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# Phytochemical content and antioxidant properties of colored and non colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia

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**Abstract**

Two non colored and one colored rice varieties from Minahasa Regency, North Sulawesi, Indonesia were analyzed to determine the phytochemicals and antioxidant properties as natural antioxidant sources. Different brans of rice varieties divided into two groups: red rice and non colored (Superwin and Cigeulis) were macerated by ethanol (70%) and extracted with organic solvents (butanol, ethyl acetate and hexane). Antioxidant properties were determined by means of radical, 1,1-diphenyl-2-picrylhydrazyn (DPPH) assay, total phenol content (TPC), total anthocyanin content, and thiobarbituric acid (TBA) assay. The phytochemical analysis indicated rice bran crude extracts contained phenolic, flavonoid, alkaloid, triterpenoid, steroid and saponin compounds. Red variety had the highest DPPH scavenging radical activity (88.29 ± 5.62%), with the lowest $IC_{50}$ value (26.26 ± 0.95 µg/ml) and highest total anthocyanin content (68.61 ± 1.98 mg/g). The colored varieties had better antioxidant properties than non colored varieties. It can be concluded that colored varieties could be used as a natural antioxidant source.



## Introduction

Rice bran is one of the most abundant co-products produced in the rice milling industry. Rice consumption in Indonesia is higher than the others commodities, and making Indonesia as the largest producer in the world after China and India (FAOSTAT, 2013). Rice bran has been recognized as an excellent source of vitamins and minerals, but has been under-utilized as human food and has traditionally been used primarily in animal feeds. Research conducted in the last two decades has shown that it contains a unique complex of naturally occurring antioxidant compounds (Moldenhauer *et al*., 2003).

Rice bran as a waste product of paddy milling contained protein, carbohydrate, dietary fiber, ash, fat, vitamin, mineral and natural antioxidant compounds (Chen *et al*., 2008; Saenjum *et al*., 2012). Rice bran also contains phytochemical compounds in significant amount and these compounds have been considered as natural antioxidant. Rice bran oil according to Xu and Godber (1999) and Chen *et al.* (2008) contained 95.6% saponified lipid such as glycolipid and phospholipid and 4.2% unsaponified lipid such as tocopherol, tocotrienol, γ-oryzanol, sterol and carotenoid.

All phytochemical compounds would accumulate in the pericarp and testa or bran of the rice kernel. Amongst rice varieties there are rice varieties that contain color pigments. The cultivars of pigmented rice have a long history for human consumptions, especially in South East Asia (Hu *et al*., 2003). These compounds are pigment containing related to distinct colors such as red, purple and black. Antioxidant activities of paddy varieties containing colour pigments such as red Thai, black rice, red brown and dark purple had been intensively studied by Muntana and Prasong (2010) and Yodmanee *et al.* (2011), and they reported that rice with non color pigments contained lower phenolic content and antioxidant activities. Many studies have reported that black rice contains rich of anthocyanin and other polyphenolic compounds more abundantly than white rice (Ryu *et al*., 1998; Zhang *et al*., 2006). Furthermore, it has been reported that colored rice varieties contains more

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phenolic compounds and exhibits higher antioxidant activity than white rice varieties (Fujita *et al*., 2010; Muntana and Prasong, 2010; Sompong *et al*., 2011).

Previous research about antioxidant properties in colored rice bran indicated that rice bran with certain color that contains anthocyanin has a reductase enzyme inhibitory and anti diabetic activity (Yawadio *et al*., 2007; Kim *et al*., 2008; Park *et al*., 2008). Further studies reported that dark purple rice variety had higher iron, polyphenol and antioxidant properties than the red rice variety, while red rice contains higher phenolic compounds. It has also been reported that black rice has a scavenging activities higher than red rice variety, while non colored rice had phenolic content and antioxidant activities which are lower than the colored rice variety (Muntana and Prasong, 2010; Yodmanee *et al*., 2011).

However, no study has been reported on the phytochemical compounds and antioxidant properties in colored and non colored native rice bran from Minahasa, North Sulawesi, Indonesia. Therefore, the objective of this study was to determine the phytochemical compounds and antioxidant properties in the crude extract of colored and non colored varieties of rice bran from Minahasa Regency, North Sulawesi, Indonesia and to uncover its potential as a natural antioxidant source.

## Material and Methods

### *Preparation of rice bran samples*

Minahasa rice varieties used in this study were non colored rice (Superwin and Cigeulis) and colored rice (Red variety). All samples were obtained from the fields of Minahasa Regency, North Sulawesi and milled to obtain the rice bran flour. The fresh milled bran samples were collected immediately from the milling system in polyethylene bags. Stabilization of rice bran was carried out according to the previous method reported by Malekian *et al*. (2000) with a slight modification. Each sample was packed in polyethylene bags and heated in autoclave for 3 min at 120°C and then cooled down to room temperature overnight. The sample were then stored at 4°C in refrigerator for further analysis.

### *Extraction of rice bran*

The extraction of rice bran was carried out according to the method of Lai *et al*. (2009) with a slight modification. The flour of three varieties of rice bran samples 5 kg were macerated and extracted with 70% ethanol three times and each for overnight at room temperature. The ethanol extracts were fractionated with organic solvents (hexane, ethyl acetate and n-butanol) according to their polarity level. Each extract was prefiltered with whatman paper No. 42 and then evaporated by rotary evaporator (Buchi rotavapor) under vacuum to obtain the hexane, ethyl acetate and n-butanol crude extract of rice bran. The crude extracts were then stored under freezing temperature (-4°C) until used for further analysis.

### *The phytochemicals analysis*

The phytochemicals content in the crude extracts of the rice bran was determined according to the procedures of phenolic, flavonoid, alkaloid, steroid, triterpenoid and saponin tests (Harborne, 1987).

### *DPPH radical scavenging assay*

Antioxidant activity test of crude extract of rice bran was determined by the free radical-scavenging 1,1-diphenyl-2- picrylhydrazyn (Lee *et al*., 2006). Antioxidant activity from rice bran crude extract in various concentrations of 0-100 ppm was calculated with the DPPH assay (Kim *et al*., 2004). Absorbance was measured by UV-Vis spectrophotometer at 517 nm. While inhibition was calculated with the following formula:

$$\text{DPPH scavenging (\%)} = \frac{1}{A517\ nm.sample - A517\ nm.control} \times 100\%$$

While the $IC_{50}$ value was calculated from inhibition percentage and absorbance value in a linear regression of some concentrations of crude extract of the rice bran samples.

### *Determination of total phenolic content*

The total phenolic content of crude extract rice bran was determined by spectrophotometric method using the Follin-Ciocalteu reagent (Singleton and Rossii, 1965; Iqbal *et al*., 2005). One hundred microliters of the crude extract rice bran solution (5 mg/ml) was mixed with 1.5 ml 10% sodium carbonate solution and then a-3 ml of 10% of Follin-Ciocalteu reagent was added. The final mixture was kept in the dark at ambient conditions for 2 hours to complete the reaction. The absorbance was measured by a spectrophotometer at 765 nm. All measurements were determined triplicate and the data were expressed as mg Gallic Acid Equivalent (GAE) per 100 g of crude extract of rice bran.

### *Determination of total anthocyanin content*

Total anthocyanin content in crude extract of rice bran was determined by pH differential method (Yodmanee *et al*., 2011; Chakuton *et al*., 2012). Twenty microliters of crude extract was added into 2 mL of potassium chloride buffer (pH 1.0) and 2

mL of sodium acetate buffer (pH 4.5). Absorbance was measured at 550 and 700 nm wavelengths using a spectrophotometer. Distilled water was used as a blank. The difference in absorbance between pH values and wavelengths was calculated as follow:

$$A = (A_{550\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{550\ nm} - A_{700\ nm})_{pH\ 4.5}$$

The anthocyanin concentration of crude extract rice bran was calculated according to the following formula and expressed as cyanidin-3-glucoside equivalents:

$$\frac{A \times MW \times DF \times 1000}{MA \times 1}$$

A = absorbance of samples
MW = molecular weight of cyanidin-3-glucoside (449.2 g/mol)
DF = dilution factor of sample
MA = molar absorptivity

*Thiobarbituric acid assay*

Thiobarbituric acid assay was determined according to the method described by Pegg (2001) with modification. Fifty milligrams of samples was added with 25 ml n-butanol. The solution was mixed thoroughly. Five milliliters of the solution was then mixed with 5.0 ml of 0.2 g/100 ml TBA in n-butanol. The solution was incubated for 2 hours at 95°C. The absorbance of the solution was measured at 528 nm wavelength. TBA value was expressed as the increasing absorbance due to reaction of the equivalent of 1 mg sample per 1 ml volume with TBA which was calculated by the following equation :

$$\text{TBA value} = [50 \times (A_{sample} - A_{reagent\ blank})] / m$$

where m represents mass of sample (mg).

## Results and Discussion

*The phytochemical analysis*

Phytochemical screening of three rice bran varieties indicated that these rice bran varieties contained almost all secondary metabolites found in plants, such as phenolic compounds, flavonoids using NaOH 10% test, triterpenoids, alkaloids, and saponins, but did not indicated any traces of steroids.

*Determination of antioxidant properties of colored and non colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia*

The overall results of the antioxidant properties of crude extract of colored and non colored varieties of rice bran from Minahasa, North Sulawesi are shown in Table 1.

Table 1. Antioxidant properties of colored and non colored varieties of rice bran from Minahasa, North Sulawesi, Indonesia

| Rice Bran Varieties | DPPH (%) | $IC_{50}$ (µg/ml) | Total Phenolic Content (mg/g) | Total Anthocyanin (mg/g) | TBA (mg/g) |
|---|---|---|---|---|---|
| Cigeulis | | | | | |
| Hexane | 73.81 ± 2.32 | | 0.00 | 0.66 ± 0.21 | 0.56 ± 0.01 |
| Ethyl acetate | 64.74 ± 1.00 | 363.17± 91.21 | 261.96 ± 6.52 | 54.45 ± 2.08 | 20.29 ± 0.88 |
| n-Butanol | 51.02 ± 1.10 | | 41.80 ± 1.77 | 18.17 ± 0.75 | 0.004 ± 0.01 |
| Superwin | | | | | |
| Hexane | 67.96 ± 1.10 | | 0.00 | 0.49 ± 0.02 | 0.19 ± 0.01 |
| Ethyl acetate | 76.10 ± 1.20 | 341.88 ± 74.10 | 239.04 ± 8.16 | 43.30 ± 1.28 | 15.01 ± 0.85 |
| n-Butanol | 51.29 ± 1.57 | | 147.00 ± 2.53 | 34.17 ± 1.74 | 0.00 |
| Red | | | | | |
| Hexane | 82.83 ± 0.92 | 26.26 ± 0.95 | 63.18 ± 2.28 | 4.58 ± 0.13 | 4.82 ± 0.28 |
| Ethyl acetate | 82.36 ± 1.33 | | 258.23 ± 2.83 | 68.61 ± 1.98 | 8.47 ± 0.06 |
| n-Butanol | 88.29 ± 5.62 | | 58.55 ± 5.42 | 42.25 ± 0.55 | 2.62 ± 2.07 |

Data are expressed as the Mean ± standard deviation of triplicate analyses

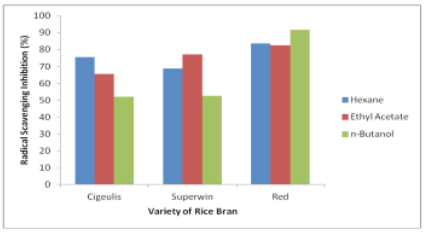


Figure 1. Percentage of radical scavenging inhibition

*Determination of DPPH radical scavenging activity*

The antioxidant activity was determined with DPPH free radical scavenging for each fraction of the crude extract of rice bran and the average radical scavenging activity varied from 51.02 ± 1.10 - 88.29 ± 5.62%. The highest percentage of radical scavenging inhibition were of non colored and colored varieties, respectively, Cigeulis 73.81 ± 2.32% (non polar fraction), Superwin 76.10 ± 1.20% (semi polar fraction) and red variety 88.29 ± 5.62% (polar fraction). The highest radical scavenging activity was obtained by extraction with polar solvents, n-butanol. The percentage of radical scavenging inhibition of crude exctract are shown in Figure 1.

Linear regressions of inhibition percentage of colored and non colored crude extract of rice bran varieties at various concentrations are shown in Figures 2, 3, and 4. Hence the $IC_{50}$ value of crude extract of colored rice bran varieties, red variety was 26.26 ± 0.95 µg/ml and $IC_{50}$ values of non colored rice bran, Superwin and Cigeulis, $IC_{50}$ values were 341.88 ± 74.10 µg/ml and 363.17 ± 91.21 µg/ml respectively. The colored crude exctract of rice bran had a lower $IC_{50}$ value than the non colored varieties. The lower the $IC_{50}$ indicated the stronger capability of samples to catch free radical of DPPH.

A similar study was reported by Rao *et al.* (2010), investigating the methanolic extract of rice bran from four varieties in India, and they noted that the Njavara had the highest DPPH scavenging

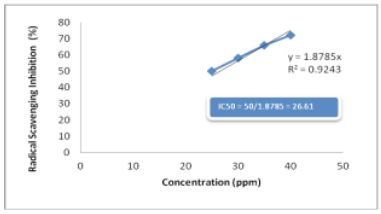


Figure 2. $IC_{50}$ value of red variety, colored varieties of rice bran

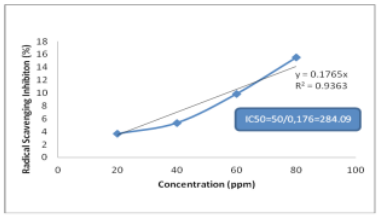


Figure 3. $IC_{50}$ value of Cigeulis, non colored varieties of rice bran

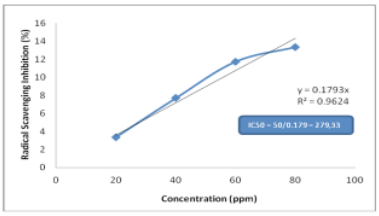


Figure 4. $IC_{50}$ value of Superwin, non colored varieties of rice bran

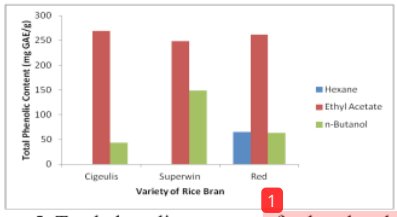


Figure 5. Total phenolic contents of colored and non colored varieties of rice bran

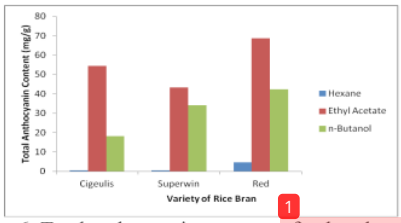


Figure 6. Total anthocyanin contents of colored and non colored varieties of rice bran

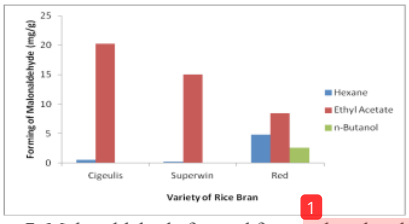


Figure 7. Malonaldehyde formed from colored and non colored varieties of rice bran

activity with an $IC_{50}$ value was 30.85 µg/ml, and the $IC_{50}$ values of the other varieties were 48.88, 70.58 and 87.72 µg/ml for Jyothi, Yamini and Vasumathi, respectively. Arab *et al.* (2011) and Chakuton *et al.* (2012) reported that DPPH scavenging activity ($IC_{50}$ values) of colored rice extract was better than that of non colored extracts, while the methanol extracts of Fajr rice bran had a higher inhibition (93.91%) than ethanol and ethyl acetate extracts. In another study, Zubair *et al.* (2012) reported that DPPH radical scavenging activity of the 80% isopropanol extract had a higher activity than the 100% methanol and the 100% ethanol extract. The wide variability of $IC_{50}$ values of isopropanol, methanol and ethanol extracts of the Pakistani rice cultivar, were 2.22 ± 0.11 – 3.88 ± 0.16 mg/ml, 3.59 ± 0.12 – 4.99 ± 0.16 mg/ml, 5.09 ± 0,21 – 6.26 ± 0.23 mg/ml respectively. Lum and Chong (2012), observed the antioxidant properties of pigmented rice from Sabah, Malaysia, and reported that the red rice variety had the highest DPPH radical scavenging activity (65.54 ± 0.57%) than the black and brown rice varieties, 37.66 ± 3.85% and 13.74 ± 11.77%, respectively, and the scavenging activity of white variety cannot be determined, and this could be due to the low content of phytochemical compounds.

A previous study on DPPH scavenging activity of Japonica rice bran extract was reported by Lai *et al.* (2009), where the methanol extract had a higher scavenging activity than the ethyl acetate and hexane extracts. The methanol extract of Japonica rice bran had a 93% inhibition of DPPH radical scavenging. The better activity of methanol extract, may be explained by the possibility of more polar phenolic compounds and lipids eluted in the methanol extract than in the ethyl acetate extract (Lai *et al.*, 2009).

*Determination of total phenolic content*

The total phenolic content was determined according to the Follin-Ciocalteu method and the results were expressed as gallic acid equivalents. Total phenolic contents of the rice bran varieties at different fraction are presented in Table 1 and Figure 5. The differences were observed among the solvents. Phenolic compounds were dissolved in the semi polar solvent, ethyl acetate for all varieties of the rice bran, and the Cigeulis and Superwin extracts were not dissolved in the non polar solvent, hexane, while red variety would have been dissolved by polar solvents, 63.18 ± 2.28 mg/g gallic acid. The

highest of total phenolic content was Cigeulis 261.96 ± 6.52 mg/g gallic acid, red variety 258.23 ± 2.83 mg/g gallic acid and the lowest was Superwin variety 239.04 ± 8.16 mg/g gallic acid. A previous study on antioxidant activity of colored and non colored Thai rice cultivars with various solvents reported that the total phenolic content of methanolic extract had the higher value than distilled water, hexane and ethyl acetate extract (Chakuton *et al.*, 2012), and a similar study was reported by Lai *et al*. (2009), and they noted that the ethyl acetate extract of rice bran had a highest total phenolic content (19.7 ± 0.8 g GAE/kg) than the methanol and hexane extracts, 15.7 ± 0.6 g GAE/kg and 14.7 ± 1.2 g GAE/kg, respectively. While, the study about antioxidant activity of Iranian rice bran varieties, extracted with three different solvents (methanol, ethanol and ethyl acetate) reported that the methanolic extract of Fajr variety had a higher total phenolic content (3.31 ± 0.03 mg GAE/g) than those of ethanol and ethyl acetate extracts, 1.67 ± 0.01 mg GAE/g and 1.29 ± 0.03 mg GAE/g, respectively (Arab *et al*., 2011). In a previous study, Lum and Chong (2012), observed the antioxidant properties of pigmented rice from Sabah, Malaysia, and found that red rice variety contained the highest quantity of phenolic acids (329.93 ± 19.17 mg/100 g) than the black rice (290.77 ± 13.72 mg/100 g), brown rice (69.63 ± 5.58 mg/100 g), and the white rice variety (22.59 ± 1.31 mg/100 g).

*Determination of total anthocyanin*

Anthocyanin contents of the samples are shown in Table 1 and Figure 6. The colored varieties had more higher anthocyanin content than the non colored varieties and the highest anthocyanin content dissolved in semi polar solvent, ethyl acetate. Total anthocyanin content of colored varieties, red variety was 68.61 ± 1.98 mg/g and non colored varieties Cigeulis and Superwin, were 54.45 ± 2.08 mg/g and 43.30 ± 1.28 mg/g, respectively.

A previous study on anthocyanin of colored rice in Thailand, China and Srilanka was reported by Sompong *et al.* (2011), where all rice varieties with black colored pigments had the highest amount of total anthocyanin compared to 10 red pigment varieties. Black pigmented varieties have 109.52-256.61 mg/100 g anthocyanin, while the total anthocyanin contents of red varieties vary between 0.33-1.38 mg/100 g. A study on 8 different pigmented varieties in Thailand by Yodmanee *et al*. (2011), reported that rice varieties with dark purple color contained a higher amount of anthocyanin ranging between 208.42-329.24 mg/100 g, compared to the red pigmented varieties ranging between 58.89-84.43 mg/100 g. Sutharut and Sudarat (2012), also reported that three rice varieties in Thailand, where a non pigmented variety contained anthocyanin at a range between 1.09-10.83 mg/100 g, and a range of 17.89-99.53 mg/100 g was reported for the two colored varieties.

*Thiobarbituric acid assay*

Antioxidant activity using thiobarbituric acid (TBA) assay was used for measuring formed malonaldehyde (MDA), while MDA was the product of the oxidation of polyunsaturated fatty acids, considered as an index of lipid peroxidation. The basic principles of the method is the reaction of one molecule of malonaldehyde and two molecules TBA, leading to the formation of a pink pigment malonaldehyde-TBA complex, which can be quantified by spectrophotometry (Tokur *et al*., 2006).

MDA products of crude extract rice bran for each fraction differed, the lowest of MDA products were formed in polar fraction for all varieties of rice bran crude extract and the highest was observed in semi polar fraction, respectively 20.29 ± 0.88 mg/g, 15.01 ± 0.85 mg/g, 8.47 ± 0.06 for Cigeulis, Superwin and red variety. The MDA products of colored and non colored varieties of rice bran for each fraction are shown in Figure 7. A study on thiobarbituric acid assay from pigmented rice variety in Sabah, Malaysia by Lum and Chong (2012) reported that the red rice variety had the highest antioxidant activity compared with three other varieties with the lowest absorbance (0.329), black rice (0.364), brown rice (0.411) and white rice variety had a lowest antioxidant activity (0.420).

The result of this study indicated that the antioxidant properties of rice bran from Minahasa, North Sulawesi were broadly comparable with previous studies. Studies on antioxidant properties of colored rice and non colored rice was determined by Hu *et al.* (2003) and Chakuton *et al.* (2012) showed a significant positive correlation between pigmented varieties and their antioxidant activity. Antioxidant properties of colored rice bran were better than that of non colored rice bran. The antioxidant properties of colored rice bran varieties is due to their pigment compounds of anthocyanin. Pigmented rice variety had a better scavenging activity than non pigmented rice variety because pigmented variety had a higher anthocyanin content which is a potent reducing agents and possesses strong radical scavenging activity (Nam *et al*., 2006). According to a study of antioxidant capacity, screening in 591 rice cultivars including white rice, weedy red rice and pigmented

rice, blackish purple rice cultivars showed twice stronger activity than the white rice cultivars (Lee *et al.*, 2011). Many studies have been reported that the colored rice variety contains rich of anthocyanin and other polyphenolic compounds much more abundantly than non colored rice variety (Ryu *et al.*, 1998; Zhang *et al.*, 2006).

## Conclusion

Crude extracts of rice bran samples contained phenolic, flavonoid, alkaloid, triterpenoid and saponin compounds and the result of all parameters of antioxidant activities showed that Minahasa colored rice bran varieties had a better properties than the non colored rice bran. It can be concluded that crude extract of colored rice bran might act as a potential natural antioxidant source.

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