

Original Article

ANTIOXIDANT ACTIVITIES AND CYTOTOXICITY OF THAI PIGMENTED RICE

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ABSTRACT

**Objective:** This research quantified the phytochemical profile, antioxidant activities and cytotoxicity of Thai pigmented rice.

**Methods:** Acidified ethanol extracts from seeds of twelve Thai pigmented rice cultivars (six black and six red rice) were investigated for their content of bioactive compounds (phenolics, anthocyanins, and procyanidins), antioxidant activities (ferric reducing antioxidant power; FRAP, DPPH radical scavenging activity; DPPH, and inhibition of lipid peroxidation; TBARS), and cytotoxicity on human promyelocytic leukemia (HL-60) cells.

**Results:** Black rice cultivars (0.05–0.54 mg GAE/ml extract) and red rice cultivars showed total phenolic content (0.21–0.99 mg GAE/ml extract). The predominant phenolic pigments were anthocyanins (1.51±1.70 mg C3G/l extract) for black rice and procyanidins (0.35±0.23 mg EPE/ml) for red rice. Antioxidant activity ranged within 0.06–1.36 mg AAE/ml for FRAP, IC<sub>50</sub> 0.10–1.12 mg/ml for DPPH and 7.57–40.48 % for TBARS. Red rice tended to exhibit higher antioxidant activities than black rice in the FRAP assay, but there were no significant differences between the rich colors in the DPPH and TBARS assays. Pigmented rice extracts containing the highest total phenolic content were selected to assess their cytotoxicity on human promyelocytic leukemia (HL-60) cells. Black rice showed higher cytotoxicity, corresponding with the lower IC<sub>50</sub> compared to red rice.

**Conclusion:** The results revealed that Thai pigmented rice is a natural antioxidant source with potential for use as an active ingredient in cosmetics, functional food and pharmaceuticals.

**Keywords:** Antioxidant activities, Cytotoxicity, Pigmented rice, Phenolic compounds.

INTRODUCTION

Free radicals are highly reactive chemical entities that contain one or more unpaired electron(s), and that occur in living systems as a result of normal metabolic activity or environmental exposure [1, 2]. Free radicals can react with and damage biomolecules, including DNA, proteins, and membrane lipids, thereby contributing to development of a large number of chronic diseases such as cardiovascular disease, neurodegenerative disorders, cancer, diabetes, and aging [3]. Antioxidants play an important role in disease prevention by neutralizing excess free radicals, protecting cells against the toxic effects of free radical damage [4, 5]. Antioxidants have also been used for maintaining the quality and durability of food, pharmaceutical and cosmetic products. Natural phenolic antioxidants can effectively scavenge free radicals, absorb light in the ultraviolet (UV) region (100 to 400 nm), and chelate transition metals, thus stopping progressive autoxidative damage and production of off-odors and off-tastes[6]. Antioxidant activity has been demonstrated for natural bioactive phytochemicals such as phenolic compounds, flavonoids, carotenoids, vitamin C, and vitamin E [6, 7].

Rice (*Oryza sativa* L.) is the major food of the world's population and is becoming increasingly popular because of its nutritional and beneficial health properties [8]. Unpolished grain that is obtained by removing the husk contains significant quantities of all the major nutrients, including carbohydrate (32.1 g/100 g grain), protein (2.6 g/100 g grain), fat (1.1 g/100 g grain), and fiber (0.8 g/100 g grain)

[9]. Some important health benefits of unpolished rice are related to its phytochemical content (mainly phenolic compounds), especially pigmented rice varieties which have higher amounts of phenolic compounds and stronger antioxidant activities in the aleurone layers [10, 11]. The phytochemicals in rice include  $\gamma$ -oryzanol, vitamin E homologues, phenolic acids, anthocyanins, procyanidins, etc. [11-14]. The major flavonoid pigments in rice extracts were identified as anthocyanins and procyanidins for black and red rice, respectively [15, 16]. These phytochemicals are powerful antioxidants and have been shown to reduce atherosclerotic plaque formation[17], inhibit aldose reductase activity [14], decrease hyperlipidemia [18] and suppress cancer cell proliferation [19].

The aims of this study were to evaluate total phenolic, anthocyanin, and procyanidin contents of 12 cultivars of Thai pigmented rice, including 6 black and 6 red rice samples. After extraction of these bioactive compounds from rice samples, their potential to neutralize free radicals was assessed by measuring the ferric ion reducing (antioxidant) power, DPPH radical scavenging activity and inhibition of lipid peroxidation. Moreover, pigmented rice extracts containing the highest total phenolic content (two each for black and red varieties) were evaluated for their cytotoxicity on HL-60 human cancer cells. The results from this study could provide a better understanding of the potential health benefits of Thai pigmented rice extract and promote new commercial opportunities for specific strains of pigmented rice containing high levels of bioactive compounds.

Table 1: Description of rice samples used in this study

Color	Rice variety	Abbreviation	Cultivation		
Black	Hom nin	HN1	Chang Rai		
		HN2	Chang Rai		
		HN3	Chang Mai		
		HN4	Phayao		
	Niaw dum	ND1	Chang Rai		
		ND2	Phayao		
		Red	Mun pu	MP1	Chang Rai
				MP2	Phayao
MP3	Prachinburi				
Sang yod	SY1		Phatthalung		
	SY2		Phatthalung		
	SY3		Songkhla		

## MATERIALS AND METHODS

Twelve commercial pigmented rice cultivars were purchased from Thailand markets. The description of these rice samples is shown in table 1. Hydrochloric acid (HCl) was purchased from J. T Baker. Ethanol, sulfuric acid, and trichloroacetic acid were purchased from Merck. 1,1-diphenyl-2-picrylhydrazyl (DPPH), cyanidin-3-glucoside, epicatechin, folin-ciocalteu reagent, gallic acid, sodium acetate, sodium carbonate, thiobarbituric acid, vanillin, and trypan blue solution were purchased from Sigma. Ferric chloride and potassium ferricyanide were purchased from Fisher scientific. Linoleic acid was purchased from Fluka. Potassium chloride was purchased from Ajax Finechem. Fetal bovine serum, phosphate buffered saline, penicillin streptomycin, and RPMI-1640 media were purchased from Gibco. Dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Bio Basic Inc. Human promyelocytic leukemia cell line (HL-60) was purchased from the American Type Culture Collection (ATCC).

### Extraction of rice samples

A portion (50 g) of each pigmented rice sample was extracted with 100 ml of HCl in ethanol (0.01 ml/ml) at room temperature for 1 hour. The extracts were filtered with Whatman® filter paper no.1 and stored at 4 °C until analysis.

### Absorption spectrum

The visible absorption spectrum (400–700 nm) of each pigmented rice extract solution was determined using a UV-Vis spectrophotometer (Biochrom, USA).

### Determination of total phenolic content

The total phenolic content (TPC) was analyzed using the Folin-Ciocalteu assay [20]. Briefly, the pigmented rice extracts (20 µl) were mixed with 50 µl of deionized water, 20 µl of Folin-Ciocalteu reagent and 125 µl of 0.07 mg/ml sodium carbonate. Samples were incubated for 90 min at room temperature. The absorbance was measured at 750 nm using a microplate reader (Biochrom, USA). The TPC of samples was determined using a gallic acid standard solution and expressed as gallic acid equivalents (mg GAE/ml extract).

### Determination of total anthocyanins content

The total anthocyanins content (TAC) was determined by using the pH-differential method [21]. The buffer systems were prepared as 0.025 M potassium chloride buffer, pH 1.0, and 0.4 M sodium acetate buffer, pH 4.5. Pigmented rice extracts (20 µl) were mixed with 180 µl of each buffer and incubated for 10 min at room temperature. The absorbance was measured at 520 and 700 nm using a microplate reader. The TAC was calculated using the following equation and expressed as cyanidin-3-glucoside equivalents (mg C3G/l extract).

$$\text{Total anthocyanins content} = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1}$$

Where  $A = (A_{520} - A_{700})_{\text{pH}=1.0} - (A_{520} - A_{700})_{\text{pH}=4.5}$ , MW = molecular weight, DF = dilution factor, 1 = path length in cm,  $\epsilon = 26900$  molar extinction coefficient in  $\text{L mol}^{-1} \text{cm}^{-1}$  for cyanidin-3-glucoside, 1000 = conversion from g to mg

### Determination of total procyanidins content

The total procyanidins content (TPCC) was quantified using the Vanillin assay [22]. In brief, the pigmented rice extracts (20 µl) were mixed with 100 µl of vanillin in sulfuric acid (0.01 mg/ml). The mixture was incubated for 15 min at room temperature and the absorbance was determined at 500 nm using a microplate reader. A standard curve for TPCC was developed using an epicatechin standard solution. The results were expressed as epicatechin equivalents (mg EPE/ml extract).

### Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) was determined according to the ferric reducing power method [23]. Pigmented rice extracts (25 µl) were mixed with 50 µl of 0.01 mg/ml potassium ferricyanide and allow standing for 60 min at room temperature.

Next, 25 µl of 0.1 mg/ml trichloroacetic acid and 75 µl of deionized water were added and the absorbance was measured at 700 nm using a microplate reader and designated as absorbance 1 (A1). Then, 25 µl of 0.001 mg/ml ferric chloride was added and the absorbance at 700 nm was measured again and designated as absorbance 2 (A2). The optical density (OD) of each sample was calculated using the following equation:

$$\text{Optical density} = (A2 - A1)_{\text{sample}} - (A2 - A1)_{\text{control}}$$

The reducing power activity was determined using an ascorbic acid calibration curve and results were expressed as ascorbic acid equivalents (mg AAE/ml extract).

### DPPH radical scavenging activity assay (DPPH)

The scavenging activity of pigmented rice extracts for 1,1-diphenyl-2-picrylhydrazyl free radicals was determined according to Rangkadilok *et al.* [24]. Reaction mixtures containing 5 µl of sample and 195 µl of 0.1 mmol/l DPPH solution were incubated for 30 min at room temperature. Absorbance was measured at 515 nm using a microplate reader. The scavenging activity was derived as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The scavenging activity of pigmented rice extract was expressed as 50% inhibition concentration (IC<sub>50</sub>, mg/ml).

### Inhibition of lipid peroxidation (TBARS)

The inhibition of lipid peroxidation was determined by the thio barbituric acid-reactive substances (TBARS) method [25]. For each pigmented rice extract, 100 µl of samples were mixed with 900 µl of 0.25 ml/ml linoleic acid and incubated for 20 min at 95 °C. Then, 1 ml of 0.01 mg/ml thiobarbituric acid in trichloroacetic acid was added and the mixtures were incubated 30 min at 95 °C. The absorbance was measured at 532 nm using a microplate reader. The inhibition of lipid peroxidation activity was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

### Cell culture

A human promyelocytic leukemia cell line (HL-60) was used in this study. These cell lines were grown in RPMI-1640 medium, supplemented with 0.1 ml/ml fetal bovine serum, and 0.01 ml/ml penicillin streptomycin solution. The cell lines were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C.

### Cytotoxicity

Pigmented rice extracts containing the highest total phenolic content (HN3, ND1, MP3 and SY3) were selected to investigate cytotoxicity on HL-60 cells. The cytotoxicity assessment was performed by MTT assay [26]. HL-60 cells (100 µl), suspended in complete culture medium at a density of  $1 \times 10^6$  cells/ml, were placed in wells of a 96-well plate. Cells were treated with diluted pigmented rice extracts at concentrations of 0.08, 0.16, 0.31, 0.63, 1.25, and 2.50 mg/ml for 24 hours. Afterward, 0.5 mg/ml of MTT solution (100 µl) was added to each well and incubated for 4 hours. The plate was centrifuge at 400 xg for 10 min to pellet the cells and the supernatant (200 µl) was aspirated. DMSO (200 µl) was added to all wells and incubated for 30 min at room temperature. The absorbance of each sample was measured at 570 nm against a reference wavelength of 650 nm using an ELISA plate reader (Sunrise, Tecan). The percentage of cell viability was calculated using the formula,

$$\text{Viable cell (\%)} = \frac{A_{\text{treated group}}}{A_{\text{untreated group}}} \times 100$$

The cytotoxic activity of pigmented rice extracts was expressed as the IC<sub>50</sub> (mg/ml).

### Statistical analysis

All measurements were performed in triplicate. The obtained data were statistically analyzed using the SPSS program version 11.5 for

Windows (SPSS Inc, Chicago, IL, USA). The comparison of data between each sample was analyzed by using One Way Analysis of Variance (ANOVA) and the differences were considered significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

The acidic ethanol extracts from black and red rice both exhibited a red-brown color, but red rice extracts appeared lighter in color with

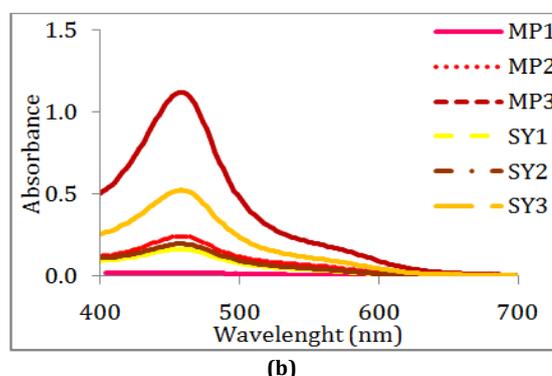
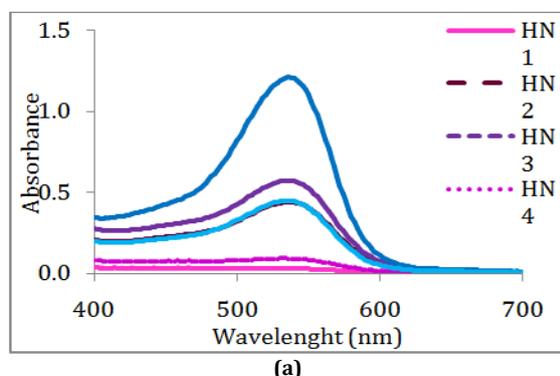


Fig. 1: Absorption spectrum of (a) black rice extracts and (b) red rice extracts

Phenolic compounds have been found to be a major source of antioxidant activity, with the ability to neutralize free radicals and play a crucial role in stabilizing lipid membrane by preventing lipid peroxidation [1]. Moreover, they have the potential to reduce the risk of cardiovascular diseases, cancer and aging [29]. In this study, total phenolic content (TPC) of pigmented rice extracts was determined using the Folin-Ciocalteu assay and expressed as gallic acid equivalents (mg GAE/ml extract). As shown in table 2, TPC ranged from 0.05 to 0.54 mg GAE/ml for black rice extracts and from 0.21 to 0.99 mg GAE/ml for red rice extracts. Significant differences were found among rice samples, of which the highest TPC values were red MP3 (0.99 mg GAE/ml), followed by red SY3 (0.65 mg GAE/ml), black ND1 (0.54 mg GAE/ml), and black HN3 (0.42 mg GAE/ml) ( $p < 0.05$ ). These results showed red rice had tend to higher phenolic content than black rice, it similar the result of Min *et al.* [30]. Although these results differ from those of previous studies [31, 32], they are consistent with red rice having a higher phenolic content than black rice. This might be because the phenolic content may depend on pigmented rice varieties, cultivated location, growing condition, climate conditions, harvesting time, and production process [33].

Anthocyanins are a group of hydrophilic flavonoids which are responsible for red and purple color in plants [34]. Numerous reports have described the potential of these compounds to reduce hyperlipidemia, atherosclerotic plaque formation and cancer [18, 35-37]. The major anthocyanins in pigmented rice were previously identified as cyanidin-3-glucoside and peonidin-3-glucoside [14] (fig. 2).

In this study, total anthocyanins content (TAC) was analyzed using the pH-differential method and the results are shown in table 2. TAC in black rice extracts ranged between 0.13 and 4.64 mg C3G/l, while red rice extracts ranged between 0.03 and 0.29 mg C3G/l. The mean of anthocyanin content in black rice (1.51 mg C3G/l) was about 14-fold higher than that of red rice (0.11 mg C3G/l). This result is in close agreement with Abdel-Aal *et al.* [38], who reported that the mean anthocyanins content in black rice (3.276 mg/g) was about 35-fold higher than that of red rice (0.094 mg/g).

Procyanidins, known as condensed tannins, are polymers of flavan-3-ol monomer units (catechin and epicatechin diastereoisomers) [39] (fig. 3). They are the main precursors of the blue-violet and red pigments in plants [40]. Procyanidins have been reported to be highly effective in protecting against cardiovascular diseases and cancer [41].

a yellow tint. The absorption spectra of all extracts are shown in fig. 1. The visible spectra of black rice extracts showed a  $\lambda_{max}$  at 530-536 nm, which is consistent with the presence of anthocyanins as the predominant pigments [21, 27]. In contrast, red rice extracts showed a different  $\lambda_{max}$  at 458 nm, characteristic of procyanidins as the predominant pigments [28]. The ND1 extract showed the highest absorbance value at  $\lambda_{max}$  in the black rice group, while the MP3 extract exhibited the highest absorbance in the red group.

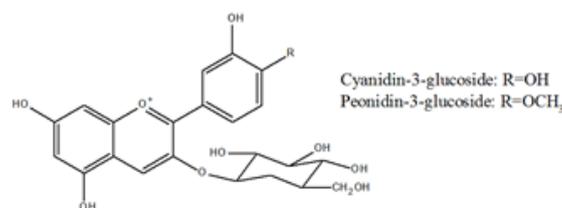


Fig. 2: Structures of cyanidin 3-glucoside and peonidin 3-glucoside

In this study, total procyanidin content (TPCC) was determined using the vanillin assay and the results were expressed as epicatechin equivalents (table 2). The TPCC of black rice extracts varied over a 3.3-fold range (from 0.10 to 0.33 mg EPE/ml); likewise, TPCC of red rice extracts varied over a 3.8-fold range (from 0.18 to 0.69 mg EPE/ml). Although the two highest TPCC values were found in red rice cultivars (MP3 and SY3), one black cultivar (ND2) exhibited a higher TPCC than the four remaining red rice cultivars. These results are in agreement with the study of Finocchiaro *et al.* [11], which reported that one black rice cultivar had no procyanidins and another cultivar had a large amount of procyanidins, significantly higher than that of the red rice cultivars they tested. A possible explanation for this might be that the content of procyanidins not only depended on rice color phenotype but also depended on genotype of rice. There was a very high positive correlation between TPC and TPCC among the red rice cultivars ( $r = 0.956$ ), strongly suggesting that procyanidins are the major phenolic compounds in red rice; this result is consistent with the previous findings of Oki *et al.* [28] In contrast, the correlation between TPC and TPCC among the black rice samples was very low ( $r = 0.194$ ), suggesting that procyanidins are not the predominant phenolic compounds in black rice varieties. This latter result is consistent with the conclusion from the preceding section, i.e., that anthocyanins are the major phenolic compounds in black rice.

The reducing power of pigmented rice extracts was determined by the ferric reducing antioxidant power assay (FRAP). An antioxidant present in a sample can directly donate an electron to the  $Fe^{3+}$ /ferricyanide complex, reducing it to the ferrous form which can be monitored by measuring the Prussian blue color at 700 nm [1]. The antioxidant power of black rice extracts ranged from 0.06 to 0.63 mg AAE/ml, while red rice extracts ranged from 0.23 to 1.36 mg AAE/ml (table 2). The highest FRAP activities were observed for two

red rice cultivars (MP3 and SY3), followed by two black rice samples (ND1 and HN3). Overall, the color of rice showed no significant effect on the ferric ion reducing ability, supporting the previous finding of Sompong *et al.* [42]. In addition, pair-wise correlations between TPC and FRAP were highly positive ( $p < 0.01$ ) among all rice groups ( $r = 0.960$ ), black rice groups ( $r = 0.943$ ) and red rice groups ( $r = 0.963$ ). This result indicates that the major source of antioxidant activity in pigmented rice is phenolic compounds, which are known to act as powerful reducing agents.

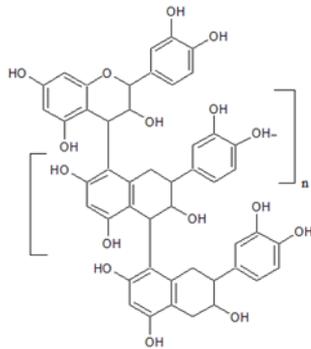


Fig. 3: Structure of procyanidins

Table 2: Total phenolic, anthocyanin, procyanidin content and antioxidant activities of pigmented rice\*

Rice sample	TPC (mg GAE/ml)	TAC (mg C3G/l)	TPCC (mg EPE/ml)	FRAP (mg AAE/ml)	DPPH (IC <sub>50</sub> ; mg/ml)	TBARS (% inhibition)
Black rice						
HN1	0.05±0.01 <sup>h</sup>	0.13±0.02 <sup>d,e</sup>	0.12±0.01 <sup>f,g</sup>	0.06±0.01 <sup>h</sup>	0.14±0.00 <sup>b</sup>	40.48±1.44 <sup>a</sup>
HN2	0.16±0.03 <sup>g</sup>	2.18±0.21 <sup>b</sup>	0.13±0.00 <sup>f,g</sup>	0.23±0.01 <sup>g</sup>	0.59±0.01 <sup>g</sup>	14.79±1.31 <sup>c,d,e</sup>
HN3	0.42±0.05 <sup>d</sup>	0.48±0.06 <sup>d</sup>	0.23±0.02 <sup>d</sup>	0.48±0.07 <sup>d</sup>	0.57±0.00 <sup>f</sup>	20.92±5.01 <sup>b</sup>
HN4	0.15±0.00 <sup>g</sup>	0.41±0.01 <sup>d,e</sup>	0.10±0.01 <sup>g</sup>	0.30±0.00 <sup>g</sup>	0.26±0.02 <sup>c</sup>	15.44±0.39 <sup>c,d</sup>
ND1	0.54±0.03 <sup>c</sup>	1.24±0.51 <sup>c</sup>	0.15±0.03 <sup>e,f</sup>	0.63±0.00 <sup>c</sup>	0.55±0.00 <sup>f</sup>	16.16±0.21 <sup>c</sup>
ND2	0.19±0.03 <sup>f,g</sup>	4.64±0.18 <sup>a</sup>	0.33±0.00 <sup>c</sup>	0.37±0.09 <sup>e,f</sup>	0.53±0.01 <sup>e</sup>	15.11±0.10 <sup>c,d,e</sup>
Average	0.25±0.19	1.51±1.70	0.18±0.09	0.34±0.20	0.44±0.19	20.48±10.06
Red rice						
MP1	0.21±0.02 <sup>f,g</sup>	0.11±0.06 <sup>d,e</sup>	0.18±0.02 <sup>e</sup>	0.47±0.01 <sup>d</sup>	0.10±0.00 <sup>a</sup>	11.19±2.52 <sup>d,e,f</sup>
MP2	0.33±0.01 <sup>e</sup>	0.29±0.00 <sup>d,e</sup>	0.18±0.01 <sup>e</sup>	0.42±0.04 <sup>d,e</sup>	0.12±0.01 <sup>a</sup>	12.81±2.58 <sup>c,d,e</sup>
MP3	0.99±0.07 <sup>a</sup>	0.03±0.00 <sup>e</sup>	0.69±0.01 <sup>a</sup>	1.36±0.04 <sup>a</sup>	0.15±0.00 <sup>b</sup>	20.98±1.61 <sup>b</sup>
SY1	0.25±0.02 <sup>f</sup>	0.13±0.03 <sup>d,e</sup>	0.22±0.00 <sup>d</sup>	0.26±0.03 <sup>g</sup>	1.12±0.02 <sup>h</sup>	10.51±0.67 <sup>e,f</sup>
SY2	0.27±0.02 <sup>e,f</sup>	0.05±0.01 <sup>e</sup>	0.22±0.02 <sup>d</sup>	0.23±0.00 <sup>g</sup>	0.34±0.00 <sup>d</sup>	7.57±0.78 <sup>f</sup>
SY3	0.65±0.02 <sup>b</sup>	0.08±0.02 <sup>d,e</sup>	0.60±0.03 <sup>b</sup>	0.78±0.03 <sup>b</sup>	0.17±0.01 <sup>b</sup>	20.87±0.15 <sup>b</sup>
Average	0.45±0.31	0.11±0.09	0.35±0.23	0.59±0.43	0.33±0.40	13.99±5.63

\*Mean±SD (n=3); different superscript letters in the same column indicate values that are significantly different at  $p \leq 0.05$ .

HL-60 cells have been reported as a valid model to determine the anticarcinogenic potential of various chemicals [46]. The demonstration of cytotoxic effects on cancer cells can indicate a chemical's antiproliferation activity, as well as its potential use in a strategy for cancer prevention [47]. In this study, the four pigmented rice extracts containing the highest total phenolic content were selected (MP3 and SY3 for red rice; ND1 and HN3 for black rice) to investigate cytotoxicity on HL-60 cells. Cells were exposed to various concentrations (up to 2.5 mg/ml) from the selected pigmented rice extracts for 24 h, and the number of viable cells was determined using an MTT assay. All extracts at concentrations lower than 0.31 mg/ml had no toxicity effect on HL-60 cells. The 50% inhibitory concentration (IC<sub>50</sub>) values obtained were 1.31, 1.14, 1.22 and 1.74 mg/ml for HN3 (black), ND1 (black), MP3 (red) and SY3 (red) extracts, respectively. Since lower IC<sub>50</sub> values indicate higher cytotoxicity, the ND1 (black) extracts showed more potent cytotoxicity than other. While, IC<sub>50</sub> of positive standard, cyanidin-3-glucoside, was 0.67 mg/ml. Dreiseitel [48] previously reported that the IC<sub>50</sub> of cyanidin-3-glucoside on HL-60 cells was 12.6 μmol/l, while the IC<sub>50</sub> of procyanidin B1 (a procyanidin dimer) was higher than 100 μmol/l. In addition, Jo *et al.* [49] suggested that cytotoxicity

The DPPH radical scavenging activity assay is one of the most commonly used methods to evaluate the free radical scavenging activity of samples. This method determines the electron- or hydrogen-donating ability of a sample, based on the reduction of a purple DPPH radical to become a stable, essentially colorless diamagnetic molecule (non-radical) [43]. The IC<sub>50</sub> values ranged from 0.14 to 0.59 mg/ml for black rice extracts and from 0.10 to 1.12 mg/ml for red rice extracts, and there was no significant difference between the two groups. Moreover, the pigmented rice extract showed good inhibitory activity against DPPH radicals when compared with ascorbic acid as positive standard (0.16 mg/ml).

Lipid peroxidation is one factor contributing to ageing and classical oxidative stress-linked diseases, such as neurodegenerative diseases, diabetes, atherosclerosis and cancer [44]. The high inhibition of lipid peroxidation by some plant extracts may represent an indicator of their high therapeutic potential [2]. Using the TBARS method, the inhibition of lipid peroxidation by extracts from black and red rice groups was not significantly different. The highest inhibition of TBARS was black HN1 (40.48%), followed by red MP3 (20.98%), black HN3 (20.92%), and red SY2 (20.87%), and there was no significant difference between the two color groups. Although, previous studies also reported that red rice had higher inhibition of lipid peroxidation than black rice [45]. The different results were possibly due to different sources and varieties of rice used in the experiments. Moreover, the pigmented rice extract showed significant TBARS compared with the values obtained for 0.1 mg/ml ascorbic acid as positive standard (18.69%).

of procyanidins may be proportional to the degree of polymerization. Thus, a more complete characterization of the bioactive compounds in pigmented rice samples will be important for future studies of cytotoxicity on cancer cells.

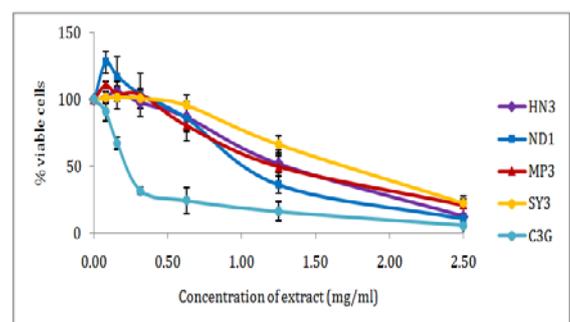


Fig. 4: The percentage of HL-60 cells viability after exposure to pigmented rice extracts

## CONCLUSION

Pigmented rice is one of the good sources of antioxidant compounds including phenolics, anthocyanins and procyanins. The highest TPC values were red MP3, followed by red SY3, black ND1, and black HN3. The anthocyanin content in black rice was about 14-fold higher than that of red rice, while, the procyanidin content in red rice was about 2-fold higher than that of black rice. In antioxidant assays, the highest FRAP, DPPH, and TBRAS was found red MP3, red MP1, and black HN1, respectively. Moreover, red rice showed lower cytotoxicity on HL-60 cells than black rice. Although, there was no significant difference between two color groups in antioxidant activities. In overall, red rice varieties tend to higher antioxidant potential than that of black rice varieties. Thus, there are interesting to further study about an effect of pigmented rice to human cells and the health promoting properties of them, especially anti-cancer.

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## CONFLICT OF INTERESTS

Declared None

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