

# การเปรียบเทียบตัวทำละลายอินทรีย์สำหรับการสกัดสารประกอบฟีนอลิกจากรำข้าว

## Comparison of Different Solvents for Extraction of Phenolic Compounds from Rice Bran

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### บทคัดย่อ

ผลของตัวทำละลาย 6 ชนิด (เมทานอล, เอทานอล, เอ็น-เฮกเซน, เอทิลอะซิเตท, ตัวทำละลายผสมของเอทานอลและเฮกเซน (เอทานอล: เฮกเซน, 1:1 ปริมาตรต่อปริมาตร) และตัวทำละลายผสมของเอทิลอะซิเตท:เฮกเซน (เอทิลอะซิเตท: เฮกเซน, 1:1 ปริมาตรต่อปริมาตร) ต่อปริมาณฟีนอลิก, ปริมาณฟลาโวนอยด์ การต้านอนุมูลอิสระและคุณสมบัติรีดิวซ์ของสารสกัดจากรำข้าวเจ๊กเซยและเหลืองทองได้ถูกทำการศึกษา รำข้าวสกัดจากเมทานอลมีปริมาณฟีนอลิก (2.80 มิลลิกรัมต่อกรัมน้ำหนักแห้งของรำข้าวสายพันธุ์เจ๊กเซย และ 2.47 มิลลิกรัมต่อกรัมน้ำหนักแห้งของรำข้าวสายพันธุ์เหลืองทอง) และฟลาโวนอยด์ (1.34 มิลลิกรัมต่อกรัมน้ำหนักแห้งของรำข้าวสายพันธุ์เจ๊กเซย และ 1.31 มิลลิกรัมต่อกรัมน้ำหนักแห้งของรำข้าวสายพันธุ์เหลืองทอง) ในปริมาณสูง และสารละลายสกัดยังมีคุณสมบัติรีดิวซ์ และการต้านอนุมูลอิสระของ DPPH สูง (SC<sub>50</sub> เท่ากับ 673.69 มิลลิกรัมต่อลิตร รำข้าวสายพันธุ์เจ๊กเซย และ 815.02 มิลลิกรัมต่อลิตร สำหรับรำข้าวสายพันธุ์เหลืองทอง) โดยผลการศึกษาพบว่าปริมาณสารสกัดโดยเอทานอลมีปริมาณสูงกว่าสารละลายอินทรีย์ชนิดอื่น ผลการศึกษานี้แสดงว่ามีสารฟลูเคมิและคุณสมบัติการต้านอนุมูลอิสระและคุณสมบัติการรีดิวซ์ต่างกันในสารละลายแต่ละชนิด สารสกัดเมทานอลมีปริมาณสารประกอบฟีนอลิก และฟลาโวนอยด์ และคุณสมบัติการต้านอนุมูลอิสระและคุณสมบัติการรีดิวซ์สูงกว่าสารละลายสกัดชนิดอื่น

**คำสำคัญ:** การสกัด, สารฟีนอลิก, ฟลาโวนอยด์, การต้านอนุมูลอิสระ, รำข้าว

### Abstract

The effects of six extracting solvents [methanol, ethanol, n-hexane, ethyl acetate, mixed solvent of ethanol and hexane (ethanol:hexane, 1:1 v/v) and mixed solvent of ethyl acetate and hexane (ethyl acetate:hexane, 1:1 v/v) on the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and reducing power of extracts of rice brans of *Oryza sativa* L. cv. Jekchuey and cv. Leuangthong were investigated. The methanolic rice bran extract contained appreciable amounts of TPC (2.80 mg/g of DW for Jekchuey rice bran

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and 2.47 mg/g of DW for Leuangthong rice bran), TFC (1.34 mg/g of DW for Jekchuey rice bran and 1.31 mg/g of DW for Leuangthong rice bran) and it also contained high reducing power and DPPH scavenging capacity ( $SC_{50}$  of 673.69 mg/L for Jekchuey rice bran and 815.02 mg/L for Leuangthong rice bran). Generally higher extract yields was obtained using ethanol, while phenolic contents and rice bran antioxidant activity were obtained using methanol, as compared to the respective the other organic solvents. These results indicate that there are differences in phytochemical content and antioxidant activity and reducing power among the different solvent extract. Methanolic extract has higher content of phenolics and flavonoids, and has higher antioxidant activity and reducing power when compared to the other solvent extracts.

**Keywords:** extraction, phenolic, flavonoid, antioxidant, rice bran

## Introduction

Today's plant secondary metabolites are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. During the extraction of plant material, it is important to minimize interference from compounds that may co-extract with the chemicals, and to avoid contamination of the extract, as well as to prevent decomposition of metabolites or artifact formation as a result of extraction conditions or solvent impurities. The technique of phenolic components isolation from a plant material, including the methods and type of extracting solvent, depends generally on the type of phenolic compound and the solvents.<sup>1</sup> Results of extraction yield of phenolic and flavonoid content is greatly depending on the solvent polarity.<sup>2,3</sup> Recovery of antioxidant compounds from plant materials is typically accomplished through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. Solvent extraction is most frequently used technique for isolation of plant antioxidant compounds. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence

of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix.

Rice bran is a source of natural, which is the predominant byproduct of rice bran and a good source of insoluble dietary fiber, protein, phytic acid, inositol and vitamin B.<sup>4</sup> The antioxidant potential of rice bran was also determined,<sup>5,6</sup> but it still remains a relatively unexplored source material, which demands further investigation especially with regards to its phytochemical composition related to possible health benefits as antioxidants. It can be concluded that it is not clear which type of solvent is more effective for extracting total phenolics and flavonoids of rice and evaluating the antioxidant activity of them. On the other hand, little is known about phenolic and flavonoid contents and antioxidant activity of rice bran by different solvents with different polarity. Therefore, the objectives of this research were to investigate the effect of different extracting solvents with different polarity on total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity [1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay] and reducing power of two varieties of Thai rice (*Oryza sativa* L.) brans.

## Methodology

### Rice sample

Twenty kilograms of the paddy rice cv. Jekchuey and Leuangthong were purchased from local market, Saraburi province, Thailand. They were dehusked on a Satake Rice Machine. Rice bran was obtained by polishing using a Grainman polisher model 60-115-60-2AT for 90% milling

### Moisture content determination

The method uses weight measurements. Rice bran with a certain moisture content was weighed, then the water was evaporated from the sample using hot air oven at 105°C until no weight change was observed anymore, and the remaining dry matter was weighed again. The moisture content was then calculated.

### Percentage of rice bran

Rice bran is weighed to calculate the % rice bran was derived following Eq. :

$$\% \text{ rice bran (w/w)} = \frac{\text{Weight of rice bran}}{\text{Weight of paddy rice}} \times 100$$

### Extraction of rice bran samples

Ten grams of rice bran were extracted with 50 mL of methanol (MeOH), ethanol (EtOH), n-hexane, ethyl acetate (EtOAc) ethanol:n-hexane (1:1) and ethyl acetate:n-hexane (1:1) at room temperature for 24 h in an electrical shaker. The extract was filtered through the paper filter Whatman no. 1 connected with a vacuum pump. The precipitates were re-extracted by the same process for 3 times. All filtrates were collected, pooled and dried by a rotary evaporator (Rotavapor R210, Buchi, Switzerland) at 50 °C. The dried extracts were kept at -4 °C until use.

### Total phenolic content (TPC)

The total phenolic content was determined using a modified Folin–Ciocalteu method.<sup>7</sup> Each test sample (250 µL) was added to a test tube that contained 6.0 mL of distilled water. After vortexing 500 µL of Folin–Ciocalteu's phenol reagent (Fluka) was added. The tubes were vortexed for 2 min, 2.0 mL of 15% Na<sub>2</sub>CO<sub>3</sub> was added each tube and 1.25 mL of distilled water was added to each tube. The tubes were vortexed again and then allowed to stand for 2 h at room temperature. Thereafter, the absorbance of each sample was measured against a blank at 750 nm using a spectrophotometer. A calibration curve was constructed using 50, 100, 150, 200 and 250 mg/L gallic acid (Fluka) as a standard. The total phenolic content is expressed as milligrams of gallic acid per gram.

### Determination of total flavonoid content

Total flavonoid content was determined using aluminium chloride (AlCl<sub>3</sub>) according to the method of Zhishen *et al.*<sup>8</sup> (1999) using quercetin as a standard. The rice bran extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% NaNO<sub>2</sub> (0.03 ml). After 5 min at 25°C, AlCl<sub>3</sub> (0.03 ml, 10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as mg quercetin (QE)/g bran.

### DPPH assay

The free radical scavenging activity of the extractions was measured by the DPPH (1-1 Diphenyl-2-picryl hydrazyl).<sup>9</sup> 2.0 ML of 2.5 x 10<sup>-4</sup> M DPPH was added to 2.0 mL of rice bran

extracts obtained from paddy rice stored in different conditions. The mixture was shaken and left in the dark for 30 min. Then, the absorbance was measured at 517 nm by spectrophotometer. The DPPH radical-scavenging activity was calculated according to the following: % of DPPH scavenging activity =  $\{1 - (AbS/AbC)\} \times 100$ , where AbC was the absorbance of the control and AbS was the absorbance in the presence of the test compound.  $SC_{50}$  is the effective concentration in mg extract/mL which inhibits the DPPH activity by 50%. 1,1-diphenyl-2-picrylhydrazyl, butylated hydroxytoluene (BHT) was used for comparison.

#### **Reducing power of rice brans by ferricyanide test**

The reducing power of each extract was determined by the method of Oyaizu<sup>10</sup> with some modifications as follows: An aliquot (20  $\mu$ L) of the extracts was diluted to a desired final concentration (0.16–3.3 mg/mL of rice bran) with 0.7 mL of 50 mM phosphate buffer (pH 6.6). The dilute sample was then mixed with 0.5 ml of 1% potassium ferricyanide  $[K_3Fe(CN)_6]$  and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (10%; 0.5 ml) was added to the mixture, which was then centrifuged at 1000g for 10 min. Aliquots (0.5 mL) from the upper layer of the solution were mixed with the same 0.5 ml volume of deionized water and 0.1%  $FeCl_3$  solution (0.1 mL). The absorbance ( $\lambda_{max} = 700$  nm) was then measured using a UV/VIS spectrophotometer.

### **Results and Discussion**

Moisture content calculated from the weight of water contained in rice bran expressed in percent and percentage of rice bran after polishing were

shown in Table 1. Both rice cultivar had almost the same percentage of rice bran and moisture content.

#### **Effects of extracting solvent on the extracts yields from rice brans**

Amounts (mg/g of dried rice bran) of the total phenolics, flavonoids and antioxidant extract determined, using six different solvents (methanol, ethanol, n-hexane, ethyl acetate, mixed solvent of ethanol and hexane (ethanol:hexane, 1:1 v/v) and mixed solvent of ethanol and hexane (ethyl acetate:hexane, 1:1 v/v) by maceration extracting with shaking techniques are shown in Table 1.

Our findings reported that maximum extract yield (mg/g) from rice bran was obtained with methanol. The differences in the extract yields from the rice brans in the present analysis might be ascribed to the different availability of extractable components, resulting from the varied chemical composition of rice brans.<sup>11</sup> The amount of the components that can be extracted from rice bran is mainly affected by the solubility of the extraction procedure, which may probably vary from rice bran cultivar. Efficiency of the extracting solvent to dissolve endogenous compounds might also be very important.<sup>12,13</sup> For the effectiveness of extracting technique, the results showed that yields of the extract were better when extraction was done material and solvent used. This indicated that ethanol was more efficient for the recovery of components, thus offering higher extract yields.

#### **Effects of extracting solvent on the total phenolic contents of rice brans**

Total phenolic contents (TPC) of rice bran, using six solvent systems: (methanol, ethanol, n-

hexane, ethyl acetate, mixed solvent of ethanol and hexane (ethanol:hexane, 1:1 v/v) and mixed solvent of ethanol and hexane (ethyl acetate:hexane, 1:1 v/v). are presented in Table 2.

Among the different solvent systems, methanolic extract offered the highest TPC (2.80 mg/g of DW for Jekchuey rice bran and 2.47 mg/g of DW for Leuangthong rice bran), followed by ethyl acetate, ethanol:hexane (1:1) ethanol, ethyl acetate:hexane (1:1), n-hexane. Results of the present study showed that among all the solvent extracts; the methanol extracts had the highest TPC. This may be due to the fact that phenolics are often extracted in higher amounts in more polar.<sup>14</sup>

In contrast to the trends noted for extraction yields, the TPC of all rice bran extracted using solvent extraction technique decreased, regardless of the nature of the extracting solvent used. The decrease in the amounts of TP extracts, prepared might have been due to the solubility of some phenolic for extraction.

The results showed that maceration extracting with shaking techniques attributed to an effective extraction conditions leading to higher release of some bound phenolics.

#### **Effects of extracting solvent on the total flavonoids of rice brans**

Total flavonoid contents (TFC) of rice brans, extracted with six different solvent systems, using shaking extracting techniques, are given in Table 1. TFC were determined as gallic acid equivalents (GAE). Among different solvent methanolic extracts offered the highest TFC (1.34 mg/g of DW for Jekchuey rice bran and 1.31 mg/g of DW for Leuangthong rice bran) followed by ethanol, ethanol:hexane (1:1), ethyl acetate, ethyl

acetate:hexane (1:1), n-hexane. Amount of TF in all extracts generally decreased when hydrophobicity of solvent extract increase.

#### **Effects of extracting solvent/technique on the DPPH Scavenging activity (SC<sub>50</sub>)**

DPPH scavenging activity of rice bran as affected by extracting solvents is depicted in Table 1.

A lower of SC<sub>50</sub> DPPH scavenging is correlated to a higher antioxidant activity.<sup>15</sup> The extracts of all the extracts possessed SC<sub>50</sub> free radical scavenging properties, but to varying degrees, ranging from 673.69 to 2818.24 mg/L. A maximum scavenging activity was offered by methanol, followed by ethanol, ethyl acetate:hexane (1:1), ethanol:hexane (1:1), ethyl acetate and n-hexane. It has well established that free radical scavenging activity of rice bran is mainly due to phenolic compounds. This reduction in the radical scavenging activity of the extracts, obtained by the solvent extraction technique might be ascribed to the decomposition of phenolics.<sup>16</sup>

#### **Effects of extracting solvent/technique on the reducing power of rice brans**

The results showing the effects of extracting solvent/techniques on the reducing potential of extracts of rice brans at concentration of 50-1000 mg/L, are shown in Fig. 1. The reducing power of the extracts increased in a concentration dependent manner. The values of absorbance for the rice bran extract solutions at concentration of 50-1000 mg/L determined in this assay, ranged from 0.048 to 0.548 and followed the order of effectiveness as: methanol > ethanol > n-hexane > ethyl acetate:hexane (1:1). In general, the organic solvent extracts of the rice bran, exhibiting

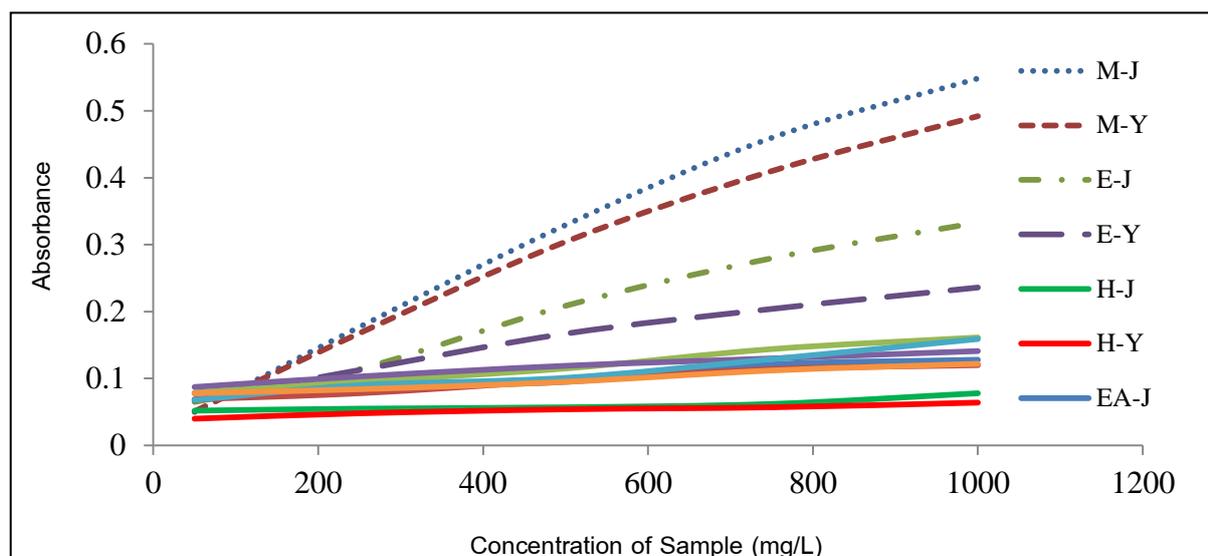
**Table 1** Comparison of rice bran yield (w/w) and the moisture content from the paddy milling process of Jekchuey and Leuangthong rice

Rice variety	Rice bran yield (g/g paddy rice)	Moisture content (g/g rice bran)
Jekchuey	3.80	10.08
Leuangthong	3.73	11.07

**Table 2** TPC, TFC and 50% radical scavenging concentration (SC<sub>50</sub>) of Jekchuey and Leuangthong rice bran by various solvent extraction

Sample		% yield (w/w)	TPC <sup>a</sup> (mg/g)	TFC <sup>b</sup> (mg/g)	SC <sub>50</sub> <sup>c</sup> (mg/l)
BHT <sup>d</sup>					27.30
Rice variety	Solvents				
Jekchuey	Methanol	13.20	2.80	1.34	673.69
	Ethanol	22.93	1.35	0.64	2427.79
	n-Hexane	20.57	0.86	0.14	2818.24
	Ethyl acetate	21.61	1.48	0.34	2593.57
	Ethanol:Hexane (1:1)	19.49	1.42	0.39	2463.33
	Ethyl acetate:Hexane (1:1)	20.72	0.94	0.32	2429.84
Leuangthong	Methanol	13.41	2.47	1.31	815.02
	Ethanol	25.56	1.34	0.62	2285.90
	n-Hexane	21.51	0.81	0.12	2782.38
	Ethyl acetate	22.75	1.40	0.32	2310.63
	Ethanol:Hexane (1:1)	20.77	1.40	0.39	2433.33
	Ethyl acetate:Hexane (1:1)	22.11	0.91	0.31	2566.33

<sup>a</sup> TPC = total polyphenol contents (mg/g)<sup>b</sup> TFC = total flavonoid contents (mg/g)<sup>c</sup> SC<sub>50</sub> = 50% radical scavenging concentration (mg/L)<sup>d</sup> BHT = butylated hydroxytoluene



**Figure 1** Reducing power of the rice bran extracts of rice bran cv. Jekchuey and cv. Leuangthong by various solvents (Consonant in front of the dash is various solvents : M = Methanol, E = Ethanol, H = Hexane, EA = Ethyl acetate, ET = Ethanol:Hexane (1:1) and EH = Ethyl acetate:Hexane (1:1). For, consonant in back of the dash is species of rice bran: J = Jekchuey, Y = Lueangthong).

greater TPC, also depicted good reducing power in the present analysis. The reducing potential of antioxidant components is very much associated with their TPC. The rice bran extracts with higher levels of total phenolics also exhibit greater reducing power.<sup>16</sup> As far as the effects of extraction techniques on the antioxidant activity is concerned, However, each material tested retained the same efficacy order as displayed in the case of maceration extracting shaking techniques.

## Conclusions

The results of the present investigation revealed that solvent extracts of rice bran, prepared by the shaking extraction techniques, exhibited better antioxidant activities and higher phenolic contents. Moreover, higher antioxidant extracts yields from the rice bran were obtained using extraction technique by shaking, with different solvent

system used. Contrarily to extraction yield, TPC and antioxidant activities of the rice bran decreased when these were extracted using the high hydrophobic solvent. The present data would certainly help to ascertain the potency of the rice bran as potential source of natural antioxidants to be used for nutraceutical and functional food applications. However, further research is needed to identify individual components forming antioxidative system and develop their applications for food and pharmaceutical industries.

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