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The Effect of Extraction Methods on Phenolic, Anthocyanin, and Antioxidant Activities of Riceberry Bran

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Abstract

Riceberry bran, byproduct from the rice milling process, is one of bioactive compounds sources. In order to increase Riceberry bran value, the purpose of this study was to compare the effect of different Riceberry bran extraction methods, including accelerated solvent extraction (ASE), ultrasonic-assisted extraction (UAE), soxhlet extraction (SE), and maceration method (MC) on bioactive compounds and antioxidant activities. It was found that the Riceberry bran extract from ASE presented the highest total phenolic, total anthocyanin content (55.45 mg GAE/g and 3.06 mg/g) and antioxidant properties including IC_{50} of DPPH assay (0.109 mg/mL), FRAP value (688.04 mmole Fe(II)/kg) and IC_{50} of ABTS assay (3.42 mg/mL) among the four methods. These results imply the potential to use the ASE method for extraction of phenolic and anthocyanin compounds from Riceberry bran.

Keywords: Accelerated solvent extraction, Maceration, Riceberry bran, Soxhlet, Ultrasonic-assisted extraction

1. Introduction

Riceberry, deep purple grain; (*Oryza sativa* L.), a cross-bred strain from the Khao Hom Nin Rice variety which is well known as containing high antioxidant properties and Khao Hom Mali 105 well known as fragrant rice (Leardkamolkarn et al., 2011). Consumption of Riceberry is becoming popular in Thailand because of high nutrition and so as to meet the increasing market demand, the Thai government is supporting farmers to grow Riceberry (Peanparkdee, Yamauchi, & Iwamoto, 2018). Riceberry bran is a byproduct of the rice milling process. It is always extracted oil and defatted Riceberry bran is used an ingredient in animal feed. A number of studies have reported the presence of bioactive compounds in Riceberry bran. For instance, Riceberry bran is a rich source of phenolic compounds, anthocyanins, vitamin E and oryzanol which have shown great antioxidant activities (Peanparkdee et al., 2018). In addition, several studies reported that Riceberry possessed chemopreventive properties including decreasing inflammation, managing diabetes and improving the regenerative changes of the pancreas, kidneys, heart and liver (Leardkamolkarn et al., 2011; Posuwan et al., 2013; Prangthip et al., 2013). Extraction, the first step of bioactive compounds study, plays a significant and important role in the final quality of the extract. There are many extraction techniques that have been developed for the extraction of bioactive components from rice bran such as UAE, ASE microwave and ohmic heating-assisted extraction, etc. Peanparkdee et al. (2018) extracted phenolic and anthocyanins from Riceberry bran using UAE. Suttiarporn, Sookwong, and Mahatheeranont (2016) studied fractionation and identification of antioxidant compounds from Riceberry bran using solvent extraction (hexane, dichloromethane and methanol). However, there is little published information to compare extraction methods of bioactive compound from Riceberry bran. Conventional solvent extraction is used in extracting bioactive compounds from plants, based on many parameters, including the amount of solvents, extraction time, polarity of the antioxidant, and temperature (Peanparkdee et al., 2018). The conventional methods such as SE, and MC are still considered to be compared with new

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extraction methods. Recently, new extraction methods have obtained increasing attentiveness to produce more environmentally sustainable, more effective, decreasing cost and faster extraction (Barros, Dykes, Awika, & Rooney, 2013). New extraction methods were used in various bioactive compounds, such as phenolic from rice bran (Tabaraki & Nateghi, 2011) using UAE since it can increase the efficiency of solvent extraction. ASE was also used to extract phenolic compounds from sorghum brans (Barros et al., 2013), and anthocyanin composition from blue wheat, purple corn, and black rice (Abdel-Aal, Akhtar, Rabalski, & Bryan, 2014). This method uses a combination of elevated temperature and pressure with common solvents to increase the efficiency of the extraction process. The result is faster run times and a significant reduction in solvent use. Therefore, the main objective of the present study was to extract Riceberry bran using ASE, UAE, SE, and MC followed by the evaluation of the total phenolic content, total anthocyanin content and antioxidant activities in order to increase the utilization of Riceberry bran.

2. Methodology

2.1 Raw material

Defatted Riceberry bran (*Oryza sativa* L.) obtained from Sunfood Corp Limited (Samutprakan, Thailand). It was sieved through 20–100 mesh screen. It was kept in a sealed container at -18°C. All measurements were performed in triplicate.

2.2 Chemicals

Folin-Ciocaltue reagent gallic acid, 2,2'-azaino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2'-dipheny-l-picrylhydrazyl (DPPH), , ascorbic acid (vitamin C), α-tocopherol and 2,6-di-tert-butyl-4-methyl-phenol were purchased from Sigma-Aldrich (St. Louis, MO). Potassium chloride and sodium carbonate were purchased from Ajax Finechem (NSW, Australia). Sodium acetate hydrate and furrous sulphate were purchased from Carlo Erba (Chaussee du Vexin, France). Hydrochloric acid was purchased from Merck (Darmstadt, Germany).

2.3 Determination of extract yield

The yield of extracts on a dry weight basis was calculated from equation (1) as shown below:

$$\text{Extract yield (\%)} = (W_1 \times 100) / W_2 \quad (1)$$

W_1 was the weight of extract after evaporation of ethanol
W_2 was the dry weight of the fresh plant sample

2.4 Determination of moisture content

The moisture content of the Riceberry bran was analyzed according to AOAC methods (Association of Official Analytical Chemists [AOAC], 2019).

2.5 Determination of water activity (a_w)

a_w was determined at 25°C using a_w meter, Aqua Lab (Series 3 TE (Decagon Devices Inc., USA). Approximately 2 g of the ground sample was used for the analysis.

2.6 Determination of color value

The color of rice bran was measured with spectrophotometer (Hunter Lab, Color Quest XE, USA) equipped with a D65 illuminant using the CIE L* a* b* system.

2.7 Sample extraction

Four extraction methods, ASE, UAE, SE, and MC were investigated. Riceberry bran was extracted with ethanol using UAE method according to Tabaraki and Nateghi (2011) method with some modifications. The bran (10 g) was dissolved in 200 mL of 67% (v/v) aqueous ethanol. The sample was sonicated (37 kHz) in an ultrasonic cleaner (Ultrasonic bath, Elama siries Elmasonic P, Germany) at 55°C for 40 min. The resulting extract was filtered through a Whatman No.1 filter paper and vacuum-dried in a rotary evaporator (Rotavapor R-100, Buchi, Switzerland) at 40°C. The crude extract was stored at -18ºC until use.

ASE was performed on a Dionex ASE 350 system (Thermo Scientific, USA). The bran sample of 10 g was mixed with diatomaceous earth (DE) in a proportion of 1:1 and placed in a 66 mL stainless steel extraction cell. A cellulose D28 filter (Dionex Corporation) was placed at the bottom of the extraction cell to avoid the collection of suspended particles in the collection vial. The extraction cells

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were arranged in the sample carousel and prefilled with the solvent (67% ethanol), static time 10 min, and heated at 55°C. The extraction was done using 5 cycles and the cell was rinsed with 100% flush. The extract was collected in 250 mL collection vials. The resulting extract was filtered through a Whatman No. 1 filter paper and vacuum-dried in a rotary evaporator at 40°C. The crude extract was stored at -18ºC until use.

The SE was based on the UAE method. The bran (10 g) was placed in a thimble and put it in the extractor that dissolved in 50 mL of 67% ethanol. The distillation flask was filled with 250 mL of 67% ethanol, and heat source. The sample was placed in a thimble-holder that was gradually filled with condensed fresh extractant from a distillation flask. When the solvent reached the overflow level, a siphon aspirates the solute from the thimble-holder and unloaded it back into the distillation flask, thus carrying the extracted analyses into the bulk liquid. This operation was repeated until the solvent in the extractor was clear. The resulting extract was filtered through a Whatman No.1 filter paper and vacuum-dried in a rotary evaporator at 40°C. The crude extract was stored at -18ºC until use.

MC was investigated at room temperature. The bran (10 g) was dissolved in 200 mL of 67% ethanol in closed bottles. After 48 h of extraction, the extract was filtered through a Whatman No. 1 filter paper and repeat 3 times until the extract was clear. The collection of the extract was vacuum-dried in a rotary evaporator at 40°C. The crude extract was stored at -18ºC until use.

2.8 Determination of total phenolic content

The total phenolic content of Riceberry bran extracts was determined by the Folin-Ciocalteu reagent using different concentration (0-200 μg/mL) of Gallic acid as standard (Wolfe, Wu, & Liu, 2003) with some modifications. 125 μL of samples were mixed with 500 μL distilled water in a test tube followed by the addition of 125 μL of Folin-Ciocalteu reagent and allowed to stand at room temperature for 6 min. Then it was mixed with 1,250 μL of 7% Na_2CO_3 and followed by the addition of 1,000 μL of distilled water and allowed to stand at room temperature for 90 min. The absorbance of the mixtures was determined at 760 nm in spectrophotometer (UV mini-1240, Shimadzu, USA). Total phenolic content in the extract was expressed in mg Gallic acid equivalent/g.

2.9 Determination of anthocyanin content

The analysis method for anthocyanin content was modified from the method used by Giusti & Wrolstad (2005). The Riceberry bran extracts (1 mL) in 25 mL of volume metric flasks were adjusted with potassium chloride buffer (0.03 mol/L, pH 1.0) and the other was adjusted with sodium acetate buffer (0.4 mol/L, pH 4.5). Each of them was left for 15 min in the dark at room temperature. The absorbance of the mixtures was determined at 510 nm and 700 nm in spectrophotometer. The anthocyanin concentration (mg/L) of sample was calculated according to the following formula (2) and expressed as Cyanidin-3-glucoside equivalents:

$$\text{Total anthocyanin content (mg/g)} = (A \times MW \times DF \times V) / (\varepsilon \times 1 \times G) \quad (2)$$

A was $(A\lambda_{700} - \lambda_{510})$ pH 1.0 - $(A\lambda_{700} - \lambda_{510})$ pH 4.5
MW was the molecular weight of Cyanindin-3glu coside (449.2 g/mol)
DF was the dilution factor (20 μL sample is diluted to 2 mL, DF = 1000)
ε was the extinction coefficient (L × cm-1 × mol-1) = 26,900 for Cyanindin-3-glucoside where L (path length in cm) = 1
l was the volume of solvent
G was the weight of sample

2.10 Determination of antioxidant activities

- IC_{50} of DPPH scavenging activity

The free radical scavenging activity of Riceberry bran extracts will be evaluated using the stable radical DPPH according to the method of Re et al. (1999). Various concentration of each extract was pipetted into 1 mL DPPH working solution. The mixture was shaken and incubated for 30 min in the dark at room temperature. Ascorbic acid, α-tocopherol, and BHT were used as standard. The analysis was done in triplicate for standard and each extract. The absorbance of the mixtures was determined at 517 nm relative to the control (as 100%) using a spectrophotometer. The percentage of radical scavenging ability was calculated by using the formula (3):

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Scavenging ability (%) =

$$\frac{(\text{Absorbance 517 nm of control} - \text{Absorbance 517 nm of sample}) \times 100}{\text{Absorbance 517 nm of control}} \quad (3)$$

IC_{50} of DPPH scavenging activity of each extract could be calculated using its calibration curve.

- Ferric reducing antioxidant power (FRAP)

The FRAP assay was modified from Benzie and Strain (1999). 60 µL of samples were mixed with 1.8 mL of the FRAP reagent and 180 µL of distilled water. The absorbance of the mixtures was determined at 593 nm using a spectrophotometer after 4 min incubation at 37°C. FRAP reagent was prepared daily and consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and distilled water in a ratio of 10:1:1:1.2 (v/v/v/v). FRAP value was obtained by comparing the absorbance change in the test mixture with doses obtained from increasing concentrations of Fe(III) and expressed as mmol of Fe(II) equivalents per g extract. Ascorbic acid, α-tocopherol, and BHT were used as standard.

- IC_{50} of ABTS radical scavenging assay

ABTS radical scavenging activity was determined according to Re et al. (1999) with some modification. A stable stock solution of ABTS radical cation was produced by reacting a 7 mM aqueous solution of ABTS with potassium persulfate in the dark at room temperature for 12-16 h before use. The working solution was diluted with 95% ethanol to reach and absorbance of 0.7±0.02 at 734 nm. 20 µL of samples were mixed with 2 mL of working solution, and the absorbance was measured immediately at 734 nm after 6 min at room temperature in the dark. Ascorbic acid, α-tocopherol, and BHT were used as standard. The analysis was done in triplicate for standard and each sample. Antioxidant capacity of each sample was determined based on the reduction of ABTS absorbance by calculating the percentage of antioxidant activity. IC_{50} of ABTS scavenging activity of each extract could be calculated using its calibration curve.

3. Results and Discussion

3.1 Quality of raw material

Chemical and physical properties of defatted Riceberry bran are presented in Table 1. Defatted Riceberry bran had low moisture content and aw and dark purple in color. The particle size of Riceberry bran was mostly 60-80 mesh.

Table 1. Chemical and physical properties of Riceberry bran.

Chemical and physical properties	Measurement values
Moisture (%)	5.65±0.12
a_w	0.33±0.01
L*	35.33±0.17
a*	5.69±0.11
b*	4.28±0.12
Particle size 20-40 Mesh (%)	4.55±0.78
40-60	19.24±0.95
60-80	65.16±0.86
80-100	7.80±0.84
≥ 100	3.25±0.75

3.2 Effect of extraction methods on total phenolic and total anthocyanin content

The extracts obtained from ASE, UAE, SE and MC were investigated. Percentage of extraction yield, total phenolic and total anthocyanin contents are shown in Table 2. There were no significantly differences in the percentage of extraction yield ($p>0.05$). The percentages of extraction yield from each method were in the range of 18.07–21.73%. Total phenolic content was determined in comparison with gallic acid and the results were shown in terms of mg GAE/g extract. The results showed that the Riceberry bran extract from ASE had significantly higher total phenolic and total anthocyanin content than the other methods. This method uses high pressures during the extraction process so they allow the solvent to be heated at higher temperatures than their boiling point which increases diffusion rates, disturbs the strong solute–matrix interactions and reduces liquid solvent viscosity, allowing better penetration into the matrix and then improving extraction (Barros et al., 2013). Total phenolic content of the Riceberry bran extract from SE was 54.18 mg GAE/g extract while total anthocyanin content was 0.91 mg/g extract. The increase of total phenolic content in the Riceberry bran extract from SE due to high temperature and long extraction time. During extraction, heating of the sample might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions, leading to more polyphenols diffusion into the solvent (Das, Goud, & Das, 2017; Tao, Wu, Zhang, & Sun, 2014). The Riceberry bran extract from UAE had

lower total phenolic content than other methods because it took a shorter extraction time than MC and lower temperature than SE.

There were significant differences in total anthocyanin content ($p \leq 0.05$). The results obtained from four methods showed that the Riceberry bran extract from ASE had higher total anthocyanin content than the other methods because this method took a short time to heat. This result was in agreement with previous reports that ASE was more appropriate in extracting anthocyanin from colored grains as being comparable with the commonly used solvent extraction method based on changes in anthocyanin composition (Abdel-Aal et al., 2014). The Riceberry bran extract from UAE had higher anthocyanin than the Riceberry bran extract from SE and MC since ultrasound induced swelling of plant cells or breakdown of cell walls during sonication.

Table 2. Effect of extraction methods on a percentage of extract yield, total phenolic content and total anthocyanin content.

Method	Extract yield^{ns} (%)	Total phenolic (mgGAE/g extract)	Total anthocyanin (mg/g extract)
ASE	20.97±0.07	55.45±0.11^{a}	3.06±0.13^{a}
UAE	19.57±0.11	48.52±0.50^{d}	2.28±0.13^{b}
SE	18.07±0.45	54.18±0.60^{b}	0.91±0.07^{d}
MC	21.73±0.25	50.03±0.17^{c}	1.44±0.21^{c}

$^{a-c}$ Means in same columns followed by different letter are significantly different ($p \leq 0.05$).

^{ns} Means not significant ($p > 0.05$).

Therefore, antioxidants can be released from plant cells into the solvent. A decrease in anthocyanin of the Riceberry bran extract from SE because the extract was obtained high temperature and long extraction time as a result that anthocyanin was decomposed. An increase in temperature up to 74°C could increase phenolic content but it decreased anthocyanin content (Cacace & Mazza, 2003; Sripum, Kukreja, Charoenkiatkul, Kriengsinyos, & Suttisansanee, 2017). The degradation rate of anthocyanin depends on time and temperature. Therefore, high temperature and short extraction time were used successfully to retard anthocyanin degradation in plants (Ju & Howard, 2003).

3.3 Effect of extraction methods on radical scavenging activities

The radical scavenging activities of Riceberry bran were evaluated for DPPH, ABTS and FRAP assay as shown in Table 3. DPPH, ABTS radical scavenging activity of different extraction methods which express in terms of IC_{50} value. Lower IC_{50} value means more antioxidant potential. The chelating ability on ferrous ion in Riceberry bran extract was determined by FRAP assay. The radical scavenging activities were compared with ascorbic acid, α-tocopherol and BHT as standard. There were significantly different in IC50 of DPPH and ABTS assay and FRAP assay amongst the extraction methods ($p \leq 0.05$).

Table 3. Effect of extraction methods on radical scavenging activities (DPPH, ABTS and FRAP assay).

Method	DPPH assay IC_{50} (mg/mL)	ABTS assay IC_{50} (mg/mL)	FRAP assay mmole Fe(II)/kg
ASE	0.109±0.000^{a}	3.42±0.05^{a}	688.04±1.12^{a}
UAE	0.144±0.004^{d}	4.16±0.04^{c}	543.51±0.67^{c}
SE	0.127±0.000^{b}	3.45±0.06^{a}	649.76±0.99^{b}
MC	0.137±0.002^{c}	3.75±0.06^{b}	668.65±1.22^{ab}
BHT	0.135±0.002	0.499±0.002	3,937.00±10.00
Ascorbic acid	0.004±0.001	0.207±0.002	5,943.00±10.07
α-tocopherol	0.117±0.000	0.779±0.014	15,471.00±23.01

$^{a-d}$ Means in same columns followed by different letter are significantly different ($p \leq 0.05$).

^{ns} Means not significant ($p > 0.05$).

The results showed that the Riceberry bran extract from ASE had lower IC_{50} of DPPH and ABTS assay than the other methods. The IC_{50} of DPPH assay in the Riceberry bran extract from ASE was 0.109 mg/mL which was comparatively lower than the IC_{50} of DPPH assay in BHT and α-tocopherol, the IC_{50} showed that the Riceberry bran extract from ASE had more effective antioxidant activities than antioxidant compared to BHT and α-tocopherol. Previously, Soradech et al. (2016) compared IC_{50} of six species of colored rice and it was found that the extract of Gum-Doy-Moo-Ser and Riceberry had an effective antioxidant activities than other species of colored rice in that study.

The IC_{50} of ABTS in the Riceberry extract from ASE was not significantly different from SE but significantly lower than UAE and MC ($p \leq 0.05$). The Riceberry bran extract from ASE and SE has more effective antioxidant activities than UAE and MC. The FRAP values from each method were in the range of 543.51–688.04 mmole Fe(II)/kg. The highest FRAP value was the Riceberry bran extract from ASE. There were no significant differences in FRAP value of the Riceberry bran extract from ASE and MC method ($p > 0.05$). The Riceberry bran extraction from UAE showed weak radical scavenging activities despite of high total anthocyanin content. The results indicated that

phenolic gave antioxidant activity greater than anthocyanin. This result is in agreement with the previous report by Chen, Nagao, Itani, & Irifune (2012) that anthocyanin pigments of colored rice gave a low antioxidant activities.

Table 4. The Pearson's correlation coefficient (r) between total phenolic content and antioxidant activities measured by DPPH, ABTS and FRAP assays.

	TPC	DPPH	ABTS	FRAP
TPC	1			
DPPH	-0.933*	1		
ABTS	-0.936*	0.841*	1	
FRAP	0.853*	-0.822*	-0.959*	1

* Means correlation is significant ($p \leq 0.01$).

Pearson's correlation coefficients between total phenolic content and antioxidant activities measured by DPPH, ABTS and FRAP assays were computed and the results are shown in table 4. Significant correlations were found between total phenolic content and antioxidant activity ($p \leq 0.01$) but the results of antioxidant activities were not significantly correlate to total anthocyanin content. Total phenolic content had negative correlation with IC_{50} of DPPH assay and IC_{50} of ABTS assay (r = -0.933 and r = -0.936). Significant positive correlation was obtained for total phenolic content with FRAP value (r = 0.853). The highest correlation was found between FRAP value and IC_{50} of ABTS assay (r = -0.959). The lowest correlation was found between the IC_{50} of DPPH assay and FRAP value (r = -0.822). These results corresponded to the previous research (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009), which reported that significant correlations were found between DPPH, ABTS, and FRAP assays and total phenolic content determined by the Folin-Ciocalteu method. Therefore, phenolic compounds in plant extracts contribute significantly to their antioxidant potential.

4. Conclusion

Analysis of the total phenolic and anthocyanin content and radical scavenging activities of Riceberry extracts showed differences depending on extraction method. Amongst the extraction methods, The Riceberry bran extraction from ASE was the most effective method in antioxidative reactions and high total phenolic and total anthocyanin content. It can be concluded that the Riceberry bran extraction SE had high total phenolic content and strong antioxidative reactions but it had a long extraction time and high costing. The Riceberry bran extract from UAE had high total anthocyanin content and it used the lower energy input. The Riceberry bran extract from MC had low bioactive compounds and antioxidant activities but it took a long extraction time.

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