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Research Article

Antioxidant Capacities of Various Grains Extracts of Three Kinds of Rice Grown in Central Java-Indonesia

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ABSTRACT

The goals of this research were to determine antioxidant activity from different polarities grains extract of three kinds of rice using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) and correlation of total phenolic, flavonoid and carotenoid content with their IC_{50} of DPPH and IC_{50} of FRAP antioxidant activities. Extraction was carried out by reflux using different polarities solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and FRAP assays, determination of total phenolic, flavonoid and carotenoid content were performed by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities were analyzed by Pearson's method. Ethyl acetate and ethanolic extracts of black rice and red rice grains were very strong antioxidant, using DPPH assays. Phenolic compounds in black rice and red rice grains extracts were the major contributor in IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity. Grains extract of black rice, red rice and white rice had linear result in DPPH and FRAP assays.

Keywords: Antioxidant, DPPH, FRAP, three kinds, rice, grains

INTRODUCTION

Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, included antibacterial and antioxidant activity[1,2]. Previous study[3,4] demonstrated that phenolic and flavonoid content could be correlated to their antioxidant activities. Oxidative stress related with many diseases, can be prevented by antioxidant. Plants included sweet potatoes, rice, guava, tea and citrus contained phenolic and flavonoid compounds[5-9].

DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) can be used to evaluate antioxidant activity of vegetables, fruits and food[6,10]. Previous study[6,9] exhibited that DPPH, FRAP and ABTS methods could be used to determine antioxidant activity in many plants extracts. The previous study[5,11,12] stated that rice had antioxidant activities by using DPPH, FRAP and ABTS assays. The objective of this research were to determine antioxidant activities of different polarities grains extract (n-hexane, ethyl acetate and ethanol) of three kinds of rice grown in Central Java-Indonesia using DPPH and FRAP assays, and correlations of total phenolic, flavonoid and carotenoid content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), TPTZ (TPTZ (2,4,6-tripyridyltriazine), gallic acid, quercetin, beta carotene were purchased from Sigma-Aldrich (MO, USA), grains of three kind of rice. All of other reagents were analytical grades.

Preparation of sample

Grains of three kinds of rice (*Oryza sp*.) were: black rice namely as BLA, red rice as RED and white rice as WHI were collected from Magelang- Central Java, were thoroughly washed with tap water, sorted while wet, cut, dried and grinded into powder.

Extraction

Three hundred gram of powdered sample was extracted by reflux using different polarities solvents. Extraction using n-hexane was repeated three times. The remaining residue was then extracted three times by using ethyl acetate. Finally the remaining residue was extracted three times using ethanol. Therefore, there were nine extracts: three n-hexane extracts (namely BLA1, RED1 and WHI1), three ethyl acetate extracts (BLA2, RED2 and WHI2) and three ethanolic extracts (BLA3, RED3 and WHI3).

DPPH scavenging activity

DPPH solution was prepared by using modification of Blois's method[13]. Various concentration of each extract was mixed with DPPH solution 50 µg/ml (volume 1:1) to obtain a calibration curve. The absorbance was measured after 30 minutes incubation at wavelength 515 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank, DPPH 50 µg/ml as control and ascorbic acid as standard. Analysis was done in triplicate for standard and each extract. Antioxidant activity of each extract by DPPH method was determined by calculating percentage of antioxidant activity using

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reduction of DPPH absorbance[14]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

FRAP capacity

Method of Benzi[15] was used in preparing of FRAP solution. The FRAP solution was prepared in acetate buffer pH 3.6. Various concentration of each extract was added into FRAP solution 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 593 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Acetate buffer was used as a blank, FRAP 50 µg/ml as control and ascorbic acid as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increasing in Fe (II)-TPTZ absorbance by calculating percentage of antioxidant capacity[15].

Total phenolic content (TPC)

Folin-Ciolcalteu reagent was used in total phenolic content determination[16]. The absorbance was measured at wavelength 765 nm. Analysis was carried out in triplicate for each extract. Gallic acid standard solution (105-200 µg/ml) was used to obtain a calibration curve. Total phenolic content was figured as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Total flavonoid content (TFC)

Chang *et al*[17] was done for evaluating total flavonoid content. The absorbance was read at wavelength 415 nm. Analysis was conducted in triplicate for each extract. Quercetin standard solution (36-100 µg/ml) was used to obtain a calibration curve. The total flavonoid content was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

Total carotenoid content was measured using modified method from Thaipong *et al*[6]. Each extract was diluted in n-hexane[9]. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Beta carotene standard solution (30-100 µg/ml) was used to obtain a calibration curve. The total carotenoid content was exposed as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical Analysis

Each sample analysis was done in triplicate. All of presented results are means (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure was carried out with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant activities, and correlation between two antioxidant activity methods were performed using the Pearson's method.

RESULTS

IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity

The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacity in various extracts of rice grains using DPPH and FRAP assays were shown in Fig 1 and Fig 2. IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacity of each extract were compared to IC_{50} and EC_{50} of ascorbic acid as standard. The lowest value of IC_{50} means had the highest antioxidant activity.

TPC, TFC and TCC in rice grains extract

TPC among the various extracts were expressed in term of gallic acid equivalent using the standard curve equation $y = 0.005 x - 0.198$, $R^2 = 0.9971$. The TPC in various extracts of rice grains gave different result in the range of 0.84-6.36 g GAE/100 g. The highest phenolic content (6.36 g GAE/100 g) was given by ethanolic grains extract of black rice (BLA3) and the lowest given by ethanolic extract of white rice grains (WHI3). TFC among the various extracts were revealed in term of quercetin equivalent using the standard curve equation $y = 0.007 x + 0.001$, $R^2 = 0.9991$. The TFC in various extracts of rice grains showed different result ranged from 0.48-3.22 g QE/100 g (Tabel 1). Ethyl acetate extract of black rice grains (BLA2) had the highest total flavonoid content (3.22 g QE/100 g), while ethanolic grains extract of black rice (BLA3) gave the lowest total flavonoid content (0.48 g QE/100 g). TCC among the various extracts were exhibited in term of beta carotene equivalent using the standard curve equation $y = 0.007x - 0.002$, $R^2 = 0.9979$. The TCC in various extracts of rice grains had result in the range of 0.05 – 2.06 g BE/100 g (Table 1). The highest carotenoid content (2.06 g BE/100 g) was showed by n-hexane extract of white rice grains (WHI1), while the lowest carotenoid (0.05 g BE/100 g) for ethanolic extract of black rice grains (BLA3).

Correlations between total phenolic, flavonoid, carotenoid content in various rice grains extracts and IC_{50} of DPPH scavenging activities, EC_{50} of FRAP capacities

TPC in various grains extracts of black rice and red rice gave negative and significant correlation with their IC_{50} of DPPH scavenging activities were ($r = -0.723$, $p<0.05$; $r = -0.969$, $p<0.01$, respectively), while with their EC_{50} of FRAP capacities, all of various rice grains extracts had negatively significant correlation. TFC in white rice grains extract had negative and significant correlation with their EC_{50} of FRAP capacities ($r = -0.629$, $p<0.05$) and only TCC in black rice and red rice grains extract had negative and significant correlation with their IC_{50} of DPPH scavenging activities ($r = -0.756$; $r = -.0.956$, $p<0.01$, respectively) (Table 2).

DISCUSSION

The previous research[5,12] reported that rice had antioxidant capacity. There was no study regarding antioxidant activity of various grains extracts (which were n-hexane, ethyl acetate and ethanol) of three kinds of rice from Central Java- Indonesia using DPPH and FRAP assays. The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various grains extract from three kinds of rice using DPPH and FRAP assays were shown in Fig 1 and Fig 2. The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various grains extracts compared to IC_{50} or EC_{50} of ascorbic acid standard. The lowest IC_{50} means showed the highest antioxidant activity. Sample which had IC_{50} or EC_{50} lower than 50 µg/ml was a very strong antioxidant, 50-100 µg/ml was a strong antioxidant, 101-150 µg/ml was a medium antioxidant, while a weak

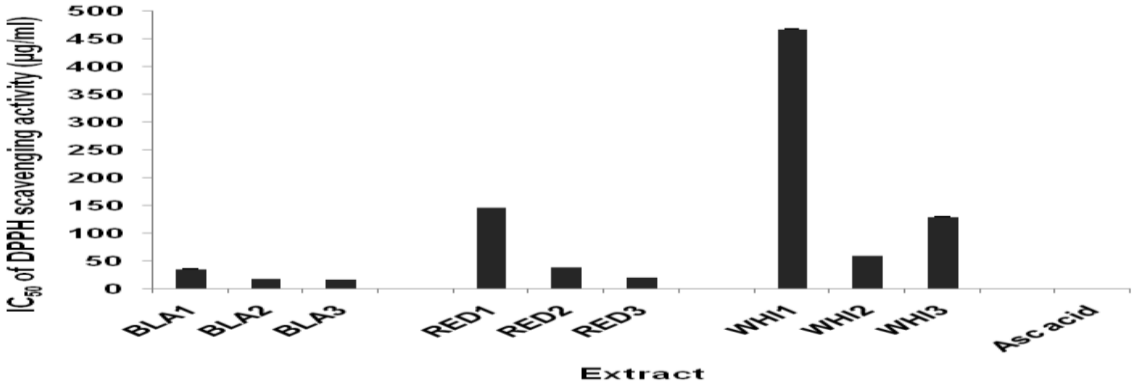

Figure 1: IC_{50} of DPPH scavenging activities in rice grains extract

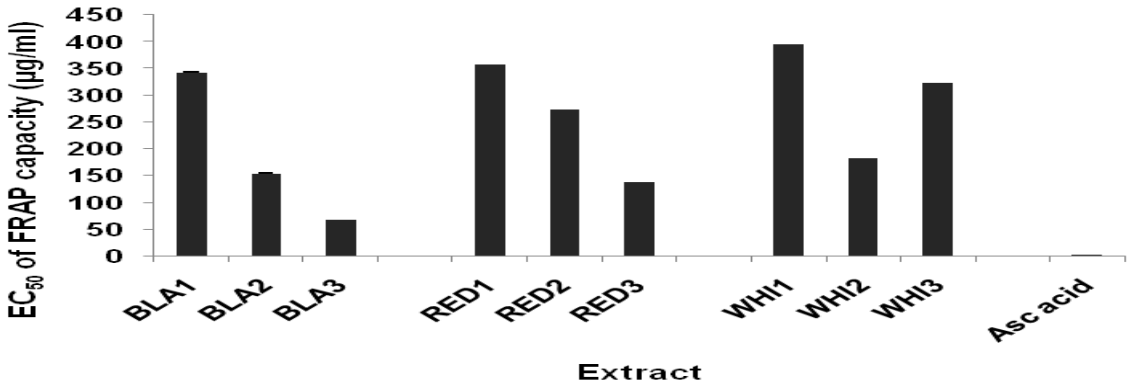

Figure 2: EC_{50} of FRAP capacities in rice grains extract

Table 1: Total phenol, flavonoid, carotenoid content in various rice grains extracts

Sample	TPC (g GAE/100 g)	TFC (g QE/100 g)	TCC (g BE/100 g)
BLA1	1.0221 ± 0.0284	2.2852 ± 0.0508	0.7195 ± 0.0080
RED1	0.9750 ± 0.0539	0.6790 ± 0.0106	0.0454 ± 0.003
WHI1	1.1150 ± 0.0331	1.9782 ± 0.0053	0.1706 ± 0.0014
BLA2	2.2907 ± 0.0454	3.2197 ± 0.0395	2.0564 ± 0.0200
RED2	1.6445 ± 0.0133	2.4695 ± 0.0659	0.3466 ± 0.0061
WHI2	1.7299 ± 0.0260	2.8846 ± 0.0307	0.2121 ± 0.0048
BLA3	6.3573 ± 0.267	0.4803 ± 0.0119	1.2369 ± 0.0080
RED3	2.049 ± 0.0115	1.0351 ± 0.0965	0.5719 ± 0.0063
WHI3	0.8389 ± 0.0095	1.4361 ± 0.0070	0.5071 ± 0.0063

BLA = black rice, RED = red rice, WHI = white rice, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanolic extract, TPC = total phenolic content, TFC = total flavonoid content, TCC = total carotenoid content, GAE = gallic acid equivalent, QE = quercetin equivalent, BE = betacaroten equivalent

antioxidant with IC_{50} greater than 150 µg/ml[13]. In the present study revealed that IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities of various grains extract of three kinds of rice in varied from 35.27 to 467.12 µg/ml and 68.93 to 396.03 µg/ml, respectively. The previous study[18] demonstrated that IC_{50} of DPPH scavenging capacity of methanolic extract of eight cultivars of colored rice in the range of 535 – 49,746 µg/ml, which was categorized as weak antioxidant. It was contrary with the present study which exposed that various extracts (n-hexane, ethyl acetate and ethanol) of black rice ranging from 16.37 to 35.27 µg/ml which was classified as very strong antioxidant. Research by Vichit[19] reported that IC_{50} of DPPH scavenging activities of HCl in ethanol extract of six black rice cultivar and six red rice cultivar varied from 140 to 590 µg/ml and 100 to 1,120 µg/ml, respectively, while in the present study gave different result which figured that IC_{50} of DPPH various grains extracts of red rice at the low value in the range of 19.71-145.69 µg/ml. Previous study regarding ten selected rice from Pakistan expressed that 80% methanolic extract of Basmati Pak variety had the lowest IC_{50} of DPPH scavenging activity (2,340 µg/ml) compared to the other varieties and its 100 % methanol extract[20], while its 80% ethanolic extract and 100 % ethanolic extract showed IC_{50} of DPPH 5,150 and 5,130 µg/ml, respectivley. Sompong[12] exhibited that percentage of DPPH scavenging activities of 85% aqueous methanol extract of three varieties of black rice and ten varieties of red rice in the range of 16.04 - 30.25% and 12.99 – 76.38 %, respectively, while study by

Table 2: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in various rice grains extracts with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities

Antioxidant activities	Coefficient correlation Pearson (r)					
	TPC	TFC	TCC	EC_{50} FRAP BLA	EC_{50} FRAP RED	EC_{50} FRAP WHI
IC_{50} DPPH BLA	-0.723*	0.230 ns	-0.756*	0.968**		
IC_{50} DPPH RED	-0.969**	-0.544 ns	-0.956**		0.863**	
IC_{50} DPPH WHI	-0.367 ns	-0.303 ns	-0.455 ns			0.853**
EC_{50} FRAP BLA	-0.873**	0.471 ns	-0.570 ns			
EC_{50} FRAP RED	-0.959**	-0.048 ns	-0.973**			
EC_{50} FRAP WHI	-0.802**	-0.629 *	0.069 ns			

IC_{50} DPPH = IC_{50} of DPPH scavenging activity, EC_{50} FRAP = EC_{50} of FRAP capacity, BLA = black rice, RED = red rice, WHI = white rice, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

Moko[5] showed that n-hexane fraction, ethyl acetate fraction and n-butanol fraction of red rice had percentage of DPPH scavenging capacities were 82.83, 82.96 and 88.29% respectively. The present research reported that ethanolic extract of black rice and red rice had EC_{50} of FRAP capacity 68.93 µg/ml and 137.82 µg/ml, respectively. The previous study[19] stated that EC_{50} of FRAP capacity of HCl in ethanol extract of six black rice in the range of 0.06 -0.63 mg ascorbic acid equivalent (AAE)/ml and the red rice 0.23-1.36 mg AAE/ml, while the other study[12] exposed that 85% aqueous methanol extract of black rice gave EC_{50} of FRAP varied from 3.65 to 7.58 mmol Fe(II)/100 g and the red rice 0.85 to 8.08 mmol Fe(II)/100 g. This research also expressed that the 85% aqueous methanol extract of red rice had antioxidant activity in the range of 2.08-12.29 mmol Trolox/100 g and the black rice 4.98-12.03 mmol Trolox/100 g by TEAC method. Total phenolic content can be correlated with antioxidant activity[21]. Cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid[22]. Du[23] revealed that rice contained phenolic compounds which were included flavonoid compounds likes catechin, kaempferol, myricetin and quercetin. The previous research[5] reported that ethyl acetate fraction of red rice had the highest TPC (258 mg GAE/100 g) compared to n-hexane fraction (63 mg GAE/100 g) and n-butanol fraction (58 mg GAE/100 g). It was contrast with the present study which stated that the highest TPC was given by ethanolic grains extract of black rice (6.36 g GAE/100 g), while the ethyl acetate grains extract of black rice, red rice and white rice had TPC 2.29, 1.64 and 1.73 g GAE/100 g, respectively. The other study[12] figured that 85% aqueous methanol extract red rice showed TPC ranged from 79-691 mg ferulic acid equivalent (FAE)/100 g, and the black rice 336-665 mg FAE/100 g. Study by Vichit and Saewan[19] exposed that TPC in HCl in ethanol extracts of six red rice ranging from 0.21 to 0.99 mg GAE/ml which was higher than the six black rice 0.05 to 0.54 mg GAE/ml. It was contrary with the present study which exposed that TPC in ethanolic extract of black rice (6.36 g GAE/100 g) higher than the red rice (2.05 g GAE/100 g). Research by Chakuton[18] exposed that TPC in methanolic extract of cultivar 53 (7.40 mg GAE/100 g) was the highest among eight colored rice from Thailand. Zubair[20] demonstrated that TPC in 80% methanolic extract of Basmati Pak variety showed the highest TPC (275 mg GAE/kg) compared to the other varieties and its 80% and 100% ethanolic extract (224.8 mg GAE/kg). In the present study reported that TFC ethyl acetate grain extract of three kinds of rice (black rice, red rice and white rice) 3.22, 2.47, 288 g QE/100 g, respectively which was higher than their n-hexane and ethanolic extracts. Rice contained many anthocyanin compounds which were cyanidin-3-glucoside and peonidin-3-glucoside[24]. These anthocyanin soluble in ethyl acetate, ethanol, methanol and n-butanol solvents. Therefore, some previous researches needed to evaluate total anthocyanin content (TAC) in rice grains extract. Study by Chakuton[18] figured that methanolic extract of cultivar 53 gave the highest TAC (1045 mg malvidin/100 g) among eight colored rice, while the other research[12] showed that 85% aqueous methanol extract of Niaw Dam Pleuak Khao variety of black rice had the highest TAC 256 mg cyanidin- 3- glucoside equivalent (C3GE)/100 g, and TAC in ten varieties of red rice ranged from 0.33 to 1.39 mg C3GE/100 g. Previous research[5] expressed that TAC in n-hexane fraction, ethyl acetate fraction and n-butanol fraction of red rice were 4.58, 68.61, 42.25 mg C3GE/g, respectively, TAC in HCl in ethanolic ectract of six varieties of red rice in the range of 0.03-0.29 mg C3GE/l extract and six varieties of black rice 0.13-4.64 mg C3GE/l extract. Rice contained carotenoid compound such as beta carotene, lutein and zeaxanthin[25], which act as antioxidant, soluble in n-hexane and ethyl acetate solvent. In the present study it can be seen that TCC in n-hexane grains extract of black rice (0.72 g BE/100 g) higher than the red rice and white rice. The TCC in ethyl acetate and ethanolic grains extracts of black rice (2.06 g BE/100 g and 0.35 g BE/100 g, respectively) higher than the red rice and white rice. Pearson's correlation coefficient was negatively significant if $-0.61 \leq r \leq -0.97$ and positively high if $0.61 \leq r \leq 0.97$[6]. Sample which had the lowest IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity had the highest antioxidant activity. Increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was stated by lower IC_{50} of DPPH scavenging activity and or EC_{50} of FRAP capacity. Therefore the good correlation between TPC, TFC and or TCC with their IC_{50} of DPPH or EC_{50} of FRAP is significantly negative correlation[26]. Data in Table 2 revealed that there were negatively significant correlation between TPC in grains extracts of black rice and red rice with their IC_{50} of DPPH scavenging activities ($r = -0.723$, $p<0.05$; $r = - 0.969$, $p<0.01$) and TPC

in all of grains extracts of black rice, red rice and white rice with their EC_{50} of FRAP capacities (r = -0.873; r = -0.959; r = -0.802, p<0.01). Based on the result it can be concluded phenolic compounds were the major contributor in antioxidant activities of grains extracts of black rice and red rice using DPPH method and major contributor in all of grains extracts of black rice, red rice and white rice using FRAP method. It means antioxidant capacities of black rice and red rice grains extracts using DPPH method can be estimated indirectly by determining TPC. The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant[27]. Reagent of FRAP is $FeCl_3$ that combined with TPTZ in acetate buffer pH 3.6. Fe (III) will be reduced to Fe (II). Sample will act as antioxidant in FRAP assay if sample had reduction potential lower than reduction potential of Fe (III)/Fe (II) which was 0.77 V, so the sample had the reducing power to reduce Fe (III) to Fe (II), this sample will be oxidized and act as antioxidant[15]. Complex of Fe (II) - TPTZ shows blue color and gave characteristic absorption at wavelength 593 nm. Intensity of blue color depends on amount of Fe (III) which is reduced to Fe (II). Flavonoid and phenolic acid were included in phenolic groups. Flavonoid which had ortho di OH at C-3'-C4', OH at C-3, oxo function at C-4, double bond at C-2 and C-3 have high antioxidant activity. The ortho di OH at C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides. Flavonoid had greater antioxidant activity than phenolic acid[22]. In Table 1 it could be seen that TPC in ethyl acetate grains extract of black rice (BLA2) 2.29 g GAE/100 g higher than TPC in ethyl acetate of white rice (WHI2) 1.73 g GAE/100 g, but IC_{50} of DPPH scavenging activities of BLA2 (17.55 μg/ml) which was categorized as very strong antioxidant, lower than IC_{50} of DPPH of WHI2 (58.94 μg/ml) as strong antioxidant. Based on the result it might be predicted that BLA2 contained many phenolic compounds such as catechin, myricetin and quercetin which have high antioxidant activity, while WHI2 contained only a little phenolic compounds with high antioxidant activity. TPC in ethanolic grains extract of black rice (BLA3) 6.36 g GAE/100 g was higher than TPC in ethanolic grains extract of white rice (WHI3) 0.84 g GAE/100 g, but antioxidant capacity of BLA3 by FRAP method (68.93 μg/ml) was lower than EC_{50} of FRAP of WHI3 (324.13 μg/ml). Based on the result it could be seen that BLA3 contained many phenolic compounds with reduction potential lower than reduction potential of Fe(III)/Fe(II) 0.77 V, so it could reduce Fe(III)/Fe(II) and then Fe(II) react with TPTZ form blue color complex, while WHI3 contained phenolic compounds with reduction potential higher than 0.77 V. TFC in ethyl acetate grains extract of red rice (RED2) 2.47 g QE/100 g was higher than TFC in ethanolic grains extract of red rice (RED3) 1.04 g QE/100 g, but IC_{50} of DPPH of RED3 (19.71 μg/ml) was lower than IC_{50} of DPPH of RED2 (38.02 μg/ml). It can be estimated that RED3 contained flavonoid glycoside compounds such as cyanidin-3-glucoside, the anthocyanin compound which gave color in black rice. Cyanidin-3-glucoside has ortho di OH at C-3'-C-4' which have high antioxidant activity. RED2 contained many flavonoid aglycone compounds such as kaempferol, which has no OH at C-3'and C-4' and explanation regarding antioxidant activity showed that it would give low antioxidant activity. Carotenoid have antioxidant capacity by scavenging free radical[28]. The higher scavenging radical activity will be given by carotenoid which contained more than 7 double bonds[29]. Charles[30] reported that beta carotene was used as standard because it had conjugation double bonds which had ability to scavenge free radicals. Increasing in lipophilicity of carotenoid would increase scavenging radical activity and will give the lower IC_{50} of DPPH scavenging capacity[31]. TCC in n-hexane grains extract of black rice (BLA1) 0.72 g BE/100 g was higher than n-hexane grains extract of white rice (WHI1) 0.17 g BE/100 g. The IC_{50} of DPPH of BLA1 (35.27 μg/ml) which was very strong antioxidant, lower than IC_{50} of DPPH of WHI1 (467.12 μg/ml). It can be supposed that many carotenoid compounds in WHI had maximum 7 double bonds which had low antioxidant activity and many carotenoid compounds in BLA1 were beta carotene, lutein and zeaxanthin which were contained more than 7 double bonds and had high antioxidant activity. DPPH and FRAP had different mechanism reaction. Mechanism of FRAP was redox assay[15] whereas DPPH that was electron transfer assay[32]. The Pearson's correlation coefficient indicated that IC_{50} of DPPH scavenging activities of grains extracts of black rice, red rice and white rice had positive and significant correlation with their EC_{50} of FRAP capacities (r = 0.968; r = 0.863; r = 0.853, p<0.01, respectively). It could be seen that antioxidant activities of grains extracts of black rice, red rice and white rice by DPPH and FRAP assays gave linear results.

CONCLUSION

Antioxidant activity of sample should be determined using different methods in parallel because various methods could give different results. Ethyl acetate and ethanolic grains extracts of black rice and red rice were very strong antioxidant, using DPPH assays. Phenolic compounds in black rice and red rice grains extract were the major contributor in IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity. There were linear correlation in antioxidant activities of black rice, red rice and white rice grains extracts using DPPH and FRAP methods. Black rice and red rice may be exploited as natural antioxidant sources to reduce oxidative stress.

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