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Free radical scavenging and metal ion chelating properties of differently pigmented rice varieties

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ABSTRACT: Pigmented rice (*Oryza sativa* L.) becomes interestingly important in agroindustry because it contains high phytochemical compounds that can be antioxidants and metal ion chelating. This study attempted to relate the content of phenols, flavonoids, anthocyanin, and bioactive phytochemicals activity from differently pigmented rice (red and black) varieties. The bioactive phytochemical activities were evaluated by measuring free radical scavenging, hydroxyl scavenging, and metal ion chelating activity. The result showed that the highest phenolic, flavonoids, and anthocyanin content found in black rice, followed by red rice and non-pigmented rice. The anthocyanin in black rice was significantly higher ($p \leq 0.05$) than in red and non-pigmented rice. The chlorophyll and carotenoids contents in all samples varied from 0.03 to 1.42 µg g^{-1} and 0.26 to 0.53 µg g^{-1}, respectively. The antioxidant and metal ion chelating activities in black and red rice were significantly higher than non-pigmented rice. The correlation analysis showed that the phenols, flavonoids, and phytochemical pigments have a positive correlation ($p \leq 0.05$) with antioxidant and metal chelating activity. In this study, black and red rice varieties showed the potential natural resources for the important effect of nutraceutical food products and pharmaceuticals.

Keywords: Anthocyanins, antioxidants, carotenoids, flavonoid, phytochemical.

INTRODUCTION

Rice (*Oryza sativa*) is the best staple food in all cereal grains, which provides the primary source of calories for half of the world's population (Falade and Christopher, 2015). The research report that rice is a potential of functional macronutrients and micronutrients such as carbohydrates, protein, lipids, vitamins, phenolics, and ferulic acids (Zubair et al., 2012). Rice can be classified into non-pigmented and pigmented rice based on the pigment in the outer layer of rice. Although non-pigmented rice is a more popular staple food in Asia than pigmented rice, few people know that pigmented rice potentially has health benefits because it has a higher phytochemical compound (Kraithong et al., 2018). Phytochemicals are natural bioactive compounds from the plant such as vegetables, fruits, and whole grains, which play an important role in human health. Specifically, phytochemical compounds such as carotenoids, anthocyanin, and flavonoids are the most studied phytochemicals for antioxidants and have potential pharmacological activities (Ashraf, 2020).

In biological systems, the two biggest problems in body health are reactive oxygen species (ROS) and metal toxicity. These reactive species play an important role in the pathogenesis of several oxidative stress-related diseases like carcinogenesis, cardiovascular diseases, rheumatoid arthritis, ulcerative colitis, and neurological degenerative diseases (Pavithra and Vadivukkarasi, 2015). Hydroxyl radical (OH*) is the most potent oxidant, and one of the most reactive natural, free radicals known. Hydroxyl radical has a short half-life and is continuously forming in the process of reducing oxygen to water (Treml and Smejkal, 2016). In the Haber-Weiss reaction, hydroxyl radicals are generated in hydrogen peroxide and iron ions (Lipinski, 2011). Iron is an essential element for life, but in excess, it can be very toxic because of its potential ability to generate free radicals. Humans do not have a physiological mechanism in the excretion of iron (Crisponi et al., 2013). The human body has an enzymatic defense mechanism to fight oxidative stress involving superoxide dismutase, catalase, and glutathione peroxidase (Sudan et al., 2014). Apart from the innate defense system, a natural product from pigmented rice can improve the body's capacity to counter oxidative stress. Cereal grains of pigmented rice contains higher phytochemicals, such as carotenoids, phenolics, flavonoids, and anthocyanin (Deng et al., 2013). The research revealed that pigmented rice has antioxidant activity (Ghasemzadeh et al., 2018), anti-inflammatory (Limtrakul et al., 2016), anticancer (Baek et al., 2015), antimutagenic (Punvittayagul et al., 2014), and antidiabetic (Hemamalini et al., 2018).

Pigmented rice varieties such as black and red rice generally used in various food products such as pudding, flatbread, beverages, ingredients in the meat processing, salad dressing, and low gluten bread (Falade and Christopher, 2015). Besides, the extract of pigmented rice used as colorants in cookies, ice creams, and liquors (Deng et al., 2013). The development of novel food products usually involves phytochemical components and beneficial antioxidant activities that enhance functional food products.

Increasing consumer interest in health-promoting food products creates a large market for pigmented rice that produces health while simultaneously producing economic benefits for producers (Mbanjo et al., 2020). The quality of rice varieties is currently more focused on evaluating production and resistance to biotic and abiotic stress, and there is a lack of antioxidant characters. Therefore, this research objective was to investigate the potential of phenolic and plant phytochemical pigment in black and red rice to antioxidant and metal ion chelating activities. These results could provide information on the pigmented rice varieties with high nutraceuticals products and help the breeders to create a new line with high-quality varieties.

MATERIALS AND METHODS

Materials

Five rice (*Oryza sativa* L.) varieties were used in this study. All varieties were grown under average climate conditions in the Agrotechnopark Research Center, Jember University, East Java, Indonesia. The rice varieties include the following: two red rice varieties, namely Bulu Hideung (Red-BH) and MS Pendek (Red-MP); two black rice varieties namely Gogo Niti (Black-GN) and Hitam Padang (Black-HP); and one non-pigmented rice varieties namely IR-64 as control (Figure 1). After harvest, the samples were dried to 14% using the oven method and stored at 4°C. Rice grains were dehulled using a rice husker machine and grounded to pass through a 60 sieve mesh for further analyses (Figure 1). The chemical reagents used were selenium quercetin ($C_5H_1O_7$), gallic acid ($C_7H_6O_5$), folin-ciocalteu ($H_3PO_4(Mo_3)_{12}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid ($C_6H_8O_6$), 2-deoxy-d-ribose ($C_5H_{10}O_4$), hydrogen peroxide (H_2O_2), tertiary butyl alcohol (TBA), trichloroacetic acid (TCA), and ethylenediaminetetraacetic acid (EDTA) procured from Sigma-Aldrich, Singapore.

Extraction sample

The five rice (*Oryza sativa* L.) varieties were extracted using methanol as a solvent. Flour sample (2 g) each sample added methanol (80%, 8 ml) and macerated on the stirrer (4ºC, 1 day). The solution was centrifuged (10000 rpm, 10 minutes), and then the supernatant was taken and stored at -20ºC.

Phenolic and flavonoid contents

The total phenolic was determined by Taga et al. (1984) method. Sample extract (5 µl) was loaded into the tube. Solutions of Folin-Ciocalteu (50%, 50 µl), and methanol (100%, 45 µl) were added, and the tube was incubated (24ºC, 30 minutes). Absorbance was measured at 750 nm using UV-Spectrophotometer (U-2900, Hitachi, Japan), and the total phenolic was calculated in gallic acid equivalent (mg GAE g^{-1}) against the gallic acid standard curve.

Total flavonoid was determined using the method described by Mudoi and Das (2019). The sample extract (10 µl) was loaded into the tube. The solution of methanol (100%, 40 µl), distilled water (400 µl) and $NaNO_2$ (5%, 30 µl) were added, and the tube was incubated (24ºC, 5 minutes) then added $AlCl_3$ (10%, 30 µl) and re-incubated (24ºC, 5 minutes). After the incubation, NaOH (1 N, 200 µl) and distilled water (240 µl) was added. Absorbance was measured at 415 nm using UV-Spectrophotometer (U-2900, Hitachi, Japan), and the total flavonoid was calculated in quercetin equivalent (mg QE g^{-1}) against the quercetin standard curve.

Chlorophylls and carotenoids contents

Chlorophylls and carotenoids were determined using the method reported by Henry and Grime (1993). The absorbance of sample extract (3 ml) was measured using Spectrophotometer (U-2900, Hitachi, Japan) at 480, 645, and 663 nm. Chlorophyll and carotenoid were calculated using the following formula:

$$\text{Chlorophylls } (\mu g/ml) = (8.02 \times A663) + (20.2 \times A645)$$

$$\text{Carotenoids } (\mu mol/l) = [A480 + (0.114 \times A663) - (0.638 \times A645)] / (112.5 \times W)$$

Figure 1. Grain of pigmented rice varieties (A) Red-BH, (B) Red-MP, (C) Black-GN, (D) Black-HP, (E) IR-64; and Flour of rice (a) Red-BH, (b) Red-MP, (c) Black-GN, (d) Black-HP, (e) IR64.

Where A480 is absorbance at 480 nm, A645 is absorbance at 645 nm, A663 is absorbance at 663 nm, V is volume of extracts (ml), and W is weight of sample (g).

Anthocyanins contents

Anthocyanin contents were determined by the pH differential method (Abdel-Aal and Hucl*,* 1999). Extracts solutions were dissolved in two different buffer solutions. The first extract solution (1 ml) was dissolved in KCl (0.025 M, 2 ml, pH 1.0), and the other extract solution (1 ml) was dissolved in sodium acetate (0.4 M, 2 ml, pH 4.5). The absorbance of the solution measured using Spectrophotometer (U-2900, Hitachi, Japan) at 510 and 700 nm. Concentration of anthocyanins was determined using the following formula:

$A= [(A510 – A700)_{pH1.0} – (A510 – A700)_{pH4.5}]$ and

AC (mg/ml) = [(A x MW x DF x 1000) / (ε x1)].

Where: A is total absorbance value, A510 is the absorbance at 510 nm, A700 is the absorbance at 700 nm, AC is anthocyanins content, MW is the molecular weight of cyanidin 3-glucoside, DF is dilution factor, and ε is molar absorption of cyanidin 3-glucoside.

2,2-diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was measured as described by Palombini et al. (2013) Serial extract sample (10, 20, 40, 60 and 80 µg GAE ml^{-1}) was reacted with a methanolic solution of DPPH (90 µM, 400 µl) and distilled water until a volume of 500 µl, then incubated in a dark room (24ºC, 5 minutes). After incubation, the solution was recorded at 517 nm using a Spectrophotometer (U-2900, Hitachi, Japan), and ascorbic acid was used as a positive control. The scavenging activity was calculated using the following formula:

Inhibition (%) = $[(A_0 – A_S)/A_0] \times 100$ %.

Where: A_0 is the absorbance of the blank, and A_S is the absorbance of the sample extracts

Hydroxyl radical scavenging activity

The scavenging activity of hydroxyl free radicals was measured as described by Halliwell et al. (1987). Serial extract sample (20, 40, 60, 80 and 100 µg GAE ml^{-1}) was reacted with 2-deoxy-D-ribose (2,8 mM, 20 µl), Ethylenediaminetetraacetic acid (1 mM, 100 µl), $FeCl_3$ (10 mM, 10 µl), H_2O_2 (1 mM, 10 µl), ascorbic acid (1 mM, 100 µl) and phosphate buffer until a volume 1 ml. The solution was then incubated (37ºC, 1 hour), then TBA (1%, 500 µl) and TCA (2.8%, 500 µl) were added. The solution was re-incubated (80ºC, 30 minutes). The absorbance of the solution was recorded at 532 nm using a Spectrophotometer (U-2900, Hitachi, Japan), and ascorbic acid was used as a positive control. The scavenging activity was calculated using the following formula:

Inhibition (%) = $[(A_0 – A_S)/A_0] \times 100$ %.

Where A_0 is the absorbance of the blank, and A_S is the absorbance of the sample extracts.

Metal Ion chelating activity

Fe^{2+} chelating activity was measured by Chew et al. (2000) method with little modification. The serial extract sample

(30, 60, 90, 120, and 150 µg GAE ml^{-1}) was reacted with $FeCl_2$ (100 µl, 2 mM), $FeSO_4$ and distilled water until 5 ml. The solution was incubated (25ºC, 3 minutes). After incubation, ferrozine (100 µl, 5 mM) was added. The absorbance was recorded at 562 nm using a Spectrophotometer (U-2900, Hitachi, Japan), and EDTA was used as a positive control. The chelating activity was calculated using the following formula:

$$\text{Chelating (\%)} = [(A_0 – A_S)/A_0] \times 100\ \%.$$

Where is A_0 = absorbance of the blank;, and A_S = absorbance of the sample extracts.

Statistical analysis

The analysis was conducted using statistical software SPSS 6.0 (Inc., Wacker Drive, Chicago, IL, USA), and each data point represents the mean of three replicate ± displayed on the standard deviation (SD). The variance analysis from the result received with a 5% level significance was used for ANOVA analysis. Following Tukey test was used to compare samples. The calculate of correlation statistical using Microsoft Excel (Microsoft Inc., Redmond, WA, USA).

RESULT AND DISCUSSION

Phenolic and flavonoid contents

Phenolic compounds are secondary metabolites in plants that have a wide range of therapeutic uses. The results of the phenolic and flavonoid content of the sample were presented in Table 1. The phenolic content of samples ranged from 1.93 to 9.51 mg GAE g^{-1}. The highest phenolic found in black rice (Black-GN and Black HP) followed by red rice (Red-BH and Red-MP) and non-pigmented rice (IR-64). Black rice has four times the amount of phenolic than non-pigmented rice and about twice than red rice. The result is similar to Shen et al. (2016), who reported the total phenolic in red rice ranged from 1.65 to 7.31 mg GAE g^{-1}, and black rice ranged from 8.41 to 12.44 mg GAE g^{-1}. Phenolic in pigmented rice provides benefits for human health. According to Heuberger et al. (2010), phenolic compounds can decrease the oxidative rate of organic materials by transferring hydrocarbon atoms to radical molecules. The phenolic compound's ability in rice extracts depends on the number and position of the hydroxyl group on the phenolic ring. Characteristics of phenolic antioxidants have been shown in health studies to anti-inflammatory (Limtrakul et al., 2016), anticancer (Baek et al., 2015), antimutagenic (Punvittayagul et al., 2014), and antidiabetic (Hemamalini et al., 2018).

Similar to phenolic content, flavonoids are secondary metabolites in plants with 18 to 25% of the total phenolic. The flavonoids content of samples ranged from 0.36 to 2.32 mg QE g^{-1}. The highest flavonoid found in black rice, followed by red rice and non-pigmented rice. Flavonoids are bioactive compounds that are synthesized from the phenylpropanoid pathway. Flavonoid is a compound that consists of fifteen carbon with two aromatic rings (rings A and B) and interlinked by a three-carbon chain (structure C6-C3-C6) (Samyor et al., 2016). Flavonoids can donate electrons and stop chain reactions. The flavonoid's ability is associated with phenolic hydroxyl, specifically in the 3′OH and 4′OH of the three-carbon chain. In this study, Black-GN has the highest flavonoid content (2.32 mg QE g^{-1}), and IR64 has the lowest flavonoid content (0.36 mg QE g^{-1}).

Phytochemical pigment (chlorophyll, carotenoid, and anthocyanin)

The results of the phytochemical pigment of the sample were presented in Table 2. The chlorophyll is a phytochemical pigment of the plant, which is responsible for green color. The result of chlorophyll content in all samples ranged from 0.03 to 1.42 µg g^{-1}. IR-64, as control, has the lowest chlorophyll content. The amount of chlorophyll in non-pigmented rice (IR-64) is minimal. According to Rahman et al. (2015), chlorophyll accumulation in non-pigmented rice grains begins after fertilization and reaches a maximum level during the drought stage of seed development. The pigment decreases until nothing in the mature seeds. The chlorophyll content in non-pigmented rice grains after harvesting is smaller than pigmented rice because the outer layer of non-pigmented rice contains fiber and protein, not contain pigment, while the pigmented rice has contained pigment with purple, red, green or brown (Rahman et al., 2013). The accumulation of pigments in the outer layer of pigmented rice causes more chlorophyll. Non-pigmented rice tends only to focus on accumulation carbohydrate in grains, while the pigmented rice not only accumulation carbohydrate but also accumulation pigments in the outer layer such as chlorophyll, anthocyanins, and carotenoids during maturation. In this study, chlorophyll pigmented rice grains (black and red rice) have more quantities than non-pigmented rice so that it can be used as an additional source of chlorophyll for humans.

The carotenoid is a phytochemical pigment of the plant responsible for red, yellow, and orange. In this study, the carotenoid contents of Red-BH, Red-MP, Black-GN, and Black-HP were 0.25, 0.37, 0.51, and 0.26 µg g^{-1}, respectively. IR-64, as control, does not contain carotenoid content. Black-GN has the highest carotenoid followed by Red-MP, while the carotenoid content of Black-HP and Red-HP not significantly different. The differences in carotenoid content mainly influenced by genetic variations and other factors, such as climate and geographical

Table 1. Phenolic and flavonoid content of pigmented rice varieties.

Varieties	PC (mg GAE g^{-1})	FC (mg QE g^{-1})
Red-BH	$2.92^{c} \pm 0.02$	$0.54^{b} \pm 0.04$
Red-MP	$2.69^{b} \pm 0.05$	$0.61^{b} \pm 0.03$
Black-GN	$9.51^{e} \pm 0.17$	$2.32^{d} \pm 0.03$
Black-HP	$7.19^{d} \pm 0.06$	$1.83^{c} \pm 0.06$
IR-64	$1.93^{a} \pm 0.05$	$0.36^{a} \pm 0.02$

Data are the mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$), PC: Phenolic content, and FC: Flavonoid content.

Table 2. Phytochemical pigment (chlorophyll, carotenoid, and anthocyanin) content of pigmented rice varieties.

Varieties	ChC (μg g^{-1})	CrC (μg g^{-1})	AC (μg g^{-1})
Red-BH	$1.32^{c} \pm 0.03$	$0.25^{b} \pm 0.02$	$30.42^{b} \pm 0.42$
Red-MP	$0.97^{b} \pm 0.04$	$0.37^{c} \pm 0.02$	$28.78^{a} \pm 0.33$
Black-GN	$1.42^{e} \pm 0.01$	$0.51^{d} \pm 0.01$	$254.62^{c} \pm 2.31$
Black-HP	$1.11^{d} \pm 0.02$	$0.26^{b} \pm 0.01$	$328.22^{d} \pm 1.22$
IR-64	$0.03^{a} \pm 0.03$	$0.00^{a} \pm 0.00$	ND

Data are the mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$), ChC: Chlorophyll, CrC; Carotenoid content, AC: Anthocyanin content and ND; Not-determined.

conditions (Maiani et al., 2009). The carotenoid concentration correlates with grains pigment. The result was in agreement with the work of Ashraf et al. (2017), who reported the carotenoid content of red and black rice higher than non-pigmented rice. Melini and Rita (2017) reported that the types of carotenoid rice are lutein and zeaxanthin (0.19 to 1.88 μg g^{-1}). The carotenoid can provide health effects such as optical enhancement (Mozaffarieh et al., 2003), immunomodulatory (Pechinskii and Kuregyan, 2014) and antioxidant function (Fiedor and Burda, 2014). Humans cannot synthesize carotenoid molecules; therefore, they must be obtained through the diet. In this study, the carotenoids of pigmented rice can contribute to the daily needs of carotenoids. Compared to other carotenoid sources (green vegetables and colored fruits), pigmented rice has advantages such as staple food, can be consumed throughout the year, and long shelf life.

As shown in Table 2, the anthocyanin contents of Red-BH, Red-MP, Black-GN, and Black-HP were 30.42, 28.78, 254.62, and 328.33 μg g^{-1}, respectively. IR-64, as a control, anthocyanin content was not detected. The anthocyanin content in black rice is the highest difference significant from red rice. Black rice has ten times anthocyanin than red rice because it has a high level of expression than red rice (Kim et al., 2007). The main anthocyanins in grain rice are found in the structure of cyanidin 3-O-glucoside and peonidin-3-O-glucoside (Pereira-Caro et al., 2013). The structural characteristics of anthocyanins are highly reactive to reactive oxygen species (ROS). According to Goufo and Trindade (2015), anthocyanin content influenced by genetic, geographical, and climatic conditions.

2,2-diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging test is based on electron transfer from the donor molecule to the molecule radical. The colors change from purple to yellow indicate the sample's ability to reduce harmful reactive oxygen species (Yaqoob et al., 2014). The DPPH radical scavenging effect of rice grain extracts increases with increasing concentration (Figure 2). Black rice extract (Black-HP and Black GN) showed the highest DPPH activity, followed by red rice (Red-BH and Red-MP). IR-64, as control, showed the lowest antioxidant activity. The findings in this study is similar to that of Ghasemzadeh et al. (2018) who reported the DPPH activity effected by pigments. All data have shown that samples extract have lower DPPH activity than the positive controls (ascorbic acid). The IC_{50} of Red-BH, Red-MP, Black-GN, Black-HP, and IR-64 is 52.17, 62.00, 35.82, 28.14, and 129,31 μg GAE ml^{-1}, respectively. The extract of black and red rice is the potential resource of nature. According to Supriyadi et al. (2019), natural resources with IC_{50} values bellow 500 μg ml^{-1} have great potential as nutraceutical resources.

Hydroxyl radical scavenging activity

Hydroxyl radicals are important active oxygen species causing lipid peroxidation and enormous biological damage (Gayathri et al., 2014). Hydroxyl Radical HO* is the most reactive and interacts with the purine and pyrimidine bases of DNA. It can also abstract hydrogen

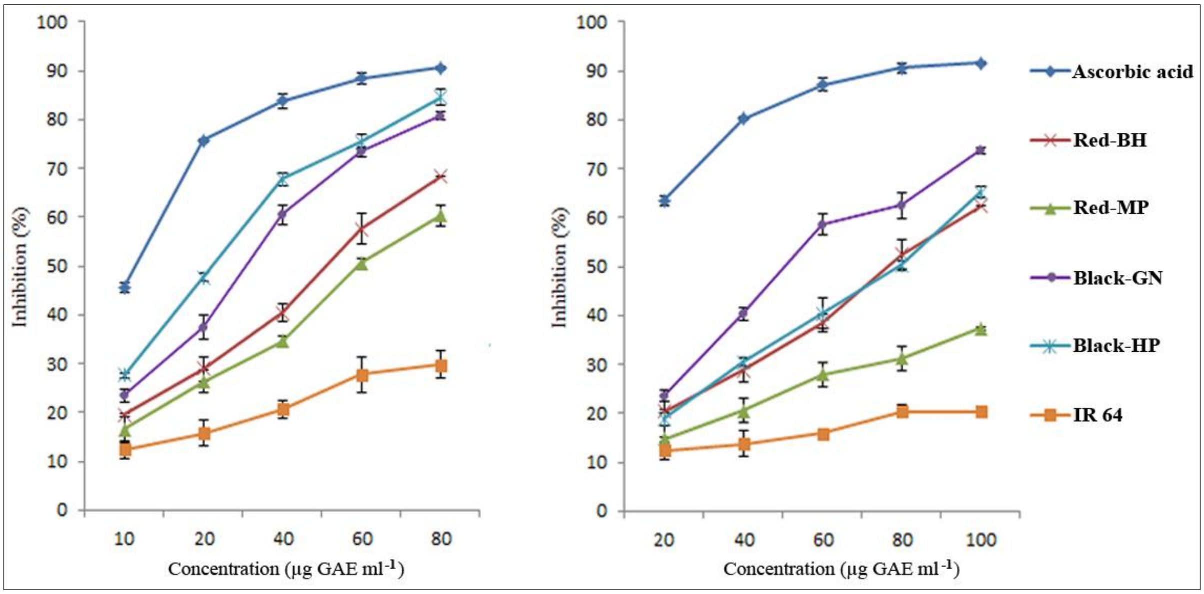

Figure 2. The antioxidant activity of pigmented rice variety extracts (A) DPPH and (B) Hydroxyl scavenging activity.

atoms from biologic molecules, including thiols, which leads to the formation of sulfur radicals capable of combining oxygen to generate oxysulfur radicals and damage biologic molecules (Halliwell, 1987). Hydroxyl radical produced by Fenton reaction ($Fe^2+ + H^2O^2 \rightarrow Fe^{3+} + OH^- + OH^*$), then hydroxyl reacted with 2-deoxy-D-ribose to form malondialdehyde which caused pink color. The presence of phenolic compounds caused the competition between phenolic and 2-deoxy-D-ribose to reacted with hydroxyl radical. The reaction of hydroxyl and phenolic can reduce malondialdehyde and decrease the intensity of the pink color (Nam et al., 2006). According to Treml and Smejkal (2016), three main types of reactions of OH* are hydrogen abstraction, addition to the double bond, and electron transfer. All of these reactions lead to the formation of new radicals and thus propagate chain reactions. The hydroxyl radical scavenging effect of rice grain extracts increases with increasing concentration (Figure 2). Black rice extract (Black-HP and Black GN) showed the highest hydroxyl scavenging activity, followed by red rice (Red-BH and Red-MP). IR-64, as a control, showed the lowest antioxidant activity. Black HP and Red BH not significantly different in hydroxyl scavenging activity. The IC_{50} of Red-BH, Red-MP, Black-GN, Black-HP, and IR-64 are 78.89, 144.73, 57.31, 76.37, and 366,36 µg GAE ml^{-1}, respectively. All sample extracts have a higher IC_{50} than the positive control (ascorbic acid, 15.76 µg ml^{-1}). The extract of black and red rice is the potential resource of nature. All varieties have higher antioxidant activity in DPPH than hydroxyl scavenging activity.

Metal ion chelating activity

Ferric ion chelation is an important antioxidant effect to inhibit metal-catalyzed oxidation. Fe^{2+} chelating uses the basis that ferrozine can form complex bonds with Fe^{2+} with phytochemical pigments (phenolic, anthocyanin, and carotenoid), which act as chelating agents. The red color reduction in the sample shows that there is damage to the complex bonds of ferrozine and Fe^{2+} (Muller et al., 2011). The Fe^{2+} chelating activity effect of rice grain extracts increases with increasing concentration (Figure 3). Black rice extract (Black-HP and Black GN) showed the highest Fe^{2+} chelating activity, followed by red rice (Red-BH and Red-MP). IR-64, as control, showed the lowest antioxidant activity. The IC_{50} of Red-BH, Red-MP, Black-GN, Black-HP, and IR-64 were 116.43, 127.72, 89.75, 74.56, and 299.58 µg GAE ml^{-1}, respectively. Black rice has the highest Fe^{2+} chelating activity because it has high in phenolic and anthocyanin content. All sample extract has higher IC_{50} than the positive control (EDTA) with IC_{50} of 28.76 µg GAE ml^{-1}. The extract of black and red rice is the potential resource of nature.

Correlation of phytochemical pigment, antioxidant activity and Fe^{2+} chelating in rice

The correlation of phytochemical pigments to antioxidant activity and Fe^{2+} chelating using antioxidant activity at concentrations of 60.00 µg ml^{-1} of extracts is shown in Table 3. The results of the correlation display is shown in Table 4. The phenolic, flavonoid, and phytochemical pigment have a positive correlation with antioxidant and metal chelating. The value of correlation (r) of all components to antioxidant and metal chelating is more than 0.7. It indicates a strong correlation. The highest correlation is anthocyanin content to Fe^{2+} chelating (r = 0.92), following by phenolic content to DPPH activity (r =

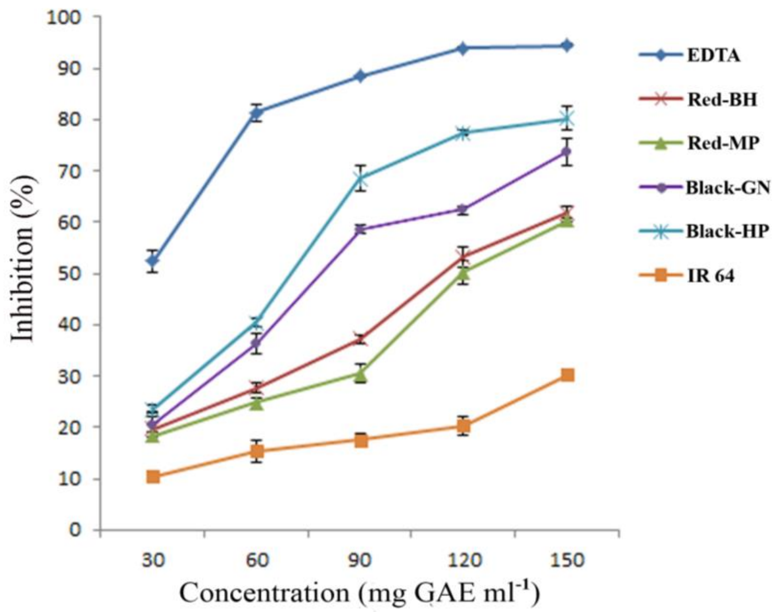

Figure 3. The Fe^{2+} chelating activity of rice pigmented rice variety extracts.

Table 3. Free radical scavenging and Fe^{2+} chelating in pigmented rice varieties*

Varieties	DPPH radical scavenging (%)	Hydroxyl radical scavenging (%)	Fe^{2+} Chelating (%)
Red-BH	57,88 ± 3,39^{c}	37,59 ± 0,99^{c}	27,41 ± 0,27^{c}
Red-MP	49,77 ± 1,08^{b}	27,30 ± 2,03^{b}	25,38 ± 1,20^{b}
Black-GN	71,97 ± 1,66^{d}	58,55 ± 1,80^{e}	35,94 ± 0,36^{d}
Black-HP	75,78 ± 0,45^{e}	41,74 ± 1,65^{d}	40,63 ± 0,79^{e}
IR-64	27,75 ± 2,57^{a}	15,99 ± 0,34^{a}	15,65 ± 0,52^{a}
Ascorbic acid	87,99 ± 0,59^{f}	89,74 ± 1,15^{f}	-
EDTA	-	-	81,27 ± 0,84^{f}

Data are the mean ± SD; n = 3, values followed by the same letters in the same column are not significantly different ($p > 0.05$). * The concentration of the samples was 60.00 µg GAE ml^{-1}.

Table 4. Pearson correlation of phytochemical pigment, antioxidant activity and Fe^{2+} chelating in pigmented rice varieties.

	PC	FC	ChC	CrC	AC	DPPH	Hydroxyl	Fe^{2+}Chelating
PC	1.00							
FC	1.00	1.00						
ChC	0.61	0.59	1.00					
CrC	0.68	0.67	0.85	1.00				
AC	0.91	0.93	0.51	0.48	1.00			
DPPH	0.84	0.85	0.82	0.76	0.86	1.00		
Hydroxyl	0.89	0.86	0.84	0.84	0.71	0.89	1.00	
Fe Chelating	0.85	0.86	0.79	0.67	0.92	0.93	0.83	1.00

PC: Phenolic content, FC: Flavonoid content, ChC: Chlorophyll content, CrC; Carotenoid content, AC: Anthocyanin content.

0.89), and flavonoid content to DPPH and Fe^{2+} chelating ($r = 0.86$). The positive correlation between phytochemical and antioxidant activity was reported by several authors (Zheng and Wang, 2015; Ghasemzadeh et al., 2018; Djeridane et al., 2006, and Oki et al., 2002).

The antioxidant activity and Fe^{2+} chelating observed in

Black rice contained the highest phenols, flavonoids, and anthocyanin). Phenolic and flavonoid structures affect antioxidant activity. Flavonoids have a mechanism to capture free radicals and inhibit various oxidation reactions. In other words, flavonoids stabilize reactive oxygen species by reacting with radical reactive compounds (Hamidu et al., 2018). According to Lin et al. (2009), the number and position of the hydroxyl group (OH) bonds in phenolic and flavonoids influence antioxidant activity. The increasing number of hydroxyl groups substituted in molecules causes a higher antioxidant ability because many hydrogen atoms can be donated, and carotenoids, chlorophyll, and anthocyanins also have OH groups that can be donated to radicals so that they also have radical scavenging activity. Besides, the anthocyanins have methoxyl groups and glycosylated B-ring structure to substantially increase the antioxidant activity (Khoo et al., 2017). The highest positive correlation indicates that the phytochemical of pigmented rice has high hydroxyl groups that can be donated to radical scavenging activity.

Phytochemicals show a positive correlation with Fe^{2+} chelating, and this indicates that Phenolic, flavonoids, chlorophyll, carotene, and anthocyanin are chelating agents of organic compounds that can bind metal ions to form ring-like structures called 'chelates' (Adusei et al., 2019). The Chelating agent has a "ligand" binding atom that forms two covalent or one covalent bond and one or two coordination bonds in the chelate bidentate (Flora and Pachauri, 2010).

Conclusion

In this study, pigmented rice has rich in phenolic, flavonoids, and phytochemical pigment, especially in black rice. The phytochemical in pigmented rice showed a positive correlation with antioxidant and metal ion chelating activity. This study showed that pigmented rice (black and red rice) has potential natural resources for the important effect in the field of nutraceutical food products and pharmaceuticals. Further work on cultivation methods that can add value to rice varieties and utilization of pigmented rice in food product formulations are highly recommended.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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