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# Phenolic Compounds and Potential Health Benefits of Pigmented Rice

Prinya Wongsu

## Abstract

Rice (*Oryza sativa* L.) is one of the most important staple plant foods for global population especially in Asian countries. Pigmented rice including red rice, black, and purple contains a range of bioactive compounds including phenolics acids and flavonoids. Anthocyanins and proanthocyanidins are recognized as a major functional component in pigmented rice. Recently, pigmented rice varieties have received increasing attention from consumers due to its high nutritional values and bioactive compounds, providing its potential health benefits including antioxidant, anti-inflammatory, anticancer, and antidiabetic. Therefore, the objective of this chapter is to provide an up-to-date coverage of a systematic and advanced isolation, extraction and analytical methods, and potential health benefit studies related to antioxidant, anti-inflammatory, antidiabetic cardiovascular disease risk inhibition potential and anti-neurodegenerative potential of pigmented rice.

**Keywords:** black rice, red rice, phenolics, anthocyanins, potential health benefits

## 1. Introduction

Rice (*Oryza sativa* L.) is a cereal food and consumed, providing energy and nutrients for more than half of the world's population, particularly in Asian countries [1]. An increasing in rice consumption in Africa and Latin America has been observed in the past decade, mainly due to urbanization and changes in eating habits. In addition, European, US and Australian citizens are eating more rice, possibly due to an increased interest in global traveling and Asian cuisines. The world production of rice (paddy) was 782.00 million metric tons in 2018 [2]. The most common rice consumed by human is white rice (85%) and the rest is pigmented rice.

Rice paddy consists of hull and caryopsis. The hull or the outer layer constitutes about 20% of the total paddy rice contain minerals and cellulose. The hulling process also separated the hull from the grain or kernel. After removing of husk, whole-grain rice is the unpolished version of the grains consisting of the germ (2-3%), bran (6-7%), and endosperm (about 90%), and is also called brown rice [3]. Consequently, the process of milling is carried out to obtained white rice, also referred to as milled or polished rice, by removing 8-10% of external layers (mainly bran) from brown rice [4]. The endosperm of rice contains water 12%, carbohydrate 75-80%, and protein 7% [5]. Rice carbohydrate is primarily a starch which is made

up of linear amylose and branched amylopectin [6–8]. According to the amylose content in rice, rice cultivars can be classified into five groups including waxy (1–2%), very low (2–9%), low (10–20%), intermediate (20–25%) and high (25–33%) [9].

White rice is a common source of starches and phytochemicals including phenolic compounds, sterols,  $\gamma$ -oryzanol, tocotrienols and tocopherols which locate particularly in the outer layer such as pericarp and aleurone of rice grains [10]. In addition, pigmented or colored rice including black, red and dark purple rice is well known by the rice pericarp or grain having a black, red brown or dark purple color in its covering layers. The pigments, which are located in the aleuronic layer of rice grain, have been reported to consist of a mixture of anthocyanin compounds [6, 10–13]. The pigmented rice has been cultivated and consumed in Asia including China, Japan, Korea, and South East Asian countries for a long time and is widely known as “forbidden rice.” Royal families and kings used to consume these special varieties of rice in order to have their health improvement and to enhance their wealth [14]. In addition, many varieties of dark pigmented rice including Japonica black rice, Chinese black rice, Thai black rice and Indonesian black rice have been widely studied. Similar to white rice, pigmented rice is found in short-grained and long-grained, waxy (glutinous) and non-waxy (non-glutinous), husked and early maturing stages. The black and red rice have been used as a functional food and their extracts are also used as food colorants in breads, ice cream and liquor [8, 15].

Interestingly, an increased incidence in non-communicable including heart disease, Type II diabetes, obesity, and cancers in both developing and developed countries has been observed. Recently, pigmented rice varieties have gained an increase in attention from consumers due to its high nutritional value and bioactive compounds, providing its potential health benefits including antioxidant, anti-inflammatory, anticancer, and antidiabetic [8, 11, 16, 17]. In addition, many epidemiological and interventional studies have reported that the consumption of fruits and vegetables, cereal, whole grains, wine, cocoa, coffee and tea can reduce the risks of non-communicable disease including obesity, neurodegenerative diseases, Type II diabetes, and cardiovascular diseases [18, 19]. For the most part, the biological functional effects in acute and chronic diseases of plants have been traced to their phenolic compounds. Various studies have shown that phenolic compounds act as antioxidants [20]. Antioxidants control and reduce the oxidative damage in foods by delaying or inhibiting oxidation caused by reactive oxygen species (ROS), extending the shelf-life and quality of food products [21]. In addition, beta carotene, ascorbic acid, and phenolic compounds play important roles in delaying aging, reducing inflammation, and preventing certain cancers in human. The health benefits of whole grains are mainly contributed by one of its major constituents of phenolic compounds including phenolic acids, anthocyanins, and proanthocyanidins, which are the most common secondary metabolites in cereal grains, exist in pigmented grains and are considered to be the most effective antioxidants in nature [12]. Therefore, the objective of this chapter is to provide an up-to-date coverage of pigmented rice in regard to bioactive constituents, extraction and analytical methods, and their potential health benefits. Special attention is paid to bioactivities of pigmented rice and its main anthocyanins.

## **2. Phytochemicals in cereal grains**

Cereal grains are composed of nutrients and phytochemicals. Phytochemicals are bioactive, non-nutritional plant compounds, which typically occur in small quantities in plants foods, for example, fruits and vegetables, whole grains and cereals. The most important groups of dietary phytochemicals can be divided into general categories as phenolics, alkaloids, nitrogen-containing compounds, organosulfur compounds,

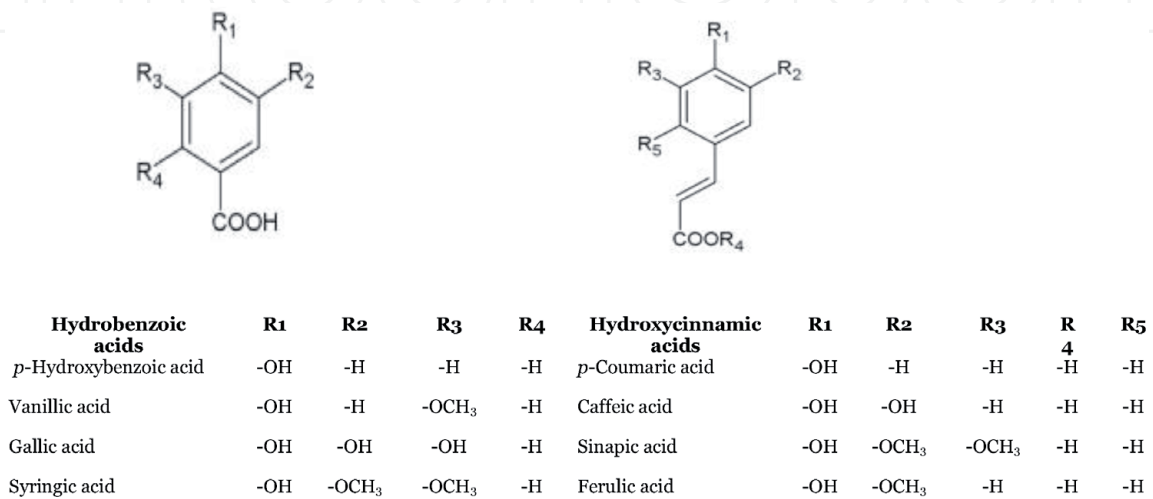
phytosterols, and carotenoids [18]. In addition, bioactive compounds including phenolic compounds, phytosterols, tocols, dietary fibers (mainly beta-glucan), lignans, alkylresorcinols, phytic acid,  $\gamma$ -oryzanols, avenanthramides, inositols and betaine are obtained from whole grain cereals [19, 22]. Some bioactive compounds are quite specific to certain cereals, for example,  $\gamma$ -oryzanol in rice, avenanthramide and saponins in oats, beta glucans in oats and barley and alkylresorcinol in rye, although these are also present in other cereals like wheat but relatively in fewer amounts.

### 3. Phenolic compounds in pigmented rice

Phenolics compounds or polyphenols contain one or more aromatic rings with one or more hydroxyl groups and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins and tannins [18]. Phenolic compounds are the secondary metabolites plants, providing essential function in the reproduction and growth of the plant, acting as defense mechanisms against pathogens, parasites and environments (light, chilling, pollution, etc.), and contributing to the color of plant [23, 24]. In addition to their roles in plants, phenolic compounds in human diet provide health benefits associated with reduced risk of chronic diseases [22].

#### 3.1 Phenolic acids

Phenolic acids in rice grain present in two forms including soluble and insoluble forms. The soluble form, including free and conjugated forms, the former of which can be extracted by solvent, such as aqueous methanol, ethanol, and acetone, and the latter can be hydrolyzed from soluble phenolics by alkali. However, the insoluble form, also called bound phenolics, which esterify to the cell walls. Among these three phenolic fractions, the most abundant is insoluble bound phenolic acids, followed by soluble conjugated phenolic acids and the least is soluble free phenolic acids [25]. In addition, phenolic acids mainly exist in rice bran with trace amounts in endosperm in different rice genotypes. Phenolic acids are widely distributed into two sub-classes including hydrobenzoic and hydroxycinnamic acids and derivatives. **Figure 1** shows the structure of some common phenolic acids detected in rice. Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic acid, vanillic, syringic and gallic acids. In addition, hydroxycinnamic acid derivatives like *p*-coumaric, caffeic, ferulic and sinapic acids. Phenolic acids in many plants are



**Figure 1.**  
The chemical structures of the main phenolic acids.

polymerized into larger molecules such as the proanthocyanins. Moreover, phenolic acids may arise in food plants as glycosides or esters with other natural compounds such as sterols, alcohols, glucosides and hydroxy fatty acids.

### 3.2 Flavonoids

Like as phenolic acids, flavonoids are secondary metabolites of plants with polyphenolic structure. Flavonoids consist of a 15-carbon skeleton organized by a three-carbon chain (C6–C3–C6 structure) and they are the most diverse compounds in the plant kingdom. Flavonoids can be classified in to several sub-classes including flavanols, flavones, flavones, isoflavones and anthocyanins. The most common flavonoids of rice belong to a wide variety of sub-families such as flavonols, flavones, flavanols, flavanons, and anthocyanins.

#### 3.2.1 Anthocyanins and proanthocyanidins

Anthocyanins and proanthocyanidins are known as color pigments found in several varieties of rice as bioactive compounds. These colorful pigment bioactive compounds are located in the aleurone layer of rice grain [26]. Pigmented rice is diverse in the color, mainly due to the grain's high anthocyanin content. Several pigmented rice including black, brown, dark brown, dark purple and red-grain rice have been reported have been reported, which its color is depend upon the kinds of pigment colors [17].

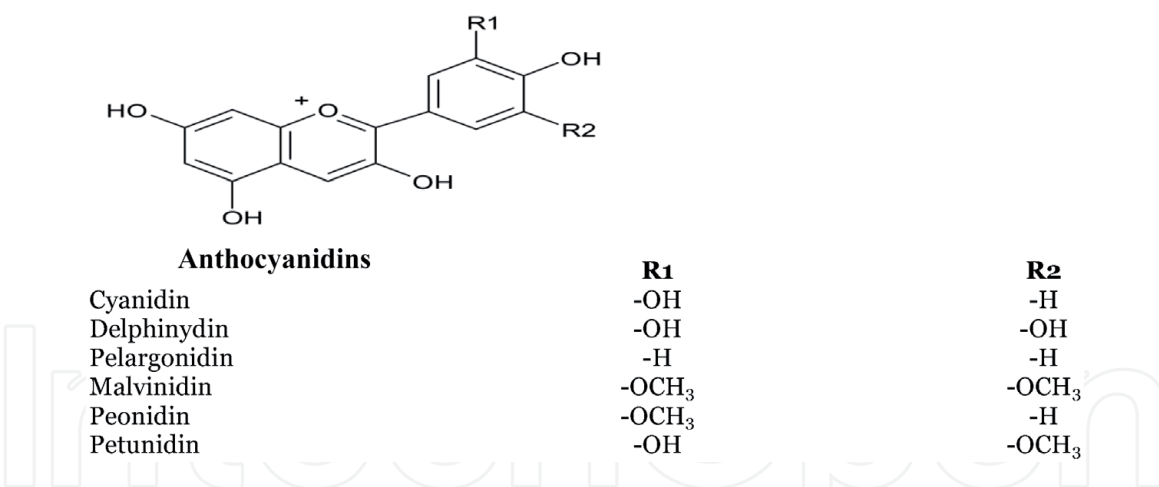
##### 3.2.1.1 Anthocyanins

Anthocyanins is a group of reddish to purple water-soluble flavonoids existing in pigmented rice and other cereal grains [27, 28]. The anthocyanidins or aglycons, the basic structure of anthocyanins, consist of an aromatic C6 (A ring) that bonded to a heterocyclic C3 (C ring) that contains oxygen, which is bonded by a carbon-carbon bond to a third aromatic C6 (B ring). When the anthocyanidins are bonded to a sugar moiety in the glycosidic linkage, they are known as anthocyanins [26]. In plants, they are found in mono, di, or tri of O-glycosides and acylglycosides of anthocyanidins [17]. Individual differences in anthocyanidins are related to the number of hydroxyl groups; the nature, number and position of sugars linked to the molecule; and the presence of aliphatic or aromatic acids attached to the sugar molecule. Anthocyanins are derived from the most common six anthocyanidins (aglycones) including cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin. Several anthocyanins have been isolated and identified from pigmented rice including cyaniding 3-glucoside, cyaniding 3-galactoside, cyaniding 3-rutinoside, cyaniding 3, 5-diglucoside, malvidin 3-galactoside, peonidin 3-glucoside, and pelargonidin 3, 5-diglucoside [6, 13, 28] and the basic chemical structures of the main anthocyanidins are shown in **Figure 2**. Cyanidin-3-O-glucoside has been identified in black rice as the significantly higher than others [6, 13].

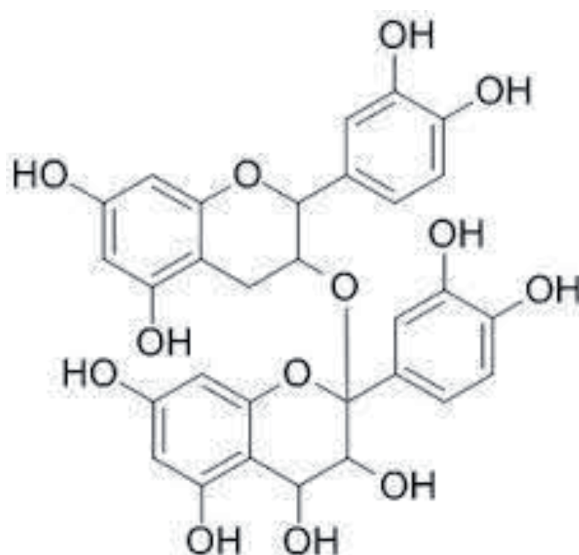
##### 3.2.1.2 Proanthocyanidins

Proanthocyanidins are a group of polymeric phenolic compounds consisting mainly of flavan-3-ol units such as afzelechin, epiafzelechin, catechin, epicatechin, gallocatechin, and epigallocatechin (**Figure 3**) [26]. More complex proanthocyanidins, having the same polymeric building block, form the group of tannins. Proanthocyanidins can be A-type or B type structure with flavan-3-ol units doubly linked by C4-C8 and C2-O7 or C4-C6 and C2-O7 for the former, and linked mainly





**Figure 2.**  
 The chemical structures of the main anthocyanidins.



**Figure 3.**  
 The chemical structures of the main proanthocyanidins.

through C4-C8 or C4-C6 for the latter B-type proanthocyanidin is very common in nature. In red rice, the block unit of proanthocyanidin consists of catechin and epicatechin. Proanthocyanidins are synthesized in plants by using anthocyanidins as key intermediates. These pigmented compounds are also responsible for red and purple color in rice.

#### 4. Extraction, identification, and quantification

The determination of phenolic compounds is a necessary prerequisite not only to define the nutritional qualities of whole grain rice, but mostly to investigate on the health benefits associated to the consumption of these food plants [29]. Therefore, the most recent techniques for the extraction of the target compounds from rice along with the analytical approaches adopted for the separation, identification and quantification of phenolic acids, flavonoids, anthocyanins, and proanthocyanidins must be fully studied. Extraction is a process used for separating bioactive compounds from solutions using specific solvents by applying standard procedures. In addition, extraction of bioactive compounds can be obtained by using either conventional or non-conventional methods [30, 31].

#### 4.1 Conventional solvent extraction bioactive compounds

Conventional extraction is being used at a small-scale level to extract bioactive components from several plant materials. This technique is usually based on the extraction efficiency of different solvents, which are being used for this purpose. The manual solvent extraction at ambient temperature is the most commonly used method in extracting bioactive compounds from grains. The solvents included acidified methanol with 1.0 N HCl (85:15, v/v), acidified methanol with 1 M phosphoric acid (95:5 v/v), acidified methanol with trifluoro acetic acid (99.8:0.2, v/v), acidified methanol with glacial acetic acid (95:5, v/v), and acetone/water (80:20, v/v). The extraction ratio was a material to solvent at 1:10 (w/v) [30]. In addition, in cold conditions, methanol (85%) and HCl (1 mol/L) was found to be an appropriate extraction solvent for anthocyanins, along with 85% methanol or 70:29.5:0.5, v/v acetone:water:acetic acid for free proanthocyanidins [17].

According to Shao et al., [12] soluble-free, soluble-conjugated and insoluble-bound phenolics of white, red and black rice were extracted by using 80% methanol. The soluble phenolics mixture was extracted and concentrated to obtain soluble phenolics. In order to get soluble-free phenolics, the concentrated soluble phenolics were further extracted by ethyl acetate three times, and then dried by a rotary evaporator, and dissolved in 5 mL of 50% methanol. To get soluble-conjugated phenolics, the concentrated soluble phenolics were hydrolyzed using 4 M NaOH for 2 h followed by adjusting pH to 1.5–2.0, extraction with ethyl acetate, drying using a vacuum evaporator, and then dissolving in 5 mL of 50% methanol. After the extraction of soluble phenolics, the residues were used to extract insoluble-bound phenolics. Similarly, the soluble-conjugated phenolics could be prepared from the concentrated soluble phenolics extracts by using 4 M NaOH and ethyl acetate.

In addition, our group [13] also used solid phase extraction (SPE) techniques to purify and prepare soluble-free (unbound fraction) and soluble-conjugated (polyphenol-rich bound fraction) phenolic compounds of pigmented rice. The crude extracts of colored rice were purified by applied to C<sub>18</sub> solid phase extraction unit. The solid phase cartridge was pre-washed in 0.2% (v/v) formic acid in acetonitrile and then pre-equilibrated in 0.2% (v/v) formic acid in water. The unbound materials including free sugars, organic acids and vitamin C were collected. The SPE unit was then washed with a unit volume of 0.2% (v/v) aqueous formic acid and then with 2 volumes of ultra-pure water. The polyphenol-rich bound fraction was eluted with a unit volume of 80% (v/v) acetonitrile in water.

#### 4.2 Non-conventional extraction techniques

The longer extraction time, costly and high purity solvent, evaporation of the huge amount of solvent, low extraction selectivity, and thermal decomposition of thermolabile compounds are major challenges of conventional extraction. These limitations of conventional extraction methods can be improved by introducing the promising techniques or non-conventional extraction techniques, for example, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) [31].

##### 4.2.1 Microwave-assisted extraction (MAE)

**Microwave-assisted extraction** has been implemented as an alternative technique for extracting anthocyanins from pigmented rice because of its ability to reduce both consumption time and solvent volume. For the MAE method, a combination of 70°C, 300 W, with 10 min in MAE was the most effective in extracting

anthocyanins from blue wheat and purple corn compared with 50°C, 1200 W, and 20 min for black rice [30]. Moreover, this technique reduced the losses of the biochemical compounds being extracted.

#### 4.2.2 Ultrasonic-assisted extraction

Ultrasound-assisted extraction (UAE) has been used in applications of food-processing technology to extract bioactive compounds from plant materials. Ultrasound at levels greater than 20 kHz is used to disrupt plant cell walls. It helps to improve the solvent's ability to penetrate the cells and obtain a higher extraction yield. The UAE operates at a low operating temperature through processing and maintain a high extract quality for compounds. Recently, Setyaningsiha et al. (2019) reported the optimization of the UAE conditions for individual phenolic compounds extraction from rice grains using 80% methanol in water for 25 min at 45°C with amplitude 47%, cycle 0.4 s – 1, pH 4.25 and sample-to solvent ratio of 1:5 [32]. The developed method presented the acceptable value for linearity and precision (RSD). Therefore, the proposed UAE method is an effective technique for the determination of individual phenolic compounds including caffeic, *p*-coumaric, syringic, chlorogenic, isovanillic, isoferulic ferulic, *p*-hydroxybenzoic, sinapic, *p*-hydroxybenzaldehyde, protocatechuic, vanillic acids, protocatechuic aldehyde and quercetin in rice samples. However, the UAE has two main negative properties mainly related to experimental repeatability and reproducibility [31].

#### 4.3 Identification

After the extraction of bioactive compounds, the separation, identification and quantitation are necessary to studied. In the past few decades, there are a huge number of published reports on HPLC analysis of extracted bioactive compounds from rice grains describe as the most widely used analytical method. Recently, Prabhakaran et al. (2019) reported the analyzed method of selected phenolic compounds in rice grains and its by-products using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) [33].

In addition, our group developed an identification and quantification techniques for phenolic acids and anthocyanins in pigmented rice by using UPLC-ESI-QqQ-MS/MS analysis [13]. The analysis was performed using a UPLC coupled with a mass spectrometer. The separation was carried out by UPLC HSS T3 column 1.8  $\mu$ m, 2.1  $\times$  100 mm. Column temperature was maintained at 35°C. The mobile phase consisted of 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) and the flow rate was set at 0.4 mL/min. The injection volume was 2.0  $\mu$ L. A stepwise gradients B (%) including an initial isocratic at 2.0% for 1 min, then linear gradient to 98% in 5 min, and by return to the initial condition of 2% B in 7 min. Therefore, the total operation time was 12 min. The solvents and extracts were previously filtered through a 0.45  $\mu$ m filter membrane. Mass spectral data were obtained in positive or negative mode with a mass range between  $m/z$  0 to  $m/z$  500. The Multiple Reaction Monitoring (MRM) transitions and compound parameters for the target phenolic compounds were developed. Identification was confirmed by comparing  $m/z$  values, retention times and fragmentation patterns with those of references standards. In addition, the concentration of phenolic compounds was quantified using external standard method. Our study showed that eight target phenolic compounds were detected and identified in both the unbound and polyphenol-rich bound fractions of pigmented rice [13]. The identification of compounds was carried out by applying one quantification transition (quantifier ion) and/or one or two confirmation transitions (qualifier ions) to assess the detection



and quantification specific to each compound (**Table 1**). Positive ionization mode was selected for caffeic, ferulic acids, (+)-catechin and anthocyanins while negative ionization mode was applied for *p*-coumaric acid and quercetin, due to the chemical structures of the analyses and their ionization behavior observed in ion mode.

4.4 Quantitation

Pigmented rice is diverse in color, mainly due to the grain’s high anthocyanins content. The contents of phenolic compounds and anthocyanins are summarized in **Tables 2** and **3**, respectively, as examples of the phytochemicals that were isolated and analyzed from pigmented rice including black, red, and purple rice varieties. A range of phenolic compounds including vanillic, *p*-coumaric, protocatechuic, caffeic and ferulic acids has been detected in pigmented rice. In pigmented rice, the major phenolic acid was ferulic acid [6, 13, 25, 37]. Moreover, major flavonoids present in pigmented rice are quercetin and catechin. In addition, cyanidin-3-O-glucoside is the most predominant anthocyanins in pigmented rice. A mean of cyanidin-3-O-glucoside content in black rice was about higher than that of red rice [6, 13, 25, 37]. However, malvidin-3-O-glucoside was not observed in pigmented rice.

Our UPLC-ESI-QqQ-MS/MS analysis for phenolic acids and anthocyanins in pigmented rice showed that the retention times for the target phenolic compounds ranged between 2.9 and 4.6 min An example of a UPLC-ESI-QqQ-MS/MS chromatogram for cyanidin-3-O-glucoside for the polyphenol-rich bound fraction of black rice cv. *Hom nil* is shown in **Figure 4**. Hydroxycinnamic acids including caffeic, *p*-coumaric and ferulic acids were characterized by the loss of the carboxylic acid group (-COO; -44 *m/z*) [38]. The loss of the carboxylic acid group was observed for *p*-courmaric acid giving *m/z* at 119 [M-H-44]–, as a characteristic ion. For acids with a methoxy moiety, such as ferulic acid, the -COO loss was found by a loss of the methyl group (-CH3; -15 *m/z*, -COO; - 44 *m/z* and -CH3; -59 *m/z*). The peak with *m/z* 195 was contributed to the ferulic acid’s precursor ion while the peak of product ion at *m/z* 136 was characterized to -COO and -CH3 moiety. In addition, the characterization of anthocyanin was mostly based on the loss of glucose (162 *m/z*). A molecular ion at *m/z* 449 [M + H] + corresponding with glycoside derivative of cyanidin and a major fragmentation occurring at *m/z* 287 [M + H-162] + corresponding with a cyanidin aglycone was observed in the polyphenol-rich bound fraction of black rice cv. *Hom nil* (**Figure 5(a)**). The MS/MS spectrum of pelargonidin-3-glucoside in

Qualified compound	Retention time (min)	m/z Precursor ion	m/z Quantifier	m/z Qualifier
(+)-Catechin	3.04 ± 0.01	291.10	130.01	123.03
Caffeic acid	3.29 ± 0.01	181.02	163.03	145.01
<i>p</i> -Coumaric acid	3.72 ± 0.01	162.95	118.96	92.95
Ferulic acid	3.87 ± 0.01	195.02	134.00	145.02
Quercetin	4.59 ± 0.01	300.90	178.05	150.92
Cyanidin-3-glucoside	2.87 ± 0.01	449.10	287.05	137.00
Pelargonidin-3-glucoside	3.03 ± 0.01	433.10	271.05	121.00
Peonidin-3-glucoside	3.10 ± 0.01	463.10	301.08	286.03

Reference: [13].

**Table 1.**  
Retention time and fragment of phenolic compounds.

Phenolic acids	Pigmented rice varieties	Extracting solvent	Analysis method	Content (mg/g)	Reference
Vanillic acid	Purple Rice	Methanol	HPLC	0.19 ± 0.06	[34]
		95% Ethanol	HPLC	0.77 ± 0.02	
		Deionized water	HPLC	1.15 ± 0.08	
	Purple rice	80% Methanol	Reversed-phase HPLC	0.34 ± 0.02	[35]
	Red rice	80% Methanol	Reversed-phase HPLC	nd	
p-Coumaric acid	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.05 ± 0.01	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.04 ± 0.00	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.02 ± 0.00	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.06 ± 0.00	
	Purple rice	80% Methanol	Reversed-phase HPLC	0.31 ± 0.02	[34]
	Red rice	80% Methanol	Reversed-phase HPLC	0.01 ± 0.20	
Protocatechuic acid	Purple Rice	Methanol	HPLC	0.24 ± 0.05	[34]
		95% Ethanol	HPLC	1.02 ± 0.08	
		Deionized water	HPLC	1.26 ± 0.01	
Protocatechuic acid	Purple rice	80% Methanol	Reversed-phase HPLC	1.60 ± 0.02	[35]
	Red rice	80% Methanol	Reversed-phase HPLC	0.31 ± 0.02	
Caffeic acid	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	nd	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	nd	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	nd	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.02 ± 0.00	

(Continued)

Phenolic acids	Pigmented rice varieties	Extracting solvent	Analysis method	Content (mg/g)	Reference
Ferulic acid	Purple rice	80% Methanol	Reversed-phase HPLC	0.98 ± 0.32	[35]
	Red rice	80% Methanol	Reversed-phase HPLC	nd	
	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.25 ± 0.01	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.40 ± 0.03	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.24 ± 0.02	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.57 ± 0.02	[12]
	Purple rice	80% Methanol	Reversed-phase HPLC	1.03 ± 0.02	
	Red rice	80% Methanol	Reversed-phase HPLC	0.34 ± 0.05	
	Purple Rice	Methanol	HPLC	0.09 ± 0.01	[34]
		95% Ethanol	HPLC	1.73 ± 0.05	
Catechin		Deionized water	HPLC	0.37 ± 0.09	
	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.12 ± 0.01	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.10 ± 0.01	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.01 ± 0.00	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.91 ± 0.08	
	Purple rice	80% Methanol	Reversed-phase HPLC	0.29 ± 0.22	[35]
	Red rice	80% Methanol		n.d	
	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.07 ± 0.03	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.08 ± 0.00	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.10 ± 0.01	

n.d: not detectable.

**Table 2.**  
*Phenolic compound contents in pigmented rice.*

Anthocyanins	Pigmented rice varieties	Extracting solvent	Analysis method HPLC	Content (mg/g)	Reference
Cyanidin 3-glucoside	Black rice cv. Niaw Dam Pleuak Khao	Acidified methanol	HPLC	137.41 ± 16.66	[6]
	Black rice cv. Niaw Dam Pleuak Dam	Acidified methanol	HPLC	19.39 ± 0.09	
	Purple Rice	Methanol	HPLC	21.09 ± 1.09	[34]
		Ethanol	HPLC	8.40 ± 0.00	
		Deionized water	HPLC	n.d	
	Thai black rice	Acidified methanol	HPLC	142 ± 1.5	[36]
	Thai Jasmine red rice	Acidified methanol	UPLC-ESI-QqQ-MS/MS	n.d	
	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	132.96 ± 393	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	47.80 ± 0.44	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	11.75 ± 0.32	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.06 ± 0.00	
Pelargonidin 3-glucoside	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.23 ± 0.01	[13]
	Black rice cv. Rice berry		UPLC-ESI-QqQ-MS/MS	0.07 ± 0.00	
	Purple rice cv. Khoa kum		UPLC-ESI-QqQ-MS/MS	0.03 ± 0.00	
	Red rice cv. Hom deang			n.d	
Malvidin 3-glucoside	Purple Rice	Methanol	HPLC	n.d	[34]
		Ethanol	HPLC	n.d	
		Deionized water	HPLC	n.d	

(Continued)



Anthocyanins	Pigmented rice varieties	Extracting solvent	Analysis method HPLC	Content (mg/g)	Reference
Peonidin-3-glucoside	Black rice cv. Niaw Dam Pleuak Khao	Acidified methanol	HPLC	11.07 ± 0.97	[6]
	Black rice cv. Niaw Dam Pleuak Dam	Acidified methanol	HPLC	12.75 ± 0.51	
	Black rice cv. Hom nil	Acidified methanol	UPLC-ESI-QqQ-MS/MS	19.56 ± 0.39	[13]
	Black rice cv. Rice berry	Acidified methanol	UPLC-ESI-QqQ-MS/MS	6.94 ± 0.26	
	Purple rice cv. Khoa kum	Acidified methanol	UPLC-ESI-QqQ-MS/MS	5.29 ± 0.10	
	Red rice cv. Hom deang	Acidified methanol	UPLC-ESI-QqQ-MS/MS	0.01 ± 0.0	
	Thai black rice	Acidified methanol	HPLC	98 ± 0.5	[36]
				n.d	
	Thai Jasmine red rice	Acidified methanol	HPLC	n.d	

n.d: not detectable.

Table 3. Anthocyanins contents in pigmented rice.

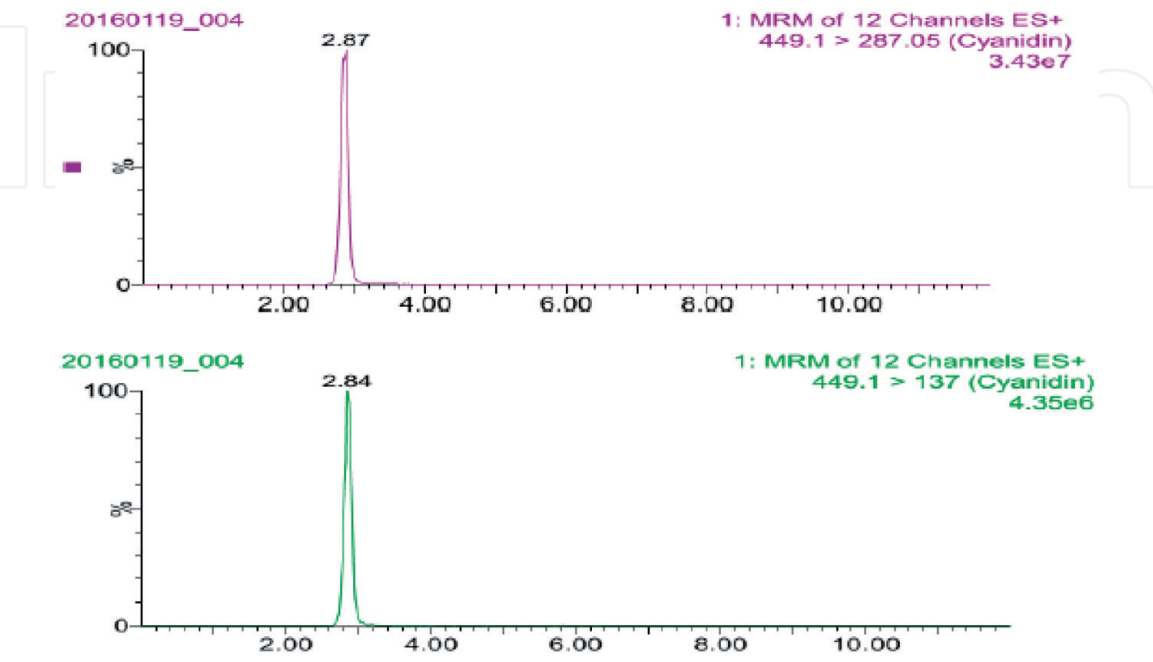
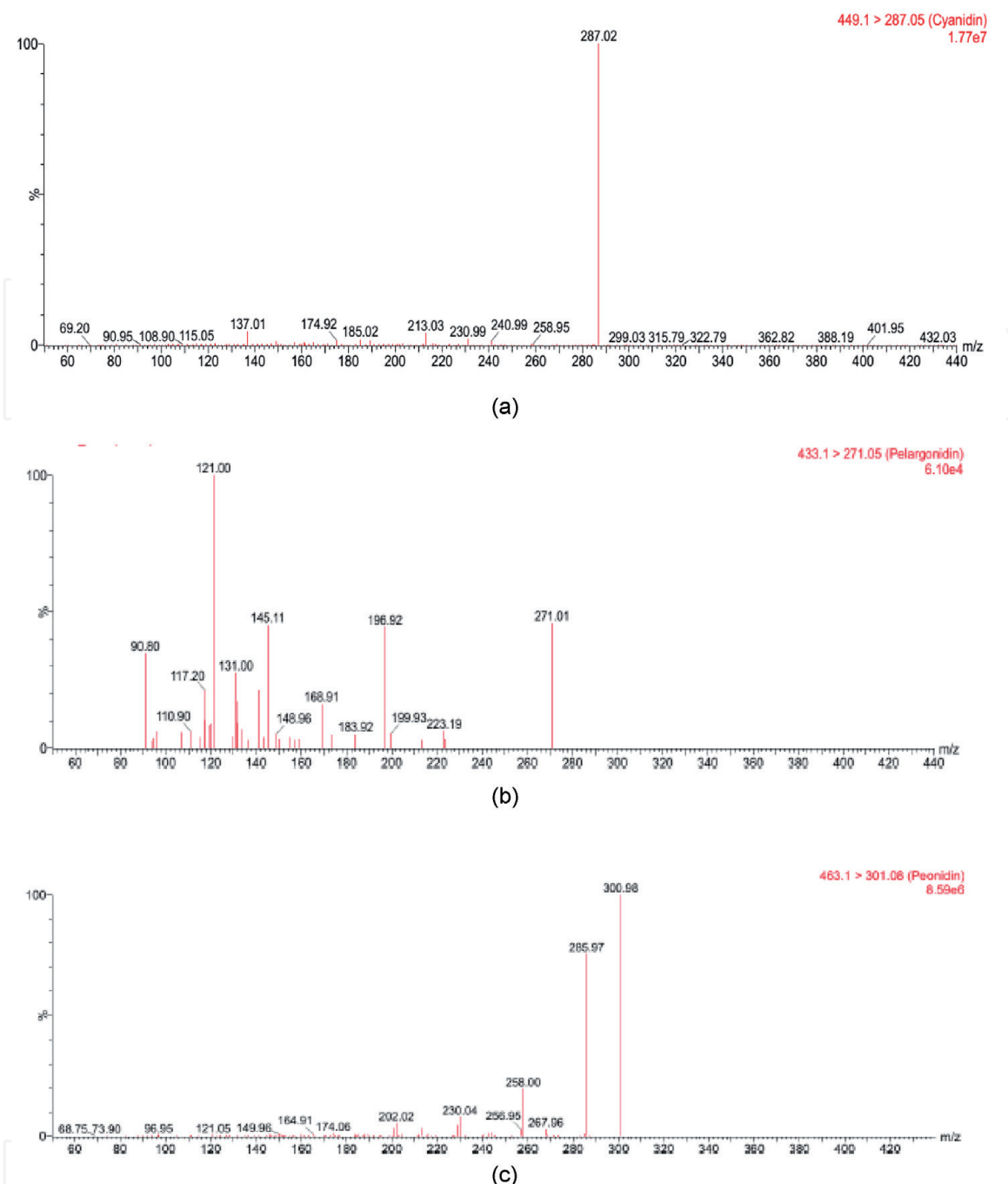


Figure 4. Chromatogram of cyanidin 3-O-glucoside [13].



**Figure 5.**  
(a) MS/MS spectrum of cyanidin 3-O-glucoside. (b) MS/MS spectrum of pelargonidin 3-O-glucoside.  
(c) MS/MS spectrum of peonidin 3-O-glucoside [13].

the positive ionization mode (**Figure 5(b)**) of the polyphenol rich bound fraction of black rice cv. *Hom nil* showed protonated molecular ions at  $m/z$  433  $[M + H]^+$ . The product ion of aglycone at  $m/z$  271  $[M + H - 162]^+$  corresponds to the loss of a pelargonidin moiety. The positive ion mass spectrum of the polyphenol-rich bound fraction of black rice cv. *Hom nil* (**Figure 5(c)**) showed its molecular ion at  $m/z$  463  $[M + H]^+$  and a major fragment at  $m/z$  301  $[M + H - 162]^+$ . These data indicated the presence of a peonidin aglycone and a hexose moiety.

Proanthocyanidins are high molecular weight of flavan-3-ol polymers that consist of catechin, epicatechin, gallocatechin, and epigallocatechin units that can also be found in rice germ and bran, particularly in pigmented rice. Proanthocyanidins were only detected in red and black rice, and their contents were dependent on the genotypes which differed from 15.57 mg/kg to 1417.12 mg/kg, respectively. In red rice, the majority of proanthocyanidins are oligomers of 5–8 mers (40%), whereas the

polymers (DP > 10) accounted for 29% [12]. Moreover, total proanthocyanidins of the traditional Sri Lankan red-grained rice varieties ranged from 11.95 to 24.70 mg/g in bran samples and from 1.07 to 2.27 mg/g in brown rice samples [39].

## 5. Health benefit potentials of pigmented rice

Phenolic compounds have many biological activities, for instance, antioxidant [40, 41] and anti-inflammatory properties [42]. Several epidemiological studies suggested that high dietary consumption of polyphenols is associated with decreased risk of a range of diseases including antidiabetic [43, 44], cardiovascular disease (CVD) [45], and neurodegenerative diseases [46].

### 5.1 Antioxidant activity

Phenolic acids and flavonoids function as reducing agents, free radical scavengers, and quenchers of singlet oxygen formation. The antioxidant property of the phenolic ring depends on the number and location of the hydroxy groups [47]. Pigmented rice varieties showed high antioxidant capacity along with the highest flavonoid and polyphenol content [42]. Flavonoids and phenolic acids components play important roles in the control of oxidative stress, which is considered to be substantial in an initiation and development of many current conditions and diseases including inflammation, hyperglycemia, hypertension, heart diseases, neurodegenerative diseases, cancer and in the aging process. However, antioxidants are substances that significantly delay or prevent the oxidation of an oxidisable substrate when present in low concentrations compared to the substrate.

### 5.2 Antidiabetic activity

Hyperglycaemia, or high blood sugar, is one of the most common disorders. Antidiabetic activity of phenolic acids and flavonoids has been reported by several studies. It has been reported that these anthocyanins have various beneficial effects, including inhibitory effects against carbohydrate hydrolysing enzymes, including in  $\alpha$ -amylase and  $\alpha$ -glucosidase. Our study on  $\alpha$ -amylase inhibitory action of cyanidin-3-glucoside, ranged from 0.1 mg/100 g DM in the red rice sample to 138.8 mg/100 g DM in the black rice sample, showed that the IC<sub>50</sub> value for potential inhibition against  $\alpha$ -amylase activity of crude extract of colored rice samples ranged from 2.5  $\mu$ g/mL to 14.4  $\mu$ g/mL. The potential inhibition against  $\alpha$ -amylase activity in black rice samples were higher than those in red and purple rice samples, respectively [13]. In addition, the percentage inhibition on  $\alpha$ -glucosidase and  $\alpha$ -amylase of fragrant black rice fractions had higher potential than acarbose, a synthetic antidiabetic drug [48]. However, variation in the structure of anthocyanins makes it difficult to determine their effects on Type II diabetes. Understanding the absorption and metabolism of anthocyanins is important for understanding their role in the improvement of this disease. Previous literature suggested that anthocyanins may lower blood glucose by improving insulin resistance, protecting  $\beta$  cells, increasing secretion of insulin and reducing digestion of sugars in the small intestine. The mechanisms of action are primarily related to their antioxidant properties, but enzymatic inhibition and other pathways may also be relevant [49].

### 5.3 Anti-inflammatory potential

Overproduction of free radicals and/or oxidants can cause oxidative stress and oxidative damage to biological macromolecules including lipid, protein and nucleic

acids, which are associated with chronic diseases such as cancer, cardiovascular disease and neurological disease [42]. In addition, free radicals including superoxide anion, (NO) and peroxynitrite play important roles in the inflammatory process. According to the study carried out by Chalermpong et al. (2012) on the anti-inflammatory activities of bioactive rich extracts from Thai glutinous purple rice bran [50], the extracts of five purple rice bran sample showed a strong anti-inflammatory activity through inhibitory effect on nitric/oxide (NO) production in combined LPS-IFN- $\gamma$ -activated RAW 264.7 murine macrophage cells. Moreover, the results also indicated that  $\gamma$ -oryzanol rich extracts from Thai glutinous purple rice bran were acting as a lipophilic radical scavenger better than a hydrophilic radical scavenger. Moreover, these extracts exert a potent anti-inflammatory activity.

#### **5.4 Cardiovascular disease risk inhibition potential**

Cardiovascular disease (CVD) is a chronic disease related to a range of genetic and environmental factors including smoking, high saturated fat diets and physical inactivity. Moreover, coronary heart disease and stroke are a major cause of mortality in developed countries. Dyslipidemia is an abnormal high level of dietary lipids such as plasma cholesterol, triglycerides, or a low level of high-density lipoproteins (HDL). In addition, dyslipidemia often increases the risk factor for atherosclerosis, which further increases the risk for developing coronary heart diseases that are the leading cause of death among the aged population [51]. Several studies indicated a correlation between the intake of flavonols, flavones and flavanols and a reduced risk of coronary artery disease and anthocyanin and flavanone intake and reduced CVD related mortality. Lo et al., (2016) demonstrated the effects of the newly bred Korean non-germinated and germinated pigmented rice cultivars on ovariectomized (OVX) rats in comparison with the nonpigmented normal brown rice [45]. All rat groups were fed with 20% rice per total diet and showed that the supplementation of germinated rice for some groups, particularly pigmented rice cultivars, resulted in better a lipid profile compared to the groups that consumed non-germinated rice cultivars. In addition, germination increased the quantities of the bioactive compounds that are responsible for the hypolipidemic activities of these rice grains. Showed a low total cholesterol levels, high levels of high-density lipoproteins-cholesterol, high fecal lipid output, low hepatic lipid values, and low hepatic adipocyte accumulation. There were also an increase in the rate of lipolysis and decrease in lipogenesis based on the lipid-regulating enzyme activity profiles obtained for the groups that fed on germinated rice. Also, results revealed that pigmented rice cultivars had superior effects in improving the lipid metabolism relative to the non-pigmented normal brown rice variety. The application of germinated rice, blackish-purple cultivars enhanced potential for the prevention and occurrence of dyslipidemias.

#### **5.5 Anti-neurodegenerative potential**

Neurodegenerative disorders including Parkinson's and Alzheimer's diseases have been increasing in our aging societies. Flavonoids may act to protect the brain in a number of ways, including by protection of vulnerable neurons, the enhancement of existing neuronal function or by stimulating neuronal regeneration. Vargas et al. (2018) reported the of studies using neuron-like cells, such as the SH-SY5Y cell line, allowed the evaluation of the antioxidant activity of compounds in human cells with biochemical characteristics similar to neurons, indicated the extract of bioactive compounds in red and black rice brans extracts was able to prevent H<sub>2</sub>O<sub>2</sub>-induced oxidative damage in SH-SY5Y cells [46].



## 6. Conclusion

Pigmented rice especially black rice contains a wide range of biologically active compounds for example cyanidin O-glucoside that can be used in various functional foods and in nutraceuticals applications. The advancement of isolation and quantification techniques provides more opportunities for *in-vitro* and *in vivo* studies on potential health benefits, given by pigmented rice and its by-products. In addition, most of the studies on the biological properties of black or red rice bioactive compounds have been conducted through an *in vitro* approach; however, more preclinical or in animal studies should be considered. Further investigations will be needed to study evidence on the efficacy of bioactive compounds including phenolic acids and anthocyanins in pigmented rice in terms of antagonistic, additive/non-interactive, or synergistic effect on potential health benefits.

## Acknowledgements

The author would like to thank Mae Fah Luang University for a financial support of this work.

## Conflict of interest

The author declares no conflict of interest.

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