

# Phytochemicals and Antioxidant Activity of Thai Rice Flowers

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## Abstract

The purpose of this study was to determine the phytochemicals (total phenolic, flavonoid and anthocyanin contents) and antioxidant activity of 7 Thai rice flowers cultivar: 2 white rice, 2 red rice and 3 black rice. The total phenolic, flavonoid and anthocyanin contents were measured by using Folin-Ciocalteu, colorimetric aluminum chloride and pH differential methods, respectively. The antioxidant activities were determined by ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assays. The results of the phytochemical screening of Thai rice flower cultivars revealed the total phenolic and flavonoid contents in all the extracts, but anthocyanin was no detectable in this rice flowers. The total phenolic and flavonoid contents were in the range of 1225.73-2160.43 mg GAE/100g of sample and 1121.96-1803.19 mg quercetin/100 g of sample, respectively. The antioxidant activity by DPPH and FRAP assay were in the range of 1.060-6.928 ppm (IC<sub>50</sub>) and 11203.01-983.71 mM Fe(II)/100 g dry weight, respectively. The rice flower extract from white rice (Jasmine 105) had significantly higher phytochemicals and antioxidant activity than the extracts from other rice flowers. The results suggested that the Jasmine 105 rice flowers might indeed be potential sources for future novel antioxidants in food and pharmaceutical formulations.

**Keywords:** Phytochemicals, Antioxidant Activity, Thai Rice Flowers

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## Introduction

Rice is cereal which its scientific name is *Oryza sativa* L. It is in a family of Poaceae. Rice grown, in Thailand is Indica. It is an important food of the world. There are different types of rice that contain color pigments, such as purple rice, black rice and red rice. The pigmented rice varieties (red, black or purple) are traditionally known to have health benefits and are particularly valued in local markets. Recently, Thai pigmented rice varieties have been increasingly popular and demand higher prices in the Asian rice market (Ahuja et al., 2007). Many studies have reported that pigmented rice contains higher amount of phytochemicals and possesses higher antioxidant activity than white rice (Zhang et al., 2010; Yodmanee et al., 2011; Ti et al., 2014. Su et al., 2004) In addition, a significant positive correlation between the pigmented rice extract and their antioxidant activity has been observed (Hu et al., 2003; Ling et al., 2001). Generally, it has been reported that most of these pigments serve vital functions for plants, but they could also benefit human health in two meaningful ways. Their important bioactivities include free-radical scavenging enhancement of the immune system and protection against heart disease, cardiovascular disease, glycemic control, diabetes, and cancer (Pedro et al., 2016; Ti et al., 2014; Gunaratne et al., 2013; Okarter et al., 2010; Rattanachitthawat et al., 2010; Chiang et al., 2006). Pigmented rice is, thus, anticipated to possess a greater functional dietary potential than white rice (Nam et al., 2005). In addition colored rices are important and rich sources of natural antioxidants, such as phenolics, flavonoids, anthocyanins,  $\beta$ -carotene, vitamin E and  $\gamma$ -oryzanol (Goufo & Trindade, 2014; Butsat & Siriamornpun, 2010; Artht et al., 2009; Carlos et al., 2007; Abdel-Aal et al., 2006). Many studies have reported the antioxidant activity and phytochemical compounds in each part of rice and rice product such as seed, bran, root, tillering, panicle initiation, booting, milking, maturation stage, the four stages of development after flowering and at maturity, bran powder, germinated brown rice and fermented germinated brown rice, etc. (Er et al., 2017; Inket & Phugan, 2017; Hiran et al., 2015; Yafang et al., 2014; Kerdchoechuen et al., 2013; Krasaetep et al., 2011) There are research studies on flowers that have high phytochemical and antioxidant activity (Mlcek & Rop, 2011; Onanong et al., 2011; Li et al., 2014; Xinfeng et al., 2017). However, there are no studies that have reported the phytochemical and antioxidant activity of rice flower. The rice flower refers to the stamens and pistils used for breeding and consists of two outer sheaths that wrap the inner part. The outer shell is

called the lemma, while the small outer shell is called the palea. In each flower rice, there are 6 stamens and a single pistil, which consists of two stigma-like stigma receptors. Each ovary has a style attached to it. When pollinated, it becomes a seed that is a good source of nutrients. Thus, rice flower should be a large source of other nutrients and could be used as an easily accessible source of natural antioxidants for potential use in cosmetic, food supplement or in the pharmaceutical industries.

## Objective

This research aimed to study the antioxidant activity and phytochemical composition of Thai rice flower cultivar extracts (using different methods of extraction). In addition, this study provides a basis for future studies of rice flower cultivars.

## Materials and methods

### 1. Instrumentation

Absorbance was measured in 1 cm quartz cuvettes using an ultraviolet-visible spectrophotometer (Shimadzu, Japan)

### 2. Material

All chemicals used were analytical grade reagents. Chemical suppliers were as follows: Folin-Ciocalteu reagent, 2, 2-diphenyl-1-picrylhydrazyl, gallic acid, sodium carbonate, , ethanol, sodium nitrite, aluminium chloride, cyaniding-3-glucoside, butylated hydroxyanisole, ferric chloride and all solvents (HPLC grade) were purchased from Fluka (Switzerland).

### 3. Methods

#### 3.1 Plant Materials

Seeds of the seven flowers of Thai rice cultivars were collected in December 2015 from the Chumphae Rice Research Center, Khonkhen province, Thailand. The varieties were white rice (Jasmine 105, Goh-koh 6), red rice (Taptim-Chumphae rice), and black rice (Entry 1, Entry 2, SRN2007.NO.8). The seeds of the seven rice cultivars were grown in a field managed by the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham province, Thailand, in February 2016.

### 3.2 Extraction

The flowers of the rice cultivars from the field were washed with distilled water, then cut into small pieces and dried in an oven at 25 °C before being ground to a fine powder in a mechanical blender. Dried sample powder (5.0 g) was extracted by 95% ethanol containing 1.0% HCl (3×100 mL) for 60 min with intermittent shaking at room temperature. The extracts were combined and filtered through a whatman filter membrane no.1. After that, the extracts were slowly concentrated under reduced pressure at temperature below 40 °C in a rotary evaporator to yield the crude flower extracts. The crude flower extracts were stored at 4 °C in storage vials for the determination of phytochemicals and antioxidant activity.

### 3.3 Phytochemical reference

Phytochemical references of seed extracts from Thai red rice were carried out on the three extracts, total phenolic flavonoids and anthocyanin contents.

### 3.4 Total phenolic contents

The total phenolic contents of the crude Thai rice flowers extracts were determined by a spectrophotometric method using Folin–Ciocalteu’s phenol reagent, as described earlier, with some modifications by Krasaetep (2012). The crude extract (0.5 mL) was diluted to 5.0 mL with distilled water. Folin–Ciocalteu’s reagent (5.0 mL) was added, and mixed thoroughly. After 15.0 min, 5 mL of 10% sodium carbonate solution was added, and the mixture was allowed to stand for 1 h with intermittent shaking. The mixture solution was measured at 750 nm, in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada). The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per 100 g of sample.

### 3.5 Total flavonoid contents

The total flavonoid contents of the crude extract of Thai rice flowers were determined by a spectrophotometric method using the aluminium chloride colorimetric method, as described earlier with some modifications by Krasaetep (2012). 250 µL Crude extracts were mixed with 1.25 mL of distilled water and 75.0 µL of 5% NaNO<sub>2</sub> solution and shaken well. After 5 minutes, 150 µL of 10% aluminum chloride (AlCl<sub>3</sub>) was added. The mixture was allowed to stand for 6 min. Then, 500 µL of 1 M NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water.

The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada). The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per 100 g of sample.

### 3.6 Total anthocyanin contents

The total anthocyanin content (TAC) was determined by the pH-differential method (Giusti & Wrolstad, 2001). 1 mL of crude flower extract solutions were pipetted into 10 mL volumetric flasks in order to prepare two dilutions of each sample; in one sample the volume was adjusted with a 0.025 M potassium chloride buffer, pH 1.0, and in the other a 0.4 M sodium acetate buffer, pH 4.5, was the diluent. These dilutions were allowed to equilibrate for 15 min. The absorbance of each dilution was measured at 510 and 700 nm, against a blank cell filled with ethanol. The results were expressed as micrograms of cyanidin 3-glucoside equivalents (Cy3-GE)/ 100 g of dry weight. The anthocyanin content was calculated as follows:

$$\text{The total anthocyanin content (mg/L)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times l)$$

$$\text{Where } A = (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5}$$

MW = molecular mass cyaniding-3-glucoside (449.2)

DF = dilution factor

$\epsilon$  = molar absorptivity of cyanidine-3-glucoside (26,900)

### 3.7 Antioxidant activities

#### 3.7.1 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The radical scavenging activity of crude flower extracts from Thai rice was measured using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described earlier, with some modifications by Krasaetep (2012). 1 mL of the crude extract (0.01-10.0 mg/mL) was mixed with 3.0 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol. The mixture was shaken vigorously and was left to stand for 30 min at room temperature in the dark. The absorbance was measured at 517 nm, in a Shimadzu UV-2101PC Spectrophotometer (Bio-Tek Instrument INC, Canada). The control reaction contained all reagents except for the crude samples. The radical scavenging effect was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [(A_c - A_s)/A_c] \times 100,$$

where  $A_c$  is the absorbance of the control at 517 nm, and  $A_s$  is the absorbance of the extract/standard at 517nm.

### 3.7.2 Ferric Reducing Antioxidant Power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was performed according to the methods, with slight modification, proposed by Xiao et al. (2015). The FRAP reagent was freshly prepared by adding 10 mM of 2,4,6-Tris (2-pyridyl)-1,3,5-triazine (TPTZ) (dissolved in 40 mM of HCl), 20 mM of FeCl<sub>3</sub> in water and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. 100  $\mu$ L crude extracts were mixed with 3 mL FRAP reagent, and mixed with 300  $\mu$ L of distilled water. Both samples and blank were incubated for 4 minutes at 37°C. The absorbance was measured at 593 nm, in a Shimadzu UV-2101PC spectrophotometer (Bio-Tek Instrument INC, Canada). The values obtained were expressed as  $\mu$ M of ferrous equivalent Fe (II) per 100 gram of dry weight.

### 3.8 Statistical analysis

Results obtained were reported as mean  $\pm$  SD of triplicate measurements. One-way ANOVA and significant differences for multiple comparisons were determined by Turkey's Honest Significant Difference (HSD) at 0.05 probability level.

## Results and Discussion

The phytochemical reference of Thai rice flower cultivars revealed the flavonoids and phenolics were present in all extracts; anthocyanin was undetectable in any of the samples. It was suggested that rice flower lacks anthocyanin compounds responsible for red, purple and blue pigmentation. There are no studies that have reported phytochemical and antioxidant activity of rice flower. Many studies have been carried out on the nutritional composition of flowers, but most of them reported that the content of common characteristics, such as antioxidant activity, and phytochemical compounds, are not different from other plant organs (Xinfeng et al., 2017; An-Na et al., 2014; Mlcek & Rop, 2011). It has been reported that, from a nutritional point of view, flowers can be divided in pollen, nectar, petals and other parts. Pollen is a source of proteins and carbohydrates, saturated and unsaturated lipids, carotenoids and flavonoids. Nectar contains a balanced mixture of sugars (fructose, glucose and sucrose), together with free amino acids, proteins,

inorganic ions, lipids, organic acids, phenolic substances, alkaloids and terpenoids, among others. Petals and other parts of flowers are rich in vitamins, minerals and antioxidants (Mlcek & Rop, 2011).

### 1. Total Phenolic Compounds

Total phenolic content (TPC) of rice flower extract was measured by the Folin-Ciocalteu reagent method using gallic acid as the standard. A linear calibration curve of gallic acid was used with a linear regression equation for the calibration curve ( $Y=2.8549X+0.10$ ,  $R^2=0.999$ ). The average quantity of the total phenolic compounds found in the rice flower extracts are shown in Table 1. The amount of total phenolic contents of the rice flower extracts were in the range of  $1225.73\pm 81.66 - 2160.43\pm 325.39$  mg GAE/100g of sample. Among the rice flower extracts, the extract from white rice presented the highest amount of TPC (Jasmine 105). Generally, phenolic compounds are the main constituents in plants and characterized by having at least one aromatic ring with one or more hydroxyl groups attached.

### 2. Total Flavonoid Compounds

Total flavonoid content of rice flower extract was determined by the aluminum chloride colorimetric method, using quercetin as the standard. A linear calibration curve of quercetin was used with a linear regression equation of calibration curve ( $Y=2.039X+0.048$ ,  $R^2=0.995$ ). The average quantity of the total flavonoids compounds found in the rice flower extracts are shown in Table 1. The flavonoids are polyphenolic, which are the most numerous of the phenolics, and are found throughout the plant kingdom. The amount of total flavonoids of the rice flower extracts were in the range of  $1121.96\pm 156.39 - 1803.19\pm 159.24$  mg quercetin/100 g of sample, with white rice (Jasmine 105) presenting the highest amount of TFC. It is suggested that the pollen grains of rice flower contain a number of large nutritional compounds such as chlorophylls, riboflavin, carotenoids, and polyphenol.

### 3. Total anthocyanin contents

The total anthocyanin content (TAC) was determined by the pH-differential method using cyanidin 3-glucoside as the standard. A linear calibration curve of cyanidin 3-glucoside was used with a linear regression equation for the calibration curve ( $Y=1.416X+0.92$ ,  $R^2=0.991$ ). Anthocyanin was not found in the rice flower extracts. Anthocyanins are phenolic compounds that contribute to the red, blue, or purple coloration. It is suggested that during the flowering stage of rice there is active anthocyanin production.

**Table 1** The total phenolic, flavonoids and anthocyanin contents of Thai rice flower cultivars

Rice flower cultivars	TPC (mg GAE/100g of sample)	TFC (mg Quercetin/100 g of sample)	TAC (mg Cyanidin-3-glucoside/100g of sample)
white rice (Jasmine 105)	2160.43 ± 325.39 <sup>a</sup>	1803.19±159.24 <sup>b</sup>	nd
white rice (Goh-koh 6)	1565.82 ± 501.95 <sup>a</sup>	17950.72±80.87 <sup>b</sup>	nd
red rice (Taptim-Chumphae rice)	1269.67 ± 430.53 <sup>a</sup>	1648.03±74.51 <sup>b</sup>	nd
black rice (Entry 1)	1334.97 ± 565.74 <sup>a</sup>	1511.11±72.41 <sup>b</sup>	nd
black rice (Entry 2)	1316.04 ± 430.53 <sup>a</sup>	1416.47±173.08 <sup>a</sup>	nd
black rice (SRN2007.NO.8)	1225.73 ± 81.66 <sup>b</sup>	1121.96±156.39 <sup>a</sup>	nd

Remark: nd (not detected): the total anthocyanin contents were not found

#### 4. Antioxidant Activities

The antioxidant activity of the flower extracts from Thai rice cultivars were measured by DPPH-radical-scavenging and FRAP assay. The average quantity of the DPPH value in the rice flower extracts are shown in Table 2. The radical-scavenging activity of each rice flower extract was measured by using the DPPH assay. When the DPPH radical is scavenged by an antioxidant through the donation of H<sup>•</sup> to form the reduced DPPH-H, the color changes from purple to yellow. For the DPPH radical-scavenging assay, the concentrations of BHA and rice flower extract for which 50% of the DPPH radicals were scavenged (IC<sub>50</sub>) were reported - the lower the IC<sub>50</sub>, the higher the antioxidant activity. BHA was used as a reference. The order of the IC<sub>50</sub> values was BHA < white rice (Jasmine 105) < red rice (Taptim-Chumphae rice) < black rice (SRN2007.NO.8) < black rice (Entry 2) < black rice (Entry 1) < white rice (Goh-koh 6). The white rice (Jasmine 105) showed the highest activity (IC<sub>50</sub> = 1.05 ± 0.45 ppm), and the white rice (Goh-koh 6) showed the lowest (IC<sub>50</sub> = 10.67 ± 2.84 ppm). A possible mechanism is that the radical-scavenging effects of rice flower might be due to the hydroxyl groups in the polyphenol compounds of the extract, which promote chain reactions during the reducing system. The average ferric reducing antioxidant power (FRAP) values of the rice flower extracts are shown in Table 2. The FRAP method measures the reduction of ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>) in the presence of antioxidants. The order of the reducing ability of the extracts is white rice



(Jasmine 105) > black rice (Entry 1) > red rice (Taptim-Chumphae rice > black rice (SRN2007.NO.8) > white rice (Goh-koh 6) > black rice (Entry 2). The white rice (Jasmine 105) showed the highest FRAP value ( $11203.01 \pm 3178.14$  mM Fe (II)/100g of DW), and the black rice (Entry 2) showed the lowest ( $983.71 \pm 75.97$  mM Fe (II)/100g of DW).

**Table 2** The antioxidant activity of the flower extracts from Thai rice cultivars were measured by DPPH-radical-scavenging and FRAP assay

Rice flower cultivars	Radical-scavenging activity by DPPH assay (IC <sub>50</sub> )	The FRAP value (mM Fe (II)/100g of DW)
white rice (Jasmine 105)	$1.05 \pm 0.086^b$	$11203.01 \pm 3178.14^a$
white rice (Goh-koh 6)	$10.67 \pm 2.84^g$	$1002.51 \pm 912.06^b$
red rice (Taptim-Chumphae rice)	$2.29 \pm 1.22^c$	$3164.16 \pm 1399.76^b$
black rice (Entry 1)	$6.93 \pm 1.48^f$	$8203.02 \pm 2354.84^a$
black rice (Entry 2)	$6.12 \pm 1.79^e$	$983.71 \pm 75.97^b$
black rice (SRN2007.NO.8)	$5.93 \pm 0.19^a$	$6802.14 \pm 1024.35^b$
BHA	$0.71 \pm 0.02^b$	-

The correlation of phytochemical contents (TPC and TFC) and antioxidant activity of the seven Thai rice flowers are shown in Table 3. On the other hand, our results show a high and significant correlation between TPC and antioxidant activity, evaluated by the DPPH and FRAP assay ( $R^2 = 0.781$ ,  $R^2 = 0.541$ , respectively). It is suggested that phenolic compounds are likely to contribute to the radical scavenging activity of these rice flowers. However, the correlation coefficients between the TFC and antioxidant activity (DPPH and FRAP) were determined to be  $R^2 = 0.211$ ,  $R^2 = 0.309$ , respectively, which were much smaller than those determined between total phenolics and ABTS values. These results suggest that, apart from flavonoids, there might be other phenolic compounds such as phenolic acids, tannic acid, etc, contributing to the antioxidant activity of these rice flowers.

**Table 3** The Pearson correlation between the antioxidant activity and total phenolic and flavonoid of the extract of the rice flowers. Correlation is significant at the 0.01 level (2-tailed)

Assays	Correlation $R^2$			
	TPC	TFC	FRAP	IC <sub>50</sub>
TPC	1	0.673	0.541	0.781
TFC	0.673	1	0.309	0.2.11
FRAP	0.541	0.309	1	0.509
IC <sub>50</sub>	0.781	0.2.11	0.509	1

**Remark:** TPC: Total phenolic content; TFC: Total flavonoids content

## Conclusion

The results presented in this study reported the antioxidant activity (DPPH and FRAP assay) and phytochemical compounds (total phenolic and flavonoid contents) of seven Thai rice flowers (white rice; Jasmine 105, Goh-koh 6, red rice; Taptim-Chumphae rice, and black rice; Entry 1, Entry 2, SRN2007.NO.8). The results showed that Thai rice flower cultivars possessed relatively strong antioxidant activity. The rice flower extract from cultivated jasmine 105 showed the highest values for both phytochemical compounds and antioxidant activities. There was also a high correlation between only the total phenol content and antioxidant activities, determined by DPPH ( $R^2 = 0.781$ ) and FRAP ( $R^2 = 0.541$ ). Prior to this research, there were no reports about phytochemical and antioxidant activity of rice flower. However, many studies have reported the antioxidant activity and phytochemical compounds in rice and plant flowers, such as Li et al. (2014) that reported the FRAP values of 51 edible and wild flowers from China varied from  $0.17 \pm 0.00$  to  $178.43 \pm 14.31$  mmol Fe(II)/g wet weight and the total phenolic contents varied from  $0.13 \pm 0.02$  to  $11.48 \pm 0.56$  mg GAE/g wet weight. Onanong et al. (2011) reported that the TPC, TFC, DPPH and FRAP of soluble and bound of edible flowers from Thailand ranged from 28.37- 148.73 mg of GAE/g dry weight and 4.20 to 68.0 mg GAE/g dry weight, 18.12-97.64, % inhibition and 203.8 mmol FeSO<sub>4</sub>/100 g dry weight, respectively.

Xinfeng et al. (2017) identified and evaluated the antioxidant components in the flowers of five *Chimonanthus* species. Yafang et al. (2018) reported that the TPC, TFC and DPPH in non-pigmented, red, and black rice ranged from 0.79 to 62.55 mg GAE/100 g, 63.06–415.10 mg CE/100 g and 0.05–5.69  $\mu$ M TE/ g, respectively. The anthocyanins were only detected in red and black rice. Relative to these above data, the values obtained in this study were higher. The results suggest that phenolic compounds are the major contributors to the antioxidant activities of rice flowers, which are a potential source of antioxidants and phytochemicals, which have applications in nutraceuticals, the food industry, and health products or functional food products. Moreover, further studies should work on isolation, purification, identification and quantification of each phenolic and flavonoid compounds of these rice flowers.

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