Research Article

Total flavonoids and anthocyanins content of pigmented rice

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ABSTRACT

Pigmented rice/glutinous rice consisting of black rice (BR) (*Oryza sativa*), black glutinous rice (BGR) (*O. sativa* Var. *glutinosa*), and brown rice (RR) (*Oryza nivara*) is currently becoming the popular ingredients because it is a food as carbohydrate source that has functional properties. Pigmented rice naturally contains one or more compounds that are considered to have physiological functions that are beneficial to health, especially as antioxidants, such as polyphenols and anthocyanin pigments. In West Java, there are several local varieties of BR, glutinous BR, and RR which have the potential to be developed as a functional food. The objective of this study was to determine total flavonoids and total anthocyanins, as well as the antioxidant activity of local variety of BR, BGR, and RR. Rice samples were evaluated for color intensity, the yield of extract, total flavonoids, total anthocyanin, and antioxidant activity used kinetic assays of diphenyl-2-picrylhydrazyl. The result showed that the increase in the color intensity was increase the number of total flavonoids, total anthocyanin, and antioxidant activity. BR and BGR have total flavonoids content 18.68 mg 100 g^{-1} and 15.45 mg 100 g^{-1}, respectively. The total anthocyanin content of BR and BGR was 39.61 mg 100 g^{-1} and 28.63 mg 100 g^{-1}, respectively. Based on IC_{50} values, BR contains the bioactive compounds that are classified as strong antioxidants. Local variety of BR and BGR has potential to be an alternative of functional food.

KEY WORDS: Anthocyanin, Black glutinous rice, Black rice, Brown rice, Flavonoids, Functional food

INTRODUCTION

Based on the color, there are several types of rice known as white rice, black rice (BR), brown rice (RR), and black glutinous rice (BGR). Recently, pigmented rice has become popular and is consumed by some people as a functional food, because naturally or through certain processes contain one or more compounds that are considered to have physiological functions that are beneficial to health. Pigmented rice contains high phenolic and anthocyanin compounds which are located in the pericarp layer, which gives red to dark purple.[1] Anthocyanin has been recognized as a health functional food ingredient due to its antioxidant activity, anticancer, hypoglycemia, and anti-inflammatory effects.[2-6] These functions provide a synergistic effect with various nutrients *in vivo*. Anthocyanin pigments are also effective in reducing cholesterol levels.[7]

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Pigmented rice and glutinous rice contain a very potential source of antioxidants. RR is very popularly used as a functional food due to its very high polyphenol content and anthocyanin content. The most abundant types of anthocyanin are cyanidin-3-o-glucoside and peonidin-3-o-glucoside (P3G).[8] From the high-performance liquid chromatography profile showed that the anthocyanin extracted in BR was cyanidin-3-O-b-glucoside as the first peak (85%) and peonidin-3-O-b-D-glucoside the second peak (15%).[9]

Anthocyanin is a pigment with the ability as an antioxidant that can reduce cholesterol levels. Anthocyanin is a phenolic compound that belongs to the flavonoid group which plays an important role, both for the plant itself and human health, namely as an antioxidant. Antioxidants are defined as organic molecules that promote health by protecting the body's cells from damage caused by free radicals and reactive oxygen species that may otherwise exert harmful metabolic effects.[10] The molecules with antioxidant activity contained in rice include phenolic

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acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, c-oryzanol, and phytic acid.[10]

In general, all phytochemical compounds accumulate in the pericarp and testa or rice kernel bran. The main characteristic that determines the type of phenolic compounds in grains is the color of the pericarp.[9] The color of the pericarp is related to the concentration of phenolic in grains, and the concentration is usually higher in grains that have red and black pigment of the pericarp. Phenolic compounds are associated with pigment contents such as red, purple, and black.[11] BR has a high anthocyanin content in the pericarp layer which gives a deep purple color.[1]

There is a very good linear correlation between the total anthocyanin content and the antioxidant activity that extracted with acidified solvents.[12] The antioxidant activity of RR bran is greater than BR bran and white rice bran in Thai rice cultivars.[11] RR obtained in China, Thailand, and Sri Lanka contains polyphenol and anthocyanin compounds that can act as good antioxidants.[13] The objective of the study was to determine the content of total flavonoids, anthocyanin pigments, and antioxidant capacity of pigmented rice and glutinous rice.

MATERIALS AND METHODS

Materials

The research materials are eight pigmented rice varieties, which are local varieties of West Java and the superior varieties obtained from previous studies. The eight pigmented rice varieties are as follows: Local BGR Tanjungsari, local BR Jatinangor, local RR Mareum Tanjungsari, Aek Sibundong (RR), Impari 24 (RR), Impari 25 (RR), Impara 7 (RR), and Impago 7 (RR) from the Food Crops Research Institute Sukamandi, West Java. The chemical used is methanol (Merck), ethanol (Merck), citric acid (Merck), and diphenyl-2-picrylhydrazyl (DPPH) reagents (Sigma-Aldrich).

Measurement of Rice Color

The color was expressed in L*, a*, b*, C*, and h. The coordinate of L* represents lightness (L* = 0 yields black and L* = 100 indicates diffuse white). The coordinate of a*represents redness to greenness, and b* represents yellowness to blueness.[14] The chroma (C*) represents color intensity which is the distance of color from the origin (a* = b* = 0) in a* and b* plane. Hue angle (h) expresses in degrees ranged from 0° to 360°, where 0° (red) locates on the +a* axis, then rotating anticlockwise to 90° (yellow) for the +b* axis, 180° (green) for −a*, and 270° (blue) for −b*.[15] The colors observed using Konica Minolta CM-2006 Chroma Meter and calibrated with standard white-color.

Extraction of Bioactive Components

Extraction of bioactive components of rice and glutinous rice was used methanol solvent.[16] The ratio of the sample with solvents is 1:6 b/v. The filtrate obtained from the extraction was dried using a vacuum drier. The yield was obtained from the weight ratio of the extract with the dry weight base of the sample.

Determination of Total Flavonoids and Anthocyanins[17]

About 1 g of pigmented rice was added to a mixture of ethanol 98% and citric acid 1.0 mol/L in the ratio 80:20. The extraction was performed in a thermostated cell, protected from light, and under constant stirring. After processing, the extract was filtered used filter paper (qualitative Whatman No: 1), and the residue was rinsed using the extraction solvent until completing a volume of 50 mL. The extract was stored at −20°C in polyethylene bottles. Total flavonoids and total anthocyanin of pigmented rice were quantified by ultraviolet (UV)-Vis spectrophotometry at 374 nm and 535 nm, respectively. The content of total flavonoids was expressed as quercetin equivalents (mg 100 g^{-1}) of dry weight rice sample and total anthocyanins expressed as cyaniding-3-glucoside (C3G) equivalents (mg 100 g^{-1}) of dry weight rice sample. The content of flavonoids and anthocyanins was determined using equations (1) and (2).

$$Total\ flavonoids\,(TF) = \frac{A_{374\,nm} \times dilution\ factor}{76.6} \quad (1)$$

$$Total\ anthocyanins\,(TA) = \frac{A_{535\,nm} \times dilution\ factor}{98.2} \quad (2)$$

Determination of Antioxidant Activity[13]

The sample extract was dissolved with methanol to a concentration of 10 ppm. Then, a 50 ppm stock DPPH solution (5 mg DPPH in 100 mL of methanol) was prepared. The comparison solution also prepared, 2 mL of methanol was mixed with 1 mL of 50 ppm DPPH solution. For the test sample, each 2 mL sample solution was mixed with 2 mL DPPH solution. All samples are made triple. Then, the mixed solutions were incubated for 30 min at a temperature of 27°C until there was a color change from purple to bright yellow. All the incubated extract samples were tested using UV-Vis spectrophotometer for absorbance values at 517 nm. The effective concentration value is a number indicating the concentration of extract (μg mL^{-1}) which is able to inhibit 50% oxidation. The effective concentration value (IC50) was determined using the following formula:

$$\%\ Antioksidan = \frac{A_C - A}{A_C} \times 100\% \quad \textbf{(3)}$$

Where *Ac* was the absorbance of control and *A* was the absorbance of the sample. The compounds that have IC_{50} values <50 are very strong antioxidants, strong (50–100), moderate (100–150), and weak (151–200). The smaller the IC50 value, the higher the antioxidant activity.[18]

RESULTS AND DISCUSSION

The Color of Pigmented Rice

The results of measurements of rice color using Chroma Meter are shown in Table 1. The coordinates L* indicated the smaller the value, the color of the sample darker. The coordinates of a* showed the difference between red and green, the smaller the value, the color of the sample more green. The coordinates b* showed the difference between yellow and blue, the smaller the value showed the color of the sample more blue. The C* coordinates indicate the brightness of the sample color, the smaller the value, the color of the sample duller while the h coordinates indicated the strength of the color, the higher the value, the color of the sample stronger. The table showed that the sample of local BGR and local BR has a value of L*, a*, b*, C* smaller, and h larger, meaning that the samples have a darker color than RR.

The darker color of rice as shown by local BR and glutinous rice was thought to contain high bioactive compounds such as phenolic and anthocyanin. The previous study reported that the red and purple rice varieties showed higher total phenolics, flavonoids, and antioxidant activity than those of light-colored varieties, such as white and brown varieties.[19,20] The color parameters L*, a*, and b* are related to polyphenols and antioxidant activity.[21] The negative correlation in the color parameters a* and b* with total anthocyanin can occur because the value of a* is negative indicating the color green and leads to red when the value was heading in the positive direction. The value of b* will show a negative value if it is closed to blue and the color leads to yellow if the value leads to positive. Therefore, the lower the value of a* and b*, the higher the anthocyanin content.[22]

Total Flavonoids and Anthocyanins Content

Identification of the content of bioactive compounds in several types of pigmented rice was carried out on the content of flavonoids and anthocyanin compounds. The pigments that contain in BR, BGR, and RR are thought to be anthocyanin compounds. Table 2 shows the results of the yield extract, total flavonoids, and total anthocyanins content of pigmented rice.

The yield extracts of bioactive compounds obtained from pigmented rice samples were in the range of 4.56 ± 0.99% – 9.34 ± 0.49% [Table 2]. Impara seven RR produces the highest amount of bioactive compounds compared to other types of rice, while Impari 25 RR produces the lowest yield extract. However, during the extraction of selected rice samples, some browning occurred, and anthocyanin coloration was lost suggesting enzymatic degradation. Anthocyanins can be degraded by a number of enzymes classified as glycosidases (anthocyanases), polyphenoloxidases (PPO), and peroxidases.[23] Glycosidases act directly on anthocyanins hydrolyzing the pigments to sugars and anthocyanidins (aglycone).[23,24] Anthocyanidins are very unstable and degrade to colorless derivatives or condense with other phenolics to form polymeric colored compounds.[25] The action of PPO and peroxidases on anthocyanins is indirect.[26]

Table 1: The rice color (L*, A*, B*, C*, and H) using Chroma Meter

Pigmented rice	L*	a*	b*	C*	h
Local BGR	9.29±0.40	5.29±0.28	−2.70±0.32	5.95±0.11	332.96±3.91
Local BR	18.63±2.66	11.88±1.63	−3.34±0.09	12.35±1.56	344.10±2.37
Local RR (Mareum)	37.98±8.38	22.46±1.25	19.23±2.85	29.66±1.04	40.45±5.63
Aek Sibundong RR	35.10±2.70	21.06±1.06	17.45±0.48	27.35±0.96	39.67±1.38
Impari 24 RR	32.46±3.31	19.46±1.73	16.25±1.23	25.41±0.50	39.99±4.80
Impari 25 RR	32.79±5.12	16.70±1.32	15.52±1.25	22.81±1.56	42.91±2.31
Impara 7 RR	30.10±3.68	19.31±0.56	15.68±1.68	24.90±0.98	39.01±3.44
Impago 7 RR	30.51±1.38	20.71±1.64	15.30±0.42	25.76±1.29	36.53±2.48

L*: Lightness, a*: Redness-greenness, b*: Yellowness-blueness, C*: Chroma, h*: Hue angle, BGR: Black glutinous rice, BR: Black rice, RR: Brown rice

Table 2: The yield extract, total flavonoids, and total anthocyanins content of pigmented rice

Pigmented rice	Yield extract (%)	Total flavonoids (mg 100^{-1})	Total anthocyanins (mg 100^{-1})
Local BGR	5.85±0.53^{ab}	15.45±1.41^{c}	28.63±0.48^{b}
Local BR	8.65±1.20^{cd}	18.68±0.00^{d}	39.61±0.11^{c}
Local RR (Mareum)	4.85±0.04^{a}	6.13±0.25^{ab}	0.78±0.11^{a}
Aek Sibundong RR	7.12±0.93^{bc}	5.91±0.00^{a}	0.64±0.00^{a}
Impari 24 RR	5.04±0.39^{a}	7.34±0.03^{b}	0.92±0.09^{a}
Impari 25 RR	4.56±0.99^{a}	6.29±0.36^{ab}	0.79±0.26^{a}
Impara 7 RR	9.34±0.49^{d}	6.70±0.11^{ab}	0.79±0.04^{a}
Impago 7 RR	4.58±0.47^{a}	7.19±0.03^{ab}	0.93±0.02^{a}

BGR: Black glutinous rice, BR: Black rice, RR: Brown rice, Means within rows followed by the same letter are not significant different at $P<0.05$

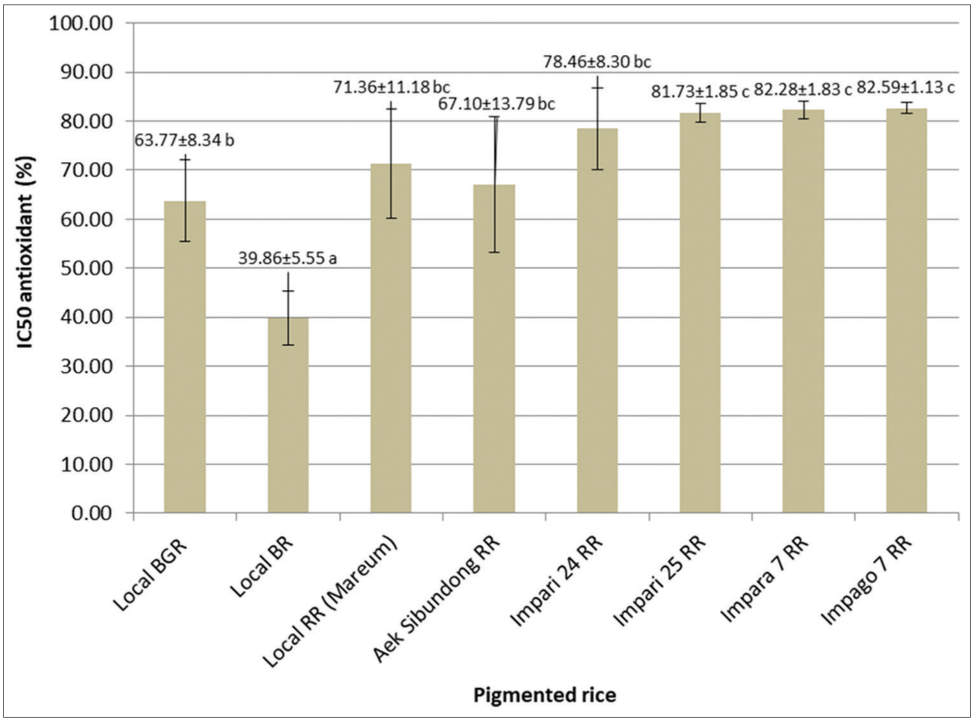

Figure 1: Antioxidant activity

The results of the determination of flavonoids and anthocyanin compounds showed that BR and BGR contained higher levels of flavonoids and anthocyanins compared to the RR [Table 2]. That is proved that the more black color of the rice shows higher levels of bioactive compounds (flavonoids and anthocyanins). The total flavonoids content of BGR and BR was 15.45 ± 1.41 mg 100 g^{-1} and 18.68 ± 0.00 mg100g^{-1}, respectively, while for RR the range was 5.91 ± 0.00 – 7.34 ± 0.03 mg 100 g^{-1}. The total anthocyanin content of BGR and BR was 28.63 ± 0.48 mg 100 g^{-1} and 39.61 ± 0.11 mg 100 g^{-1}, respectively, whereas for RR had a significant difference, there was in the range of 0.64 ± 0.00 – 0.93 ± 0.02 mg 100 g^{-1}. Many studies have reported that BR is more abundant in anthocyanin and other phenolic compounds compared to that of white rice.[27]

Anthocyanins are part of flavonoids that act as pigments and antioxidant. Anthocyanin compounds in the form of C3G and P3G and their derivatives are compounds that can affect the color of rice.[28] The total anthocyanins were expressed in equivalent units of C3G because about 93% of the total anthocyanin in BR and RR is C3G. In general, anthocyanins are found in pigmented rice in the form of ethylated procydin structures that have free radical scavenging activity.[27] Flavonoids are distinguished based on their substitution patterns, namely anthocyanins, flavones, isoflavones, flavonols, flavanones, and flavanols.[29] The determination of total flavonoid based on the effectiveness of flavonoid compounds to capture free radicals and ferrous-iron chelating activity.[10,30] The highest and dominant levels of C3G are in BR.[31] Black and RR contain sianidin-3-glucosidasa (C3G) and P3G,[13,27] and anthocyanins content in rice is related to the color of the pigmented rice.[11]

Antioxidant Activity

The ability to capture DPPH free radicals from pigmented rice extracts is shown in Figure 1. The data showed that the inhibitory ability of phytochemical compounds in BR extract was higher than the other types of pigmented rice. Based on IC_{50} values, BR contains the bioactive compounds that are classified as strong antioxidants (39.86 ± 5.55%). A compound is said to be a very strong antioxidant if IC_{50} values are <50, strong (50–100), moderate (100–150), and weak (151–200). The smaller the IC_{50} value, the higher the antioxidant activity.[18]

The results showed that BR had the highest total flavonoids and anthocyanin levels. Phenolic compounds and flavonoids have been shown to be able to donate hydrogen atoms to purple DPPH free radicals which form yellow DPPH compounds. BR has higher anthocyanin pigments and phenolic compounds than RR and white rice that is made it a potential source of antioxidants.[11,27]

CONCLUSION

The increase in the color intensity of pigmented rice was increase the number of total flavonoids, total anthocyanins, and antioxidant activity. BR and BGR have total flavonoids content 15.45 ± 1.41 mg

100 g^{-1} and 18.68 ± 0.00 mg 100 g^{-1}, respectively. The total anthocyanin content of BR and BGR was 28.63 ± 0.48 mg 100 g^{-1} and 39.61 ± 0.11 mg 100 g^{-1}, respectively. Based on IC_{50} values, BR contains the bioactive compounds that are classified as strong antioxidants. Local variety of BR and BGR rice has the potential to be an alternative of functional food.

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REFERENCES

1. Takashi I, Bing X, Yoichi Y, Masaharu N, Tetsuya K. Antioxidant activity of anthocyanin extract from purple black rice. J Med Food 2001;4:211-8.
2. Nam S, Choi SP, Kang MY, Koh HJ, Kozukue N, Friedman M. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chem 2006;94:613-20.
3. Philpott M, Gould KS, Lim C, Ferguson LR. *In situ* and *in vitro* antioxidant activity of sweetpotato anthocyanins. J Agric Food Chem 2004;52:1511-3.
4. Hyun JW, Chung HS. Cyanidin and malvidin from *Oryza sativa* Cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. J Agric Food Chem 2004;52:2213-7.
5. Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. J Nutr 2003;133:2125-30.
6. Tsuda T, Horio F, Osawa T. Cyanidin 3-O-beta-D-glucoside suppresses nitric oxide production during a zymosan treatment in rats. J Nutr Sci Vitaminol (Tokyo) 2002;48:305-10.
7. Lee JC, Kim JD, Hsieh FH, Eun JB. Production of black rice cake using ground black rice and medium-grain brown rice. Int J Food Sci Technol 2008;43:1078-82.
8. Lee JH. Identification and quantification of anthocyanins from the grains of black rice (*Oryza sativa* L.) varieties. Food Sci Biotechnol 2010;19:391-7.
9. Yawadio R, Tanimori S, Morita N. Identification of phenolic compounds isolated. J Food Chem 2007;101:1616-25.
10. Goufo P, Trindade H. Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Sci Nutr 2014;2:75-104.
11. Muntana N, Prasong S. Study on total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. Pak J Biol Sci 2010;13:170-4.
12. Awika JM, Rooney LW, Waniska RD. Anthocyanins from black sorghum and their antioxidant properties. Food Chem 2004;90:293-301.
13. Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G, Berghofer E. Phsyco-chemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. J Food Chem 2011;124:132-40.
14. Bao J, Cai Y, Sun M, Wang G, Corke H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. J Agric Food Chem 2005;53:2327-32.
15. Nontasana S, Moongngarma A, Deeseenthum S. Application of functional colorant prepared from black rice bran in yogurt. APCBEE Procedia 2012;2:62-7.
16. Chakuton K, Puangpropintag D, Nakornriab M. Phytochemical content and antioxidant activity of colored and non-colored Thai rice cultivars. Asian J Plant Sci 2012;11:285-93.
17. Lees DH, Francis FJ. Standardization of pigment analysis in cranberries. HortScience 1972;7:83-4.
18. Badarinath A, Rao K, Chetty CS, Ramkanth S, Rajan T, Gnanaprakash KA. Review on *in-vitro* antioxidant methods: Comparisons, correlations, and considerations. Int J PharmTech Res 2010;2:1276-85.
19. Shao Y, Xu F, Sun X, Bao J, Beta T. Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). J Cereal Sci 2014;59:211-8.
20. Walter M, Marchesan E, Massoni PF, Silva LP, Sartori GM, Ferreira RB. Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. Food Res Int 2013;50:698-703.
21. Shen Y, Jin L, Xiao P, Lu Y, Bao JS. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. J Cereal Sci 2009;49:106-11.
22. Kristamtini T, Basunanda P, Murti RH. Genetic variability of rice pericarp color parameters and total anthocyanine content of eleven local black rice and their correlation. Ilmu Pertanian 2014;17:57-70.
23. Francis FJ. Food colorants: Anthocyanins. Crit Rev Food Sci Nutr 1989;28:273-314.
24. Schwartz SJ, von Elbe JH, Giusti MM, Colorants. In: Fennema OR, Damodaran S, Parkin K, editors. Food Chemistry. 4th ed. Boca Raton, FL: CRC Press/Taylor and Francis; 2008.
25. Shiroma-Kian C, Tay D, Manrique I, Giusti MM, Rodriguez-Saona LE. Improving the screening process for the selection of potato breeding lines with enhanced polyphenolics content. J Agric Food Chem 2008;56:9835-42.
26. Wrolstad RE, Durst RW, Lee J. Tracking color and pigment changes in anthocyanin products. Trends Food Sci Technol 2005;16:423-8.
27. Sutharut J, Sudarat J. Total anthocyanin content and antioxidant activity ofgerminated colobrown rice. Int Food Res J 2012;19:215-21.
28. Escribano-Bailón MT, Santos-Buelga C, Rivas-Gonzalo JC. Anthocyanins in cereals. J Chromatogr A 2004;1054:129-41.
29. Ghosh D, Scheepens A. Vascular action of polyphenols. Mol Nutr Food Res 2009;53:322-31.
30. Tapas A, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: A review. Tropic J Pharm Res 2008;7:1089-99.
31. Widyawati PS, Suteja AM, Suseno TI, Monika P, Saputrajaya W, Liguori C. Effect of pigment color difference in organic rice on antioxidant activity. Agritech 2014;34:399-406.

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