were conducted through the tyrosinase activity inhibition. In this study, PPE contained saponin, tannin, triterpenoid, and terpenoid while the PTE showed the presence of flavonoid, saponin, phenol, tannin, and alkaloid in phytochemical analysis. In the antioxidant assay, PPE presented a higher DPPH scavenging activities ($IC_{50} = 84.09 \mu\text{g/mL}$) when compared to PTE ($IC_{50} = 98.30 \mu\text{g/mL}$) ($p < 0.05$). In antiaging assay, PPE showed a higher tyrosinase inhibitory activities when compared to PTE with $=97.05 \mu\text{g/mL}$ and $194.51 \mu\text{g/mL}$, respectively. It can be concluded that PPE has antioxidant and antiaging activities effective for preventing skin aging.

Key words: Aging, antioxidant, hyperpigmentation, *Pachyrhizuserosus*, tyrosinase

Aktivitas Antioksidan dan Antitirosinase Ekstrak Etanol Kulit dan Daging *Pachyrhizuserosus*

Abstrak

Proses penuaan merupakan suatu proses fisiologis yang terjadi pada makhluk hidup yang dapat disebabkan oleh radikal bebas. Hiperpigmentasi kulit merupakan salah satu masalah penuaan yang disebabkan oleh radikal bebas melalui peningkatan aktivitas enzim tirosinase. Bengkuang (*Pachyrhizus erosus*) diketahui mengandung berbagai senyawa aktif yang dapat menangkal radikal bebas serta mengurangi risiko penuaan kulit. Penelitian dilaksanakan pada September 2018 di Aretha Medika Utama, *Biomolecular and Biomedical Research Center*, Bandung, Indonesia untuk menguji kemampuan aktivitas antioksidan dan antitirosinase pada ekstrak etanol kulit bengkuang (EEKB) dan ekstrak etanol daging bengkuang (EEDB). Pembuatan ekstrak kulit dan daging bengkuang dilakukan dengan cara mengekstraksi bahan dengan etanol 70% menggunakan metode maserasi, kemudian dilanjutkan dengan analisis fitokimia ekstrak dengan modifikasi metode Farnsworth. Aktivitas antioksidan diuji dengan mengukur pemerangkapan 2,2-Diphenyl-1-picrylhydrazil (DPPH) sedangkan *antiaging* diuji dengan mengukur aktivitas penghambatan tirosinase. Pada uji fitokimia menunjukkan EEKB memiliki kandungan senyawa saponin, tanin, triterpenoid dan terpenoid, sedangkan EEDB menunjukkan kandungan senyawa flavonoid, saponin, fenol, tanin, dan alkaloid. Pada uji antioksidan, EEKB memiliki aktivitas tertinggi pada pemerangkapan DPPH ($IC_{50} = 84.09 \mu\text{g/mL}$) dibanding dengan EEDB ($IC_{50} = 98.30 \mu\text{g/mL}$) ($p \leq 0.05$). Pada pengujian antiaging, EEKB memiliki nilai yang lebih tinggi pada aktivitas penghambatan tirosinase dibandingkan dengan EEDB ($IC_{50} = 97.05 \mu\text{g/mL}$; $194.51 \mu\text{g/mL}$) ($p \leq 0.05$). Simpulan, EEKB memiliki aktivitas antioksidan dan antiaging sehingga efektif dalam mencegah penuaan kulit.

Kata kunci: Antioksidan, *Pachyrhizuserosus*, penuaan, tirosinase

Corresponding Author: I Nyoman Enrich Lister, Faculty of Medicine, Universitas Prima Indonesia, Medan, Jalan Belanga No.1, Sei Putih Tengah, Medan Petisah, North Sumatera, 20118, Indonesia, Email: yysunpri@gmail.com

Introduction

Tyrosinase enzyme has to play a major role in melanin synthesis being responsible as a dark pigment in the skin.¹ Overactivity of tyrosinase enzyme also leads to overproduction of melanin leading to hyperpigmentation of the skin.¹ Melanin is the pigment in the human and animal skin that is synthesized by tyrosinase from L-tyrosine to L-DOPA, following the oxidation of L-DOPA to L-DOPA quinone.²

Ultraviolet radiation, inflammatory mediators and hormones can upregulate melanogenesis inducing skin hyperpigmentation.³ Free radicals such as UV radiation have played roles in tyrosinase activation of human skin through melanocyte proliferation. Thus, antioxidant as reactive oxygen species (ROS) inhibitors may decrease hyperpigmentation and that can be used as whitening products.³

Many cosmetics or skin lightening agents contain tyrosinase inhibitors and they have been sold commercially. Some of them have been available as chemical and fungal derived skin-lightening agents having chronic, cytotoxic, and mutagenic effects in humans.⁵ Inhibition of melanogenesis in cells without side effects may be a good solution using inhibitor-target tyrosinase, as tyrosinase is produced by melanocytes. This inhibitor-target tyrosinase can be used to treat skin diseases caused by the accumulation of melanin such as melasma, solar lentigo, and freckles.^{4,5} Skin hyperpigmentation can trigger diseases of the skin so that the natural sources of tyrosinase inhibitors were needed.¹

Pachyrhizuserosus L. (Fabaceae), commonly known as bengkuang grows in many areas of tropical and sub-tropical regions, especially in Indonesia.⁶ *P. erosus* tuber has brown-skinned that has been used as whitening agents through inhibition of melanogenesis and usually used in cosmetic products.² *P. erosus* has some properties such as anti-inflammatory, antibacterial, antioxidant, anti-tyrosinase activities.⁷

The tuber of bengkuang has tyrosinase and protein expression inhibitory activity so it can be used as skin whitening products.² In the present study, antioxidant activity of *P. erosus* peel (PPE) and tuber extracts (PTE) through DPPH scavenging activity were evaluated as well as tyrosinase inhibitory activity (antiaging).

Methods

Bengkuang (*P. erosus*) plants were collected

from Ciampea-Bogor, West Java, Indonesia. The plants were identified by herbarium staff, Department of Botanical, Indonesian Institute of Sciences, Bogor, West Java, Indonesia. This study was conducted in September 2018 in Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia. Extraction of *P. erosus* using maceration by Widowati et al.⁹ method. The tuber (100 g) and peel (150 g) of *P. erosus* were mashed, and then using 1300 mL distilled ethanol 70% for extraction each sample. Then, the ethanol filtrate was filtered and wastes were re-macerated until colorless filtrate every 24 h. Maceration were concentrated using 50°C evaporator to obtain the extract. The tuber *P. erosus* extracts (31.05 g) and peel (28.77 g) *P. erosus* extracts was stored at -20°C.⁹

Phytochemical screening assay was evaluated using a modified Farnsworth method. The ethanol extracts of peel and tuber were tested for the presence of flavonoids, saponins, phenols, tannins, steroids/triterpenoids, terpenoids, and alkaloids. The qualitative results were expressed as (+) for the presence and (-) for the absence of phytochemicals.⁹

Flavonoids assay—PPE and PTE (10 mg) was dropped into a test tube and added Magnesium (Mg) [Merck EM105815, USA] and Hydrochloric acid (HCl) 2N. The mixture sample was heated for 5 to 10 min, then it was cooled down and filtered, and then added amyl alcohol. The formation of red or orange color shows the presence of flavonoids compound.⁹

Saponins assay – PPE and PTE (10 mg) was added into the test tube with some water and boiled for 5 min, shaken it vigorously. Saponins content was indicated by the persistence of froth on the surface.⁹

Test for phenols – PPE and PTE (10 mg) was placed on a dropping plate, then added 1% FeCl₃ [Merck 103943, USA] into the sample. If resulted color formation of green/red/purple/blue/black showed phenols compound.⁹

Tannins assay—PPE and PTE (10 mg) was added with 2 mL of HCl 2N [Merck 1003171000, USA] in the test tube, then heated on a water bath for 30 min. Then, the mixture was cooled down and filtered, the filtrate was added with amyl alcohol [Merck 10979, USA]. The presence of tannins indicated of purple color formation.⁹

Steroids/triterpenoids—Ten milligram of PPE and PTE was placed on a dropping plate, and then soaked with acetate acid until the sample was covered. Then, after 10–15 min added one drop of absolute sulphate acid (H₂SO₄) [Merck 109073, USA]. The color formation of green/

blue showed the presence of steroids while red or orange sediment indicated the presence of triterpenoids.⁹

Terpenoids assay– PPE and PTE (10 mg) were added into a dropping plate, then added vanillin and H₂SO₄ [Merck 109073, USA]. Formation purple color on the mixture indicated the presence of terpenoids.⁹

Alkaloids assay– Ten milligram amount of PPE and PTE was added into a test tube. Briefly, the sample was dropped 10% ammonia solution. Then, chloroform was added to the mixture, forming two layers of liquid and the bottom layer was collected to a new test tube. HCl 1N was added to the liquid, forming two layers and the upper layer was taken and Dragendorff solution was added (1-2 drops). The color formation of yellow show the presence of alkaloid content.⁹

Samples (50 µL) was added to each well in a 96-well microplate. After that, 200 µL of 2,2-Diphenyl-1-picrylhydrazil (DPPH) [Sigma Aldrich D9132, USA] solution (0.077 mmol/L in methanol [Merck 1060092500, USA]) was added into each well, then the mixture incubated in the dark for 30 min at room temperature. The absorbance was measured using a microplate reader (Thermo Scientific, Multiskan™ GO Microplate Spectrophotometer, USA) at 517nm wavelength.⁹ The median inhibitory concentration (IC₅₀) of the extract needed to inhibit 50% of the DPPH radicals were calculated.

The percentage of inhibition was calculated using the following equation:

$$\text{Scavenging \%} = \frac{(Ac - As)}{Ac} \times 100$$

Note: Ac was negative control absorbance

As was sample absorbance is absorbance of a test sample

Tyrosinase inhibitory activity was measured spectrophotometrically based on Widowati et al.⁹ methods with some modifications. Ethanol extracts from tuber and peel of a *P. erosus* were evaluated for their inhibitory activity using L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate; 20 µL sample with various concentration and 20 µL tyrosinase enzyme from mushroom (125 U/ml in potassium phosphate buffer) were prepared in 96 well-plate. Briefly, the sample was added potassium phosphate buffer (140 µL, 20 mM, pH 6.8). Then, the mixture solution was incubated at room temperature for 15 min. A twenty microlitres L-DOPA (1.5 mM) was added, then incubated for 10 min at room temperature. Tyrosinase inhibitory activity was measured by quantifying the level of DOPA that was formed at

470 nm wavelength.

$$\text{Tyrosinase Inhibitory Activity (\%)} = \frac{A - B}{A} \times 100$$

Where A was control absorbance and B was sample absorbance. The concentration for 50% inhibition (IC₅₀) was determined.¹⁵

All data were expressed as mean±standard deviation of triplicate measurements and were subjected to statistical analysis using Analysis of variance (One Way ANOVA) followed by Tukey's HSD Post-hoc Test. Statistical analysis was performed using SPSS software (version 20.0). A value of p≤0.05 was considered as significant of the data.

Results

Phytochemical analysis was to investigate the presence of phytochemicals in PPE and PTE using qualitative test. Based on Table 1. PTE had the presence of flavonoids, saponins, phenols, tannins, steroids, and alkaloids (+), while PPE has presence some compounds, saponins, tannins, triterpenoids, and terpenoids.

DPPH is a stable free radical because of the unpaired electron that becomes paired in the presence of hydrogen donors.⁹ The DPPH scavenging activity of PPE and PTE was performed in Figure 1 and Table 2. In Figure 1, DPPH activity was increased in a dose-dependent manner. In the highest concentration (200 µg/mL), PPE had a higher activity compared to PTE (67.69% and 67.03%, respectively) (p≤0.05). This indicated that PPE had higher antioxidant activity. In Table 2 showed that PPE had a lower value (IC₅₀=84.09±4.87 µg/mL) compared to PTE value (IC₅₀=98.30±1.30 µg/mL). However, PPE had high DPPH scavenging activity among treatments.

Table 1 Phytochemical analysis of PPE and PTE

Phytochemical Content	PPE	PTE
Flavonoids	-	+
Saponins	+	+
Phenols	-	+
Tannins	+	+
Steroids/Triterpenoids	+	+
Terpenoids	+	-
Alkaloids	+	+

+: detected content, -: not detected

Table 2 The IC₅₀ Value DPPH Scavenging Activity of PPE and PTE

Sample	Linear Equation	R ²	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
PPE	y=0.1627x+35.405	0.97	89.70	84.09±4.87
	y=0.1582x+37.192	0.96	80.96	
	y=0.1654x+36.503	0.96	81.60	
PTE	y=0.1677x+34.297	0.92	93.64	98.30±1.30
	y=0.1855x+31.554	0.96	99.46	
	y=0.1808x+31.594	0.98	101.80	

*PPE= *P. erosus* peel extract, PTE= *P. erosus* tuber extract, R2= coefficient of regression, IC50= The half maximal inhibitory concentration

Table 3 The IC₅₀ Value Tyrosinase Inhibition Activity of PPE and PTE

Sample	Linear Equation	R ²	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
PPE	y=0.3335x+17.51	0.95	96.99	97.05±0.86
	y=0.3362x+16.966	0.96	98.26	
	y=0.3296x+17.495	0.98	98.62	
PTE	y=0.2289x+3.6363	0.95	202.55	194.51±7.63
	y=0.2527x+2.6511	0.98	187.37	
	y=0.2447x+2.6249	0.98	193.60	

*PPE= *P. erosus* peel extract, PTE= *P. erosus* tuber extract, R2= coefficient of regression, IC50= The half maximal inhibitory concentration

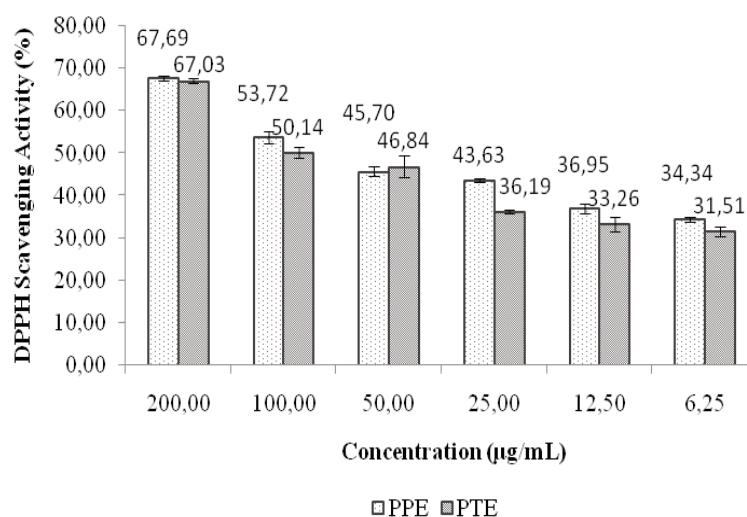


Figure 1 Effect Various Concentrations of PPE and PTE toward DPPH Scavenging Activity

*PPE=*P. erosus* peel extract; PTE=*P. erosus* tuber extract. Each value is expressed as the mean ± SD of triplicate determinations. Statistical analysis was performed using a one-way ANOVA (p≤0.05)

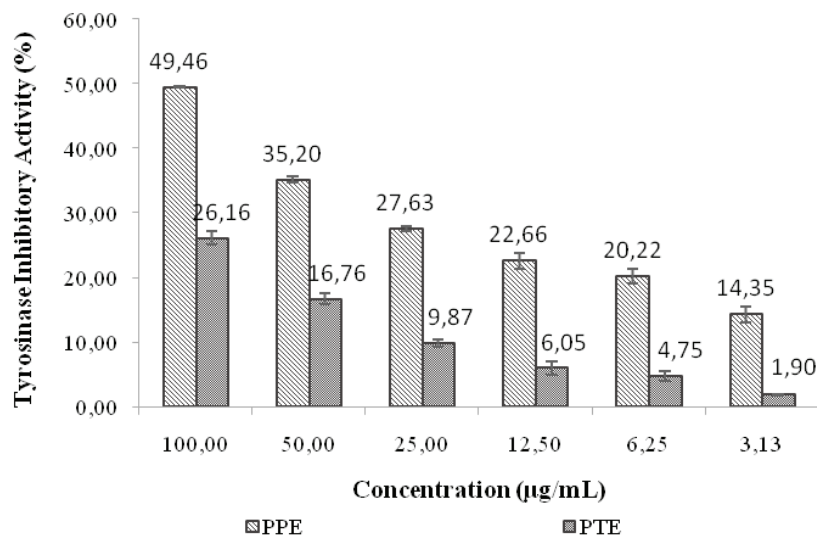


Figure 2 Effect Various Concentrations of PPE, PTE toward Tyrosinase Inhibitory Activity

*Each value is expressed as the mean \pm SD of triplicate determinations. Statistical analysis was performed using a one-way ANOVA ($p \leq 0.05$)

Tyrosinase inhibitory activity of PPE and PTE can be seen in Figure 2 and Table 3. Figure 2 showed that the tyrosinase activities of PPE and PTE were increased in a dose-dependent manner. PPE exhibited higher inhibition of tyrosinase (49.46%) compared to PTE (26.16%) at the concentration 100.00 $\mu\text{g/mL}$ (Figure 2). The higher amount of PPE show it has a stronger inhibitory effect on tyrosinase ($p \leq 0.05$).

PPE had the lower value ($IC_{50} = 84.09 \pm 4.87$ $\mu\text{g/mL}$) compared to PTE value ($IC_{50} = 98.30 \pm 1.30$ $\mu\text{g/mL}$). When IC_{50} values of the extracts were compared, PPE was found to be more effective, reaching the IC_{50} at a much lower concentration than PTE (Table 3).

Discussion

Pachyrhizuserosus a medicinal plant that has been reported to be a good source of antioxidant and antiaging.⁸ *P. erosus* had shown to inhibit skin pigmentation resulting from UV irradiation.² *P. erosus* peel extracts could be suggested as new sources of antiaging agents besides using the tuber of *P. erosus*. In the present study, the phytochemical analysis had been done to identify some compounds in *P. erosus* peel and tuber extracts. PPE had presence compounds of saponins, tannins, triterpenoids, and terpenoids, while in PTE had flavonoids, saponins, phenols,

tannins, steroids, and alkaloids. On another hand, *P. erosus* had saponin and alkaloid compounds, but not detected terpenoid, flavonoid and tannin.¹⁰ Saponins in *P. erosus* also can prevent skin damage from free radical as the result of absorption of ultra-violet rays.¹¹ Terpenoid has the ability in OH and SOD radical scavenging activity and it also can prevent aging.¹² In another study reported that PTE had phytochemical compounds such as flavonoids, retinoids, and phenylfuranocoumarin.⁷ Flavonoid and polyphenol also can inhibit the melanogenesis process.¹³ Flavonoids are natural sunscreens to prevent skin damage from free radicals and effective phenolic substances to inhibit the formation of melanin.¹⁴

The antioxidant assay can be performed using stable free radical diphenylpicrylhydrazyl (DPPH) which can estimate the antioxidant activity of food or natural material.¹⁵ The results of in vitro experiments have indicated that PPE had lower DPPH scavenging activity compared to PTE ($IC_{50} = 84.09 \pm 4.87$; 98.30 ± 1.30 $\mu\text{g/mL}$) ($p \leq 0.05$). According to the results, it suggested that compounds in *P. erosus* had an antioxidant activities. Rusmarilin et al.¹⁶ study showed that *P. erosus* had strong antioxidant activity because IC_{50} value was less than 50 ppm (39.72–11.99 mg/L). Another study suggested that *P. erosus* extract had antioxidant and inhibitory effect on melanin accumulation in B16F10 cells.¹⁷ *P. erosus*

had flavonoids that exhibit various biological activities, including antioxidative and free radical scavenging activities.¹⁸ Pineros-Hernandez et al.¹⁹ have reported that polyphenols compound show strongly correlated with antioxidant activity, more higher level of polyphenols in plant and thereby had greater antioxidant activity. The results of the study showed that the higher the concentration of PPE and PTE also show the higher DPPH scavenging activity. This result in line with Lee et al.¹⁷ performing that *P. erosus* water and 70% ethanol extracts had DPPH and ABTS scavenging activity which increased in a dose-dependent manner between 200 and 4000 µg/mL.

Tyrosinase enzyme catalyzes the hydroxylation of L-tyrosine (mohophenol activity) and the oxidation of L-DOPA (diphenolase activity) to *o*-quinone, which induced the production of melanin pigment.¹ In the present study, PPE had a higher inhibitory activity of tyrosinase compared to PTE with IC₅₀ value 97.05 µg/mL (p≤0.05). This result in line with Lukitaningsih et al.²⁰ study that PPE can suppress melanogenesis by inhibiting tyrosinase activity. Bioactive compounds of *P. erosus* can be used to decrease melanin production and can suppress tyrosinase activity.²⁰ *P. erosus* had active compounds as antioxidant and skin whitening activities such as daidzein, daidzin, genistin, (8,9)-furan-3-yl-pterocarpan-3-ol, 4-(2-(furan-2-yl)ethyl)-2-methyl-2,5-dihydro-furan-3-carbaldehyde and 2-butoxy-2,5-bis(hydroxymethyl)-tetrahydrofuran-3,4-diol.²⁰ *P. erosus* compounds may have phenol or diphenol group which can form a chelate complex with copper (Cu²⁺ ions).⁸ PTE may have a high amount of daidzein-7-O-β-glucopyranose compared to PPE causing a lower inhibitory activity.⁸ Daidzein-7-O-β-glucopyranose had only one phenol group because the other phenol groups bond to a glucose molecule which formed a weak complex resulting in lower inhibitory activity.⁸

Besides, DPPH scavenging activity and tyrosinase inhibition of PPE are higher than PTE that presumably due to the combination of activities between compounds and secondary metabolites contained therein. Therefore, *P. erosus* evidently which had the potential to be further natural source for antioxidant and antiaging therapy and can be used as skin-whitening agents in cosmetics. In summary, PTE effective to prevent skin aging, possibly through the antioxidant activity.

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