Journal of Current Science and Technology, January-March, 2025 Vol. 15 No. 1, Article 84

ISSN 2630-0656 (Online)

Cite this article: Punfa, W., Tantipaiboonwong, P., Pintha, K., Suttajit, M., & Khanaree, C. (2025). Evaluation of phytoconstituents, nutritional quality, and *in vitro* biological activities of red rice ethanolic extract from different regions of Chiang Rai and Phayao. *Journal of Current Science and Technology*, *15*(1), Article 84. https://doi.org/10.59796/jcst.V15N1.2025.84

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Evaluation of Phytoconstituents, Nutritional Quality, and *In Vitro* Biological Activities of Red Rice Ethanolic Extract from Different Regions of Chiang Rai and Phayao

Wanisa Punfa[1], Payungsak Tantipaiboonwong[2], Komsak Pintha[2], Maitree Suttajit[2], and Chakkrit Khanaree[1,*]

[1]School of Traditional and Alternative Medicine, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand
[2]Division of Biochemistry, School of Medical Sciences, University of Phayao, Phayao 56000, Thailand

[*]Corresponding author; E-mail: chakkrit.kh@gmail.com

Received 9 September 2024; Revised 26 September 2024; Accepted 26 September 2024; Published online 21 December 2024

Abstract

Red rice, a pigmented rice, is a staple food in Thailand that has beneficial biological properties for the consumer. The objective of this research was to assess the phytochemical composition, macro- and micro-nutritional quality, as well as the anti-glycation and *in vitro* antioxidant properties of three planting sites located in Chiang Rai and three planting sites located in Phayao. Raw red rice was extracted with 70% ethanol, and the phytoconstituents were evaluated using colorimetric analysis and HPLC techniques. The antioxidant activity, ROS production, lipid peroxidation, and *in vitro* anti-glycation properties were examined. According to the results, the greatest levels of total phenolic and total flavonoid contents were detected in CRR1 and PYR1. The amounts of fat, protein, fiber, and carbohydrates were comparable in red rice extracts. The PYR3 had the greatest quantities of iron and zinc, whereas the CRR3 had the highest levels of magnesium and potassium. The CRR1 and PYR1 had the highest amounts of vitamin E compounds and γ-oryzanol. Additionally, CRR1 and PYR1 had greater antioxidant capacities in comparison to the other red rice extracts. In the RAW264.7 macrophage cell, it was demonstrated that CRR1 and PYR1 prevented the generation of AGE at higher concentrations and had the strongest inhibitory effects on linoleic acid peroxidation and ROS production. The study's findings offered additional valuable sources of red rice from Chiang Rai and Phayao, giving consumers the option to choose red rice as beneficial for their health.

Keywords**:** *red rice; phytochemical contents; nutritional quality; northern Thailand; antioxidants; anti-glycation*

1. Introduction

Rice, also known as *Oryza sativa* L., is a staple grain that is grown across a large region, from temperate to tropical zones, particularly in Asia, and is consumed by over half of the world's population (Krishnan et al., 2020). There are various cultivars of rice, such as white, red, black, and purple rice, based on the color of the pericarp (Thushara et al., 2019). Previous studies have demonstrated that pigmented rice has a greater phenolic content, antioxidant activity, and variety of health-promoting phytochemicals than white rice (Sompong et al., 2011). Rice is one of Thailand's most important cereal crops and a vital source of carbohydrates. The second-largest rice-planting area is in northern Thailand (Office of Agricultural Economics, n.d.). Although there are few flat regions and most of the area is mountainous, rice is still cultivated there. There are many varieties of rice grown in northern Thailand, and red jasmine rice is widely cultivated in Chiang Rai and Phayao provinces. It has a distinctive red color and resistance

to plant diseases (Department of Foreign Trade. Ministry of Commerce, 2016).

Red rice is rich in γ-oryzanol, vitamin E, and pigmental components such as polyphenols and flavonoids, especially proanthocyanidin. These components confer a higher antioxidant capacity compared to other rice varieties (Pakuwal, & Manandhar, 2021). Furthermore, several studies showed that red rice has anti-inflammatory, anti-carcinogenic, anti-allergic, anti-hyperlipidemic, and anti-hyperglycemic effects (Pintha et al., 2014; Tantipaiboonwong et al., 2017; Veni, 2019; Jadhav, 2021). The nutritional quality of red rice is based on a carbohydrate structure with a low glycemic index and small amounts of crude protein and fat. It is also composed of minerals such as calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), manganese (Mn), and zinc (Zn) (Baptista et al., 2024). Therefore, red rice has received more attention due to its higher nutrient contents and wide range of health advantages. Red rice is currently gaining popularity in Thailand and beyond. The nutritional value of food and/or supplements is an important aspect of consumer decision-making. Nevertheless, a thorough examination of the phytoconstituents and nutrient concentrations of rice indicates that many factors, including planting locations, water, and temperature, might affect the nutritional value (Sadimantara et al., 2019). For example, the concentrations of anthocyanin, zinc, and iron in pigmented rice varieties grown in wetland and aerobic conditions differ. The nutritional quality of pigmented rice was lower when grown in aerobic soil compared to wetland soil (Jaksomsak et al., 2020).

There had been no reports on the assessment of the phytochemical content, nutritional value, and biological activity of red rice, specifically the characteristics of red rice from northern Thailand. Therefore, the current study focused on total phytochemical contents, nutritional qualities, *in vitro* antioxidants, and anti-glycation properties of three planting areas in Chiang Rai and three planting areas in Phayao.

2. Objectives

This study aimed to evaluate the total phenolic, flavonoid, proanthocyanidin contents, and macro- and micro-nutritional qualities and examine *in vitro* antioxidants, and anti-glycation properties of three planting areas in Chiang Rai and three planting areas in Phayao.

3. Materials and Methods

3.1 Plant Collection and Extraction

Raw red rice was collected from three planting areas in Chiang Rai and three planting areas in Phayao. Plant species were authenticated by comparing them to herbarium specimens from the Faculty of Pharmacy at Chiang Mai University in Thailand. The whole raw red rice was pulverized and then extracted with 70% ethanol by shaking overnight at room temperature. The ethanol solution was then filtered through filter paper to remove the residue. After the samples were filtered, they were evaporated with a rotary vacuum evaporator (BUCHI, Switzerland) and then freeze dried to get crude raw red rice extract powder. The raw red rice from three planting areas in Chiang Rai and three planting areas in Phayao is referred to as CRR1 (Mae Lao District), CRR2 (Mae Chan District), and CRR3 (Mueang Chiang Rai District), or PYR1 (Dok Kham Tai District), PYR2 (Chun District), and PYR3 (Mueang Phayao District), respectively. The six extracts were kept at -20°C until use.

3.2 Determination of Total Phenolic, Flavonoid, and Proanthocyanidin Contents

The Folin-Ciocalteu assay was used to measure the total phenolic concentration in red rice extracts (Khanaree et al., 2021). Briefly, red rice extracts were dissolved in DMSO at assigned concentrations. Twenty microliters of red rice extracts were mixed with one hundred microliters of 10% v/v Folin-Ciocalteu's reagent in a 96-well plate. The plate was then left to sit at room temperature for three minutes in the dark. Next, 80 µl of 7.5% w/v sodium carbonate (Na_2CO_3) was added to each well, followed by incubation for 30 min in the dark at room temperature. The absorbance was quantified at a wavelength of 765 nm and gallic acid was used as a standard. The total phenolic content was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of the extract.

The colorimetric technique with aluminum chloride was used to determine the total flavonoid concentration in red rice extracts (Khanaree et al., 2021). In a 96-well plate, add 25 µl red rice extracts, 125 µl deionized water, and 7.5 µl 5% $NaNO_2$, followed by incubation for 6 min in the dark at room temperature. After incubation, add 15 µl of 10% w/v aluminum chloride ($AlCl_3$) and incubate for another 6 min. To develop the color, 50 µl of 1M NaOH was added. To prepare the reaction mixture, 250 µl of deionized water was added and incubated at room

temperature for 15 min. The absorbance was quantified at a wavelength of 532 nm, and catechin was used as a standard. The total flavonoid content was calculated and expressed as milligrams of catechin equivalents (CE) per gram of dry weight of the extract.

The total proanthocyanidin content in red rice extracts was analyzed using vanillin assay (Pintha et al., 2014). Red rice extracts were dissolved in sulfuric acid/methanol and mixed with 0.1 ml of 1% w/v vanillin in methanol (0.1 ml), which was then incubated in a water bath at 30°C for 15 min. The sample's absorbance was measured at 490 nm compared to a catechin standard curve at different concentrations. The quantity of total proanthocyanidin concentration in red rice extracts was quantified as milligrams of catechin equivalents (CE) per gram of dry extract weight.

3.3 HPLC Analysis for Phenolic, Flavonoid, γ-oryzanol, and Vitamin E Derivatives in Red Rice Extracts

We used an Inertsil ODS-3-C18 column (250 x 4.6 mm, 5 µm particle diameter, GL Science Inc., Japan) to measure the phenolic and flavonoid compounds as well as γ-oryzanol in red rice extracts. To detect phenolic and flavonoid compounds, gradient elution was utilized with solvents A (0.1% trifluoroacetic acid in water) and B (100% methanol), whereas isocratic elution (methanol: acetonitrile; 65:35) was employed to detect γ-oryzanol. A total of 10 microliters of the samples were injected into the column at a flow rate of 1.0 ml/min. Standard phenolic and flavonoid compounds and γ-oryzanols were measured at 280 and 325 nm, respectively. To find the vitamin E derivatives in red rice extracts, an HPLC C30 column (250 × 4.6 mm, 5 µm particle) was used with isocratic elution and a mobile phase of methanol: H_2O (93:7). A 10 µl injection volume was utilized. Standards for Vitamin E compounds were measured at 292 nm (Pintha et al., 2014).

3.4 Nutritional Analysis

The red rice extracts were tested for their content of total carbohydrates, fat, proteins, ash, and moisture by applying the official methods of analysis of Association of Official Analytical Chemists (AOAC) (AOAC, 2016). Iron, zinc, potassium, and magnesium in red rice extracts were estimated using an atomic absorption spectrophotometer by applying the official methods of analysis of AOAC (AOAC, 2016).

3.5 Determination of Antioxidant Actives

3.5.1 DPPH Free Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging experiment (Khanaree et al., 2021), with a few modifications, was used to assess the antioxidant activity of red rice extracts. In a 96-well plate, red rice extracts at certain concentrations (20 µl) were combined with 0.2 mM DPPH reagent (180 µl). The reaction mixture was incubated at room temperature for 30 min in the dark. The measurement of absorbance was conducted at a wavelength of 517 nm using an ELISA plate reader. For the control, methanol was used as a reagent blank, whereas for the test, methanol was mixed with varying quantities of the extract. The scavenging inhibition percentage was calculated and reported as the concentration of the extracts that reduced free radicals by 50% (SC_{50}).

3.5.2 Radical Cation $ABTS^{\cdot+}$ Scavenging Activity

The antioxidant activity of red rice extracts was measured using the 2,20-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging assay (Khanaree et al., 2021) with slight modification. The red rice extracts (10 µl) at given concentrations were mixed with the working ABTS solution (990 µl). The reaction mixture was incubated at room temperature in the dark for 6 minutes before being measured using a UV-visible spectrophotometer at a wavelength of 734 nm. The control reagent blank was water, whereas the test reagent blank was a mixture of water and varying quantities of the extract. Scavenging inhibition percentage was calculated and reported as the concentration of the extracts that reduced free radicals by 50%. (SC_{50}).

3.6 Cell Lines and Culture Condition

The American Type Culture Collection provided RAW264.7 murine macrophage cells (ATCC; Manassas, VA, USA). The RAW264.7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM), which had 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) added to it. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2.

3.7 Determination of Cytotoxicity by MTT Assay

The cytotoxicity of red rice extracts on RAW264.7 cells was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as previously described (Phannasorn et al., 2017). The RAW264.7 cells (5×10^5 cells/well) were

seeded in a 96-well plate for 24 h before being treated with extracts at various doses for another 24 h. To begin, 15 µl of 5 mg/ml MTT dye was added and incubated for 4 h at 37ºC. Discard all solutions and dissolve the formazan crystal with 100 µl of DMSO. The formazan dye's absorbance (OD) was measured using an ELISA plate reader at 540/630 nm. Cell viability was determined, and non-cytotoxic concentrations ($\leq IC_{20}$) were chosen for further research.

3.8 Determination of Intracellular Reactive Oxygen Species (ROS) Level

The 2',7'-dichlorofluorescein-diacetate (DCFH-DA) dye was used to evaluate the formation of intracellular ROS in RAW264.7 cells. The RAW264.7 cells were cultured with DCFH-DA for 2 h and then treated with $FeSO_4$ and non-cytotoxic doses of the extracts. The co-incubation lasted 30 min at 37°C. In the presence of ROS, the DCFH-DA de-esterified to produce a detectable luminous product. A fluorescent microplate reader with an excitation wavelength of 480 nm and an emission wavelength of 525 nm was used to measure the fluorescent intensity that corresponded to intracellular ROS levels (Punfa et al., 2022).

3.9 Determination of Lipid Peroxidation

The linoleic acid peroxidation inhibitory ability of the extracts was tested using the method of Choi et al., (2002), with some modifications. The linoleic acid emulsion was made in phosphate buffer saline (PBS, pH 7.0) with Tween 20 as an emulsifying agent. The reaction mixture included 20 mM linoleic acid emulsion, 100 mM Tris-hydrochloric acid (HCl), 20 mM ascorbic acid, and varying amounts of sample or control γ-oryzanol. The reaction was then started by adding 40 mM $FeSO_4 \cdot H_2O$. After 30 min of incubation at 37°C in the dark, 103.5 µl of 40% v/v trichloroacetic acid was added to end the reaction. The reaction mixture was then treated with 1% w/v thiobarbituric acid in a 50 mM sodium hydroxide solution. The mixture was heated to 95°C for 10 min before centrifugation at 3,000 rpm for 10 min. The thiobarbituric acid-reacting substance (TBARS) absorbance was measured at 532 nm. All samples were examined in triplicate. The results were represented as the 50% inhibitory concentration value (IC_{50}).

3.10 Determination of *In Vitro* Glycation of Bovine Serum Albumin

BSA glycation was performed using a method described in the literature (Muñiz et al., 2018), with some modifications. The extracts were mixed with DMSO (200 µg/ml) and then incubated for two weeks at 37°C in 10 mg/ml BSA, 1.1 M glucose, 0.1 M phosphate buffer with a pH of 7.4, and 0.2% sodium azide. The production of glycated BSA was evaluated at 355 nm excitation and 460 nm emission (GENios, TECAN). Aminoguanidine (AG) was utilized as the positive control.

3.11 Statistical Analysis

All data are presented as mean±standard deviation (SD) values. Statistical analysis was analyzed with Prism version 6.0 software using one-way ANOVA with Tukey's multiple comparisons test or Dunnett's multiple comparisons test at $p<0.05$ levels. For the phytoconstituents, nutritional quality, antioxidant properties, and lipid peroxidation inhibition activity, a, b, c, d, e, f indicated statistical differences among red rice in different regions of Chiang Rai and Phayao as follows, a = significant difference compared to CRR1, b = significant difference compared to CRR2, c = significant difference compared to CRR3, d = significant difference compared to PYR1, e = significant difference compared to PYR2, and f = significant difference compared to PYR3.

4. Results

4.1 Total Phenolic, Flavonoid, and Proanthocyanidin Contents of Red Rice Extracts

The yield and phytochemical characterization data are shown in Table 1. The overall phenolic, flavonoid, and proanthocyanidin contents of six red rice extracts were different among red rice sources. The total phenolic content of six red rice extracts varied from 166.52±1.89 (PYR3) to 217.28±5.79 (CRR1) mg GAE/g extract. CRR1 extract had the highest total phenolic content, followed by CRR2, PYR1, CRR3, PYR2, and PYR3, respectively. The obtained flavonoid content varied from 49.97±6.31 (PYR3) to 88.51±3.07 (PYR1) mg CE/g extract. The extracts with the highest total flavonoid content were PYR1, followed by CRR1, CRR2, PYR2, CRR3, and PYR3, respectively. The proanthocyanidin content of red rice extracts varied from 58.06±2.64 (PYR2) to 96.21±8.44 (CRR3) mg CE/g extract. CRR3 extracts revealed the highest total proanthocyanidin content, followed by CRR2, CRR1, PYR1, PYR3, and PYR2,

respectively. This outcome can be attributed to their phenolic and flavonoid profiles.

4.2 Phytochemical Profiles in Red Rice Extracts by HPLC

The separated phytoconstituents of the HPLC test of red rice extracts showed five phenolic compounds including protocatecheuic acid, vanillic acid, chlorogenic acid, coumaric acid, and ferulic acid, and two flavonoid compounds including catechin and epicatechin. The total amount of phenolic compounds in red rice extracts was calculated to be 58.69, 48.37, 39.28, 43.10, 38.67, and 32.11 mg/kg extract in CRR1, CRR2, CRR3, PYR1, PYR2, and PYR3, respectively, which corresponded to the total phenolic content by colorimetric technique, as shown in Table 2. The total flavonoid content in red rice extracts were calculated to be 26.29, 20.53, 17.21, 29.45, 16.23, and 11.18 mg/kg extract in CRR1, CRR2, CRR3, PYR1, PYR2, and PYR3, respectively, corresponding to the total flavonoid content by colorimetry, as shown in Table 3.

Red rice is known to be rich in γ-oryzanol and vitamin E. Therefore, γ-oryzanol and vitamin E derivatives including α-tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol of red rice extracts were determined as shown in Table 4. The total vitamin E content of red rice extracts was calculated using α-tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol. The total vitamin E content in red rice extracts varied from 60.74 (CRR3) to 162.56 (PYR1) mg/kg extract. PYR1 had the highest total vitamin E contents, followed by CRR1, CRR2, PRY3, PYR2, and CRR3, respectively. Additionally, the total amount of γ-oryzanol in red rice extracts ranged from 36.37 (PYR3) to 108.74 (CRR1) mg/kg. CRR1 had the highest amount of γ-oryzanol, followed by PYR1, PYR2, CRR3, CRR2, and PYR3.

Table 1 The results of yield and phytochemical characterization in red rice extracts

Extract	%Yield	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg CE/g extract)	Total proanthocyanidin content (mg CE/g extract)
CRR1	1.68	217.28 ± 5.79^{bcdef}	84.45 ± 3.62^{bcef}	91.44 ± 7.15^{ef}
CRR2	1.37	194.93 ± 1.74^{acef}	65.24 ± 6.03^{adf}	92.11 ± 7.48^{ef}
CRR3	1.63	184.38 ± 4.52^{abf}	58.78 ± 5.33^{ad}	96.21 ± 8.44^{ef}
PYR1	1.64	188.93 ± 2.12^{af}	88.51 ± 3.07^{bcef}	81.98 ± 6.21^{ef}
PYR2	1.14	180.80 ± 1.78^{abf}	64.78 ± 3.69^{adf}	58.06 ± 2.64^{abcd}
PYR3	1.65	166.52 ± 1.89^{abce}	49.97 ± 6.31^{abde}	61.08 ± 7.53^{abcd}

The data are demonstrated in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao ($p<0.05$, One way ANOVA with Tukey's test)

Table 2 The results of total phenolic compounds in red rice extracts

Extract	The amount of phenolic compounds (mg/kg extract)					Total phenolic compounds (mg/kg extract)
	Protocatecheuic acid	Vanillic acid	Chlorogenic acid	Coumaric acid	Ferulic acid	
CRR1	25.99 ± 0.43^{bcdef}	6.78 ± 0.15	21.98 ± 6.94^{e}	1.05 ± 0.10^{e}	2.89 ± 0.73	58.69
CRR2	12.66 ± 0.25^{aef}	5.61 ± 0.06	26.95 ± 4.57^{cef}	0.96 ± 0.13^{cef}	2.19 ± 0.27^{c}	48.37
CRR3	13.22 ± 0.29^{aef}	5.81 ± 0.28	15.55 ± 4.66^{b}	1.12 ± 0.02^{b}	3.58 ± 0.93^{bf}	39.28
PYR1	9.81 ± 3.65^{ae}	6.18 ± 1.00	23.00 ± 0.58^{e}	1.10 ± 0.15^{e}	3.01 ± 0.24	43.10
PYR2	17.98 ± 0.90^{abcdf}	6.96 ± 1.64	10.10 ± 3.81^{abd}	1.18 ± 0.25^{abd}	2.45 ± 0.17	38.67
PYR3	8.17 ± 2.98^{abce}	5.59 ± 0.19	15.50 ± 4.66^{b}	0.78 ± 0.13^{b}	2.07 ± 0.08^{c}	32.11

The data are demonstrated in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao ($p<0.05$, One way ANOVA with Tukey's test)

Table 3 The results of total flavonoid compounds in red rice extracts

Extract	The amount of flavonoid compounds (mg/kg extract)		Total flavonoid compounds (mg/kg extract)
	Catechin	Epicatechin	
CRR1	21.43±4.07	4.86±2.87^{bef}	26.29
CRR2	20.53±7.71	ND^{ad}	20.53
CRR3	14.70±8.35	2.51±3.54	17.21
PYR1	25.13±6.68^{f}	4.32±1.89^{bef}	29.45
PYR2	16.23±5.36	ND^{ad}	16.23
PYR3	11.18±0.46^{d}	ND^{ad}	11.18

The data are demonstrated in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test); ND = Not detected

Table 4 The results of total vitamin E and γ-oryzanol contents in red rice extracts

Extract	The amount of vitamin E derivatives (mg/kg extract)				Total vitamin E content (mg/kg extract)	γ-oryzanol (mg/kg extract)
	α-tocopherol	γ-tocopherol	α-tocotrienol	γ-tocotrienol		
CRR1	29.66±0.31^{bcedf}	11.1±0.18^{cde}	19.27±0.25^{bcdef}	97.66±0.74^{cdef}	157.69	108.74±1.20^{bcdef}
CRR2	30.78±0.38^{acdef}	10.7±0.36^{cde}	17.65±0.16^{acdef}	96.18±0.39^{cdef}	155.31	43.76±0.55^{acdef}
CRR3	17.14±0.17^{abdef}	9.28±0.18^{abdef}	ND^{abdef}	34.32±0.34^{abdf}	60.74	57.70±0.57^{abdef}
PYR1	19.87±0.32^{abcef}	17.64±0.34^{abcef}	13.64±0.18^{abcef}	111.41±0.94^{abcef}	162.56	83.56±0.77^{abcef}
PYR2	13.62±0.34^{abcdf}	5.38±0.16^{abcdf}	9.08±0.13^{abcdf}	35.82±0.33^{abdf}	63.90	65.97±0.74^{abcdf}
PYR3	18.64±0.15^{abcde}	10.57±0.34^{cde}	10.31±0.12^{abcde}	78.7±0.40^{abcde}	118.22	36.37±0.58^{abcde}

The data are presented in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test); ND = Not detected

Table 5 The results of the proximate composition of red rice extracts

Extract	Nutritional parameters (mg/kg)					
	Total carbohydrates	Total dietary fiber	Protein	Fat	Ash	moisture
CRR1	75.61±0.39^{d}	1.16±0.02^{bdef}	7.13±0.01^{cdef}	2.49±0.11^{bcde}	1.24±0.04^{bcef}	12.38±0.29^{bcdef}
CRR2	75.93±0.33^{df}	1.24±0.09^{acde}	7.06±0.13^{cdef}	2.80±0.09^{ad}	1.42±0.16^{af}	11.56±0.32^{acdef}
CRR3	75.59±0.11^{d}	1.14±0.05^{bdef}	6.21±0.12^{abdef}	2.90±0.01^{adef}	1.40±0.08^{af}	12.76±0.10^{abde}
PYR1	74.21±0.23^{abcef}	1.40±0.09^{abcef}	6.70±0.12^{abcf}	3.23±0.11^{abcef}	1.34±0.05^{f}	13.12±0.06^{abcef}
PYR2	75.73±0.31^{df}	1.30±0.01^{abcd}	6.66±0.14^{abcf}	2.74±0.16^{acdf}	1.41±0.05^{af}	12.15±0.17^{abcdf}
PYR3	75.14±0.02^{bde}	1.25±0.06^{acd}	6.87±0.07^{abcde}	2.87±0.04^{ade}	1.08±0.05^{abcd}	12.79±0.05^{abde}
Mean	75.37	1.25	6.77	2.84	1.32	12.46

The data are presented in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test)

Table 6 The results of the mineral composition of red rice extracts

Extract	Mineral (mg/kg)			
	Iron	Zinc	Magnesium	Potassium
CRR1	10.37±0.06^{bcdef}	15.34±0.89^{d}	958.00±33.49^{bde}	1522.00±13.86^{bcd}
CRR2	9.95±0.07^{acdef}	15.23±1.17	693.00±26.56^{acdef}	1076.00±34.64^{acdef}
CRR3	7.75±0.08^{abdf}	15.17±1.16	1001.00±15.01^{bde}	1755.00±115.47^{abde}
PYR1	12.8±0.10^{abcef}	12.60±1.70^{aef}	805.00±33.49^{abcef}	1322.00±57.74^{abcf}
PYR2	7.64±0.09^{abdf}	17.40±1.04^{d}	868.00±16.17^{abcdf}	1388.00±69.28^{bcf}
PYR3	13.17±0.02^{abcde}	17.80±1.03^{d}	982.00±25.40^{bde}	1645.00±34.64^{bde}
Mean	10.28	15.59	884.50	1451.33

The data are presented in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test)

4.3 Nutritional Composition in Red Rice Extracts

The results of the proximate composition of red rice extracts are presented in Table 5. The red rice extracts had similar concentrations in all components. Carbohydrates were the main macronutrient in red rice, with a mean concentration of 75.37 mg/kg. Moreover, the mean concentrations of dietary fiber, protein, fat, ash, and moisture were found to have 1.25, 6.77, 2.84, 1.32, and 12.46 mg/kg, respectively.

The mean values for iron, zinc, magnesium, and potassium were 10.28, 15.59, 884.50, and 1451.33 mg/kg, respectively. The PYR3 extract had the highest iron and zinc contents, whereas the highest magnesium and potassium contents were found in the CRR3 extract (Table 6).

4.4 Antioxidant Properties of Red Rice Extracts

Red rice's antioxidant characteristics contribute to its health benefits for humans. Therefore, the antioxidant capabilities of red rice extracts were examined using the free-radical scavenging activities of the DPPH and ABTS radicals as shown in Table 7. The CRR1 extract had the strongest DPPH radical scavenging with the lowest SC_{50} of 42.47±1.10 µg/ml, followed by PYR1, CRR2, CRR3, PYR2, and PYR3 extracts respectively. This was closely related to the total phenolic, flavonoid, proanthocyanidin, γ-oryzanol, and vitamin E derivative contents. Furthermore, CRR1 and PYR1 extracts also showed the highest ABTS radical scavenging when compared to other extracts. The CRR1 and PYR1 extracts had SC_{50} of 7.74±0.74 and 7.96±0.57 µg/ml, followed by CRR2, CRR3, PYR2, and PYR3 extracts, respectively.

This study also looked into their possible antioxidant effects on RAW264.7 cell-induced ROS. Red rice extracts at doses up to 200 µg/ml exhibited no cytotoxic impact on RAW264.7 macrophage cells after 24 h of incubation (data not shown). Therefore, red rice extracts at a concentration of 200 µg/ml were employed to study their anti-ROS properties. After $FeSO_4$ treatment, RAW264.7 cells produced a large amount of intracellular ROS. In the presence of red rice extracts (100 µg/ml), the relative levels of intracellular ROS were reduced when compared to the $FeSO_4$-treated alone. The CRR1 extract had the strongest inhibitory effect on ROS production from the RAW264.7 murine macrophage cell line (49.67±4.59% inhibition), followed by PYR1 (47.67±8.31% inhibition), CRR2 (46.33±5.39% inhibition), CRR3 (45.67±5.96% inhibition), PYR2 (44.00±7.10% inhibition), and PYR3 (39.00±4.10% inhibition), respectively (Figure 1).

Table 7 The results of DPPH and ABTS radical scavenging activity of red rice extracts

Extract	SC_{50} of DPPH scavenging activity (µg/ml)	SC_{50} of ABTS scavenging activity (µg/ml)
CRR1	$42.47±1.10^{bcdef}$	$7.74±0.74^{f}$
CRR2	$62.30±7.22^{af}$	$8.08±0.55^{f}$
CRR3	$67.93±2.39^{adf}$	$8.31±0.46^{f}$
PYR1	$56.97±0.99^{acef}$	$7.96±0.57^{f}$
PYR2	$71.17±3.65^{adf}$	$8.58±0.35^{f}$
PYR3	$104.40±4.07^{abcde}$	$10.65±0.42^{abcde}$

The data are presented in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test)

Table 8 The results of *in vitro* lipid peroxidation inhibition activity of red rice extracts

Extract	IC_{50} of lipid peroxidation inhibition (µg/ml)
CRR1	$41.33±1.83^{bcef}$
CRR2	$53.51±1.93^{af}$
CRR3	$56.57±2.76^{adf}$
PYR1	$46.66±2.28^{cef}$
PYR2	$58.81±1.87^{ad}$
PYR3	$63.85±3.98^{abcd}$

The data are presented in mean ± SD.

^{a,b,c,d,e,f} Difference letters indicated statistical differences among red rice in different regions of Chiang Rai and Phayao (p<0.05, One way ANOVA with Tukey's test)

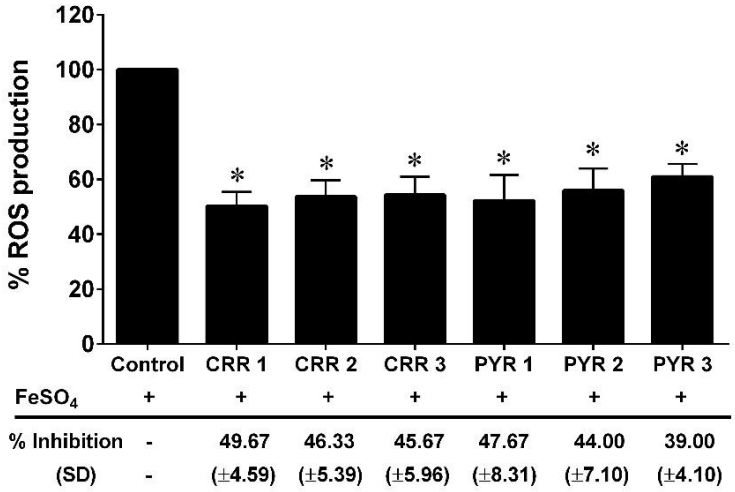

Figure 1 The effect of the extracts on intracellular ROS production in RAW264.7 cells.
The data are presented as mean±SD., *p<0.05 compared to control

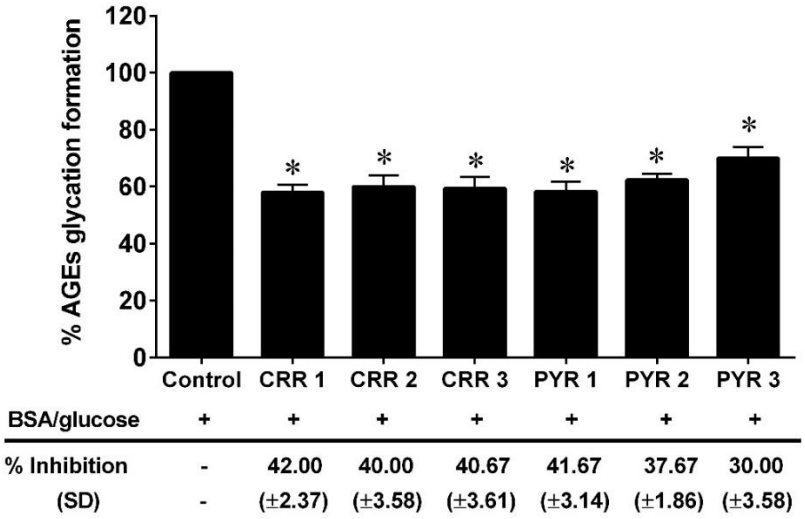

Figure 2 The effect of the extracts on AGE formation.
The data are presented as mean±SD., *p<0.05 compared to control

4.5 Effect of Red Rice Extracts on *In Vitro* Lipid Peroxidation

All extracts demonstrated the ability to suppress linoleic acid peroxidation. CRR1 exhibited the most potent inhibitory effect on linoleic acid peroxidation, with the IC_{50} value being 41.33±1.83 µg/ml. This was followed by PYR1, CRR2, CRR3, PYR2, and PYR3 showed progressively lower inhibitory effects with an IC_{50} values of 46.66±2.28, 53.51±1.93, 56.57±2.76, 58.81±1.87, and 63.85±3.98 µg/ml, respectively.

4.6 Effect of Red Rice Extracts on *In Vitro* Glycation of Bovine Serum Albumin

Glycation is a non-enzymatic interaction that occurs between sugar and the free amine groups of amino acids in protein, resulting in the generation of AGEs. Individuals with diabetes exhibit this reaction as well. In our investigation, we used the BSA-glucose model to assess rice extract's ability to suppress glycation reactions. When BSA/glucose was incubated for two weeks, fluorescence increased considerably. The addition of 100 µg/ml of red rice extracts to BSA/glucose media lowered fluorescence

intensity compared to BSA/glucose alone (Control). The CRR1 extract demonstrated considerably higher levels of the % inhibition of AGE production (42.00±2.37%) compared to control. While PYR1, CRR3, CRR2, PYR2, and PYR3 were found to suppress AGE formation by 41.67±3.14%, 40.67±3.61%, 40.00±3.58%, 37.67±1.86%, and 30.00±3.58%, respectively (Figure 2).

5. Discussion

Currently, red rice is consumed worldwide as a functional food and circuitously as a component in a variety of supplementary or alternative health foods. Red rice is a pigment-rich cereal gaining popularity among researchers due to its high nutritional value and health benefits. Nutrient quality varies between red rice cultivars grown in Thailand's northern area. In response to customer demand for functional meals, red rice cultivars with improved nutrition and health advantages might be developed. Therefore, this study focuses on the study of phytochemical contents, nutritional qualities, and *in vitro* biological activities of six selected planting places in Chiang Rai and Phayao provinces in Thailand.

The findings showed that the amounts of total phenolics, flavonoids, and proanthocyanidins in red rice extracts were different in the areas where rice was grown in Chiang Rai and Phayao provinces. The CRR1 had the highest total phenolic content (217.28±5.79 mg GAE/g extract), PYR1 had the highest total flavonoid content (88.51±3.07 mg CE/g extract), and CRR3 had the highest total proanthocyanidin content (96.21±8.44 mg CE/g extract). The active components discovered in red rice extracts were studied using HPLC. The six red rice extracts had varying quantities of protocatecheuic acid, vanillic acid, chlorogenic acid, coumaric acid, ferulic acid, catechin, and epicatechin. A previous study indicated that protocatecheuic acid, vanillic acid, chlorogenic acid, coumaric acid, ferulic acid, catechin, and epicatechin were the main phenolic and flavonoid components found in red rice (Tantipaiboonwong et al., 2017). Our findings showed that CRR1 extract had the highest levels of protocatechuic acid and epicatechin, CRR2 extract had the highest levels of chlorogenic acid, and PYR1 had the highest catechin concentrations. Interestingly, the CRR1 extract had the highest concentration of all of these chemicals, including total phenolic and flavonoid compounds (The summary of the last column of Table 2 and 3) that were determined by HPLC (84.98 mg/kg extract), followed by PYR1 (72.55 mg/kg extract), CRR2 (68.90 mg/kg extract), PYR2 (54.90 mg/kg extract), CRR3 (56.49 mg/kg extract), and PYR3 (43.29 mg/kg extract). According to prior research, phytochemical substances were present in higher concentrations in pigmented cereal grains-like red, purple, or black rice-than in non-pigmented ones (Priya et al., 2019). The same variety of rice from different places will have diverse chemical compositions when their chemical compositions are compared. Varieties, soil, and environment all contribute to this variation (Sadimantara et al., 2019).

Red rice contains vitamin E derivatives such as α-tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol in abundance (Huang, & Lai, 2016). According to the HPLC data, PYR1 extract had the highest concentration of vitamin E (162.56 mg/kg extract), followed by CRR1 (157.69 mg/kg extract), CRR2 (155.31 mg/kg extract), PYR3 (118.22 mg/kg extract), PYR2 (63.90 mg/kg extract), and CRR3 (60.74 mg/kg extract). The result showed that the red rice from several planting areas in Chiang Rai and Phayao provinces exhibited different amounts of vitamin E derivatives. This result may be due to the different environmental growing conditions of red rice, similar to findings in a previous study showing significant differences in the quantity of these vitamin E isomers amongst rice cultivars. These variations may result from variations in the ambient growing circumstances or in the genotypes that were employed (Zhang et al., 2012). The γ-oryzanol is a group of chemicals found in red rice bran oil (Chinvongamorn, & Sansenya, 2020). HPLC analysis revealed that CRR1 extract had the highest concentration of γ-oryzanol (108.74 mg/kg extract), followed by PYR1 (83.56 mg/kg extract), PYR2 (65.97 mg/kg extract), CRR3 (57.70 mg/kg extract), CRR2 (43.76 mg/kg extract), and PYR3 (36.37 mg/kg extract). Similar to vitamin E derivatives, there were differences in the levels of γ-oryzanol found in red rice from different planting locations. Furthermore, red rice is extremely nutritious and provides a significant advantage as a healthier alternative to white rice. Our findings showed that red rice extracts are high in carbohydrates, fiber, protein, fat, iron, zinc, magnesium, and potassium.

The results of DPPH and ABTS scavenging activities show that CRR1 and PYR1 had the highest antioxidant capabilities when compared to the other red rice extracts. According to the results, CRR1 and PYR1 contained the highest phenolics, flavonoids, proanthocyanidins, vitamin E, and γ-oryzanol, as well

as significant antioxidant capabilities. The antioxidant activity of red rice was related to the amount of phenolic content. Previous studies also revealed a strong correlation between the total phenolic content and the antioxidant capacity of colored rice bran extracts. This finding suggested that phenolics were the primary constituents in rice bran extracts that were responsible for their ability to scavenge free radicals (Finocchiaro et al., 2007). ROS are necessary for regular cellular activity, but excessive quantities can be detrimental. ROS-induced lipid peroxidation is crucial in cell death processes such as apoptosis, autophagy, and ferroptosis. Oxidative stress can cause inflammation by turning on the NF-κB signaling pathway, which releases cytokines that cause inflammation, such as IL-6 and TNF-α (Ramos-Tovar, & Muriel, 2020). The research showed that CRR1 and PYR1, which had the most phytochemicals, had the strongest effect on lowering ROS production and lipid peroxidation in RAW264.7 macrophage cells. AGEs, once assumed to be oxidative byproducts from diabetic hyperglycemia, are now recognized as a risk factor for islet β-cell damage, and diabetes. The current study showed that PYR1 and CRR1 reduced the production of AGE more strongly than the other red rice extracts. This aligns with earlier studies showing that proanthocyanidins in red rice can help prevent type 2 diabetes (Chen et al., 2016). According to the health benefits of red rice, customers can choose red rice with a high value of phytochemical contents and bioactivity from various cultivation areas to consume as part of their daily diet or as specialty functional foods.

6. Conclusion

The red rice in different cultivation areas in Chiang Rai and Phayao provinces exhibited a variety of phytochemical constitutions, nutritional qualities, and *in vitro* biological activities. The CRR1 and PYR1 contain the highest total phenolic and total flavonoid contents, whereas CRR3 revealed the highest total proanthocyanidin content. The red rice extracts had similar concentrations in carbohydrates, fiber, protein, and fat. Iron and zinc levels were highest in the PYR3, while magnesium and potassium contents were highest in the CRR3. The largest concentrations of vitamin E derivatives and γ-oryzanol were found in CRR1 and PYR1. Additionally, CRR1 and PYR1 had much stronger antioxidant capacities in comparison to the other red rice extracts. CRR1 and PYR1 also showed the highest inhibitory effects on linoleic acid peroxidation, ROS generation, and AGE synthesis in the RAW264.7 macrophage cells, but these effects did not significantly differ from those of the other red rice extracts. Based on this study, red rice from Chiang Rai and Phayao had phytochemical, macro- and micro-nutrient components, and contained biological activities, making them the options for consumers looking for an alternative red rice source that will enable them to maintain better health and use fewer artificial medicines.

7. Acknowledgements

This work was supported by the Agricultural Research Development Agency (Public Organization) and the Thailand Science Research and Innovation Fund and the University of Phayao, Unit of Excellence (Grant No. FF66-UoE020).

8. References

Association of Official Analytical Chemists (AOAC). (2016). *Official Methods of Analysis of AOAC International* (20th ed.). Association of Official Analytical Chemists.

Baptista, E., Liberal, Â., Cardoso, R. V. C., Fernandes, Â., Dias, M. I., Pires, T. C. S. P., ... & Barreira, J. C. M. (2024). Chemical and bioactive properties of red rice with potential pharmaceutical use. *Molecules*, *29*(10), Article 2265. https://doi.org/10.3390/molecules29102265

Chen, M. H., McClung, A. M., & Bergman, C. J. (2016). Concentrations of ligomers and polymers of proanthocyanidins in red and purple rice bran and their relationships to total phenolics, flavonoids, antioxidant capacity and whole grain color. *Food Chemistry*, *208*, 279–287. https://doi.org/10.1016/j.foodchem.2016.04.004

Chinvongamorn, C., & Sansenya, S. (2020). The γ-oryzanol content of Thai rice cultivars and the effects of gamma irradiation on the γ-oryzanol content of germinated Thai market rice. *Oriental Journal of Chemistry*, *36*(5), Article 360503. http://dx.doi.org/10.13005/ojc/360503

Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., ... & Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science*, *163*(6),

1161-1168. https://doi.org/10.1016/S0168-9452(02)00332-1

Department of Foreign Trade, Ministry of Commerce. (2016). *Thai Rice for Life*. Retrieved from https://pubhtml5.com/pwqf/jczc/Thai_Rice_for_Life/

Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall'Asta, C., Galaverna, G., Scazzina, F., & Pellegrini, N. (2007). Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Molecular Nutrition and Food Research*, *51*(8), 1006-1019. https://doi.org/10.1002/mnfr.200700011

Huang, Y. P., & Lai, H. M. (2016). Bioactive compounds and antioxidative activity of colored rice bran. *Journal of Food and Drug Analysis*, *24*(3), 564–574. https://doi.org/10.1016/j.jfda.2016.01.004

Jadhav, S. R. (2021). The golden rice bran: red rice and its health benefits. *International Journal of Science and Research (IJSR)*, *10*(3), 775-781. https://doi.org/10.21275/SR21312201321

Jaksomsak, P., Rerkasem, B., & Prom-U-Thai, C. (2020). Variation in nutritional quality of pigmented rice varieties under different water regimes. *Plant Production Science*, *24*(2), 244–255. https://doi.org/10.1080/1343943X.2020.1819164

Khanaree, C., Punfa, W., Tantipaiboonwong, P., Suttajit, M., Chewonarin, T., Pangjit, K., & Pintha, K. (2021). The attenuation of TNF-α-mediated inflammatory responses in human lung adenocarcinoma cell line by perilla seed and seed meal extract. *Chiang Mai University Journal of Natural Sciences*, *20*(4), Article e2021074. https://doi.org/10.12982/CMUJNS.2021.074

Krishnan, V., Singh, A., Sharma, S., Bollinedi, H., Mabalirajan, U., Kota, S., ... & Praveen, S. (2020). *Nutrient dense pigmented rice: A diet for healthier people*. Retrieved from https://www.researchgate.net/publication/354620367_Nutrient_dense_Pigmented_Rice_A_diet_for_Healthier_People

Muñiz, A., Garcia, A. H., Pérez, R. M., García, E. V., & González, D. E. (2018). *In vitro* inhibitory activity of *Acca sellowiana* fruit extract on end products of advanced glycation. *Diabetes Therapy: Research, Treatment and Education of Diabetes and Related Disorders*, *9*(1), 67–74. https://doi.org/10.1007/s13300-017-0335-7

Office of Agricultural Economics. (n.d.). *Table of in-season rice details*. Retrieved from https://www.oae.go.th/view/1/

Pakuwal, E. & Manandhar, P. (2021). Comparative study of nutritional profile of rice varieties in Nepal. *Nepal Journal of Biotechnology*, *9*, 42–49. https://doi.org/10.3126/njb.v9i1.38648

Phannasorn, W., Khanaree, C., Wongnoppavich, A., & Chewonarin, T. (2017). The effect of purple rice (*Oryza sativa* L. indica) extract on the inflammatory response in a colon cancer cell line and dextran sulfate-induced tumor promotion in the rat colon. *Molecular and Cellular Toxicology*, *13*(4), 433-442. https://doi.org/10.1007/s13273-017-0047-0

Pintha, K., Yodkeeree, S., Pitchakarn, P., & Limtrakul, P. (2014). Anti-invasive activity against cancer cells of phytochemicals in red jasmine rice (*Oryza sativa* L.). *Asian Pacific Journal of Cancer Prevention*, *15*(11), 4601–4607. https://doi.org/10.7314/apjcp.2014.15.11.4601

Priya, T., Nelson, A. R. L., Ravichandran, K., & Antony, U. (2019). Nutritional and functional properties of coloured rice varieties of South India: a review. *Journal of Ethnic Foods*, *6*(11), 1-11. https://doi.org/10.1186/s42779-019-0017-3

Punfa, W., Khanaree, C., Pintha, K., Chumphukam, O., Suttajit, M., & Tantipaiboonwong, P. (2022). Protective effect of Perilla leaf extract against ROS formation and inflammation induced by TNF-α in A549 human lung carcinoma cell line. *Songklanakarin Journal of Science & Technology*, *44*(2), 361-369.

Ramos-Tovar, E., & Muriel, P. (2020). Molecular mechanisms that link oxidative stress, inflammation, and fibrosis in the liver. *Antioxidants*, *9*, 1–21. https://doi.org/10.3390/antiox9121279

Sadimantara, M. S., Asranudin, H., Sadimantara, F. N., & Asyik, N. (2019). Physicochemical and antioxidant properties of red rice varieties of wakawondu and wangkariri from North Buton, Indonesia. *International Journal of Scientific and Technology Research*, *8*(8), 1623–1627.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. & Berghofer, E. (2011). Physicochemical and antioxidative properties

of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry*, *124*(1), 132-140. https://doi.org/10.1016/j.foodchem.2010.05.115

Tantipaiboonwong, P., Pintha, K., Chaiwangyen, W., Chewonarin, T., Pangjit, K., Chumphukam, O., ... & Suttajit, M. (2017). Anti-hyperglycaemic and anti-hyperlipidaemic effects of black and red rice in streptozotocin-induced diabetic rats. *ScienceAsia*, *43*, 281–288.

Thushara, P. A. N., Godakumbura, P. I., & Prashantha, M. A. B. (2019). Importance, health benefits and bioactivities of Sri Lankan traditional rice (*Oryza sativa* L.) varieties: A review. *International Journal of Agriculture Environment and Bioresearch*, *4*(3), 119–128.

Veni, B. K. (2019). Nutrition profiles of different colored rice: A review. *Journal of Pharmacognosy and Phytochemistry*, *8*(2S), 303-305.

Zhang, G. Y., Ru-Ru, L. I. U., Zhang, P., Yong, X. U., Jiang, Z. H. U., Ming-Hong, G. U., ... & Qiao-Quan, L. I. U. (2012). Variation and distribution of vitamin E and composition in seeds among different rice varieties. *Acta Agronomica Sinica*, *38*(1), 55-61. https://doi.org/10.1016/S1875-2780(11)60098-9