

Characterization, biological activities and safety evaluation of different varieties of Thai pigmented rice extracts for cosmetic applications

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ABSTRACT

The study aimed to evaluate the total phenolic and flavonoid contents, biological activities and safety of different varieties of Thai pigmented rice for cosmetic applications. Five different varieties of Thai pigmented rice included Sang-Yod (SY), Mun-Poo (MP), Hom-Nin (HN), Rice Berry (RB), and Luem-Pua (LP). The rice grains were extracted using 50% ethanol and dried under vacuum. The total phenolic and flavonoid contents of rice extracts were in the range of 128-322 mg GAE/g extract and 86-341 mg CE/g extract, respectively. LP extract showed the highest values for the total phenolic and flavonoid contents. Based on the antioxidant activities, LP showed the highest radical scavenging activities both DPPH and ABTS assays. Concerning the anti-tyrosinase activity, all rice extracts exhibited a weak performance. In contrast, all rice extracts strongly inhibited the formation of advanced glycation end products (AGEs), the activity of collagenase and elastase enzyme. The obtained results from UV scanning showed the potential of rice extract as a UV absorber. The cytotoxicity using human fibroblast cell indicated the safe use of all rice extracts for topical administration. At the concentration of 400 µg/mL, the cell viability was higher than 50% for all rice extracts and LP showed the highest cell viability compared to others. As a result, rice extracts are a promising ingredient for cosmetics, especially for the anti-aging application.

1. INTRODUCTION

Cosmetic products which improve the skin appearance are increasingly popular among the consumers worldwide. The consumers seek to achieve a healthy, smooth, translucent skin to look young¹. They would like to prolong and reduce skin aging signs such as fine lines, wrinkles and sagging skin. Clinical signs of skin aging are dryness, skin hyperpigmentation and most importantly the loss of skin elasticity. These undesired events are aggravated by free radicals which cause inflammation and destroy

collagen and elastin fibers in the dermal extracellular matrix²⁻⁵.

In recent years, the trend of incorporating natural ingredients into cosmetic formulation has emerged. They have gained popularity mostly due to the consumer's impression that considers natural products as safer compared to the conventional synthesis products and compatible with all skin types^{6, 7}. Moreover, natural ingredients usually contain antioxidants and other biological activities which could improve skin condition. One of the natural ingredients which are well-known worldwide

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and have been incorporated into cosmetic formulations is rice extract.

Rice (*Oryza sativa* L., Poaceae) is one of the world's leading food crops. Rice is a staple food for several countries including Thailand. Thailand has a long tradition of rice production and is one of the world's largest rice exporters⁸. Thailand has a large number of rice varieties which differ in grain length, color, texture, thickness, aroma and growing condition. Rice and rice bran extracts have been reported to have biological activities which have potentials to be used in pharmaceutical⁹⁻¹², nutraceutical^{13, 14} and cosmetic industries¹⁵⁻¹⁷. Especially in the cosmetic field, rice extract has been used as a skin and hair conditioning agent. The major composition of rice extract are proteins, carbohydrates, lipids, mineral ash and water. Recently, it has been found that rice extracts contain strong antioxidants which are phenolic acids, flavonoids, anthocyanins, proanthocyanins, tocopherols, γ -oryzanol and phytic acid^{16, 18}. Although there are multiple published works related to rice extracts and its potential used in cosmetics, they have thus far only focused on identifying the compositions and the biological properties in each rice variety individually^{16, 18}. However, to the best of our knowledge, the comparison across different types of rice, especially in the cosmetic field still lacks.

In this work, five different Thai varieties of pigmented rice (Sang-Yod (SY, red color), Mun-Poo (MP, red color), Hom-Nin (HN, purple color), Rice Berry (RB, purple color), and Luem-Pua (LP, black color)) were carefully selected based on their pericarp colours and popularity. All these different varieties of rice extracts were compared in terms of total phenolic and total flavonoid contents. The biological activities which are antioxidants, anti-glycation, anti-collagenase and anti-elastase activities were evaluated. In addition, the *in vitro* cytotoxicity of these extracts was assessed to find the best variety of rice extract to be used as a natural ingredient for cosmetic purpose.

2. MATERIALS AND METHODS

2.1. Rice samples and chemicals

Five commercial varieties of colored rice were obtained from local market, Thailand including red rice (local name Sang-Yod; SY), red rice (local name Mun-Poo; MP), purple rice (local name Rice Berry; RB), purple-non-waxy rice (local name Hom-Nin; HN), and black glutinous rice (local name Luem-Pua; LP). Folin-Ciocalteu reagent, gallic acid anhydrous, sodium nitrite (NaNO_2), aluminium chloride anhydrous (AlCl_3), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium peroxodisulfate, Kojic acid, D-glucose monohydrate were obtained from Merck KGaA (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium dihydrogen phosphate dihydrate were purchased from Ajax Finechem (Auckland, New Zealand). (\pm)-Catechin hydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), mushroom tyrosinase enzyme, bovine serum albumin (BSA), aminoguanidine hydrochloride were obtained from Sigma-Aldrich (Missouri, USA). L-3,4-dihydroxyphenylalanine (L-DOPA) was brought from Alfa Aesar (Lancashire, UK). Di-sodium hydrogen phosphate dihydrate was obtained from Scharlab (Barcelona, Spain). Sodium azide (NaN_3) was obtained from Tokyo Chemical Industry (Tokyo, Japan). EnzChekTM elastase assay kit (E-12056) and EnzChekTM gelatinase / collagenase assay kit were brought from Molecular Probes (Oregon, USA). Ethanol (95%) was purchased from Liquor Distillery Organization Excise Department, Chachoengsao, Thailand. Methanol was Laboratory Reagent from Honeywell Burdick & Jackson (Ulsan, Korea). Orthophosphoric acid was purchased from Prolabo (Paris, France). Deionized (DI) water was used throughout the experiments.

2.2. Preparation of rice extracts

The Rice grains were extracted using a hydroalcoholic solvent containing water and ethanol (50:50, v/v) at the ratio of 5:8 w/w for

3 days at room temperature ($30 \pm 2^\circ\text{C}$). Then, the obtained extract was centrifuged at 4,427 g for 15 min (Hermle, Germany). The supernatant was filtered through filter paper (Whatman® No.1, Merck, Germany). The rice grain was extracted at the same procedure as mentioned above for 3 times. Then, supernatant were collected and subsequently evaporated under reduced pressure at 50°C until the weight remained unchanged. The obtained dry extracts were kept in the air-tight container in a refrigerator controlled temperature between 4 and 8°C until use.

2.3. Determination of total phenolic content

The total phenolic content of the rice extracts was determined by Folin-Ciocalteu method with some modification¹⁹. Briefly, 20 μL of the extracts and 50 μL of 10 %v/v Folin-Ciocalteu's reagent were added into the transparent 96-well plates and left to react for 3 min. The reaction mixtures were then neutralized with an addition of 7.5 %w/v Na_2CO_3 (80 μL). After 30 min at room temperature, the absorbance at 765 nm was determined using an infinite M200 Microplate reader (Tecan®, Switzerland). Gallic acid was used as a reference standard for the preparation of the calibration curve. The total phenolic content of the rice extracts was calculated and expressed as mg of gallic acid equivalent (mg GAE)/g of extract.

2.4. Determination of total flavonoid content

The total flavonoid content was determined by aluminium chloride colorimetric method with some modifications²⁰. Catechin was used as a standard reference. The samples or standard solution (20 μL), DI water (80 μL) and 5% NaNO_2 (6 μL) were added into the transparent 96-well plates, and mixed well. After 5 min of shaking, 10% AlCl_3 solution (6 μL) was added. Then, 1M NaOH (40 μL) was subsequently added to the above mixture and the final volume of solution in each well was adjusted to be 200 μL with DI water. After shaking for 10 min at room temperature, the absorbance at 510 nm was recorded by an infinite M200 Microplate reader. The total

flavonoid content of the rice extracts was calculated and expressed as mg catechin equivalent (mg CE)/g of extract.

2.5. Determination of antioxidant activities

2.5.1. DPPH radical scavenging activity

The effect of the rice extracts on DPPH radicals was assessed using DPPH assay with slight modifications²¹. In brief, the stock solution of DPPH was prepared by dissolving DPPH in methanol to obtain the final concentration of 0.2 mM. L-ascorbic acid was used as a reference standard for evaluating DPPH radical scavenging activity. L-ascorbic acid standard or test samples with different concentrations (75 μL) were added to the transparent 96-well plates along with 150 μL of DPPH solution or methanol (for control). Consequently, the samples were incubated and shaken thoroughly for 30 min at room temperature in the dark. The absorbance values were measured at 515 nm with an infinite M200 Microplate reader. The percentage of DPPH radical scavenging activity was calculated according to the equation 1:

$$\text{DPPH radical scavenging (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100 \quad (1)$$

where A_0 represents the absorbance of the control solution and A_t represents the absorbance of the samples.

The DPPH radical scavenging of test samples was expressed as an IC_{50} value which defines as the concentration of test samples necessary to achieve 50% inhibition.

2.5.2. ABTS radical scavenging activity

The ABTS radical scavenging activity of the rice extracts was determined by the decolorization of the ABTS^+ with slight modification. The ABTS cation radicals (ABTS^+) were generated by the reaction between 2.45 mM potassium persulfate and 7 mM ABTS at equal volume stored in the dark at room temperature for 12-16 h. After incubation, ABTS^+ solution was diluted in ethanol to achieve an absorbance at 734 nm of 0.70 ± 0.20 before use.

The rice extracts with different concentrations (40 μ L) were mixed with 140 μ L of the ABTS⁺ solution, incubated and shaken thoroughly at room temperature for 10 min. Then, the absorbance values of mixtures were measured at 734 nm by an infinite M200 Microplate reader. The Trolox solution with different concentrations was used as a positive control. The percentage of ABTS⁺ scavenging activity was calculated using the equation 2:

$$\text{ABTS radical scavenging (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100 \quad (2)$$

where A_0 represents the absorbance of the control solution and A_t represents the absorbance of the samples.

2.6. Determination of anti-tyrosinase activity

The tyrosinase inhibitory activity was determined by dopachrome method using mushroom tyrosinase with some modifications. The L-DOPA was used as the substrate of mushroom tyrosinase enzyme and the absorbance of a by-product, dopachrome, was detected at a wavelength of 475 nm. Firstly, the reaction mixture containing 80 μ L of 20 mM phosphate buffer saline (PBS, pH 6.8), 40 μ L of positive control (kojic acid) or samples, 40 μ L of 250 unit/mL mushroom tyrosinase was mixed and shaken for 10 min at 37 °C in the transparent 96-well plates. Then, 40 μ L of 1 mM L-DOPA were added to each well. After incubating for 10 min at 37 °C, the absorbance values of dopachrome were measured at 475 nm by an infinite M200 Microplate reader. The percentage of tyrosinase inhibition was calculated according to the equation 3:

$$\text{Tyrosinase inhibition (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100 \quad (3)$$

where A_0 represents the absorbance of the control solution and A_t represents the absorbance of the standard reference or samples at various concentrations.

2.7. In vitro determination of anti-glycation activity

Inhibition of advanced glycation end products (AGEs) was determined according

to BSA-glucose model²². The reaction mixture consisted of 500 μ L of BSA (1600 μ g/mL), 100 μ L of 2000 mM D-glucose in phosphate buffer (0.05 M, pH 7.4) containing 0.2 g/L NaN₃, 150 μ L of phosphate buffer (0.05 M, pH 7.4) and 250 μ L of aminoguanidine as a positive control or samples at various concentrations. After that, all reaction mixtures (control and test samples) were incubated at 60 °C for 40 h in the dark. The generated AGEs were determined by measuring fluorescence intensity at excitation and emission wavelengths of 370 and 440 nm, respectively. The percentage of AGE inhibition was calculated as shown in equation 4.

$$\text{Glycation inhibition (\%)} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100 \quad (4)$$

where A is the fluorescent intensity of the incubated mixture without sample as positive control, B is the fluorescent intensity of the incubated mixture of phosphate buffer and solvent as blank control, C is the fluorescent intensity of the incubated mixture with sample and D is the fluorescent intensity of the incubated mixture of phosphate buffer and solvent as sample control. The anti-glycation activity of all samples was plotted and IC₅₀ value, the concentration of the samples providing 50% inhibition, was calculated and used to compare the efficacy among samples.

2.8. Determination of collagenase inhibitor

In vitro anti-collagenase activity of the rice extracts was performed using EnzCheck E-12055 gelatinase/collagenase assay kit. The kit contains fluorescein-conjugated DQTM gelatin and collagenase from *Clostridium histolyticum* used as a substance and enzyme, respectively. The DQTM gelatin solution at the concentration of 50 μ g/mL (20 μ L), test samples at various concentrations (80 μ L) and collagenase at the concentration of 0.4 U/mL (100 μ L), which was dissolved in Tris-HCl buffer (pH 7.4), were mixed in 96-well plate and incubated for 90 min at 37 °C. After that, the fluorescent intensity was measured at excitation and emission wavelengths of 485 and 520 nm, respectively. The positive standard was 1,10-Phenanthroline.

Collagenase inhibition was calculated as shown in equation 5.

$$\text{Collagenase inhibition (\%)} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100 \quad (5)$$

where A is the fluorescent intensity of enzyme without a sample, B is the fluorescent intensity without sample and enzyme, C is the fluorescent intensity of sample with enzyme and D is the fluorescent with the sample and without enzyme. The anti-collagenase activity was plotted and the IC₅₀ value was calculated and used to compare the efficacy of among samples.

2.9. Determination of elastase inhibitor

The anti-elastase activity was evaluated using EnzCheck elastase assay kit. In brief, porcine pancreatic elastase was dissolved in reaction buffer containing 0.1 M Tris buffer (pH 8) and 0.2 mM NaN₃ to obtain the final concentration of 0.05 U/mL of porcine pancreatic elastase. The pancreatic enzyme (100 µL) was mixed with test samples (50 µL) at different concentrations and pre-incubated at 37 °C for 5 min in a black 96-well plate. Subsequently, 50 µL of DQTM elastin (0.01 mM) was added, shaken and incubated at 37 °C for 30 min. The fluorescent intensity was recorded at excitation and emission wavelengths of 485 and 535 nm, respectively. N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone was used as a positive control. The percentage of elastase inhibition was calculated according to equation 6.

$$\text{Elastase inhibition (\%)} = \frac{[(A-B)-(C-D)]}{(A-B)} \times 100 \quad (6)$$

where A is the fluorescent intensity of enzyme without a sample, B is the fluorescent intensity without sample and enzyme, C is the fluorescent intensity of sample with enzyme and D is the fluorescent with the sample without enzyme.

2.10. Determination of photoprotective activities

To evaluate the potential of rice extracts to be used as an UV absorber, the rice extracts were diluted with 50% ethanol to obtain the final concentration of 0.4 mg/mL. The same

solvent was used as a blank. The samples were scanned from 290-400 nm using a double beam UV-spectrophotometer (UV-2600, Shimadzu, Japan).

2.11. *In vitro* cytotoxicity test in human fibroblast cell

2.11.1. Cell culture

Human primary fibroblast cells, BJ (Foreskin dermal fibroblast; from ATCC[®] CRL-2522TM, USA) were maintained in minimum essential medium (MEM, Gibco[®], Life Technologies, USA). Media was supplemented with 10% fetal bovine serum (FBS, Gibco[®]), 1 mM sodium pyruvate (Gibco[®]) and penicillin/streptomycin (Gibco[®]) at the concentration of 100 U/mL and 100 µg/mL, respectively. Cells were incubated at 37 °C and 5% CO₂ and were sub-cultured before reach 80% confluence.

2.11.2. Cytotoxicity assay

In this experiment, cytotoxicity was determined by MTT assay as previously described by Pripem et al.²³. BJ cells were seeded one day prior the experiment in 96-well plates at a concentration of 1x10⁴ cells per well. Cells were exposed to rice extracts at different concentrations. Hydrogen peroxide at the concentration of 0.01- 1% v/v was used as a positive control for cytotoxicity. After 24 h of incubation, the supernatant was removed. Cells were rinsed twice with PBS (0.01 mM, pH 7.4). Fresh media containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at the concentration of 0.5 mg/mL was added and consequently incubated at 5% CO₂ at 37 °C for 4 h. After the incubation, DMSO was added in order to dissolve formazan crystals. The formazan was quantified by the colorimetric method at 570 nm using an infinite M200 Microplate reader. Finally, cell viability was calculated according to equation 7 as the following:

$$\text{Cell viability (\%)} = \frac{(A_0 - A_t)}{A_0} \times 100 \quad (7)$$

where A₀ represents the absorbance provided by untreated cells and A_t represents the absorbance of the positive control or samples at various concentrations.

2.12. Statistical analysis

The data were expressed as the mean \pm standard deviation (SD). Data were analyzed using student's *t*-test or One-way ANOVA with Bonferroni's post-test. The significant difference was set at 95% confidential level ($p < 0.05$). All statistical analyses were conducted using GraphPad Prism version for Windows (GraphPad Software, USA).

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoid contents

Both phenolic and flavonoid compounds exhibit a series of biological properties that influence the human health²⁴. The total phenolic contents of rice extract were in the range of 128–322 mg GAE/g extract as shown in Figure 1a. LP extract showed the highest total phenolic content, followed by SY, MP, RB and HN extracts, respectively. Among the Thai pigmented rice varieties, black rice varieties had higher total phenolic contents, followed by red and purples rice varieties. The higher phenolic content in black rice varieties compared with red and purples rice varieties has been widely reported^{25–28}.

For the flavonoid content, the obtained values were in between 86–341 mg CE/g extract as shown in Figure 1b. LP extract showed the highest total flavonoid content, followed by

RB, HN, MP and SY extracts, respectively. The total flavonoids content was different among Thai pigmented rice varieties, with black rice > purple rice ~ red rice. In our study, LP extract showed the highest total phenolic and total flavonoid contents compared to others with more than two-fold increase. These results were consistent with the previous literature^{26, 29, 30}. In previous studies¹⁸, twelve phenolic acids had been identified and reported in rice. The total phenolic content depended on the parts of rice (e.g. endosperm, bran, husk or whole grain) and also rice varieties of different colors. The pigmented rice varieties (e.g. black, purple and red) contain higher phenolic acids than the non-pigmented rice varieties (brown rice). Based on the scientific reports, the most abundant phenolic acid found in bran, endosperm and whole grain of rice is ferulic acid (56–77%), followed by *p*-coumaric acid (8–24%). For husk, *p*-coumaric acid (~ 71%) was reported to be dominant phenolic acid, followed by ferulic acid (23%)¹⁸. For the total flavonoid content, the pigmented rice was also higher than non-pigmented rice varieties and it is abundant in rice bran, followed by the husk, whole grain and endosperm, respectively¹⁸. The flavonoid that found most in rice bran is tricin which has potential to be used in cosmetic and nutraceutical fields³¹.

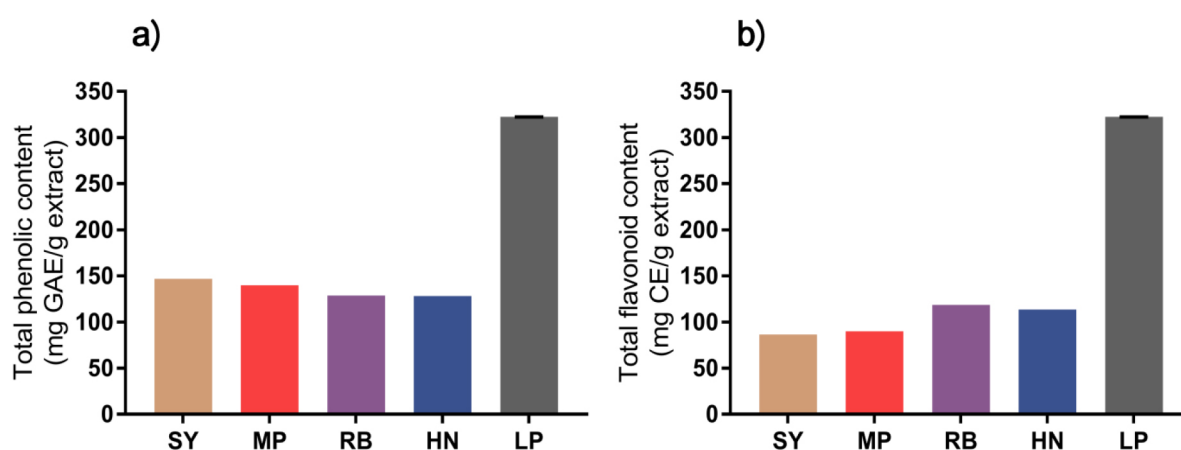


Figure 1. Total phenolic compound (a) and total flavonoid content (b) of rice extracts. The data are expressed as mean \pm SD ($n = 3$).

3.2. Antioxidant scavenging activity by DPPH and ABTS assay

Free radicals are unstable molecules that generated in the human body and derived from oxygen (reactive oxygen species, ROS) and nitrogen (reactive nitrogen species, RNS)³². Free radicals can alter lipids, proteins and DNA which lead to various diseases. The antioxidants could reduce the level of free radicals which could delay or inhibit cellular damage. Thus, antioxidants play an essential role in the healthcare system, especially in pharmaceuticals and cosmetics. Since aging process links with an imbalance between the generation of free radicals and antioxidant defenses, reduction of free radicals may delay aging³³. In this study, the scavenging activities were evaluated using DPPH and ABTS methods. DPPH and ABTS methods are widely used to evaluate antioxidant activities of plant extracts. ABTS method is based on preventing the chain initiation by antioxidants^{18,34,35}. ABTS measures the relative ability of substances to scavenge the ABTS^{•+} (blue-green color), which is generated by reacting the samples with a strong oxidizing agent, e.g. potassium persulfate, to be ABTS (colorless). The reduction in blue-green color indicates the antioxidant abilities of substances. In contrast, DPPH is a stable free radical in purple color. It is widely used for evaluating the antioxidant activities owing to its simplicity and quick analysis¹⁹. The DPPH can absorb the wavelength at 515 nm and the absorption is reduced when DPPH reacts with antioxidants²⁰. The DPPH and ABTS radical scavenging abilities of rice extracts

from different rice varieties were shown in Figure 2. It was observed that the DPPH radical scavenging of all rice extracts revealed in a dose-dependent manner. In this experiment, IC₅₀ of standard L-ascorbic acid was 4.33±0.33 µg/mL. Among all varieties tested, LP extract showed the highest DPPH radical scavenging with the lowest IC₅₀ of 0.034±0.002 mg/mL which was directly correlated with the total phenolic and the flavonoid contents. In the previous studies, it has been reported that the antioxidant activities of plant extract were related to the amount of phenolic and flavonoid contents. The higher phenolic and flavonoid contents, the higher antioxidant were observed^{36,37}.

As shown in Figure 2, LP extract also showed the highest ABTS radical scavenging when compared to other rice extracts. IC₅₀ of LP extract was 0.016±0.001 mg/mL, followed by SY, MP, HN and RB extract, respectively. IC₅₀ of standard Trolox was 6.71±0.08 µg/mL. The obtained results from DPPH and ABTS assay demonstrated that the antioxidant ability seems to be correlated with the amount of phenolic and flavonoid contents found in rice extract. It was likely due to the attribution of different kinds of antioxidant compounds, especially phenolic acids, flavonoids, anthocyanin and proanthocyanin which were found in black and red rice bran^{18,38}. Concerning the structure of phenolic and flavonoid compounds, they contain active hydroxyl groups which are good hydrogen donors. Therefore, such molecules can react with reactive oxygen species and act as an antioxidant. In addition, phenolic compounds can react with metal ions involved in the production of free radicals³⁹.

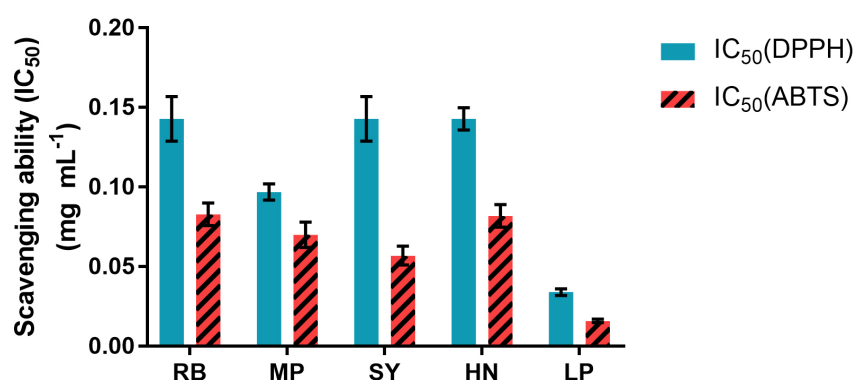


Figure 2. Scavenging ability of rice extracts in five varieties expressed as IC₅₀ measured by DPPH and ABTS assay. The data are expressed as mean ± SD (n = 3).

Moreover, the previous study reported that the extraction solvent influenced the antioxidant ability of rice bran extract. The aqueous mixture of acetone, ethanol and methanol provided the higher DPPH radical scavenging in comparison to absolute solvent solely⁴⁰. Therefore, 50% ethanol was used as the extraction solvent in this study.

3.3. Anti-tyrosinase

The melanin plays a prominent role in skin color. There are two types of melanin found in human including eumelanin and pheomelanin depending on the phenotype. Both melanins are produced from melanocyte cells located in the basal layer of the epidermis. The pheomelanin produces the yellow to reddish-brown color whereas the eumelanin originates light brown to black colour⁴¹. The melanin production can be induced by UV radiation due to the formation of ROS, leading enhanced melanin biosynthesis.

Tyrosinase, which is the rate-limiting step enzyme for melanin production, catalyzes the L-tyrosine to DOPA and further to DOPA quinone. Then, DOPA quinone is transformed to melanin⁴¹. Therefore, the inhibition of hyperpigmentation can be done using ROS scavengers such as the antioxidant or inhibitory activity of tyrosinase.

In this study, the anti-tyrosinase activity of rice extracts was investigated. The IC_{50} of kojic acid was $17.69 \pm 0.73 \mu\text{g/mL}$. All rice extracts showed the inhibitory activity of tyrosinase in a concentration-dependent fashion; however, the IC_{50} of all rice extracts could not determine due to the low potential of the extracts in the tyrosinase activity inhibition. Therefore, the anti-tyrosinase activity of rice extracts was compared at the concentration of 1 mg/mL. At this concentration, MP extract exhibited the highest tyrosinase inhibition followed by LP extract, compared with the other extracts as shown in Table 1.

Table 1. Anti-tyrosinase activities of five varieties rice extracts

Rice extract	Tyrosinase inhibitory activity (%) ^a at 1 mg/mL
SY	15.45±2.08
MP	23.51±10.88
RB	14.49±3.61
HN	14.27±6.33
LP	19.56±8.77

^a Data are expressed as mean \pm SD (n = 3).

3.4. Anti-glycation

Glycation, also known as the Millard reaction, occurs in animals and vegetables which is a non-enzymatic reaction between sugar and the free amine group of amino acids in protein, leading to the formation of AGEs. This reaction is also found in the kidney, lens, vessels, skin, etc. In the skin, one of the causes of aging is the appearance of AGEs²¹. AGEs can deposit in the skin and react with proteins, lipids and DNA⁴². AGEs cause the changes of biological activity in the skin such as an activation of the matrix metalloproteinases (MMPs), resulted in the degradation of collagen. This

reaction can also be activated by the UV radiation.

In our study, the ability of rice extract to inhibit glycation reaction was evaluated by BSA-glucose model. Aminoguanidine was used as a positive reference standard with the IC_{50} of $0.041 \pm 0.010 \text{ mg/mL}$. From Figure 3a, SY extract was the most potent in anti-glycation with the IC_{50} of $0.058 \pm 0.005 \text{ mg/mL}$, followed by LP extract with the IC_{50} of $0.069 \pm 0.015 \text{ mg/mL}$. In previous studies, it was reported that natural extracts containing polyphenolic compounds effectively inhibited the formation of AGEs. Therefore, they have been used to prevent or delay the onset of diabetic complication⁴³.

3.5. Anti-collagenase activity

Collagens are the most abundant protein found in the extracellular matrix. They are joined together by elastin. Collagens and elastin work together to give skin its shape and firmness. Collagens found in the human body have at least 16 different collagen types. The most abundant collagens are collagen I, II and III. MMPs, a group of zinc-containing proteinases, are involved in the breakdown of extracellular matrix (e.g. collagen and elastin), leading to the signs of skin aging such as wrinkles, lack of skin firmness, skin sagging⁴⁴.

In this study, the anti-collagenase activity of rice extracts was performed using EnzCheck E-12055 gelatinase/collagenase assay kit. It was found that all rice extracts could inhibit the activity of collagenase in a concentration-dependent manner.

As depicted in Figure 3b, LP extract (IC_{50} , 0.015 ± 0.008 mg/mL) showed the highest potential to inhibit the collagenase activity followed by MP (IC_{50} , 0.045 ± 0.003 mg/mL), SY, RB and HN extracts, respectively.

3.6. Anti-elastase activity

Elastin is a highly elastic protein in connective tissue which is found in the dermis. It helps skin to return to its original when it is stretched or pulled. The loss of degeneration of the elastic fiber network can cause skin aging. Elastase can cleave proteins, particularly at the amino acid valine such as elastin. The activity of elastase is increased by ROS, leading to the degradation of elastin⁴⁵.

To evaluate the potential of rice extract for the anti-aging purpose, the anti-elastase activities were evaluated using EnzCheck elastase assay kit. All rice extracts could inhibit the activity of elastase in a concentration-dependent manner. The IC_{50} of N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone, a positive control, was 3.45×10^{-4} mM. However, the IC_{50} of all rice extracts could not observe at the concentration between 0.019 and 0.625 mg/mL. Therefore, the percent elastase inhibition at a concentration of 0.625 mg/mL was used to compare among rice extracts. Among all rice extracts tested, LP and MP extracts had a high activity to inhibit the elastase enzyme, as shown in Figure 3c.

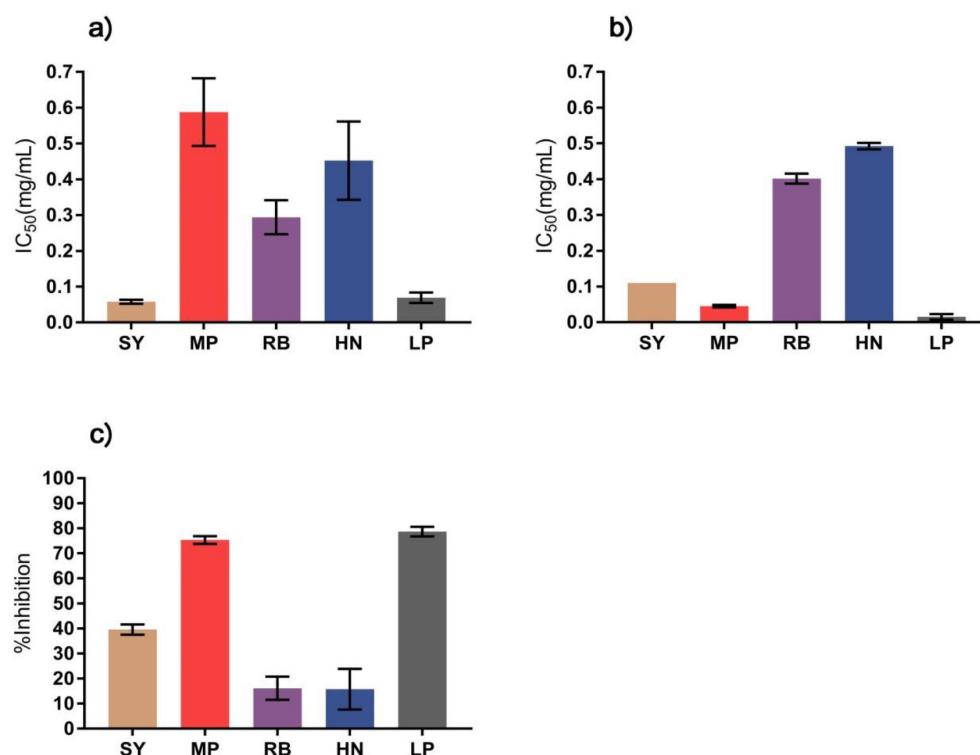


Figure 3. IC_{50} of anti-glycation activity (a), IC_{50} of anti-collagenase activity (b) and percent inhibition of anti-elastase activity at the concentration of 0.625 μ g/mL (c) of rice extracts. The data were expressed as mean \pm SD (n = 3).

3.7. Photoprotective activities

It has been reported that the plant-based ingredients (e.g., flavones) can absorb UV light⁴⁶. There are two mechanisms of sunscreens to protect skin from exposure to UV radiation including by reflecting/scattering the UV-rays such as titanium dioxide and zinc oxide and by absorbing the light before reaching the skin such as octyl methoxycinnamate (OMC) and avobenzone. With respect to the phytochemicals of flavonoids and phenolic compounds, they contain a structure that can absorb light photons that cause skin damage and rapidly return to the ground state like sunscreens. Hence, they are substances described with a photoprotective action⁴⁷.

In general, UVB ranges from 290 to

320 nm. It can penetrate into the epidermis and causes sunburn. UVA ranges from 320 to 400 nm. Due to the longer wavelength of UVA, it can penetrate into the dermis and degrade collagen leading to the skin aging⁴⁸.

According to the spectral absorption between 290–400 nm, LP extract showed the potentially good UV absorbance, especially at UVB range as shown in Figure 4. The absorbance value at 290 nm of LP extract was higher than other rice extracts approximately two- to three-folds, respectively. Among all rice extracts tested, MP extract showed the lowest ability to absorb the UV radiation in the range between 290 and 400 nm. As a result, LP extract is a promising ingredient for enhancing the efficacy of sunscreen products.

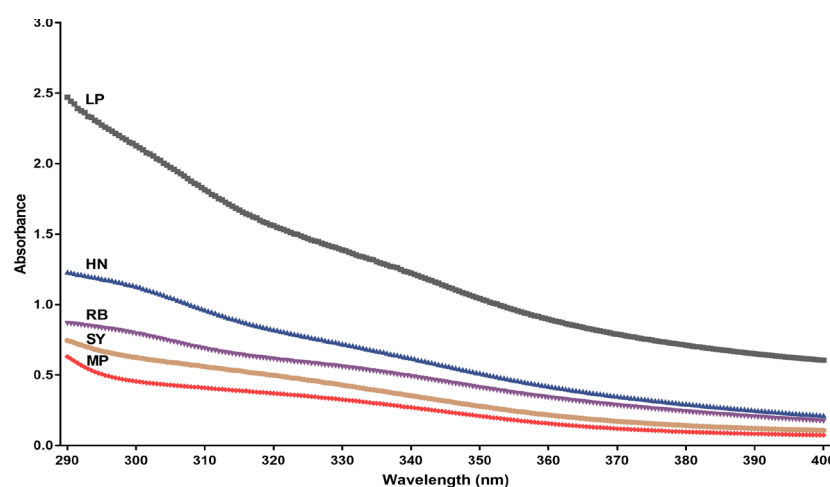


Figure 4. Absorbance of rice extracts in UVB and UVA range.

3.8. *In vitro* cytotoxicity test in human fibroblast cell

Due to the application of rice extracts for topical/dermal delivery system, *in vitro* cytotoxicity was performed using human fibroblast cell. According to Figure 5, the cell viability of fibroblast cell when exposed to rice extracts is in the concentration-dependent manner. In all concentrations tested (25–800 µg/mL of rice extracts in cell culture media), almost no toxicity was observed for LP extract since the cell viability was higher than 80% in

all concentrations tested (Figure 5e). In contrast, MP extract showed the lowest cell viability and cell viability of higher than 80% was exhibited at concentrations equal and below 100 µg/mL (Figure 5b). However, all rice extracts at the test concentrations except for MP extract at 800 µg/mL showed the cell viability of equal or higher than 50%. This indicated the low toxicity of rice extracts. Concerning the cell viability of fibroblast cell after exposure to hydrogen peroxide (positive control) from 1% to 0.01%, the cell viability was in the range of 25–35% (data not shown).

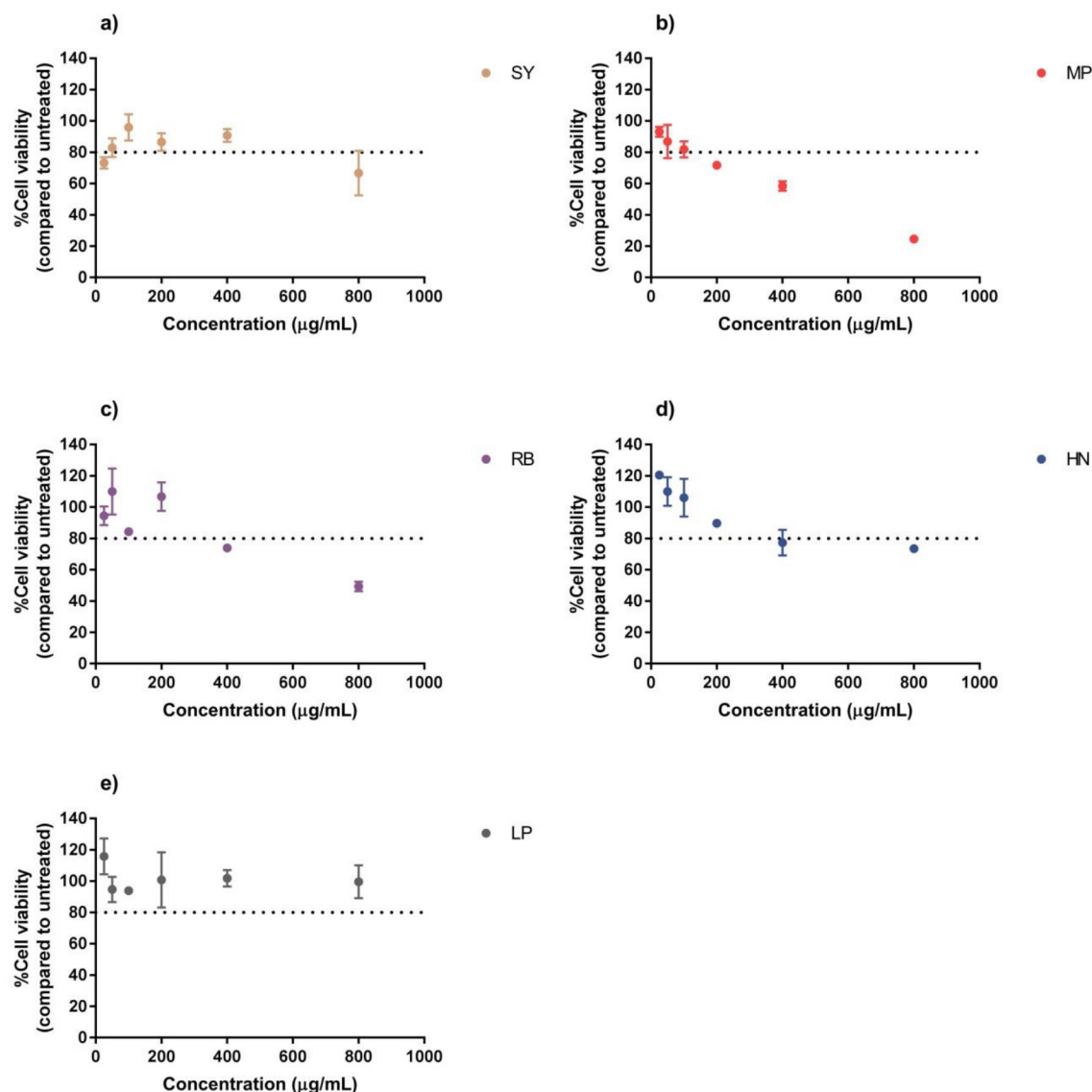


Figure 5. Relative cell viability (%) of fibroblast cells after treatment with rice extracts for 24 h according to concentration and expressed as mean \pm SD (n = 3).

4. CONCLUSIONS

We concluded that rice extracts exhibited good antioxidant activities as determined by DPPH and ABTS assays. Also, they possessed a high performance to be used for skin aging treatments as indicated by anti-glycation and anti-collagenase activities. Compared to other rice extracts, LP extract showed the best performance in term of antioxidant, anti-glycation, anti-collagenase, and anti-elastase. Moreover, LP extract could absorb both UVA and UVB;

therefore, it can be used in combination with UV filter to enhance the efficacy of sunscreen products. Concerning the cytotoxicity test on human fibroblasts, LP extract showed the highest compatible and good dermal tolerance.

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