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Research Article

ANTIOXIDANT EVALUATION AND PHYTOCHEMICAL CONTENT OF VARIOUS RICE BRAN EXTRACTS OF THREE VARIETIES RICE FROM SEMARANG, CENTRAL JAVA, INDONESIA

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ABSTRACT

Objectives: The objectives of this research were to evaluate antioxidant activity from different polarities rice bran extract of three varieties of rice using two methods of antioxidant testing which were ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and correlation of total phenolic, flavonoid and carotenoid content with their exhibitory concentration 50 (EC_{50}) of FRAP and inhibitory concentration 50 (IC_{50}) of DPPH antioxidant activities.

Methods: Extraction was conducted by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Determination of total phenolic, flavonoid and carotenoid content, antioxidant activities using FRAP and DPPH assays was performed by ultraviolet-visible spectrophotometry and its correlation with EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities was analyzed by Pearson's method.

Results: Ethanolic rice bran extract of black rice showed the lowest EC_{50} of FRAP capacity 64.35 µg/ml and IC_{50} of DPPH scavenging activity 23.92 µg/ml. The highest phenolic content, flavonoid content, and carotenoid content were also given by ethanolic rice bran extract of black rice. There was significantly negative correlation between total phenolic content and carotenoid content in rice bran extract of red rice and black rice with their IC_{50} of DPPH.

Conclusions: All of the rice bran extracts (except n-hexane rice bran extract of black rice and ethanolic rice bran extract of white rice) were very strong antioxidant by DPPH assay. Phenolic and carotenoid compounds in rice bran extracts of red rice and black rice were the major contributor in antioxidant activity by DPPH assay. Rice bran extracts of black rice had linear results by FRAP and DPPH assays.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Ferric reducing antioxidant power, Rice bran, Three varieties, Rice.

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INTRODUCTION

Oxidative stress related with many diseases can be prevented by antioxidant. In normal condition, body can prevent oxidative stress, but in excessive oxidative stress condition body needs antioxidant to inhibit negative effect of oxidative stress. Previous researches revealed that antioxidant activities could be correlated to their phenolic and flavonoid content [1,2]. Phenolic compounds are commonly found in plants, and they have been expressed to give many pharmacological effects, included antibacterial and antioxidant activity [3,4]. Many plants contained phenolic and flavonoid compounds included rice, tea and citrus [2,5-7].

Antioxidant activity of vegetables, fruits, and food could determine using antioxidant testing methods such as ferric reducing antioxidant power (FRAP) 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [5,6,8,9]. The previous studies figured that FRAP, DPPH, and ABTS methods could be used to evaluate antioxidant activity in many plants extracts [9,10]. The previous researches [11-14] exposed that rice and rice bran had antioxidant activities by using DPPH, FRAP, and ABTS assays.

The goals of this research were to determine antioxidant activities of different polarity rice bran extracts (n-hexane, ethyl acetate, and ethanol) of three varieties of rice grown in Semarang-Central Java, Indonesia, using DPPH and FRAP assays, and correlation of total phenolic, flavonoid and carotenoid content with their antioxidant activities.

METHODS

Materials

DPPH, FRAP, 2,4,6-tripyridyltriazine (TPTZ), gallic acid, quercetin, beta-carotene were purchased from Sigma-Aldrich (MO, USA), rice bran of three varieties of rice. All of other reagents were analytical grades.

Preparation of sample

Rice bran of three varieties of rice (*Oryza* sp.) was rice bran of white rice namely as PUT, rice bran of red rice as MER, and rice bran of black rice as HIT were collected from Semarang-Central Java, Indonesia, were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Extraction

About 300 g of powdered sample was extracted by reflux using different polarity 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 totally, there were three n-hexane extracts (namely PUT1, MER1, and HIT1), three ethyl acetate extracts (PUT2, MER2, and HIT2), and three ethanol extracts (PUT3, MER3, and HIT3).

Total phenolic content (TPC)

Folin–ciocalteu reagent was used for determining TPC [15]. The absorbance was observed at wavelength 765 nm using ultraviolet-vis spectrophotometer Hewlett Packard 8435. Analysis was performed in triplicate for each extract. Gallic acid standard solution (80-170 µg/ml)

was used to obtain a calibration curve. TPC was presented as percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

Total flavonoid content (TFC)

TFC was conducted using method from Chang *et al.* [16]. The absorbance was measured at wavelength 415 nm. Analysis was performed in triplicate for each extract. Quercetin standard solution (20-140 µg/ml) was used to obtain a calibration curve. The TFC was expressed as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

Modification of method from Thaipong *et al.* [9] was usedto evaluate TCC. Each extract was diluted in n-hexane. The absorbance was measured at wavelength 470 nm. Analysis was performed in triplicate for each extract. Beta carotene standard solution (40-100 µg/ml) was used to obtain a calibration curve. The TCC was represented as percentage of total beta-carotene equivalent per 100 g extract (g BE/100 g).

FRAP capacity

Method of Benzi with minor modificationwas used in preparing of FRA Psolution [17]. 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 observed at wavelength 593 nm. Acetate buffer was used as a blank, FRAP 50 µg/ml as control and ascorbic acid as standard. Analysis was performed in triplicate for each extract and standard. Antioxidant capacity of each extract was determined by calculating percentage of antioxidant capacity based on increasing in Fe (II)-TPTZ absorbance. Exhibitory concentration 50 (EC_{50}) is concentration which can increase 50% absorbance of Fe (II)-TPTZ. EC_{50} of FRAP capacity of each extract can be calculated using its calibration curve.

DPPH scavenging activity

DPPH solution was adopted from Blois's method [18] with minor modification. Various concentration of each extract was added into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction. The absorbance was measured after 30 minutes incubation at wavelength 515 nm. Methanol was used as a blank, DPPH 50 µg/ml as control, and ascorbic acid as standard. Analysis was performed in triplicate for each extract and standard. Antioxidant activity of each extract by DPPH method was evaluated using reduction of DPPH absorbance by calculating the percentage of antioxidant activity [19]. Inhibitory concentration 50 (IC_{50}) is concentration which can inhibit 50% absorbance of DPPH. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

Statistical analysis

Each sample analysis was performed in triplicate. All of the 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* Turkey procedure was performed 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

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

The EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities in various extracts of rice bran using FRAP and DPPH assays were shown in Figs. 1 and 2.

TPC, TFC, and TCC in rice bran extracts

TPC among the various extracts were reported in term of Gallic acid equivalent using the standard curve equation $y=0.0044x+0.023$, $R^2=0.9978$. The TPC in various extracts of rice bran showed different result varied from 2.33 to 12.24 g GAE/100 g. Ethanolic rice bran extract of black rice (HIT3) denoted the highest phenolic content (12.24 g GAE/100 g) and the lowest (2.33 g GAE/100 g) given by n-hexane rice bran extract of white rice (PUT1).

TFC among the various extracts were presented in term of quercetin equivalent using the standard curve equation $y=0.0037x+0.1913$, $R^2=0.9996$. The TFC in various extracts of rice bran gave different result in the range of 0.46-18.74 g QE/100 g. The highest TFC (18.74 g QE/100 g) was shown by ethanolic rice bran extract of black rice (HIT3).

TCC among the various extracts were reported in term of beta-carotene equivalent using the standard curve equation $y=0.0097x-0.1185$, $R^2=0.9988$. The TCC in various extracts of rice bran had resulted in the range of 0.27-5.80 g BE/100 g. The highest carotenoid content (5.80 g BE/100 g) was also given by ethanolic rice bran extract of black rice (HIT3), while ethanolic rice bran extract of red rice (MER3) gave the lowest carotenoid (0.27 g BE/100 g).

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

TPC in various rice bran extracts of black rice had negative and significant correlation with their EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities ($r=-1.000$, $p<0.01$), while TPC in rice bran extracts of red rice only gave significantly negative correlation with their IC_{50} of DPPH scavenging activities ($r=-0.978$, $p<0.01$). TFC in white rice bran extracts had a negative and significant correlation with their IC_{50} of DPPH scavenging activities ($r=-0.690$, $p<0.05$), while TFC in black rice bran extracts showed a significant and negative correlation with their EC_{50} of FRAP capacity ($r=-0.999$, $p<0.01$). TCC in rice bran extracts of black rice was significantly negative correlation with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities ($r=-0.857$; $r=-0.867$, $p<0.01$, respectively) (Table 1).

DISCUSSION

The previous studies [12,20] reported that rice bran had antioxidant capacity. There was no study regarding antioxidant activity of various rice bran extracts (which were n-hexane, ethyl acetate, and ethanol) of three varieties rice from Semarang-Central Java, Indonesia, using DPPH and FRAP assays.

The EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities in various rice bran extracts from three varieties rice using FRAP and DPPH assays were given in Figs. 1 and 2. The EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities in various rice bran extracts compared to EC_{50} or IC_{50} of ascorbic acid standard. The lowest EC_{50} or 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 antioxidant with IC_{50} or EC_{50} <150 µg/ml [18].

In the present research exposed that EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities of various rice bran extracts from three varieties rice in the range of 64.35 to 387.90 µg/ml and 23.92-143.59 µg/ml, respectively. All of the rice bran extracts (except n-hexane black rice bran extract and ethanolic white rice bran extract) were categorized as very strong antioxidant using DPPH method, and only ethanolic rice bran extracts of white rice, red and black rice can be classified as strong antioxidant using FRAP method. The previous study [21] stated that phytic acid extract from rice bran denoted higher antioxidant activity by DPPH and beta-carotene linoleate bleaching assays (41.5% and 93.36%) than phytic acid extract from corn (26.4% and 92.55%). In contrary with FRAP assay, phytic acid extract from corn showed higher antioxidant activity (2.78 mM $FeSO_4$) than phytic acid extract from rice bran (2.10 mM $FeSO_4$). Muntana and Prasong [20] studied regarding the antioxidant activity of rice bran extracts from 15 cultivars rice from Thailand, which was five cultivars white rice, five cultivars red rice and five cultivars glutinous black rice. The results of Muntana's research presented that the rice bran extract of red rice cultivar 5718 gave the highest antioxidant activity which had the

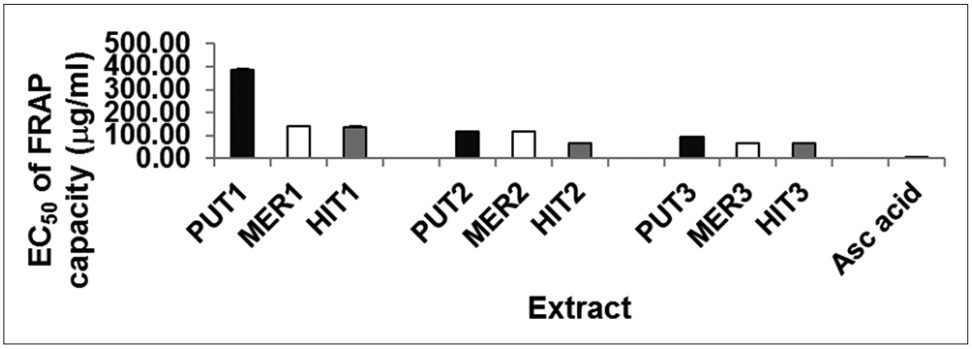

Fig. 1: EC_{50} of ferric reducing antioxidant power capacities in various rice bran extracts

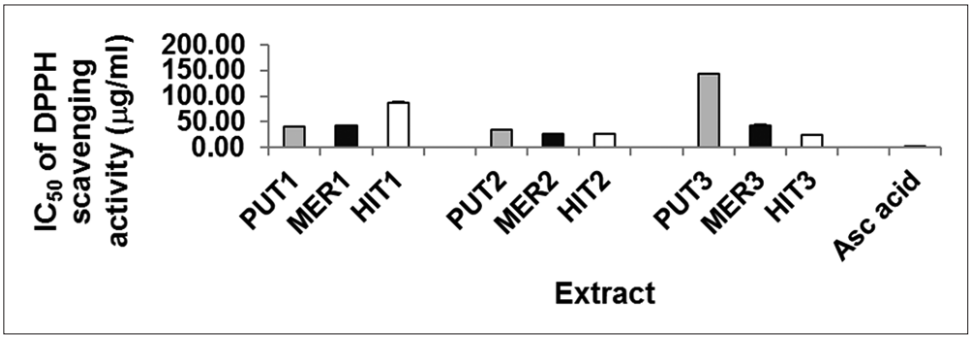

Fig. 2: Inhibitory concentration 50 of 2,2-diphenyl-1-picrylhydrazyl scavenging activities in various rice bran extracts

Table 1: Pearson's correlation coefficient of EC_{50} of FRAP capacities and IC_{50} of DPPH scavenging activities with their total phenolic, flavonoid, carotenoid content in various rice bran extracts

Parameter antioxidant	Coefficient correlation Pearson (r)					
	TPC	TFC	TCC	EC_{50} FRAP PUT	EC_{50} FRAP MER	EC_{50} FRAP HIT
EC_{50} FRAP PUT	−0.487^{ns}	−0.268^{ns}	−0.356^{ns}			
EC_{50} FRAP MER	0.411^{ns}	0.023^{ns}	0.394^{ns}			
EC_{50} FRAP HIT	−1.000**	−0.999**	−0.867**			
IC_{50} DPPH PUT	−0.501^{ns}	−0.690*	0.620*	0.512^{ns}		
IC_{50} DPPH MER	−0.978**	0.972**	−0.982**		0.212^{ns}	
IC_{50} DPPH HIT	−1.000**	0.999**	−0.857**			1.000**

PUT: White rice, MER: Red rice, HIT: Black rice, TPC: Total phenolic content, TFC: Total flavonoid content, TCC: Total carotenoid content, IC_{50} DPPH: IC_{50} of DPPH scavenging activity, EC_{50} FRAP: EC_{50} of FRAP capacity, ns: Not significant, *significant at $p<0.05$, **significant at $p<0.01$, IC_{50}: Inhibitory concentration 50, EC_{50}: Exhibitory concentration 50, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power

lowest EC_{50} of DPPH scavenging activity (5.7 µg/ml), followed by red rice cultivar 22699 (8.4 µg/ml). It was contrary with this study which exhibited that IC_{50} of DPPH of ethanolic rice bran extract of red rice and black rice were 43.43 µg/ml and 23.92 µg/ml. While antioxidant activity using thiocyanate method demonstrated that antioxidant activity of red rice cultivar 22699 higher than cultivar 5718 of red rice [20]. The previous study [14] reported that IC_{50} of DPPH scavenging activities of HCl in ethanol extracts of six cultivars of red rice and six cultivars of black rice in the range of 100-1120 µg/ml and 140-590 µg/ml, respectively. Chakuton *et al*. [13] represented that IC_{50} of DPPH scavenging capacity of methanolic extract of eight cultivars of colored rice varied from 535 to 49,746 µg/ml, which was categorized as weak antioxidant. Li *et al*. [12] extracted crude polysaccharide (CPS) from fermented rice bran using 80% methanol. The results demonstrated that CPS extract 5 mg/ml gave the same percentage of DPPH scavenging activity (96.16%) with ascorbic acid 5 mg/ml. 80% methanol CPS extract from rice bran had EC_{50} of DPPH 0.74 mg/ml, while its EC_{50} of ABTS was 0.76 mg/ml. Study by Zubair *et al*. [11] exposed that 80% methanolic extract of Basmati Pak rice variety showed the lowest IC_{50} of DPPH scavenging activity (2,340 µg/ml) compared to its 100 % methanol extract and the other varieties, while its 100 % ethanolic extract and 80% ethanolic extract had IC_{50} of DPPH 5,130 and 5,150 µg/ml, respectively. Research by Arab *et al*. [22] which extracted rice bran using methanol, ethanol and ethyl acetate by maceration method reported that percentage of radical scavenging activity of methanolic extract of Fajr rice bran was higher than its ethanolic extract and ethyl acetate extract, and percentage of DPPH scavenging activity of Fajr rice bran was higher than Tarem rice bran. Methanolic extract of Fajr rice bran 50 mg/ml showed the percentage of DPPH scavenging activity 93.91 %. It was similar to their antioxidant activity using reducing power assay which exposed that methanolic extract of Fajr rice bran gave higher antioxidant activity than the methanolic extract of Tarem rice bran. Methanolic extract of Fajr rice bran 50 mg/ml after incubation 96 h also showed higher antioxidant activity than its Tarem rice bran using linoleic acid system assay. The previous research [23] regarding antioxidant activity of rice bran extract from five varieties of rice in Pakistan, denoted that 80% methanolic extract of rice bran Super kernel (RB-kr) showed the highest antioxidant activity by DPPH, ABTS and linoleic acid system assays, compared to the other varieties (Super-2000, Super-basmati, Super-386, and Super-fine), which was similar to its γ-oryzanol content and tocopherol content. Rice bran super kernel (RB-kr) had the highest γ-oryzanol content (802 µg/ml) and tocopherol content (512 µg/ml). Moko *et al*. [6] stated that n-hexane fraction, ethyl acetate fraction and n-butanol fraction of red

rice represented the percentage of DPPH scavenging capacities were 82.83, 82.96 and 88.29%, respectively, and the highest was given by n-butanol fraction of red rice. It was different from the present study which gave DPPH scavenging activities of n-hexane, ethyl acetate and ethanolic rice bran extracts of red rice were 43.56, 25.67 and 43.43 µg/ml, respectively, and the highest was taken by ethyl acetate rice bran extract of red rice. Rice contained many anthocyanin compounds which were cyanidin-3-glucoside and peonidin-3-glucoside [24]. These anthocyanins dissolve in ethyl acetate, ethanol, methanol and n-butanol solvents. Therefore, it can be predicted that n-butanol fraction of red rice contained many anthocyanins, which can act as antioxidant and gave higher antioxidant activity than their n-hexane and ethyl acetate fractions.

TPC might be contributed in antioxidant activity [25]. Flavonoid compounds in rice such as catechin, kaempferol, myricetin, and quercetin were included phenolic compounds [26]. The study by Iqbal *et al*. [23] denoted that TPC in 80% methanolic of different rice bran varieties gave TPC ranged from 0.251 to 0.359 g GAE/100 g, and the highest was shown by rice bran-superkernel (RB-kr). It was contrary with the present study which exhibited that TPC in rice bran from white, red and black rice were 2.66, 2.42 and 12.40 g GAE/100 g. Previous research [22] stated that methanolic extract of Fajr rice bran showed the highest TPC (0.331 g GAE/100 g) compared to Tarem rice bran and the other extracts. It was related with its antioxidant activity which gave the highest antioxidant activity by DPPH, reducing power and linoleic acid system assays. Methanol extracts of rice bran from fifteen cultivars rice in Thailand gave different results in TPC. Rice bran of red rice cultivar 5718 showed the highest TPC compared to the others. 80% methanolic extract of fermented rice bran exhibited higher TPC (89.83 mg GAE/g) than imfermented rice bran (14.16 mg GAE/100 g) [12]. The other studies [11] revealed that TPC in 80% methanolic rice extract of Basmati Pak variety presented the highest TPC (0.275 g GAE/kg) compared to the other varieties and its 80% and 100% ethanolic extract. TPC in methanolic rice extract of cultivar 53 (7.40 mg GAE/100 g) was the highest among eight colored rice from Thailand [13]. Research by Sompong *et al*. [27] exposed that 85% aqueous methanol extract black rice showed TPC in the range of 336-665 mg ferulic acid equivalent (FAE)/100 g, and the red rice 79-691 mg FAE/100 g.

This study reported that TFC ethyl acetate rice bran extracts of three varieties of rice (white, red and black rice) 12.54, 0.46, and 18.73 g QE/100 g, respectively, and TFC in ethanolic rice bran extracts were 2.87, 3.58, and 18.74 g QE/100 g. Rice contained many anthocyanin compounds [24] which were cyanidin-3-glucoside and peonidin-3-glucoside. These anthocyanins dissolved in ethyl acetate, ethanol, methanol and n-butanol solvents. Therefore, some previous researches needed to determine total anthocyanin content (TAC) in rice extract. TAC in 85% aqueous methanol extract of 10 varieties of red rice ranged from 0.33 to 1.39 mg cyanidin-3-glucoside equivalent (C3GE)/100 g [27], methanolic rice extract of cultivar 53 gave the highest TAC (1045 mg malvidin/100 g) among eight colored rice [13], 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 [6]. Rice contained carotenoid compound such as beta-carotene, lutein and zeaxanthin [28], 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 and ethanolic rice bran extracts of black rice (1.83 g BE/100 g and 5.80 g BE/100 g) higher than TCC in rice bran extract of the white rice and red rice, while in ethyl acetate rice bran extract TCC in red rice (4.89 g BE/100 g) higher than the white rice and black rice.

Pearson's correlation coefficient was negatively significant if $-0.61 \leq r \leq -0.97$ and positively high if $0.61 \leq r \leq 0.97$ [9]. Sample which had the lowest EC_{50} of FRAP capacity and IC_{50} of DPPH scavenging activity had the highest antioxidant activity. Increasing in TPC, TFC and TCC caused increasing in antioxidant activities, which was stated by lower EC_{50} of FRAP capacity and or IC_{50} of DPPH scavenging activity. Therefore, the good correlation between TPC, TFC and or TCC with their EC_{50} of FRAP or IC_{50} of DPPH is significantly negative correlation [29].

Data in Table 1 expressed that TPC, TFC, and TCC in rice bran extracts of black rice red rice had negative and significant correlation with their EC_{50} of FRAP capacities ($r=-1.000$; $r=-0.999$; $r=-0.867$, $p<0.01$, respectively). It can be predicted that phenolic, flavonoid and carotenoid compounds contributed together in antioxidant activities of rice bran extract of black rice by FRAP assay. There were significantly negative correlation between TPC and TCC in rice bran extracts of red rice ($r=-0.978$; $r=-0.982$, $p<0.01$) and black rice ($r=-1.00$; $r=-0.857$, $p<0.01$). It can be supposed that phenolic and carotenoid compounds were the major contributor in antioxidant activities of rice bran extracts of red rice and black rice by DPPH assay. TFC in rice bran extracts of white rice had negative and significant correlation with their IC_{50} of DPPH scavenging activities ($r=-0.690$, $p<0.05$). Based on the result, it can be concluded that flavonoid compounds were the major contributor in antioxidant activities of rice bran extracts of white rice using DPPH method.

Sample will act as antioxidant in FRAP assay if sample had reduction potential lower than 0.77 V which was reduction potential of Fe (III)/Fe (II); therefore, the sample had ability to reduce Fe (III) to Fe (II) and this sample will be oxidized and act as antioxidant [17]. Reagent of FRAP is $FeCl_3$ that combined with TPTZ in acetate buffer pH 3.6. 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). 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 [30].

Carotenoid has antioxidant capacity by scavenging free radical [31]. Beta carotene had conjugation double bonds; therefore, it had ability to scavenge free radicals and usually used as standard [32]. Study by Kobayashi and Sakamoto [33] revealed that the higher ability to scavenge free radical activity was given by increasing in lipophilicity of carotenoid. Beutner *et al.* [34] exposed that carotenoid which contains more than 7 double bonds will express the higher scavenging radical activity. In Fig. 5, it could be seen that TCC in n-hexane rice bran extract of black rice (HIT1) 1.83 g BE/100 g was higher than n-hexane rice bran extract of red rice (MER1) 1.11 g BE/100 g, however the IC_{50} of DPPH of MER1 (43.56 µg/ml) which was very strong antioxidant (Fig. 2), lower than IC_{50} of DPPH of HIT1 (87.88 µg/ml) as strong antioxidant. It can be estimated that many carotenoid compounds in MER1 had more than 7 double bonds which had higher antioxidant activity and many carotenoid compounds in HIT1 contained maximum 7 double bonds.

Flavonoid had greater antioxidant activity than phenolic acid [35]. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides. Flavonoid and phenolic acid were included in phenolic groups. Fig. 3 demonstrated that TPC in ethanolic rice bran extract of white rice (PUT3) 2.66 g GAE/100 g was similar to TPC in ethanolic rice bran extract of red rice (MER3) 2.42 g GAE/100 g, but IC_{50} of DPPH scavenging activities of PUT3 (143.59 µg/ml) which was categorized as medium antioxidant, higher than IC_{50} of DPPH of MER3 (43.43 µg/ml) as very strong antioxidant. Based on the result, it might be supposed that MER3 contained many phenolic compounds which have high antioxidant activity, while PUT3 contained only a little phenolic compounds with high antioxidant activity. TPC in ethyl acetate rice bran extract of red rice (MER2) 8.45 g GAE/100 g was higher than TPC in ethanolic rice bran extract of red rice (MER3) 2.42 g GAE/100 g, however MER3 showed higher antioxidant capacity by FRAP method (Fig. 1) which denoted by lower EC_{50} of FRAP (66.35 µg/ml) than EC_{50} of FRAP of MER2 (118.24 µg/ml). It could be seen that MER3 contained many phenolic compounds could reduce Fe(III)/Fe(II) and then Fe(II) react with TPTZ form blue color complex because their reduction potential lower than reduction potential of Fe(III)/Fe(II) 0.77 V,

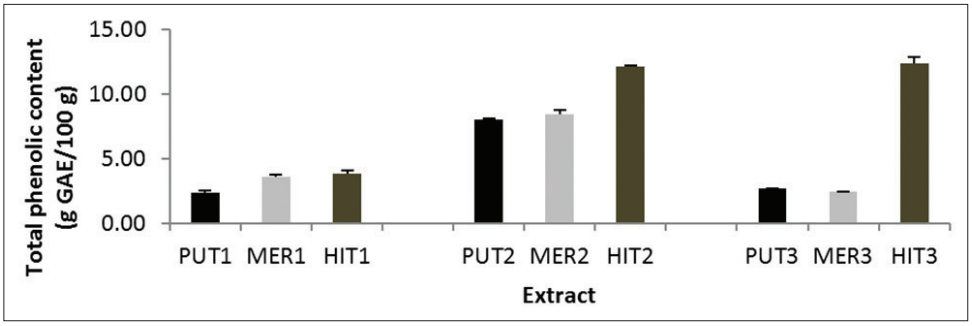

Fig. 3: Total phenolic content in various rice bran extracts

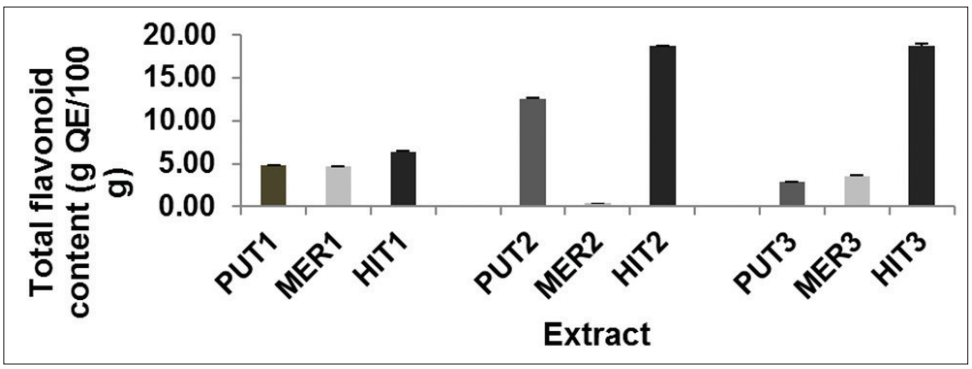

Fig. 4: Total flavonoid content in various rice bran extracts

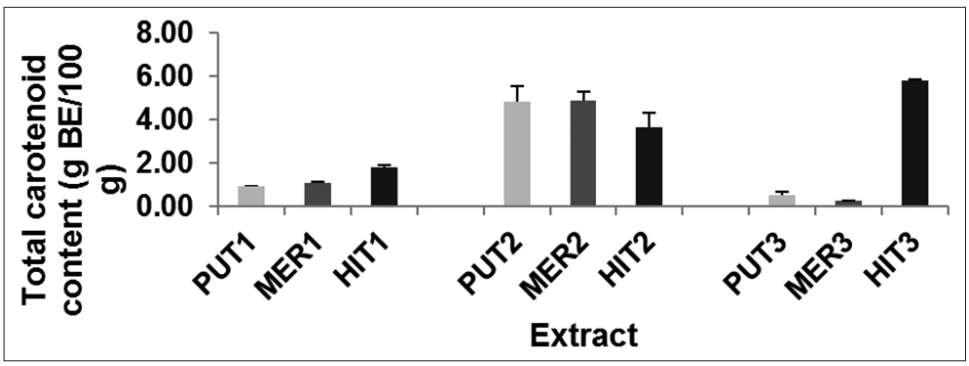

Fig. 5: Total carotenoid content in various rice bran extracts

meanwhile MER2 contained phenolic compounds with reduction potential higher than 0.77 V.

Flavonoid will have high antioxidant activity 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. The highest influence to antioxidant activity of flavonoid was given by ortho di OH at C-3'-C-4'. TFC in ethyl acetate rice bran extract of red rice (MER2) 0.46 g QE/100 g was lower than TFC in ethyl acetate rice bran extract of black rice (HIT2) 18.73 g QE/100 g, but IC_{50} of DPPH of MER2 (25.67 µg/ml) was similar to IC_{50} of DPPH of HIT2 (26.30 µg/ml). Based on the result, it can be predicted that almost all of flavonoid compounds in MER2 were flavonoid had ortho di-OH at C-3'-C-4' which gave higher antioxidant activity, meanwhile only a little flavonoid compounds in HIT2 had OH at position which can give high influence in antioxidant activity.

In general, FRAP and DPPH assays have the same mechanism reaction which is electron transfer. Electron transfer in DPPH assay, including ability to scavenge free radical of DPPH [36], meanwhile in FRAP assay depend on reduction potential of sample [17]. Data in Table 1 presented that Pearson's correlation coefficient of EC_{50} of FRAP capacity of rice bran extracts of black rice was significantly positive correlation with their IC_{50} of DPPH scavenging activities ($r=1.000$, $p<0.01$). It could be concluded that DPPH and FRAP assays gave linear results in antioxidant activities of rice bran extracts of black rice.

CONCLUSIONS

Antioxidant activity of sample using various methods could give different results; therefore, it should be determined by different methods in parallel. All of the rice bran extracts (except n-hexane rice bran extract of black rice and ethanolic rice bran extract of white rice) were very strong antioxidant by DPPH assay. Phenolic and carotenoid compounds in rice bran extracts of red rice and black rice were the major contributor in antioxidant activities using DPPH method. DPPH and FRAP methods showed the linear result in antioxidant activities of rice bran extracts of black rice. Rice bran extracts of three varieties of rice may be exploited as natural antioxidant sources to reduce oxidative stress.

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