

EFFECTS OF SOLVENT TYPES AND CITRIC ACID CONCENTRATIONS ON THE EXTRACTION OF ANTIOXIDANTS FROM THE BLACK RICE BRAN OF *ORYZA SATIVA* L. CV. HOM NIN

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ABSTRACT

Thai colored rice has been reported as a potential source of antioxidants. This research aimed to study the effects of different solvents on the antioxidant extraction of Hom Nin rice bran. The solvents (water, methanol and ethanol) combined with citric acid at concentrations of 0, 0.05 and 0.1 mol/dm³ were used for the extraction. The antioxidant activities and the contents of phenolics, flavonoids and anthocyanins were determined. The results showed that the methanolic extract with 0.1 mol/dm³ of citric acid gave the highest yield whereas phenolics, flavonoids and anthocyanin were 43.72%, 86.63 mg of GAE/g DM, 14,667.48 µg CE/g DM and 6.18 mg/g DM, respectively. Moreover, the antioxidant activities as ABTS, DPPH and FRAP were 25.76, 29.02 and 140.57 µmol TE/g DM, respectively. In addition, the total phenolic contents of the extract indicated a highly positive correlation with antioxidant activity.

Keywords: Extraction, Antioxidant, Hom Nin rice bran, Citric acid

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop cultivated in the world, which is the staple food for more than half the world's population (Danielski *et al.*, 2005; Lu *et al.*, 2008; Xia *et al.*, 2006). It is believed to provide more health benefits than any other carbohydrate-based foods since rice contains several nutrients and antioxidant compounds (Vijitra *et al.*, 2011). In addition, colored rice consumption has been rapidly incremental (Kong and Lee, 2010). It has further been reported that colored rice has a higher antioxidant content in its seed coat or pericarp than non-colored rice (Yodmanee *et al.*, 2011).

The main antioxidants in the colored rice seed coat are flavonoids, mainly anthocyanins, a subgroup of phenolic compounds (Abdel-Aal *et al.*, 2006). Furthermore, black rice has been known to provide health benefits that can reduce the risk of chronic diseases; such as, cancers and cardiovascular problems (Xia *et al.*, 2006), diabetes and its complications (Walter and Marchesan, 2011) since it contains various phytochemicals, especially phenolic compounds (Shen *et al.*, 2009). It has also been shown that black rice has a profitable contribution to nutritional and therapeutic values in comparison to white rice (Chen *et al.*, 2006; Choi *et al.*, 2007).

Hom Nin rice is a kind of black rice and it contains a higher nutritional value than that of other strains of rice including protein, vitamins and minerals (Suzuki *et al.*, 2004). In addition, the contents of vitamin B, niacin, vitamin E, calcium, magnesium, iron and zinc were higher when compared to white rice and it could also possibly be a significant potential source of anthocyanins. Anthocyanins in foods provide advantages in anti-cancer prevention, liver protection, prevention of heart disease, decrease of dyslipidemia, reduction of coronary heart disease and improvement of visual acuity (Chen *et al.*, 2006; Lee, 2010; Mazza and Miniati, 1993).

Rice bran is a by-product from rice milling and comprises about 10% of the total rice grain, which is normally used as animal feed. It also contains the same nutrients and antioxidative compounds as brown rice including tocopherols, tocotrienols, phytic acid, and tricin as well as pigments (Vijitra *et al.*, 2011) in which most of them are stored in the bran or pericarp of the rice kernel, and the different pigmented compounds are related to distinct colors; such as, red, purple and black (Hu *et al.*, 2003).

Extraction of antioxidants has become an alternative method for developing black rice waste to be a more valuable product. However, the antioxidant stability of the extract is a matter of great concern as it affects the extraction process and also

the cost of extraction. The temperature used for extraction needs to be determined due to some instability of the antioxidant compounds with heat (Benchahem *et al.*, 2015). For those antioxidants that react to alkaline and neutral solutions, acidic aqueous solvents have been used for extracting, so to disrupt the cell membranes and at the same time dissolve the water-soluble antioxidative compounds. Usually, citric acid, hydrochloric acid, and acetic acid are chosen for acidulating the extraction solvent but using citric acid is also safe for human consumption (Li *et al.*, 2012; Mateus and Freitas, 2009). The most commonly used solvents for antioxidant extraction are ethanol or methanol and water (Garcia *et al.*, 1998). The objectives of this study were to investigate the effects of extracting solvents and the concentration of citric acid on phenolic compounds as well as to evaluate the antioxidant activity from the Hom Nin rice bran extract. Thus, the natural antioxidants and also the color from the extracts of by-product materials could then be possibly used to substitute synthetic antioxidants and colors.

MATERIAL AND METHODS

Rice samples

Freshly milled Hom Nin rice bran (after milling up to 24 hours) was collected from the Nongpingkai Rice Mill Group, Amphoe Mueang, Kamphaeng Phet province, Thailand. The bran was sifted to separate the rice grains from the bran, vacuum packed in aluminum foil bags, and stored at -20°C.

Chemicals and reagents

(+)-Catechin hydrate, 2,4,6-tripyrindyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu's reagent, 2-diohenyl-1-picrylhydrazyl (DPPH), ethanol, methanol, potassium chloride, sodium acetate, and sodium carbonate were purchased from Merck (Darmstadt, Germany). Aluminum chloride hydrate, potassium persulfate, hydrochloric acid and acetic acid were purchased from Ajax Finechem Pty Ltd. (Auckland, New Zealand). All chemicals and solvents were of an analytical reagent grade.

Sample preparation

Hom Nin rice bran was sieved through a 100 mesh (150 µm) sieve and heated by a hot air oven at 100°C (Memmert UNE 500, Germany) for 15 minutes to inactivate the endogenous lipase and then cooled to room temperature (Juliano, 1985). The rice bran was defatted with hexane by using the ratio of the bran and solvent at 1:10 (w/v) for two hours in a shaker at 100 rpm (room temperature). The homogenized mixture was filtered through Whatman No. 1 filter paper and re-extracted twice using the same conditions and then dried in a hood for 12 hours to remove the solvent residual. Then, the rice bran was packed in aluminum foil bags and stored at -20°C (Kim et al., 2013).

Extraction of the Hom Nin rice bran powder

Five grams of defatted Hom Nin rice bran (DHRB) were extracted by 0, 0.05 and 0.10 mol/dm³ of citric acid in water, 99.9% methanol and absolute ethanol (1:10 w/v) with a rotary shaker (Gemmy Orbit Shaker VRN-480, Taiwan) at 100 rpm for two hours at room temperature. After pouring out the supernatant, the precipitates were re-extracted using the same procedure. Both supernatants were then combined and the methanol and ethanol were evaporated by using rotary evaporator (Buchi R 205, Switzerland) at 40°C and 100 mbar before freeze drying and the dried extracts were then used for phytochemical analyses (Graciele et al., 2011; Nontasan et al., 2012; Tananuwong and Tewaruth, 2010). For freeze drying, the samples were previously frozen and then put into a chamber (Christ alpha 1-4 LD plus, Germany) at -55 °C under pressure of 0.05 bar and were maintained under these conditions for 48 h.

Extraction yield

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. In the case of DHRB, this would provide some understanding about the extractability of the antioxidant activity under different solvents. Thus, this could be calculated according to the method of Zhang, Bi, and Liu (2007) as follows:

Extraction yield (%) = (weight of the freeze-dried extract x 100) / (weight of the original sample)

Preparation the extract solution for assays

For the determination of the bioactive compounds and antioxidant activity, the extracts were diluted with water using the ratio of 1:19 (extract : solvent, w/v) (Zhang et al., 2007).

Determination of the total phenolic content

The total phenolic content of the DHRB extract was determined using the method of Folin-Ciocalteu's reagent as described by Singleton et al (1999). Briefly, 100 µl of the extracts solution were mixed with 3 ml water, water-diluted Folin-Ciocalteu's reagent (1: 10 v/v), and 2 ml of sodium carbonate (7.5% w/v). The mixture was kept in the dark at room temperature for two hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Thermo Scientific Genesis10, USA). Gallic acid was used as a standard and expressed as milligrams (mg) of gallic acid per gram of DHRB dry matter (mg/g DM).

Determination of the total flavonoid content

The total flavonoid content was determined by Yang et al. (2009) with some modifications. 100 µl of the sample was mixed with 1.25 ml of deionized water and 75 µl of 5% of sodium nitrite and then incubated in the dark at room temperature for six minutes. 150 µl of 10% of the aluminum chloride solution was added and allowed to stand in the dark at room temperature for five minutes before the addition of 0.5 ml of 1.0 M sodium hydroxide. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Thermo Scientific Genesis10, USA), compared to the catechin standard. The total flavonoid content of the sample was expressed as mg of catechin equivalents per gram of the DHRB dry matter (mg CE/g DM).

DPPH radical scavenging assay

The DPPH method was determined as described by Brand-Williams et al. (1995) as modified by Zhang et al (2007). Briefly, 100 µl of the sample was added to 3 ml of 100 µM DPPH radical solution, which was freshly prepared. The reaction mixture was agitated and allowed to stand at room temperature in the dark for 30 minutes. The absorbance at 517 nm was used to measure the concentration of the remaining DPPH using a spectrophotometer (Thermo Scientific Genesis10, USA). The DPPH free radical scavenging activities of the extracts were expressed as µM of the Trolox equivalents (TE) per gram of the DHRB dry matter using a standard curve of Trolox (µmol TE/g DM).

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay was determined using a modified method of Benzie and Strain (1996). The FRAP reagent was performed by using 300 mM of acetate and glacial acetic acid buffer (pH 3.6), 10 mM of TPTZ (4,6- tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM of ferric chloride. The working FRAP reagent was freshly prepared by mixing the three solutions together in the ratio of 10:1:1 and the reagent was incubated at 37°C in a water bath (Memmert WB 22, Germany). Three milliliters of the FRAP reagent was mixed with 100 µl of the sample and incubated at 37°C for 30 minutes. The absorbance was read at 593 nm using a spectrophotometer (Thermo Scientific Genesis10, USA) and expressed as micromoles of the Trolox equivalents per gram of the DHRB dry matter (µmol TE/g DM).

ABTS radical scavenging assay

The ABTS assay was determined according to Arnao et al. (2001) with some modifications. The ABTS radical cation was generated from 7.4 mM of the ABTS solution with 2.6 mM of potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. The ABTS^{•+} solution was diluted with methanol to obtain an absorbance of 1.10±0.02 at 734 nm. One hundred µl sample solutions were mixed with 3 ml of the ABTS^{•+} solution and allowed to stand in the dark for 30 minutes at room temperature. The absorbance was then measured at 734 nm using a spectrophotometer (Thermo Scientific Genesis10, USA) and the results were expressed as micromoles of the Trolox equivalents per gram of the DHRB dry matter (µmol TE/g DM).

Total anthocyanin content

The total anthocyanin content was determined using the pH-differential method, which was slightly modified from Finocchiaro et al. (2010). The DHRB sample was diluted with 0.025 M of the potassium chloride buffer, pH 1.0 and separately with 0.4 M of the sodium acetate buffer, pH 4.5 and left for 15 minutes before taking the absorbance measurements. The absorbance in each buffer was measured at λ_{max} (520nm) and at 700 nm using a spectrophotometer (Thermo Scientific Genesis10, USA). Distilled water was used as a blank.

The total anthocyanin content was calculated as equivalent to cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanin content} = (\Delta A \times MW \times DF \times 1000) / e$$

Where

ΔA = (Abs_{λ520}-Abs_{λ700}) pH 1.0 - (Abs_{λ520}-Abs_{λ700}) pH 4.5

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside

DF = dilution factor

e = is the molar extinction coefficient, equaling 26,900 L/mol cm for cyanidin-3-glucoside.

1000 = conversion factor from g to mg.

The total anthocyanins were expressed as mg of cyanidin-3-glucoside equivalents per 1 gram of rice bran.

Statistical analysis

The measurements were carried out in three replicates, and all data were subjected to the analysis of variance (ANOVA). Significant differences among the treatments were analyzed by Duncan's multiple range test (DMRT) at a 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

Effects of the solvent type and citric acid concentration on the yields and antioxidant activity of DHRB

To obtain the yields and antioxidant activity with the lowest changes of the functional properties of the extract required, the extraction technique is one of the most important stages (Zhu et al., 2010). In this experiment, the effects of solvent type on the yields of extract are summarized in Table 1.

The solvents used for the extraction of DHRB showed statistically significant different yields (P<0.05). The DHRB extract yields using different solvents with various citric acid concentrations were between 2.41 to 43.72%. The yields of the DHRB extracted by using methanol were higher than those of water and ethanol, and the extraction yields rose with the increasing concentration of citric acid from 0 M to 0.1 M (P<0.05). Although, a higher yield was observed when using 0.1 M of citric acid with water and methanol, there was no significant difference (P≥0.05). Since, the viscosity of the solvent affected the extraction's efficiency, the viscosity of methanol was lower than that of water and ethanol (0.59, 0.89 and 1.07 centipoises) (Alam et al., 2018). Therefore, higher extraction yields in general were obtained by the less viscous solvents (Wijekoon et al., 2011).

The antioxidant activity of the extracts from DHRB determined as ABTS, DPPH and FRAP are presented in Table 1. It was found that both the solvent type and the citric concentration affected the ABTS value of the DHRB extract significantly ($p < 0.05$). Therefore, it was likely that the efficiency of the antioxidant activity was then increased with increasing citric acid concentration, which was the same trend as the increase of total phenolic contents (Figure 1).

Table 1 shows the efficiency of the antioxidant activity of the extract of DHRB. The results reveals that the DPPH values were significantly influenced by the type of solvent and the concentration of citric acid ($p < 0.05$). It has also been noticed that the efficiency of antioxidants was decreased when using water with a higher citric acid concentration, whereas the extraction with ethanol provided higher antioxidant efficiency when using citric acid at a higher concentration. The decrease of DPPH values of DHRB when using water with higher acid concentration may be due to the free form of phenolic compounds obtained from water extraction were destroyed by the acid (Ammar et al., 2015; Bridgers et al., 2010; Rayle and Cleland, 1992). It can be noted that at atmospheric pressure, the dielectric constant (index of polarity) of water (80.1) is higher than that of ethanol (24.5) (Cuevas et al., 2014) so that the polyphenols can be dissolved more in water. By the way, the increase of polarity of the acidified ethanol seemed to improve the solubility of polyphenols which then provided better antioxidant activity.

The efficiency tests for the antioxidant activity of the extracts from DHRB by using a FRAP assay also found that using different solvents with different citric acid concentration affected the FRAP values ($p < 0.05$). In addition, the extraction with water, ethanol and methanol would likely diminish the antioxidant activity when a higher citric acid concentration was used due to the above mentioned reasons.

Considering the antioxidant activity values obtained from the DHRB extract from the three assays, the results showed that the solvent type and the citric acid concentration had a great impact on the overall antioxidant activity. Methanol could be used to extract phenolic compounds and also antioxidants better than water and ethanol, respectively. Alcohol is normally used in antioxidant extraction and is better than water because of its smaller molecular size, less

viscosity and stronger polarity, as it could spread into a plant's cells faster and provide a higher extraction capacity. Antioxidants in most plants are normally polar substances and due to the polar nature of the water molecule itself, antioxidants are generally able to dissolve in water. The antioxidant activity of the phenolic compounds depended on a number of hydroxyl categories and properties to catch electrons among carboxylic acid in a molecule capable of providing a decrease in hydrogen (Moongngarm, 2012), as well as functional groups; such as, groups bearing polar molecules. The obtained results when using water for the extraction of antioxidants had the same trend as the number of phenolic compounds (Figure 1). The antioxidants in rice are mainly phenolic compounds and are immobilized in cellulose, lignin and proteins; such as, ferulic acid which contains 93% of the total volume (Jirum, and Srihanam, 2011; Li, et al., 2012). The acidic extraction could somehow facilitate the release of phytochemicals by breaking the plant's cells' walls and the performance of the extraction would depend upon the solvent and its concentration (Rayle and Cleland, 1992; Vadivel and Brindha, 2015). Moreover, the DHRB extract obtained from different solvents would be composed of several chemical compounds which exhibited different antioxidant activity. Extraction with water tended to decrease the antioxidant activity when using a higher concentration of citric acid, while using alcohol with higher citric acid provided higher antioxidant activity. As such, the results of this present study were in accordance with Jianmei et al. (2004) who reported the antioxidant extraction of peanut skin using water and 80% of ethanol concentration and found that extraction by using water gave better antioxidant activity than that of ethanol. Furthermore, the ABTS values were 4.10 and 3.39 μmol of the Trolox equivalents per 1 gram of the dried sample, respectively. Pinelo et al. (2004) extracted antioxidants from the bark of almonds with water, methanol and ethanol and found that the methanolic extract had better antioxidant activity than that of water and ethanolic extracts, respectively. Moreover, Anwar et al. (2013) investigated the methanolic and ethanolic extracts for the antioxidant activity of cauliflowers, which were dried using a different drying condition and found that the methanolic extract gave better antioxidant activity.

Table 1 Yields and antioxidant activities of DHRB extracts by using different extracting solvents determined by DPPH, FRAP and ABTS assays from

Solvent	Yield of powder extract (%)	Antioxidant activity (μmol Trolox equivalents/g)		
		ABTS	DPPH	FRAP
Water	22.73 \pm 0.51 ^d	24.18 \pm 1.44 ^{bc}	29.47 \pm 0.58 ^b	153.40 \pm 2.72 ^c
0.05 M of citric acid with water	32.95 \pm 2.32 ^b	23.41 \pm 0.39 ^{bc}	23.42 \pm 0.14 ^d	92.65 \pm 1.92 ^f
0.1 M of citric acid with water	41.34 \pm 2.56 ^a	22.84 \pm 0.59 ^c	22.47 \pm 0.36 ^e	65.14 \pm 1.36 ^h
Methanol	8.77 \pm 0.69 ^f	25.73 \pm 0.57 ^a	30.56 \pm 0.27 ^a	160.89 \pm 0.72 ^b
0.05 M of citric acid with Methanol	25.41 \pm 1.00 ^c	25.76 \pm 0.55 ^a	29.82 \pm 0.48 ^b	189.21 \pm 0.66 ^a
0.1 M of citric acid with Methanol	43.72 \pm 1.29 ^a	25.76 \pm 0.55 ^a	29.02 \pm 0.82 ^c	140.57 \pm 0.47 ^d
Ethanol	2.41 \pm 1.03 ^g	18.63 \pm 0.21 ^d	9.26 \pm 0.32 ^g	155.75 \pm 2.71 ^c
0.05 M of citric acid with Ethanol	15.40 \pm 0.27 ^e	24.57 \pm 1.18 ^{ab}	8.63 \pm 0.17 ^g	98.67 \pm 0.26 ^e
0.1 M of citric acid with Ethanol	22.18 \pm 1.69 ^d	25.78 \pm 1.05 ^a	11.29 \pm 0.23 ^f	70.48 \pm 0.91 ^g

*Different letters within the same row indicate statistical differences (one-way ANOVA and Duncan test, $P < 0.05$). Values are mean \pm S.D of triplicate determinations

Effects of the solvent type and citric acid concentration on the total phenolic content of DHRB

Figure 1 shows that the type of solvent and concentration of citric acid significantly affected the amount of the phenolic compounds in DHRB ($p < 0.05$). The methanolic extract gave the highest phenolic compound followed by the water and ethanolic extracts, respectively. By using methanol and ethanol for the extraction, it could be shown that the number of phenolic compounds were likely to rise with an increasing citric acid concentration while the extraction using water presented a lower number of phenolic compounds but when using a lower citric acid concentration, the obtained phenolic compounds were higher. Consequently, it seemed that alcohol and water were opposed to each other in interacting with the citric acid concentration. DHRB extracted by methanol with 0.1 M of citric acid concentration had the highest phenolic compounds of 86.63 mg of gallic acid/g and the highest phenolic compounds extracted by water only and ethanol with 0.1 M of citric acid were 52.54 and 13.35 mg of gallic acid/g, respectively. Almost all of the phenolic compounds in plants are mainly in the form of a water-soluble form (Moongngarm, 2012), and the extraction of phenolic compounds often uses organic solvents since they can be dissolved by the same principles. Additionally, the organic solvents used for the extraction of phenolic compounds usually included methanol and ethanol (Jirum and Srihanam, 2011), and ethanol was normally used because it is safer than other types of organic solvents. Thus, the obtained results were consistent with the experiments of Arab et al. (2011) who studied the type of solvent used in the extraction of phenolic compounds from two rice cultivars (Fajr and Tarem) and found that the methanolic extract gave the highest phenolic compounds followed by ethanolic and ethyl acetate extract, respectively. Tan et al. (2013) studied the use of water and methanol in the extraction of phenolic compounds from rice

(temukut) and found the methanolic extract provided a higher phenolic compound than that of water. Pinelo et al. (2004) conducted the extraction of phenolic compounds from pine sawdust using different solvents; i.e., water, methanol and ethanol and found that methanolic extract had the highest phenolic compounds followed by ethanolic and water extract, respectively. Jianmei et al. (2004) extracted phenolic compounds from the skin of peanuts using water, methanol and 80% of ethanol concentration and found that the methanolic extract provided the highest phenolic compounds followed by ethanolic and water extracts as 90.1, 89.9 and 56.7 mg of gallic acid/g, respectively. In addition, the results from this present study were consistent with Bahar et al. (2009) who studied the effect of ethanol with various amounts of 30% of citric acid on the extraction of phenolic compounds from olives and found that using 10 ml of citric acid gave the highest phenolic compounds and when using a higher amount of citric acid, the phenolic compound was reduced. This was because phenolic compounds normally found in most plants comprise three different groups; i.e., a free form, conjugated form and bound form. The bound form is the group, which is mainly found in the layer of lignin extracted by using acidic or alkaline hydrolysis. The free form is the second group, but the structure is rarely stable so when using an acidic extraction of phenolic compounds, the structure would be partially destroyed. However, when using acid at a higher concentration, the plant's cells' walls were ruptured; hence, the more active ingredients were released. The results of this present study revealed that each solvent had a certain citric acid concentration for the optimum extraction condition as well (Adom and Liu, 2002; Choi et al., 2007; Zhou et al., 2004).

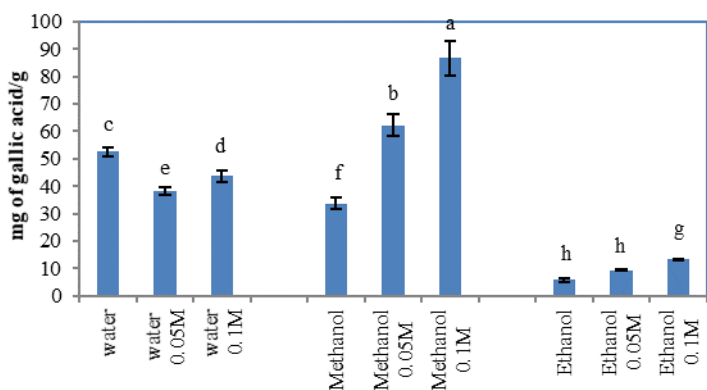


Figure 1 Total phenolic contents of DHRB extracts by using different extracting solvents. *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P < 0.05$).

Effects of the solvent type and citric acid concentration on the flavonoid content of DHRB

The study found that the type of solvents and the concentrations of citric acid statistically affected the amount of flavonoids extracted from DHRB ($p < 0.05$). The treatments extracted by using methanol gave the highest flavonoids followed by those with water and ethanol, respectively. The extraction using methanol and ethanol tended to increase the flavonoid contents when increasing the citric acid concentration while the flavonoid contents of the water extract was decreased with an increase of the citric acid concentration. The extraction using methanol with 0.1 M of citric acid concentration gave the highest flavonoid content of 14.67 mg catechin equivalents/g, and other solvents provided a flavonoid content in the range between 2.52-13.66 mg catechin equivalents/g. The obtained results were the same as the phenolic contents, as the flavonoid antioxidants were in the phenolic compound group (Liu, 2004).

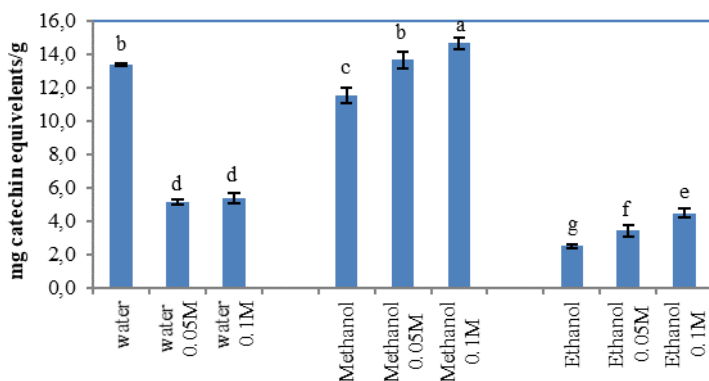


Figure 2 Flavonoid contents of DHRB extracts by using different extracting solvents. *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P < 0.05$).

Effects of the solvent type and citric acid concentration on the anthocyanin content of DHRB

The content of the anthocyanins extracted from DHRB is shown in Figure 3. The extraction conditions showed a statistically significant effect on the anthocyanin content ($p < 0.05$) in which the methanolic extract had the highest anthocyanin content followed by the water and ethanol extracts, respectively. As previously mentioned, methanol had a lower viscosity than ethanol and water, so it could better disperse into the samples and eluted more anthocyanins, which were more soluble in the polar solvents (Rezaie et al., 2015). The methanolic extract with 0.1 M of citric acid provided the highest anthocyanin content of 23.01 mg/g, and the anthocyanin contents obtained from using other solvents were found in the range of 3.69-22.42 mg/g. The number of anthocyanins was significantly increased when using acidified alcohol with a higher citric acid concentration while using water as a solvent that tended to lower the anthocyanin contents even when acidified with citric acid. Furthermore, in using a higher citric acid concentration in the water extraction, the obtained anthocyanin content was significantly higher ($p < 0.05$). This was because anthocyanin is more stable in an acid solution (Fuleki and Francis, 1968) and has the ability to bind to free radicals in the body like vitamin C, Vitamin E and beta-carotene, which are several times (Chen et al., 2006) a more powerful natural antioxidant (Lee, 2010). In addition, using acids to assist the phytochemical extraction would help digest the cell walls of the plant samples; hence, anthocyanin could be released very effectively in higher amounts. The results of this present study were

consistent with the study of Li et al. (2012) who reported the use of microwave assisted extraction of anthocyanins from grape peels with citric acid. The results showed the factors that mostly affected the anthocyanin content were the concentration of citric acid, the extraction time, the power of the microwave, and the ratio between the sample and the solvent, respectively. Moreover, the anthocyanin content was increased with increasing citric acid concentration.

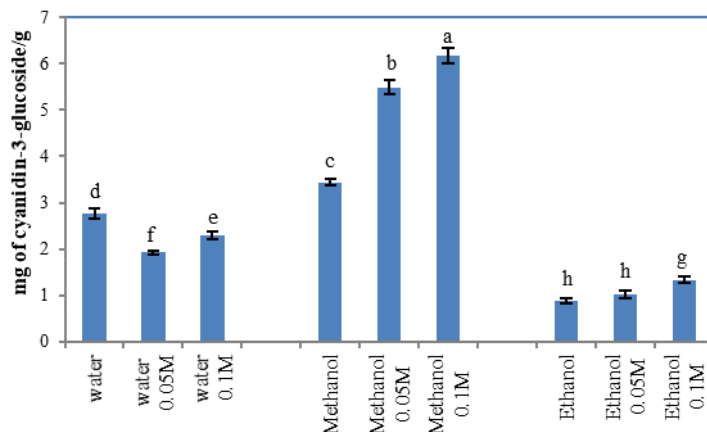


Figure 3 Anthocyanin contents of DHRB extracts by using different extracting solvents. *Different letters indicate statistical differences (one-way ANOVA and Duncan test, $P < 0.05$).

CONCLUSION

The results of this study showed that the yield of phenolic and flavonoid contents as well as the efficiency of antioxidant activity had been affected by the solvent types and citric acid concentration used for antioxidant extraction from colored rice, and the DHRB methanolic extract with 0.1 M of citric acid gave the highest antioxidants. The extraction using water and ethanol without acid found a similar efficiency of antioxidant activity. However, using citric acid in the extraction showed a trend of decreasing antioxidant activity and the anthocyanin content from DHRB extracted by using acidified water tended to increase with an increasing citric acid concentration.

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