DEVELOPMENT OF RICEBERRY EXTRACT FOR ANTIOXIDANT ACTIVITY

Wachiraporn Pukdee, Naphatsorn Kumar, Phanuphong Chaiwut and Tawanun Sripisut*

School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand
* e-mail : tawanun.sri@mfu.ac.th

Abstract
The objective of this study was to determine optimum extraction of Riceberry rice and determine its application as an antioxidant. Riceberry rice was extracted in various solvent (50:50, 60:40, 70:30, 80:20, 90:10, 100:0 ethanol-water mixture (v/v)) and various extraction time (2, 4, 6 and 12 h). The crude extract was extraction with 70:30 ethanol-water presented significantly highest of total phenolic content (TPC), total anthocyanin (TAC), and antioxidant activity as determined by ABTS and FRAP were 67.443±1.192 mg GAE/g extract (TPC), 57.611 ± 6.521 mg C3G/g extract (TAC), 0.052 ± 0.003 mg/ml (ABTS) and 2,843.243 ± 54.859 mg TEAC/g extract (FRAP). In various extraction time, 6 h was optimum extraction time for Riceberry rice. The result of 70:30 ethanol-water at 6 h of total extraction time showed the highest of TPC, TAC, ABTS and FRAP antioxidant assay were 53.354 ± 1.373 mg GAE/g extract, 42.582 ± 2.892 mg C3G/g extract, 0.015 ± 0.0003 mg/ml and 2,765.766 ± 39.844 mg TEAC/g extract respectively. The result suggested the extraction method for Riceberry rice extract can be used as standard method. The result of crude extract presented its application as antioxidant activity and highest of total phenolic content (TPC) and total anthocyanin (TAC). It can be applied as potentially active ingredient in cosmetic.

Keywords: Riceberry/total phenolic content/total anthocyanin content/antioxidant activity/riceberry extract

Introduction:
Color rice (Oriza sativa L.) is one of the main foods in Asia. Several varieties of color rice, especially red, brown and black, have been cultivated in Thailand. Color rice is rich in many nutrient components and sources of bioactive compounds, known as antioxidant, including phenolic compounds. Moreover, color rice was reported to have a greater antioxidant capacity more than non-pigment rice.

Riceberry is a thai black rice, has been recently developed with the aim of providing optimum nutrient to consumers. The Riceberry bran is believed to be high antioxidant and other significant constituents, including beta carotene, folate, vitamin E, vitamin B1, tannin, etc.

Therefore, the objective of this study was investigated to achieve the optimum extraction, for the selection of Riceberry extraction methods to use in cosmetic industry.
Materials and methods:

Chemicals
2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Sigma-Aldrich Corporation, United States), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich Corporation, United States), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich Corporation, United States), Folin-Ciocalteu (LobaChemie Pvt Ltd., India), Gallic acid (Sigma-Aldrich Corporation, United States)

Rice samples
Riceberry rice used in this study was collected from Nakhon Phanom province, Thailand. The rice sample was ground into flour and passed through a 60 mesh screen sieve.

Preparation of crude extract

Optimization of solvent extraction
The Riceberry rice flour was extracted with ethanol-water mixture (v/v) (50:50, 60:40, 70:30, 80:20, 90:10, 100:0). For each trial, 50g. of the flour sample was mixed with 500ml. of the solvent. All extractions were done in shaker of 150rpm at 50 °C for 3 h. The extract was filtered with Whatman No.1 filter paper and then evaporated solvent with vacuum rotary evaporator and freeze dry. The crude extract was stored at -20 °C until use.

Optimization of extraction time
The Riceberry flour was extracted with optimized solvent (1) by varying the extraction time (2, 4, 6, 8, 12 h) All extractions were done in shaker of 150rpm at 50 °C and then filtered with Whatman No.1 filter paper. The extract was evaporated solvent with vacuum rotary evaporator and then freeze dry. The crude extract was stored at -20 °C until use.

Determination of total phenolic contents
Total phenolic contents was determined using the Folin-Ciocalteu method. Samples were prepared within varies concentration. 20 μl of each diluted samples mixed with 100 μl of Folin-Ciocalteu reagent and 80 μl of 7.5% sodium carbonate in individual well of 96-well plate. The solutions was mixed and incubated at room temperature for 1 h. The absorbance was measured by the micro-plate reader (BMG LABTECH / SPECTROstar Nano) at 765
Gallic acid was used as standard and expressed as milligrams of gallic acid per gram crude extract (mg GAE/g of crude).

**Determination of total anthocyanin contents**

Total anthocyanin contents was determined using the pH differential method. 20 μl of riceberry extract sample was mixed with 80 μl of potassium chloride buffer solution (pH 1.0) in individual well of 96-well plate, and 20 μl of riceberry extract sample was mixed with 80 μl of sodium acetate buffer solution (pH 4.5). Both solutions were left to equilibrate for 20 min. The absorbance of each solution was measured at 510 nm and 700 nm. The content of total anthocyanin expressed as milligram cyaniding-3-glucoside per gram extract (mg C3G/g extract)

\[
\text{Total anthocyanin} = \frac{(A \times MW \times DF \times 1000)}{(e \times L)}
\]

**ABTS radical scavenging activity**

Prepared ABTS\(^+\) solution by mixed ABTS (7 mM) and potassium persulfate (2.45 mM) with a ratio of 1:1 (v/v) and kept under room temperature for 16 h in darkness. Before use, the solution was diluted with deionized water and measure an absorbance to 0.70 ± 0.02 at 734 nm that was measured by the micro-plate reader. In the assay, each sample (20 μl) was mixed with ABTS\(^+\) solution (180 μl) and incubated at room temperature for 5 min. The absorbance was measured at 734 nm. The ability of the extracts to scavenge ABTS\(^+\) was calculated. The IC\(_{50}\) of ascorbic acid was compared to the IC\(_{50}\) of the extracts.

\[
\% \text{ Inhibition} = \frac{(\text{control absorbance} - \text{extract absorbance})}{\text{control absorbance}}
\]

**FRAP radical scavenging activity**

The stock solutions included 300mM acetate buffer, 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM Iron(III) chloride hexahydrate (FeCl\(_3\)-6H\(_2\)O) solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml Iron(III) chloride hexahydrate (FeCl\(_3\)-6H\(_2\)O). FRAP assay method was performed by added sample solution (10 μl) in FRAP reagent (190 μl), mix homogeneously and solution was incubated at room temperature for 15 minute at dark light. The absorbance was measured at 593 nm. Trolox was used as standard. The reducing power activity was expressed as trolox equivalents (mg TEAC/g extract).

**UV absorbance of rice crude extract**

Riceberry crude extracts were dissolved in 70% ethanol in concentration of 1 mg in 1 ml of 70% ethanol. The UV absorption activity was measured in wavelength range between 220-700 nm.
Stability test
Heating-cooling test was the storage of crude extract in different conditions. The tests were performed on samples kept at 4 °C for 24 hr. and 45 °C for 24 hr. Total anthocyanin content were tested in each cycle until 3 cycles completed.

Statistical analysis
The results were reported as mean ± standard deviation (SD). The significance of difference was determined by ANOVA. Data subjected to independent t tests with P<0.05 were regarded as statistically significant. The analyses were performed in triplicate.

Irritation test
Single closed patch test was used to determine various concentration of crude extract causes allergic inflammation of skin. Finn chamber was used in this study, containing 5 substance (0.1% SLS, DI water, 0.01% crude extract, 0.05% crude extract and 0.1% crude extract). The tests are applied on the upper arm for 24 h, removing the tests and verified of each test.

Mean irritation index (M.I.I.) was used for evaluation criteria of irritation (Dermscan asia, 2011)

$$M.I.I. = \frac{\text{total cutaneous reactions score (erythema+oedema)}}{\text{number of volunteers}}$$

Result and discussion:
1. Optimization of solvent extraction
   1.1 The yield
   The effect of various solvent on the yields, 50:50 ethanol-water presented significantly highest of yield with the value of 3.24% whereas 80:20 ethanol-water was showed the lowest of yield with the value of 2.04% (Table 1). From the above result was showed Riceberry contained with polar molecules.

Table 1 Yield, total phenolic content, total anthocyanin content, radical scavenging activity (ABTS and FRAP) of various solvent extraction

<table>
<thead>
<tr>
<th>Ethanol : Water (v/v)</th>
<th>Yield (%)</th>
<th>TPC (mg GAE/g extract)</th>
<th>TAC (mg C3G/g extract)</th>
<th>ABTS assay IC50 (mg/ml)</th>
<th>FRAP assay (mg TEAC/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 : 50</td>
<td>3.24</td>
<td>49.093±2.378b</td>
<td>33.398 ± 5.475b</td>
<td>0.066 ± 0.003b</td>
<td>944.144 ± 51.941</td>
</tr>
<tr>
<td>60 : 40</td>
<td>2.44</td>
<td>46.481±2.933b</td>
<td>35.903 ± 6.660b</td>
<td>0.063 ± 0.001b</td>
<td>1,526.126 ± 37.966a</td>
</tr>
<tr>
<td>70 : 30</td>
<td>2.52</td>
<td>67.443±1.192a</td>
<td>57.611 ± 6.521a</td>
<td>0.052 ± 0.003a</td>
<td>2,843.243 ± 54.859a</td>
</tr>
<tr>
<td>80 : 20</td>
<td>2.04</td>
<td>46.698±2.914b</td>
<td>18.369 ± 3.010c</td>
<td>0.092 ± 0.003c</td>
<td>1,315.315 ± 36.794a</td>
</tr>
<tr>
<td>90 : 10</td>
<td>2.62</td>
<td>35.399±1.444c</td>
<td>7.236 ± 3.476d</td>
<td>0.114 ± 0.005d</td>
<td>1,036.036 ± 8.257d</td>
</tr>
<tr>
<td>100 : 0</td>
<td>2.24</td>
<td>33.489±1.523c</td>
<td>13.081 ± 2.551c</td>
<td>0.101 ± 0.009c</td>
<td>1,509.910 ± 33.028b</td>
</tr>
</tbody>
</table>

142
* Value are given as mean ± S.D. (n=3)
** Different letters in the same column show significant differences (p-value <0.05)

1.2 Determination of total phenolic content, total anthocyanin content and radical scavenging activity (ABTS and FRAP)

The effect of various solvent on total phenolic content, total anthocyanin content, and radical scavenging activity (ABTS and FRAP) from Riceberry was shown in Table 1. The result showed that the optimum ratio of ethanol:water as 70:30 obtained the highest total phenolic content and total anthocyanin content with value of 67.443 ± 1.916 mgGAE/g extract and 57.611 ± 6.521 mg C3G/g extract, respectively. This study showed the different ratio of ethanol:water obtained different total phenolic content, depend on polarity of solvent and compound (Garcia-Salas et al., 2010). The studied of Sidduraju and Becker (2003) found that highly polar solvent provide high total phenolic content and related with the studied of Nuanpun Nongyao, et al. (2014), the characterize anthocyanin composition in a diverse color rice bran with the ratio of ethanol:water as 70:30. The result of radical scavenging activity (ABTS and FRAP) showed the ratio of ethanol:water as 70:30 provide the highest result with the value of 0.052 ± 0.003 mg/ml and 2,843.243 ± 54.859 mg TEAC/g extract, respectively.

1.3 UV absorbance of rice crude extract

UV absorption patterns of Riceberry extracts are shown in Figure 2. The results indicated that extracts have two absorption peaks that are; the first one is $\lambda = 280-290$ nm and other one is $\lambda = 500-540$ nm. Both of absorption peak were confirmed the extracts are contains anthocyanin content and related with the studied of Qin et al. (2010), anthocyanin content from mulberry was found two absorption peak ($\lambda = 280$ nm and $\lambda = 520$ nm)

![Figure 2 UV-VIS spectrum of Riceberry extracts in various solvent](image)

2. Optimization of extraction time

2.1 Yield

The results showed 6 h of extraction time presented significantly highest of yield with the value of 3.61 and 2 h of extraction time showed the lowest of yield with the value of 2.27 (Table 2.). This result showed extraction time affected to the ability of the yields. If
extraction time was too short, the components didn’t come to extracts solvent (Thu Thao, et al., 2015).

**Table 2** Yield, total phenolic content, total anthocyanin content, radical scavenging activity (ABTS and FRAP) of various extraction time

<table>
<thead>
<tr>
<th>Extraction Time (Hr)</th>
<th>Yield (%)</th>
<th>TPC (mg GAE/g extract)</th>
<th>TAC (mg C3G/g extract)</th>
<th>ABTS assay IC$_{50}$ (mg/ml)</th>
<th>FRAP assay (mg TEAC/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.27</td>
<td>41.041 ± 2.702c</td>
<td>30.058 ± 1.670c</td>
<td>0.014 ± 0.0002a</td>
<td>1,882.883 ± 3.121c</td>
</tr>
<tr>
<td>4</td>
<td>2.95</td>
<td>46.574 ± 2.839b</td>
<td>30.336 ± 4.203c</td>
<td>0.021 ± 0.0002c</td>
<td>2,140.541 ± 16.216b</td>
</tr>
<tr>
<td>6</td>
<td>3.61</td>
<td>53.354 ± 1.373c</td>
<td>42.582 ± 2.892c</td>
<td>0.015 ± 0.0003a</td>
<td>2,765.766 ± 39.844a</td>
</tr>
<tr>
<td>12</td>
<td>2.62</td>
<td>38.773 ± 0.722c</td>
<td>36.459 ± 4.285b</td>
<td>0.016 ± 0.0001b</td>
<td>1,117.117 ± 34.752d</td>
</tr>
</tbody>
</table>

* Value are given as mean ± S.D. (n=3)
** Different letters in the same column show significant differences (p-value <0.05)

2.2 Determination of total phenolic content, total anthocyanin content and radical scavenging activity (ABTS and FRAP)

The effect of extraction time on total phenolic content, total anthocyanin content, and radical scavenging activity (ABTS and FRAP) from Riceberry was shown in table 2. The result showed that the optimum extraction time as 6 h obtained the highest total phenolic content and total anthocyanin content with value of 67.443 ± 1.916 mgGAE/g extract and 42.582 ± 2.892 mg C3G/g extract, resoectively. The extraction time affected to the ability of the plant constituents (Thu Thao, et al., 2015). If the extraction time was too long (12h), some plant constituents will decay. The result of radical scavenging activity (ABTS and FRAP) showed the extraction time as 6 h provide the highest result with the value of 0.015 ± 0.0003 mg/ml and 2,765.766 ± 39.844 mg TEAC/g extract, respectively, which is associated with high value of total phenolic content and total anthocyanin content. Its provide high antioxidant (Sutharut, J. and Sudarat, J., 2012)

2.3 UV absorbance of rice crude extract

UV absorption patterns of Riceberry extracts are shown in figure 3. The results indicated that extracts have two absorption peaks that are; the first one is $\lambda = 280-290$ and other one is $\lambda = 500-540$. Both of absorption peak were confirmed the extracts are contains anthocyanin content and related with the studied of Qin et al. (2010), anthocyanin content from mulberry was found two absorption peak ($\lambda = 280$ and $\lambda = 520$). The extract that provide the highest absorbance was 12 h, next as 6 h. Which it not associated to total anthocyanin content that provide the highest extraction time as 6 h. The factors that concerned could occurred from storage and pH of the extracts. It affected to stability of the extracts.
3. Stability test

The extracts were kept at 4 °C for 24 hr. and 45 °C for 24 hr (1 cycle), total 3 cycles. Total anthocyanin content was tested in each cycle, including before heating – cooling test. The results showed in table 3. After 1st cycle, total anthocyanin content was decreased that compared with before heating – cooling test. It shown the temperature affected to chemical structure and breakdown of anthocyanin (Mercadante et al., 2008). And if compared between 1st, 2nd and 3rd cycle, shown not significant change from 1st to 3rd cycle. This result was related with the studied of Mori, K., et al. (2007), they studied the effect of temperature to anthocyanin extract from graph.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Total anthocyanin content (mg C3G/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.748 ± 0.301&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>2.224 ± 0.243&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>2.458 ± 0.034&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>2.371 ± 0.188&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Value are given as mean ± S.D. (n=3)
** Different letters in the same column show significant differences (p-value <0.05)

4. Irritation test

Single closed patch test was used for irritation test. In this study, dropped 5 substance (0.1% SLS, DI water, 0.01% crude extract, 0.05% crude extract and 0.1% crude extract) in to finn chamber. The tests are applied on the upper arm of 25 volunteers for 24 h, removing the tests and verified of each test. The results shown non-irritation of all volunteers and evaluation criteria of irritation with M.I.I (Dermscan asia, 2011), shown non-irritation with the tests

Conclusions:
The optimization of solvent extract in this study was ratio of ethanol:water as 70:30 obtained the highest total phenolic content, total anthocyanin content and radical scavenging activity
(ABTS and FRAP) with value of 67.443±1.916 mg GAE/g, 57.611 ± 6.521 mg C3G/g extract, 0.052 ± 0.003 mg/ml and 2,843.243 ± 54.859 mg TEAC/g extract, respectively. And the optimization of extraction time as 6 h that provided the highest total phenolic content, total anthocyanin content and radical scavenging activity (ABTS and FRAP) with value of 67.443 ± 1.916 mgGAE/g extract, 42.582 ± 2.892 mg C3G/g extract, 0.015 ± 0.0003 mg/ml and 2,765.766 ± 39.844 mg TEAC/g extract, respectively. The results of stability of crude extract was showed the temperature affected to stability of the extracts. The result of irritation test showed non-irritation of all volunteers. In the future, it can apply to use in cosmetic industry.

Reference

10. Sutharut, J. & Sudarat, J. (2557). Total anthocyanin content and antioxidant activity of germinated colored rice