Indonesian Purple Rice Ferulic Acid as a Candidate for Anti-aging through the Inhibition of Collagenase and Tyrosinase Activities

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Abstract: *Skin aging is associated with decreased skin firmness and excessive pigmentation, which is caused by the activity of aging enzymes. This process can be prevented with powerful antioxidants from nature, such as ferulic acid which is abundant in rice. This study examines the nutritional content and phytochemicals of Indonesian purple rice and evaluates the bioactivity of ferulic acid as an anti-aging agent. Indonesian purple rice has less fat than black and white rice, more amino acids involved in aging regulation, and a similar phytochemical profile to black and white rice. Indonesian purple rice has a lower concentration of ferulic acid (4.114 ± 0.013 mg/L) than black rice but shows strong reducing power (IC_{50} 9.35 ± 1.95 μg/mL), high anti-tyrosinase (IC_{50} 59.57 ± 3.60 μg/mL), and moderate anti-collagenase activities (IC_{50} 74.18 ± 3.11 μg/mL). This study supports the use of Indonesian purple rice as a promising active ingredient in natural anti-aging cosmetics.*

Keywords: *anti-aging; collagenase; ferulic acid; purple rice; tyrosinase*

■ INTRODUCTION

Skin aging refers to the process of the skin losing its structural integrity and physiological function as a result of both internal and external influences. Genetics is an intrinsic component that is altered by time. The environment, particularly ultraviolet (UV) radiation, is an external influence [1-2]. Smoking, pollution, poor diet, lack of sleep, stress, and severe temperatures are a few more environmental factors that affect or may influence skin aging [3].

Exposure to UV radiation promotes the production of excess free radicals or reactive oxygen species (ROS), which activates collagenase and tyrosinase via the mitogen-activated protein kinase (MAPK) pathway [4-5]. Collagenase breaks down collagen and generates wrinkle formation, and tyrosinase catalyzes melanin synthesis from tyrosine, and causes hyperpigmentation [6-9].

Wrinkle formation and hyperpigmentation are signs of aging that can be prevented with an anti-aging strategy [7,9-10]. The anti-aging mechanism includes the ability to scavenge free radicals, protect the skin from UV exposure, increase skin moisture, and increase collagen production or prevent collagen degradation [3]. Collagenase and tyrosinase inhibitors have been widely used in cosmetic or pharmaceutical products as anti-aging agents [8,11].

Recently, there has been a surge of interest in naturally occurring active ingredients for anti-aging products. One potent antioxidant found in nature is ferulic acid, also known as 4-hydroxy-3-methoxycinnamic acid [12-13]. Ferulic acid can be

found in rice, oats, wheat, pineapple, seeds of coffee, beans, nuts, artichoke, and peanuts [14]. However, rice is the most common source of ferulic acid [15].

Among the rice varieties, pigmented rice is richer in nutrients than non-pigmented rice because it is produced without a grinding and polishing process [16]. There is Indonesian purple rice (IPR) produced by crossbreeding between *Mentik Wangi* black rice (BR-MW) and *Mentik Susu* white rice (WR-MS) [17]. Up to this point, there has never been an exploration of the potential for ferulic acid from rice in Indonesia, particularly the IPR. Ferulic acid levels commonly were found to correlate positively with rice pigmentation [18], but rice with a light purple color (variety YF67) obtained from a crossbreeding between black rice and white rice from China, produced higher levels of ferulic acid than black rice parental [19]. The higher concentration of ferulic acid in variety YF67 makes us wonder if the IPR is a potential source of ferulic acid, and further research into its ferulic acid content is required.

Previous research demonstrating the antimicrobial and anti-inflammatory properties of IPR ferulic acid supports its anti-aging potential [20-21]. Nevertheless, the ability to scavenge free radicals and inhibit aging enzymes needs further investigation. Moreover, the nutritional and phytochemical content, which may support anti-aging properties, is also important to explore. Therefore, this study aims to analyze the nutritional value and phytochemical profile of IPR and evaluate the potential of its ferulic acid content as an anti-aging.

■ EXPERIMENTAL SECTION

Materials

The research material used were ferulic acid (≤ 100%, Sigma-Aldrich, PHR1791), Folin-Ciocalteu (Merck, 1.09001.0500), collagenase from *Clostridium histolyticum* (≥ 125 units per mg, Type 2, Worthington), tyrosinase from *Agaricus bisporus* (1240 units per mg, Worthington), kojic acid (> 98%, TCI, KD010), L-ascorbic acid (> 99%, TCI, A0537), tricine (> 99.0%, TCI, T0682), L-tyrosine (> 98.5%, TCI, T0550), *N*-[3-(2-furyl)acryloyl)-ley-gly-pro-ala]/FALGPA (95.2%, HPLC grade, Sigma, F5135), potassium ferricyanide, and trichloroacetic acid.

Four cultivars of rice were used in this research. Indonesian purple rice (IPR), *Mentik Wangi* black rice (BR-MW), and *Mentik Susu* white rice (WR-MS) were obtained from a local farmer in the Ngawi region, East Java, Indonesia. The *Jeliteng* black rice (BR-J), the national standard black rice, was used as the positive control for rice. This rice was obtained from The Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture, Republic of Indonesia.

Instrumentation

The instruments used were a shaker water bath (Memmert), rotary evaporator (IKA HB 10), Fourier Transform Infrared spectrophotometer (FTIR, Shimadzu, IR Type Prestige 21), High-Performance Liquid Chromatography (HPLC, Prominence-I, LC-2030C 3D Plus, serial no. L214556, Shimadzu) with shim-pack GIST C18 column (5 µm, 4.6 × 150, Shimadzu), Ultra Performance Liquid Chromatography (UPLC), Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), and UV-Vis spectrophotometer (SmartSpec Plus™, BioRad Laboratories Inc., Hercules, CA, USA).

Procedure

Proximate analysis

Proximate analysis was carried out in the Central Laboratory of Life Sciences, Brawijaya University. The analysis consists of carbohydrate, protein, lipid, ash, and water contents according to Indonesian National Standard (SNI 01-2891-1992) method.

Determination of amino acids

Determination of amino acid content was conducted in PT. Saraswanti Indo Genetech, Bogor, West Java, Indonesia. The UPLC was used to analyze L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-threonine, L-valine, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-proline, L-serine, and L-tyrosine, according to the 18-5-17/MU/SMM-SIG protocol. L-methionine and L-cysteine were analyzed using LC-MS/MS according to

18-12-38/MU/SMM-SIG protocol, while L-tryptophan was analyzed using HPLC according to 18-5-63/MU/SMM-SIG. The UPLC was performed using AccQ.Tag Ultra C18 1.7 μm column (2.1 × 100 mm). The mobile phase consisted of A: Eluent A concentrate Amino Acid Analysis AccQ.Tag Ultra; B: Eluent B Amino Acid Analysis AccQ.Tag Ultra 10% in water; C: Aquabidest; D: Eluent B Amino Acid Analysis AccQ.Tag Ultra. The rate of flow was fixed at 0.5 mL/min. A photometric diode array (PDA) at the wavelength of 260 nm was used as the detector [22].

Phytochemical screening

As much as 4 g of rice powder were macerated in 96% ethanol at room temperature for 24 h and then filtrated using Whatman paper No. 1. The filtrates were screened for phytochemical content including phenolic, flavonoid, tannin, anthocyanin, leucoanthocyanidin, glycoside, and anthraquinone using the standard methods [23-24].

Extraction of ferulic acid

Rice was extracted to obtain ferulic acid using the modified method [25]. Alkaline hydrolysis was applied by adding 300 mL of 0.5 M sodium hydroxide to 50 g of rice powder, followed by constant shaking for 4 h at 60 °C. A total amount of 600 mL of 96% ethanol was added to dissolve ferulic acid then the pH was neutralized by adding 37% hydrochloric acid. The mixture was filtered with Whatman filter paper No 41 attached to a vacuum Buchner funnel and evaporated for about 30 min to obtain the concentrated extract. The brown extract indicated the existence of ferulic acid.

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content. Briefly, 200 μL of rice extract (1 mg/mL) was added with distilled water up to 3 mL and mixed with 0.5 mL of Folin-Ciocalteu reagent for 3 min. The mixture was added with 2 mL of 20% sodium carbonate and then incubated in dark conditions for 60 min. The absorbance was measured at 650 nm by a UV-Vis spectrophotometer [26]. Ferulic acid (0–10 ppm) was used as the standard calibration curve. The total phenolic content was calculated by the following formula:

$$\text{Total phenolic content} = \frac{C \times V \times DF}{m}$$

where C represents the concentration of the sample calculated using the standard calibration curve equation (ppm), V represents the volume of the sample (mL), DF represents the dilution factor of the sample, and m represents the mass of the extract (g). The total phenolic content was expressed as mg ferulic acid equivalent per gram (mg FAE/g) [27].

Identification and determination of ferulic acid content

The ferulic acid functional group was identified using FTIR based on standard methods in the Analysis and Measurement Units of the Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University. To confirm the presence of ferulic acid, we conducted HPLC. The concentrated extracts were dissolved in methanol and passed through 0.45 μm nylon filters. A 10 μL of the filtrate was then injected into an HPLC system with a shim-pack GIST C18 column. The mobile phase consisted of methanol and water (1% HAc) (65:35, v/v). The flow rate was 1 mL/min, the oven temperature was 35 °C, and the detector was set at 320 nm. Quantification of ferulic acid was performed via a calibration curve of standard (six different concentration levels range 1.17–35.1 mg/L) [25].

Reducing power assay

Reducing power was determined using Ferric Reducing Antioxidant Potential (FRAP) method modified from [28]. Briefly, 100 μL of the sample (0–10 ppm) were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% of potassium ferricyanide, then incubated in a dark condition at 50 °C for 20 min. The mixture was added with 2.5 mL of 10% of TCA. Then 5 mL of each mixture was added with 5 mL of distilled water and 1 mL of 0.1% of $FeCl_3$. The absorbance was measured at 700 nm by a UV-Vis spectrophotometer. Ascorbic acid was used as the positive control. The antioxidant activity was calculated using the following equation:

$$\%\ \text{Reducing power} = \frac{As - Ac}{As} \times 100\%$$

where As represents the absorbance of the sample and Ac represents the absorbance of control. The reducing power was expressed in terms of IC_{50} values, which determined the concentration of sample required to inhibit 50% of free radicals. The higher the reducing power, the lower the IC_{50} value.

Anti-aging activity assay

The anti-aging activity of purple rice ferulic acid extract was determined by inhibiting collagenase and tyrosinase activity. The collagenase inhibitory activity assay was performed based on [29]. A mixture of 10 µL of collagenase, 60 µL of Tricine buffer, and 30 µL of the sample (0–100 ppm) was incubated at 37 °C for 20 min. The mixture was added with 20 µL of *N*-[3-(2-furyl)acryloyl)-ley-gly-pro-ala] (1 mM in Tricine buffer) then incubated at 37 °C for 10 min. The absorbance was measured at 335 nm. Ascorbic acid was used as the positive control [11].

The tyrosinase inhibitory activity assay was performed using the modified method [30]. The mixed solution included 1.7 mM L-tyrosine, 10 mM phosphate buffer (pH 6.8), 100 µL of sample (0–100 ppm) and 125 U/mL tyrosinase was incubated at 37 °C for 10 min. The absorbance was measured at 475 nm by a UV-Vis spectrophotometer. Kojic acid was used as the positive control. The collagenase and tyrosinase inhibitory activity was calculated using the following equation [31]:

$$\%\ \text{Inhibitory activity} = \frac{Ac - As}{Ac} \times 100\%$$

where Ac represents the absorbance of the control, and As represents the absorbance of the sample. The anti-aging activity was also expressed as IC_{50} values as in antioxidant activity.

Data analysis

Statistical analysis was performed using GraphPad Prism 8 software. The one-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to determine the significant differences between TPC and IC_{50} values. We also measured the linear relationship between sample concentration and antioxidant activity, anti-aging, and the relationship between TPC and ferulic acid concentrations by Pearson correlation. Differences or correlations were considered significant when $p < 0.05$ [32].

■ RESULTS AND DISCUSSION

Nutritional Content

The nutritional content of IPR is presented in Table 1. The IPR contained lower carbohydrate and higher protein contents than BR-MW. The IPR had a lower level of fat and ash than BR-MW and BR-J. In contrast, the water level of IPR was higher than BR-MW and BR-J. In previous studies, proximate analysis of WR-MS showed carbohydrate content of 74.62%, protein 10.62%, fat 2.92%, water 11.08%, and ash 1.26% [22]. The IPR has lower fat content than both parental and BR-J, which prevents age-related diseases [33-35].

Table 1 also shows the amino acid content of IPR, including essential and non-essential amino acids. We found that IPR had a higher concentration of L-histidine, L-phenylalanine, L-threonine, L-arginine, L-serine, and L-tyrosine than BR-J and BR-MW. The concentration of amino acids is also higher when compared to the concentration of the amino acid WR-MS determined in previous studies [22]. Amino acids play a crucial role in aging regulation and help maintain healthy skin. Aspartic acid and glutamate build the DNA of skin cells, arginine restores skin damage and is a precursor to nitrogen oxides which are essential regulators for blood circulation in the dermis. Arginine is also a component of creatine that stimulates collagen and elastin production and skin function. Histidine protects against UV and smooths the skin. Tyrosine acts as an anti-melanogenic [36-38]. Phenylalanine and tyrosine are precursors of ferulic acid biosynthesis by shikimate or phenylpropanoid pathways [13].

Phytochemical Profile

The overview of the general phytochemical profile of IPR ethanol extract was performed using a color reactions test. Table 2 shows that IPR contains phenolic compounds, flavonoids, tannins, anthocyanins, leucoanthocyanidins, and glycosides. The level of these compounds varied in BR-J and BR-MW. Previously, the screening was done on an ethanol extract of WR-MS,

Table 1. The nutritional content of IPR (a) proximate analysis, (b) amino acid content

a. Component	Concentration (%)		
	BR-J	BR-MW	IPR
Carbohydrate	76.67	78.20*	77.75
Protein	8.26*	7.32	7.82
Lipid	1.98*	1.94*	0.64
Water	11.91	11.58	13.24*
Ash	1.18*	0.96	0.55
b. Amino Acid	**Concentration (ppm)**		
	BR-J	BR-MW	IPR
Essential			
L-Histidine	1940.76	1996.26*	2004.15*
L-Isoleucine	2946.15*	2828.51	2769.24
L-Leucine	6643.06*	6489.40	6197.65
L-Lysine	2872.76*	2858.04*	2639.26
L-Methionine	740.85*	712.54	691.16
L-Phenylalanine	4293.06	4271.29	4593.89*
L-Threonine	3294.73	3501.47	3594.92*
L-Tryptophan	856.48*	783.87	800.27
L-Valine	4413.15*	4401.53*	4254.92
Non-essential			
L-Alanine	3379.15	3436.69*	3187.78
L-Arginine	6751.55	6750.51	6942.86*
L-Aspartic acid	5840.53	5849.29*	5742.45
L-Cysteine	2036.69*	1806.50	729.72
L-Glutamic acid	12832.30*	12242.01	12298.76
Glycine	3627.65	3784.39*	3546.90
L-Proline	3372.86*	3359.61*	3260.84
L-Serine	4706.81	4701.81	4729.39
L-Tyrosine	2685.67	2762.49	2896.75*

Note: An asterisk notation (*) indicates the highest significant value (Tukey's HSD; $P < 0.05$). BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, IPR: Indonesian purple rice

Table 2. Phytochemical screening by color reactions

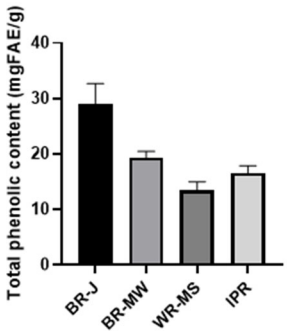
Compounds	BR-J	BR-MW	IPR
Phenolics	+++	++	++
Flavonoids	++	++	+
Tannins	+	+	+
Anthocyanins	+++	+++	+
Leucoanthocyanidins	++	++	+
Glycosides	++	++	+
Anthraquinones	-	-	-

Note: (-) absence of targeted compound, (+) low intensity of targeted compound, (++) moderate intensity of targeted compound, (+++) high intensity of targeted compound. BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, IPR: Indonesian purple rice

which revealed the presence of phenolic compounds and tannins. At the same time, no leucoanthocyanidins or glycosides were found [22]. Furthermore, IPR showed the total phenolic content (Fig. 1) at the same level as the parentals, BR-MW and WR-MS.

Identification of Ferulic Acid

The FTIR analysis was performed to identify ferulic acid functional groups in IPR. The transmittance percentage of IPR was compared to ferulic acid and plotted on the graph (Fig. 2). The FTIR spectra of IPR showed the same pattern as BR-J, BR-MW, and WR-MS.



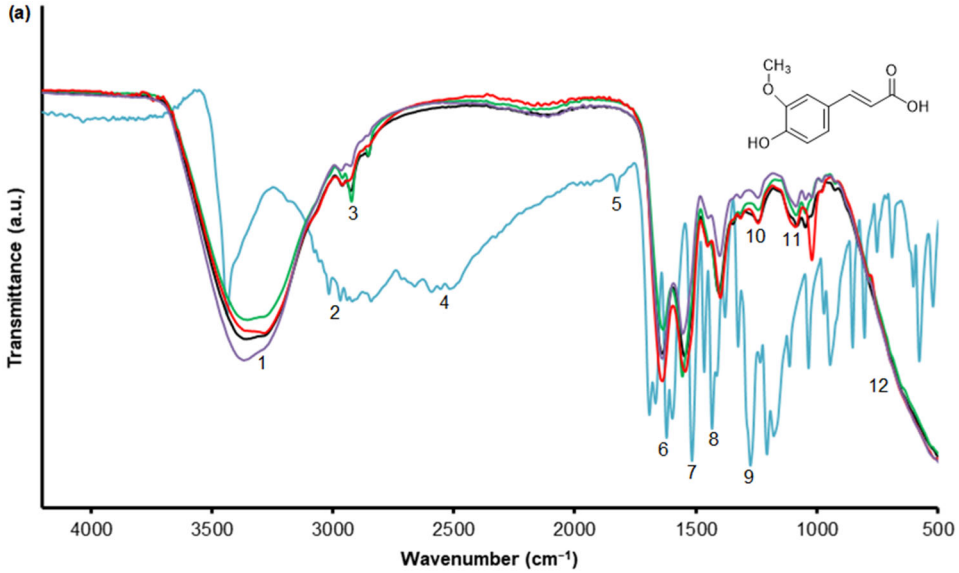
Sample	TPC (mgFAE/g)
BR-J	29.00 ± 3.68^{c}
BR-MW	19.35 ± 1.10^{b}
WR-MS	13.39 ± 1.61^{a}
IPR	16.54 ± 1.32^{ab}

Fig 1. Total phenolic content by the Folin-Ciocalteu method, the different letters indicated significant differences (Tukey's HSD; $P < 0.05$). BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, WR-MS: *Mentik susu* white rice, IPR: Indonesian purple rice

The maximum absorption differed from ferulic acid spectra which could be influenced by the presence of other compounds in the extract. However, the bands were in the same wavenumber zone as ferulic acid. Those similarities showed that several ferulic acid functional groups are present in the IPR, indicating that IPR contains ferulic acid.

The OH, C–H, C–O, and C=C are the functional groups in the structure of ferulic acid [39]. The OH functional group is the fundamental part of most phenolic compounds. The OH and the double bond in the benzene ring contribute to the antioxidant activity. In phenolic acids, the number and position of the OH group are associated with the ability to scavenge free radicals [40-42].

The HPLC analysis confirmed ferulic acid in IPR. The chromatogram (Fig. 3) showed an intense peak at retention time (RT) = 2.144 min, similar to standard

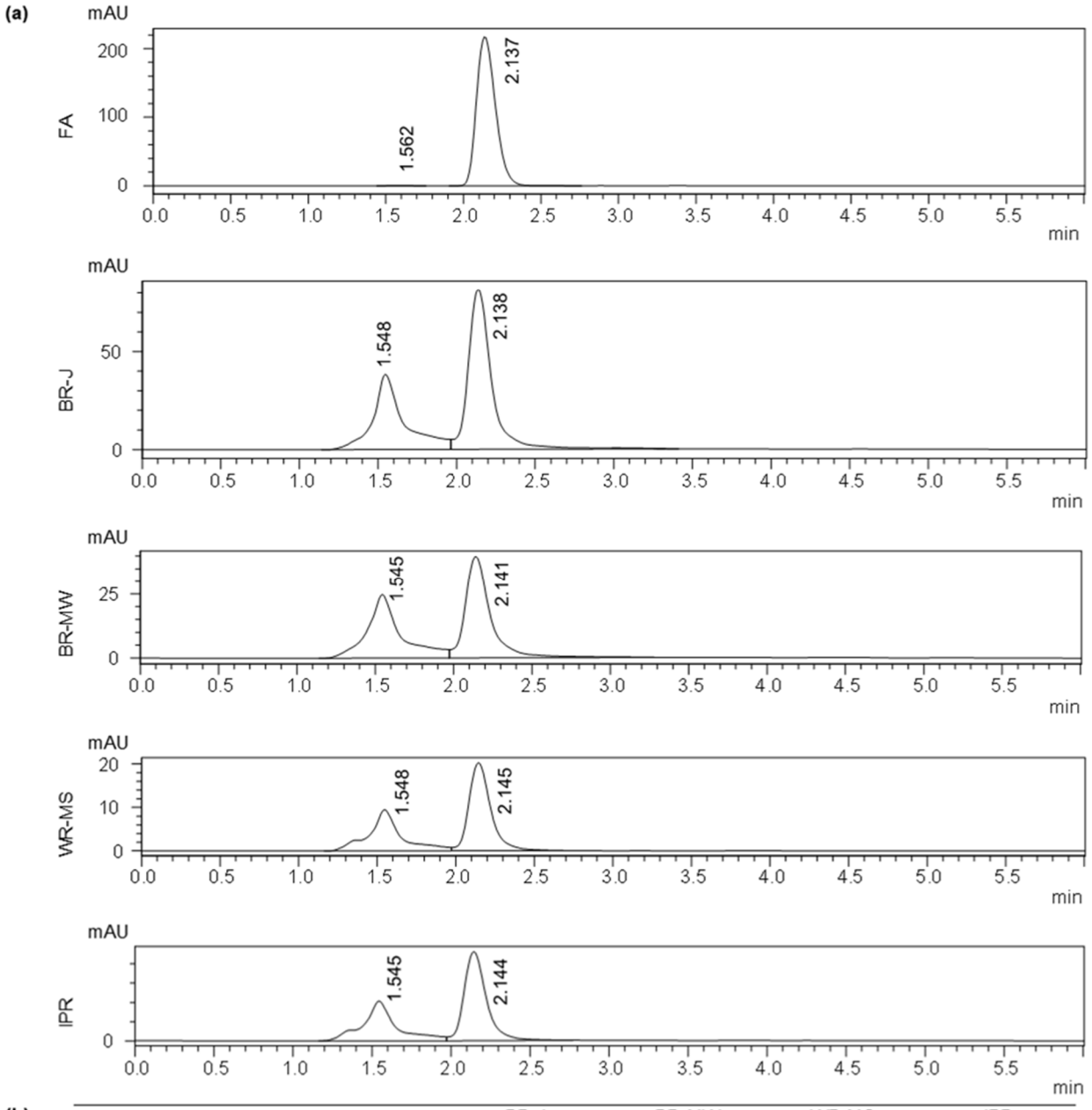


(b)

No	Zone (cm^{-1})	Functional group
1	3700–3200	OH stretch*
2	3100–3000	sp^2 C-H stretch
3	2960–2850	sp^3 C-H stretch*
4	3000–2500	OH
5	1950–1650	C=O
6	1680–1620	C=C stretch*
7	1600–1450	C=C-C Aromatic ring stretch*
8	~1450	CH_2 and CH_3 (CH rocking vibration)*
9	1410–1310	OH bend*
10	1300–1000	C-O group*
11	1300–700	Skeletal C-C vibrations*
12	1000–610	C-H bend

*Present in IPR

Fig 2. Functional group identification by FTIR. (a) FTIR spectra, (b) Table of functional group prediction. FA: Ferulic acid, BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, WR-MS: *Mentik susu* white rice, IPR: Indonesian purple rice



(b)

Ferulic acid concentration (mg/L)	BR-J	BR-MW	WR-MS	IPR
	15.296±0.019	8.081±0.076	3.569±0.003	4.114±0.013

Fig 3. Identification and determination of ferulic acid concentration in IPR. (a) HPLC chromatogram. (b) Table of ferulic acid concentration. FA: Ferulic acid, BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, WR-MS: *Mentik susu* white rice, IPR: Indonesian purple rice

ferulic acid (RT = 2.137 min). Therefore, it revealed the presence of ferulic acid in IPR. Other rice samples also showed the presence of ferulic acid with similar RT. In the chromatogram, we found another peak that appears before the RT of ferulic acid, indicating the presence of another compound in the extract. Because the extract was not purified, thus there are still other phenolic acids. The peak is predicted to be *p*-coumaric acid, the second major phenolic acid after ferulic acid in rice, Fig. 4.

Ferulic acid is found at 56–77% of the total phenolic acid in rice, followed by *p*-coumaric acid at 8–24% [42]. Some studies reported similar chromatogram

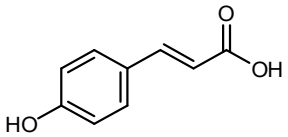


Fig 4. The structure of *p*-coumaric acid

results, in which *p*-coumaric acid appears first and adjacent to the ferulic acid peak. Among these are phenolic acid separations in potatoes, coffee, thymus, and podded bean [43-46].

The concentration of ferulic acid in IPR was determined based on a standard calibration curve. The level of ferulic acid in IPR was higher than WR-MS but lower than BR-J and BR-MW. Ferulic acid concentration positively correlated with total phenolic content (Pearson correlation; $P < 0.05$).

Reducing Power

Reducing power of IPR by the FRAP method used potassium ferricyanide as an oxidant. Potassium ferricyanide ($K_3[Fe(CN)_6]$) is a ferric salt containing the octahedrally coordinated ion $[Fe(CN)_6]^{3-}$, which is frequently used as an oxidant in electron chain reactions [47-48]. The antioxidant compounds react with potassium ferricyanide to form potassium ferrocyanide [49]. The presence of reductants, which exert antioxidant action by breaking free radical chains by donating a hydrogen atom, is commonly associated with the presence of reducing power [50].

Fig. 5 shows the graph of correlation between the sample concentrations (0–10 µg/mL) and reducing power, and table of IC_{50} values. The higher the sample concentration, the greater the increase in reducing power (Pearson correlation; $P < 0.05$). According to the IC_{50} values, IPR had the same reducing power as BR-J. The reducing power of IPR was comparable to ascorbic and ferulic acid.

The reducing power of IPR is classified as potent because the IC_{50} value is less than 50 ppm [51]. The activity level is equal to ascorbic acid and standard ferulic acid, which are powerful antioxidants [52-54]. By the FRAP method, the mechanism of IPR ferulic acid in scavenging free radicals is predicted by reducing Fe^{3+} ions to Fe^{2+}, as seen in Fig. 6. Ferulic acid acts as a chelating of metal ions such as Cu^{2+} or Fe^{2+} [55]. The phenolic rings also support strong antioxidant properties, which stabilize and delocalize unpaired electrons [15,42].

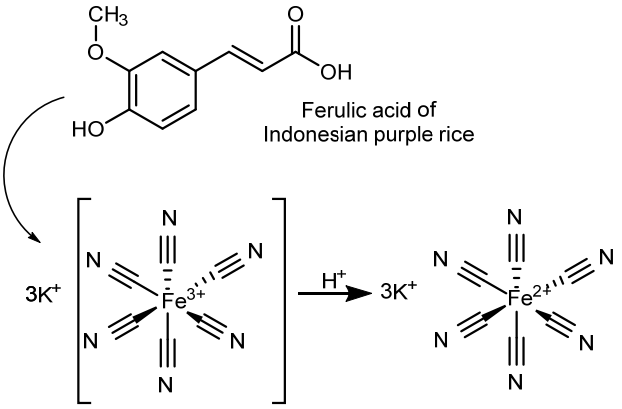
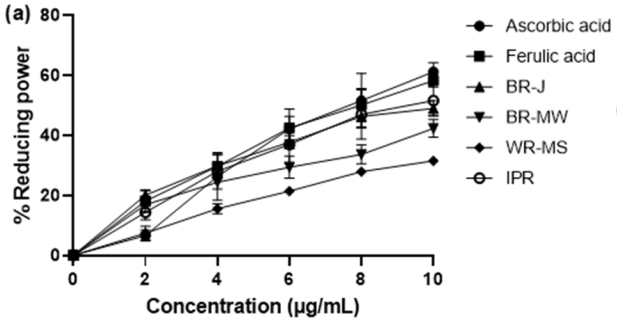


Fig 6. The prediction of IPR ferulic acid mechanism in scavenging free radicals by reducing Fe^{3+} to Fe^{2+}



(b)

Sample	IC_{50} (ppm)
Ascorbic acid	8.05 ± 1.65[a]
Ferulic acid	8.02 ± 0.91[a]
BR-J	9.20 ± 1.12[a]
BR-MW	11.88 ± 1.97[ab]
WR-MS	15.25 ± 0.95[b]
IPR	9.35 ± 1.95[a]

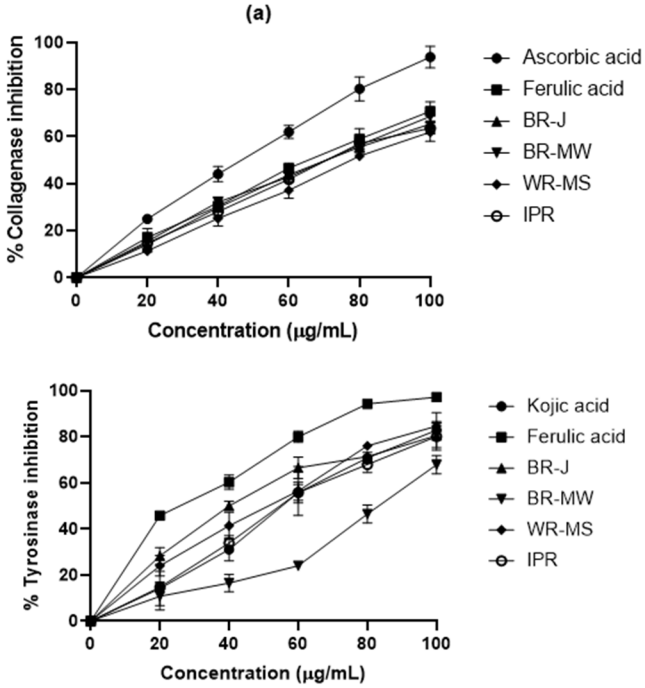
Fig 5. Reducing power of IPR. (a) Percentage of reducing power, (b) IC_{50} value, the different letters indicated significant differences (Tukey's HSD; $P < 0.05$). BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, WR-MS: *Mentik susu* white rice, IPR: Indonesian purple rice

Anti-aging Activity

The ability to inhibit collagenase and tyrosinase was used to assess anti-aging activity. The activities were compared to positive controls, ascorbic acid and kojic acid as the collagenase and tyrosinase inhibitors, respectively [8,11,56]. The activity was also compared to ferulic acid. Fig. 7 shows that the concentrations of samples (0–100 μg/mL) positively correlated with the anti-collagenase and anti-tyrosinase activity (Pearson correlation; $P < 0.05$). The IPR demonstrated the same level of collagenase inhibition as BR-J. The inhibitory power was also the same as ferulic acid but lower than ascorbic acid. In the tyrosinase inhibition, IPR was equal to BR-J. The strength was also the same as kojic acid but lower than ferulic acid. This demonstrates that IPR has a more efficient inhibitory activity than BR-J due to its lower FA concentration while producing the same activity. The IPR is more potent as a tyrosinase inhibitor than collagenase, as indicated by activity that is comparable to the positive control of the tyrosinase inhibitor, kojic acid, but lower than the positive control of the collagenase inhibitor, ascorbic acid.

For enzyme inhibitor screening, the IC_{50} value could be compared to a positive control [56]. According to the IC_{50} value, IPR showed a weaker inhibition of collagenase than ascorbic acid but the same as ferulic acid, while the tyrosinase inhibition by IPR was as strong as kojic acid. This finding indicated that IPR has high anti-tyrosinase and moderate anti-collagenase activities. The tyrosinase inhibition was supported by ferulic acid in IPR with metal ions chelating properties. Generally, copper-chelating aromatic compounds inhibit tyrosinase by mimicking the substrate of tyrosinase [56]. The interaction of hydroxyl groups and benzene rings in ferulic acid with collagenase may cause a conformational change that inhibits collagenase [11].

Compounds with antioxidant activity could prevent aging by inhibiting the activities of ROS and aging enzymes [31]. The ROS activates the MAPK pathway, which increases microphthalmia-associated transcription factor (MITF) expression and thus tyrosinase-regulated melanogenesis [5]. The MAPK activation also induces activator protein 1 (AP-1) and nuclear factor kappa-B (NF-κB), which upregulate matrix



(b)

Sample	IC_{50} (ppm)
Ascorbic acid	49.20 ± 2.92[a]
Ferulic acid	68.09 ± 3.67[b]
BR-J	73.04 ± 1.60[bc]
BR-MW	70.79 ± 2.91[bc]
WR-MS	77.23 ± 2.45[c]
IPR	74.18 ± 3.11[bc]

Sample	IC_{50} (ppm)
Kojic acid	58.60 ± 3.91[b]
Ferulic acid	36.05 ± 0.55[a]
BR-J	50.67 ± 1.64[b]
BR-MW	84.42 ± 2.59[c]
WR-MS	53.45 ± 4.99[b]
IPR	59.57 ± 3.64[b]

Fig 7. The anti-aging activity of IPR. (a) Percentage of inhibition. (b) IC_{50} value. The difference letter in the table indicated significant differences among samples (Tukey's HSD; $P < 0.05$)**.** BR-J: *Jeliteng* black rice, BR-MW: *Mentik wangi* black rice, WR-MS: *Mentik susu* white rice, IPR: Indonesian purple rice

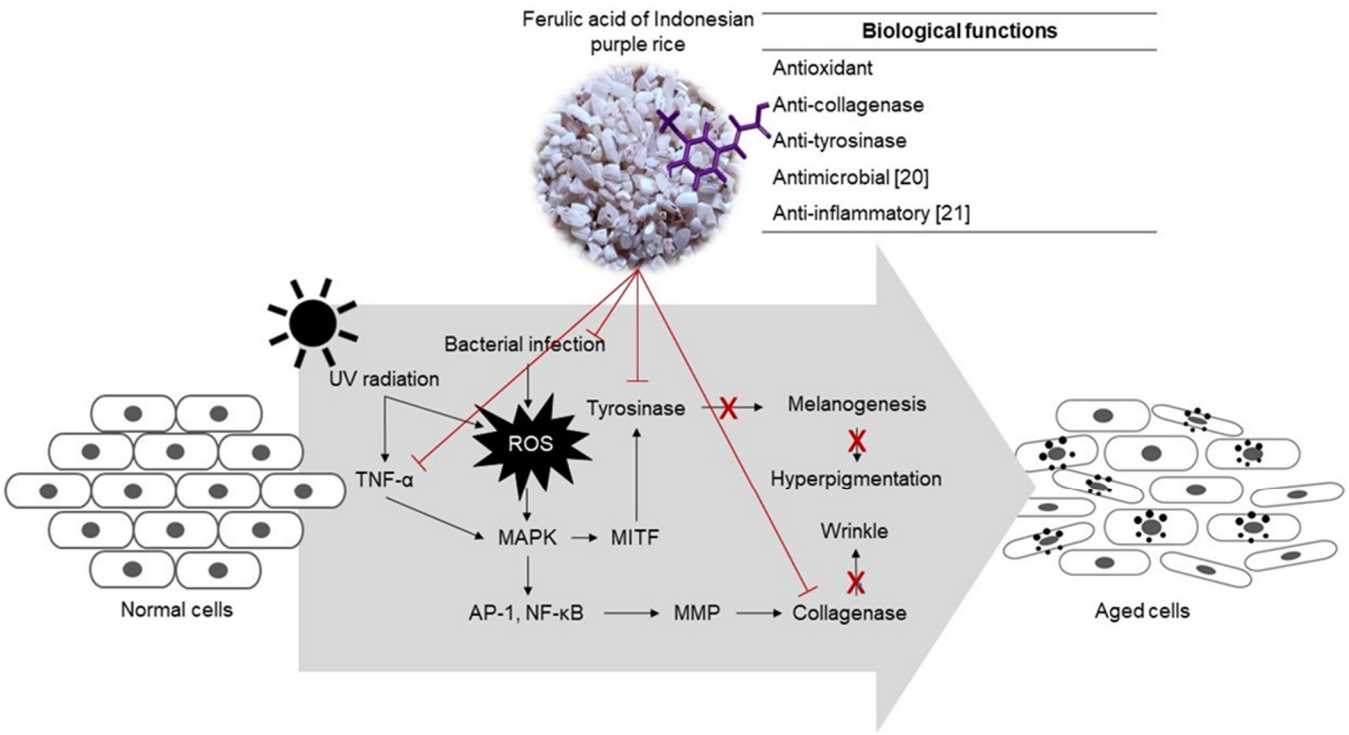


Fig 8. The anti-aging mechanism of IPR ferulic acid via the MAPK pathway. ROS: reactive oxygen species, MAPK: mitogen-activated protein kinase, AP-1: activator protein 1, NF-κB: nuclear factor kappa-B, MMP: matrix metalloproteinase, MITF: microphthalmia-associated transcription factor, TNF-α: tumor necrosis factor-alpha. →: stimulate, ⊣: inhibit, ×: not stimulate

metalloproteinases (MMPs), one of which is collagenase, which degrades collagen fibers and causes wrinkles [2].

Ferulic acid of IPR demonstrates an anti-aging mechanism through the MAPK pathway by inhibiting collagenase and tyrosinase activity, as seen in Fig. 8. In the previous study, ferulic acid of IPR acts as an antimicrobial on *Salmonella typhimurium* and *Listeria monocytogenes* [20]. Bacterial infection induces ROS production [57]. Moreover, ferulic acid also acts as an anti-inflammatory by inhibiting tumor necrosis factor-alpha (TNF-α) signaling [21]. The TNF-α stimulates MAPK pathway activation [58]. By controlling the MAPK pathway, the aging process can be suppressed [59]. In this study, we found that ferulic acid of IPR inhibited collagenase and tyrosinase activity; thus, wrinkle formation and hyperpigmentation could be prevented.

The IPR has a lower ferulic acid concentration than BR-J and BR-MW. Interestingly, IPR could provide excellent biological activity, which could be a unique characteristic of IPR, in which synergistic activity between ferulic acid and other compounds results in high activity. The synergistic effects of plant compounds increase their activity [60]. The IPR has higher reducing power than both parentals, higher anti-tyrosinase activity than BR-MW, and higher anti-collagenase activity than WR-MS. These results suggest that IPR has improved properties, resulting in higher bioactivity than its parentals. However, the IPR activity is not as great as ferulic acid, a pure compound. Ferulic acid is still present in IPR as extracts; thus, the presence of other compounds contributes to the resulting activity.

To the best of our knowledge, the exploration of ferulic acid in IPR and its biological function as anti-aging is reported here for the first time. The IPR offers excellent antioxidant and anti-aging activities with high nutritional content. These findings supported the potential of IPR as a promising active compound of nature-based skin anti-aging products.

■ CONCLUSION

The current study revealed the IPR nutritional value, phytochemical profile, and anti-aging potential of ferulic acid content. The IPR has a low lipid content, a high level of some amino acids including L-histidine, L-phenylalanine, L-threonine, L-arginine, and L-tyrosine, and some phytochemicals such as phenolics, flavonoids, tannins, anthocyanins, leucoanthocyanidins, and glycosides. The total phenolics content of IPR is related to its ferulic acid level, which is lower than black rice. Nevertheless, IPR demonstrated potent reducing power, anti-collagenase, and anti-tyrosinase activity. The combination of IPR's nutritional content and ferulic acid properties as an antioxidant, anti-collagenase, and anti-tyrosinase showed the potential of IPR as an anti-aging. Further research using an *in vivo* approach is required to confirm the findings of this study, and then IPR can be developed as a natural ingredient in skin care formulations.

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■ AUTHOR CONTRIBUTIONS

Ernanin Dyah Wijayanti contributes to conceptualization, methodology, data collection, sample analysis, validation, data curation, writing – the initial draft. Anna Safitri contributes to methodology, student supervision, writing – revisions, project management. Dian Siswanto contributes to methodology, student supervision, writing – revisions. Fatchiyah contributes to conceptualization, methodology, student supervision, project leadership; project management; and funding acquisition.

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