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Comparative Phytochemical and Bioactivity Assessment of Four Black Glutinous Rice Cultivars

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Abstract

The high content of phytochemicals and bioactive compounds in black glutinous rice varieties is well recognized; however, comparative studies using diverse bioactivity assays to evaluate their health benefits remain limited. This study examined the phytochemical compositions and biological activities of 4 black glutinous rice (*Oryza sativa* L.) cultivars in Thailand—Leum Pua rice, Rachini rice, Niaw Dam Chaw Mai Phai 49 rice, and Kum Doi Saked rice. Results showed that Niaw Dam Chaw Mai Phai 49 rice and Rachini rice had comparable total phenolic contents (17.00 ± 0.35 and 18.07±0.77 mg gallic acid equivalents/g extract, respectively), with Niaw Dam Chaw Mai Phai 49 rice exhibiting the highest flavonoid content (7.71±0.05 mg quercetin equivalents/g extract). Rachini rice contained the highest anthocyanin (1,076.93±8.52 mg/L), terpenoid (28.60±1.82 mg linalool equivalents/g extract), and β-carotene (43.40 ± 4.70 µg/g grain) levels. Kum Doi Saked rice had the highest protein concentration (22.42±0.26 µg/mL). Leum Pua rice demonstrated the strongest antioxidant activity, as determined by DPPH (IC_{50} = 48.62±2.41 µL/mL), ABTS (IC_{50} = 17.97±2.12 µL/mL), and FRAP (2.11±0.01 µM/mL) assays. The anti-inflammatory and α-glucosidase inhibitory activities of all cultivars were comparable, with IC_{50} values ranging from 16.34 to 19.53 µL/mL and 14.82 to 16.60 µL/mL, respectively. Notably, Leum Pua rice exhibited the strongest α-amylase inhibition (IC_{50} = 21.76±1.68 µL/mL). These findings suggest that black glutinous rice cultivars may serve as promising functional ingredients in diets aimed at reducing oxidative stress-related disorders, including inflammation and type 2 diabetes.

Introduction

Free radicals in the human body typically originate from metabolic processes, psychological stress, and environmental factors such as pollution, ultraviolet radiation, and pathogenic microorganisms (Dangles, 2012). The body's antioxidant defense mechanisms are categorized into enzymatic and non-enzymatic systems (Jaleel et al., 2009). These mechanisms play a crucial role in neutralizing free radicals, which are implicated

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in numerous pathological conditions including cancer, cardiovascular diseases, neurodegenerative disorders, diabetes, arteriosclerosis, and aging (Lourenço et al., 2019). Preventing oxidative stress-induced damage can be achieved through the adequate intake of antioxidant-rich diets (Heim et al., 2002). Previous studies have demonstrated that antioxidants not only help prevent or delay the progression of degenerative diseases but also significantly reduce healthcare costs associated with the treatment of various illnesses (Dirir et al., 2021; Kashtoh & Baek, 2023; Premakumara et al., 2013).

Inflammation is a physiological response of the immune system to infections, tissue injury, and toxic substances (Dharmadeva et al., 2018). These triggers can lead to both acute and chronic inflammation, potentially resulting in tissue damage and disease development (Ghasemian et al., 2016; Shallangwa et al., 2013). Inflammatory stimuli, whether infection-related or not, activate immune cells to release signaling molecules involved in inflammatory pathways such as NF-κB, MAPK, and JAK-STAT (Khatun & Mollah, 2024; Shallangwa et al., 2013). Although non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation, they can cause adverse effects such as dyspepsia, gastric and intestinal ulcers, and liver inflammation (Dharmadeva et al., 2018). As a result, the use of herbal medicines and natural products has emerged as a promising alternative to mitigate inflammation while minimizing the side effects associated with synthetic drugs (Anokwah et al., 2022; Ghasemian et al., 2016).

Diabetes mellitus is a metabolic disorder characterized by impaired insulin function, resulting in hyperglycemia and oxidative stress, which subsequently increases the risk of complications such as hyperlipidemia, hypertension, and cardiovascular disease (Choudhury et al., 2018; Kashtoh & Baek, 2023). To manage blood glucose levels, enzyme inhibitors such as α-amylase and α-glucosidase inhibitors (e.g., acarbose and miglitol) are commonly administered to diabetic patients (Khampa et al., 2023). However, these synthetic drugs are often associated with side effects and high treatment costs (Dirir et al., 2021). This has encouraged researchers to explore alternative therapies, including the use of natural compounds that are safer and potentially effective in controlling blood glucose levels (Choudhury et al., 2018; Lestari et al., 2024; Premakumara et al., 2013; Tadera et al., 2006).

A variety of indigenous rice cultivars, such as Kum Doi Saked, Silaporn, Hom Kusuma, Phet Ratri, and Wesantara rice, are cultivated in Thailand, one of the world's top producers and exporters of rice (*Oryza sativa* L.) (Itsarasook et al., 2021; Khampa et al., 2023). These cultivars are extensively cultivated across the country. Indigenous rice cultivars are well-known for their resilience to environmental stresses and pest infestations, as well as for their high levels of beneficial phytochemicals (Lestari et al., 2024). Research has indicated that certain cultivars are particularly suitable for the development of functional food products (Nakornriab, 2018). Rice is nutritionally valuable and contains a variety of phytochemicals, including phenolic compounds, flavonoids, anthocyanins, phytosterols, and vitamin E, which exhibit antioxidant, anti-inflammatory, and enzyme-inhibitory properties—particularly α-amylase and α-glucosidase inhibition (Dewan et al., 2023; Farahanah et al., 2025; Goufo & Trindade, 2014; Lestari et al., 2024; Phanthurat & Thatsanasuwan, 2023). It was found that black glutinous rice possessed the most phenolic compounds, followed by riceberry and black jasmine rice (Yodsoontorn et al., 2023). In the DPPH assay, black glutinous rice also demonstrated the most potent antioxidant activity, as indicated by the lowest IC_{50} value, followed by riceberry and black jasmine rice. Additionally, Khampa et al. (2023) revealed that 4 local rice extracts, rich in phenolics and flavonoids, had significant α-amylase inhibitory action.

Despite increasing interest in pigmented rice cultivars such as black glutinous rice for their bioactive phytochemicals and associated health benefits—including antioxidant, anti-inflammatory, and anti-diabetic properties—research has primarily focused on commercial cultivars like black rice, riceberry, and jasmine rice (Itsarasook et al., 2021; Khampa et al., 2023; Pukumpuang & Seansrimon, 2023; Yodsoontorn et al., 2023). In 2024, Thailand exported 147,782 metric tons of glutinous rice, generating a total value of 4,115 million baht (Information and Communication Technology Center, 2025). This statistic data highlights the economic significance of glutinous rice. However, limited research is available on the comparative phytochemical profiles and bioactivities of indigenous black glutinous rice cultivars. Therefore, the objective of this study was to investigate the phytochemical compositions and biological activities of four indigenous black glutinous rice cultivars: Leum Pua, Rachini, Niaw Dam Chaw Mai Phai 49, and Kum Doi Saked.

Materials and methods

1. Chemicals and reagents

Gallic acid, quercetin, linalool, bovine serum albumin (BSA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ascorbic acid, diclofenac sodium, α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (PNP-G), and α-amylase were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Folin-Ciocalteu reagent and organic solvents were obtained from Merck (Darmstadt, Germany). The reagents and chemicals used were all of analytical grade. Absorbance measurements for all assays were performed using a microplate reader (SPECTRO star Nano, BMG LabTech, Germany). Each experiment was conducted in triplicate.

2. Rice samples

Four black glutinous rice cultivars were studied (Fig. 1). Details of the growing areas, harvesting periods, and crop years of the four rice cultivars are presented in Table 1.

Fig. 1 Black rice grain samples used in the study

3. Extraction methodology

The rice extraction process was conducted following the method described by Khampa et al. (2023). Rice grains were finely ground, and 10 g of the powdered sample were weighed and transferred into a beaker. Subsequently, 100 mL of distilled water was added and mixed thoroughly. The mixture was heated in a water bath at 90°C with continuous stirring for 15 min. After heating, the extract was filtered through Whatman No. 1 filter paper, and the filtrate was stored at −20°C. Prior to analysis, the extract was thawed and centrifuged at 4,000 ×*g* at 4°C for 15 min. The resulting supernatant was collected and transferred into test tubes for further analysis. The final concentration of the extract was designated as 100% (Vattanasit et al., 2018).

Table 1 Details of the growing areas, harvesting periods, and crop years of four rice cultivars

Rice cultivars	Growing areas	Harvesting periods	Crop years
Leum Pua	Kudlad Village, Kudlad Subdistrict, Mueang District, Ubon Ratchathani Province, Thailand	December, 2023	2023
Rachini	Nong Bua Lamphu Province, Thailand	December, 2023	2023
Kum Doi Saked	Ban Tam Subdistrict, Phayao District, Phayao Province, Thailand	November, 2023	2023
Niaw Dam Chaw Mai Phai 49	Dong Keng Village, Bo Subdistrict, Borabu District, Maha Sarakham Province, Thailand	November, 2023	2023

4. Total phenolic content analysis

The total phenolic content was determined using the Folin–Ciocalteu method, as described by Khampa et al. (2023). The rice extract was diluted with distilled water to concentrations of 0, 20, 40, 60, and 80% (v/v). One milliliter of each diluted sample was mixed with 0.5 mL of 10% Folin–Ciocalteu reagent. The mixture was gently shaken and allowed to stand at room temperature for 5 min. Subsequently, 5 mL of 5% sodium carbonate (Na_2CO_3) solution was added, and the mixture was left to incubate for 60 min. The absorbance was measured at 750 nm using gallic acid as the standard. Results were expressed as mg of gallic acid equivalents per g of extract (mg GAE/g extract). All analyses were performed in triplicate for each rice cultivar.

5. Total flavonoid content analysis

Total flavonoid content was determined using the aluminum chloride colorimetric method, based on the procedure by Itsarasook et al. (2021), with slight modifications. The rice extract was diluted with distilled water to concentrations of 0, 20, 40, 60, and 80% (v/v). A volume of 600 µL of each diluted sample was mixed with 2.4 mL of distilled water and 0.18 mL of 5%

sodium nitrite ($NaNO_3$) solution. The mixture was stirred for 30 sec and left to stand for 5 min. Then, 0.18 mL of 10% aluminum chloride ($AlCl_3$) solution was added and mixed for 1 min. Following that, 1.2 mL of 1 M sodium hydroxide (NaOH) and 2.4 mL of distilled water were added, stirred, and allowed to stand for another 5 min. The absorbance was measured at 510 nm using quercetin as the standard. Results were expressed as mg of quercetin equivalents per g of extract (mg QE/g extract). All samples were analyzed in triplicate.

6. Anthocyanin content analysis

Anthocyanin content was measured using the pH differential method described by Shao et al. (2014). Briefly, 1.5 g of pulverized rice was mixed with 15 mL of 80% methanol containing 1 M hydrochloric acid (HCl) (85:15, v/v) and incubated for 24 h. The extract was then filtered using Whatman No. 1 filter paper. For measurement, 0.3 mL of the filtrate was mixed separately with 2.7 mL of pH 1.0 buffer and pH 4.5 buffer. The absorbance of each solution was measured at 520 nm and 700 nm. Each rice cultivar was tested in triplicate. The anthocyanin content was calculated as cyanidin-3-glucoside equivalent (mg/L) using the following equation:

$$\text{Total anthocyanin content (mg/L)} = \frac{A \times MW \times DF \times 1{,}000}{\varepsilon \times L} \quad (1)$$

Where, A denotes the absorbance computed from ($A_{520} - A_{700}$) at pH 1.0 – ($A_{520} - A_{700}$) at pH 4.5; MW represents the molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF indicates the dilution factor; 1,000 defines as the conversion factor from mL to L; Ɛ indicates the extinction coefficient (26,900 L/(cm × mol)); and L refers to the path length (cm).

7. Terpenoid content analysis

The terpenoid content was determined using the colorimetric spectrophotometric method described by Ponsin et al. (2025). Briefly, 10 mg of rice extract was dissolved in 10 mL of methanol and mixed thoroughly. A 200 µL aliquot of this solution was transferred into a test tube, followed by the addition of 1.5 mL of chloroform. The mixture was vortexed and allowed to stand for 3 min, after which 100 µL of concentrated sulfuric acid (H_2SO_4) was added and stirred again. The mixture was then incubated in the dark for 2 h. Following incubation, the solution was transferred to a new container, and 1.5 mL of 95% methanol was added. The absorbance was measured at 538 nm using a linalool standard curve. The terpenoid content was expressed as mg of linalool equivalents per g of extract (mg LNOLE/g extract). All analyses were conducted in triplicate.

8. Total protein content analysis

The total protein content was estimated using the Lowry method, as described by Lowry et al. (1951). A 100 µL aliquot of rice extract was mixed with 1 mL of Lowry reagent and allowed to stand at room temperature for 15 min. Afterward, 100 µL of Folin–Ciocalteu reagent was added, and the mixture was incubated in the dark for 30 min. The absorbance was measured at 750 nm using a bovine serum albumin (BSA) standard curve. The results were expressed in µg per mL (µg/mL). Each rice cultivar was analyzed in triplicate.

9. β-Carotene content analysis

The β-carotene content was determined using the UV-Visible spectrophotometric method as described by Nagata and Yamashita (1992). One gram of ground rice was extracted with 20 mL of an acetone:hexane solution (2:3 v/v) and homogenized for 1 min. The mixture was then incubated for 24 h. Absorbance was measured at 4 different wavelengths: 663, 645, 505, and 453 nm. The β-carotene content was calculated using the following formula and expressed as µg per g of rice powder (µg/g powder)

$$\beta\text{-carotene content } (\mu g/g \text{ powder}) = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (2)$$

10. Antioxidant activity assays

10.1 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging activity was evaluated following the method of Promprom et al. (2024), with slight modifications. Rice extracts were diluted to 5 concentrations (0–80% v/v) and mixed with 900 µL of DPPH solution. After 30 min of incubation in the dark, the absorbance was measured at 520 nm. The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100 \quad (3)$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test sample. The IC_{50} value was calculated and reported in µL/mL. Ascorbic acid served as the standard. Each rice cultivar was analyzed in triplicate.

10.2 The 2,2-azino-*bis*-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay

The ABTS assay was conducted based on the procedure described by Khatun and Mollah (2024), with slight modifications. Briefly, 2 mL of ABTS solution was mixed with 0.2 mL of rice extract (0–80% v/v), and the mixture was incubated for 6 min. Absorbance was recorded at 734 nm. The percentage of inhibition and IC_{50} values were determined as described in the DPPH assay and reported in µL/mL. Ascorbic acid was used as the standard. Each rice cultivar was analyzed in triplicate.

10.3 The ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1996), with some modifications. A 100 µL aliquot of diluted rice extract (0–80% v/v) was mixed with 2,000 µL of FRAP reagent. After 4 min of incubation, the absorbance was measured at 593 nm. The antioxidant capacity was expressed as µm Fe (II) equivalents per mL (µM Fe (II)/mL). Ascorbic acid was used as a standard. Each rice cultivar was analyzed in triplicate.

11. Anti-inflammatory activity

The anti-inflammatory activity was assessed using the albumin denaturation method described by Dharmadeva et al. (2018), with some modifications. Rice extract at various concentrations (0–80% v/v) was mixed with 200 µL of 1% BSA solution and incubated at 37°C for 15 min, followed by heating at 70°C for 1 min. Absorbance was measured at 680 nm. The percentage of inhibition was calculated using the same equation as the DPPH assay. IC_{50} values were calculated and expressed in µL/mL. Diclofenac sodium was used as the reference standard. Each rice cultivar was analyzed in triplicate.

12. α-Glucosidase inhibition activity

The α-glucosidase inhibition activity was determined based on the protocol by Matsui et al. (1996). Diluted rice extracts (0–80% v/v) were mixed with 1 mL of sodium phosphate buffer (pH 6.8) and 20 µL of α-glucosidase enzyme solution. After 10 min of incubation at room temperature, 50 µL of PNP-G was added, and the mixture was further incubated for 60 min. Absorbance was measured at 405 nm. The inhibition percentage was calculated as described in the DPPH assay, and IC_{50} values were reported in µL/mL. Acarbose was used as the standard. Each rice cultivar was analyzed in triplicate.

13. α-Amylase inhibition activity

The α-amylase inhibition activity was assessed using the method of Kwon et al. (2008). Rice extract (0–80% v/v) was mixed with α-amylase solution and incubated at 25°C for 10 min. A 1% starch solution was then added and incubated for an additional 10 min. The reaction was terminated by adding DNS reagent, and absorbance was measured at 540 nm. The percentage of inhibition was calculated using the same formula as in the DPPH assay. IC_{50} values were estimated and reported in µL/mL. Acarbose was used as the reference standard. Each rice cultivar was analyzed in triplicate.

14. Data analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for mean comparisons at a 95% confidence level ($P < 0.05$).

Results and discussion

1. Phytochemical composition of four black glutinous rice cultivars

There were statistically significant differences ($P < 0.05$) in the total phenolic and flavonoid contents among the rice cultivars. It was found that Niaw Dam Chaw Mai Phai 49 rice showed the highest levels of phenolic content (17.00 ± 0.35 mg GAE/g extract) and flavonoid contents (7.71 ± 0.05 mg QE/g extract), followed by Kum Doi Saked rice, Rachini rice, and Leum Pua rice, respectively. The anthocyanin contents differ significantly among the rice cultivars ($P < 0.05$). It was noted that the highest anthocyanin level was found in Rachini rice (1,076.93 ± 8.52 mg/L), followed by Niaw Dam Chaw Mai Phai 49 rice, Kum Doi Saked rice, and Leum Pua rice, respectively. Also, the terpenoid contents varied significantly ($P < 0.05$) and ranged from 19.48 to 28.60 mg LQE/g of powder. Rachini rice had the highest terpenoid content (28.60 ± 1.82 mg LNOLE/g extract), followed by Kum Doi Saked rice, Niaw Dam Chaw Mai Phai 49 rice, and Leum Pua rice, respectively. The total protein contents varied significantly among rice samples ($P < 0.05$). It was revealed that Kum Doi Saked rice showed the highest protein content (22.42 ± 0.26 µg/mL), followed by Niaw Dam Chaw Mai Phai 49 rice, Rachini rice, and Leum Pua rice, respectively. Within rice cultivars, significant differences were observed in the β-carotene content ($P < 0.05$). It was indicated that Rachini rice possessed the highest level of β-carotene (43.40 ± 4.70 µg/g powder), followed by Niaw Dam Chaw Mai Phai 49 rice, Kum Doi Saked rice, and Leum Pua rice, respectively. The data are presented in Table 2.

Table 2 Phytochemical analysis of four indigenous black glutinous rice cultivars.

Rice cultivars	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)	Anthocyanins (mg/L)	Terpenoids (mg LQE/g powder)	Total Protein (μg/mL)	β-carotene (μg/g powder)
Leum Pua	12.67±0.22[c]	3.71±0.01[c]	636.70±2.23[d]	19.48±0.61[b]	14.52±0.08[d]	3.97±2.06[d]
Rachini	18.07±0.77[a]	4.08±0.03[c]	1,076.93±8.52[a]	28.60±1.82[a]	17.67±0.18[c]	43.40±4.70[a]
Kum Doi Saked	15.30±0.14[b]	6.42±0.10[b]	810.86±10.41[c]	21.91±3.79[b]	22.42±0.26[a]	16.92±0.50[c]
Niaw Dam Chaw Mai Phai 49	17.00±0.35[a]	7.71±0.05[a]	990.54±0.99[b]	20.90±0.35[b]	21.33±0.05[b]	31.64±1.32[b]

Remark: Results are represented as mean ± standard error of the mean (SEM). Different superscripts ([a-c]) within the same column indicate a significant difference among rice cultivars ($P < 0.05$; $n = 3$).

It is generally accepted that phenolic compounds and flavonoids are natural compounds with potent antioxidant activities (Feng et al., 2023; Goufo & Trindade, 2014). Previous research has indicated that the consumption of grains may diminish the risk of cancers, cardiovascular diseases, and diabetes (Dewan et al., 2023; Farahanah et al., 2025; Goufo & Trindade, 2014). The total phenolic content of Niaw Dam Chaw Mai Pai 49 Rice (17.00±0.35 mg GAE/g extract) and Rachini Rice (18.07±0.77 mg GAE/g extract) observed in this study was comparable to, or slightly higher than, the values reported for other black glutinous rice varieties cultivated in Southeast Asia. For instance, Zhang et al. (2010) reported a total phenolic content range of 2365 to 7367 mg GAE/100 g dry weight in black rice cultivars from China. Similarly, a study by Kammapana (2023) on indigenous Thai rice found phenolic compounds ranging from 42.94 to 341.19 mg GAE/100 g dry weight. These comparisons suggest that the studied cultivars possess relatively high levels of phenolic compounds, underscoring their potential as valuable sources of natural antioxidants. In this regard, Niaw Dam Chaw Mai Phai 49 rice could be useful for the development of dietary supplement products, such black rice extract capsules or instant rice drink powder in order to reduce oxidation-related complications. Anthocyanins are flavonoid pigments that play a key role in the protection of plants from UV radiation, temperature, and pests (Lu et al., 2024). Studies on the development of anthocyanins as natural food colorants, health products, and colorants for food and beverages have been documented (He & Giusti, 2010; Lu et al., 2024; Oliveira et al., 2020). It has been reported that anthocyanins exhibit various pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, anticancer, and anti-aging abilities (Lu et al., 2024; Oliveira et al., 2020). Terpenoids, present in plants, fungi, microbes, and insects, possess a chemical structure including five carbon atoms in an isoprene unit (C_5H_8) (Gershenzon & Dudareva, 2007; Lourenço et al., 2019). Research indicates that terpenoids possess antiviral, anti-inflammatory, anticancer, antioxidant, and glycemic-regulating activities (Lourenço et al., 2019). β-carotene is a secondary plant metabolite belonging to the carotenoid group, present in plants, vegetables, cereals, and fruits (Gebregziabher et al., 2023). Recently, β-carotene has applications in the food industry, medicines, cosmetics, and textiles (Singh & Sambyal, 2022). The biological activities of β-carotene include improving vision, mitigating Alzheimer's symptoms, controlling cell proliferation and death, promoting skin health, and demonstrating antioxidant properties (Ponsin et al., 2025; Singh & Sambyal, 2022). The results of this research indicated that Rachini rice possessed the highest levels of anthocyanins, terpenoids, and β-carotene, whereas Kum Doi Saked rice showed the highest protein content compared to other rice cultivars. The findings suggest that Rachini rice may serve as an ingredient in cosmetics or health products. Moreover, Kum Doi Saked rice contained a higher content of protein compared to other rice cultivars. This result demonstrated that Kum Doi Saked rice can be utilized to create innovative health beverages that enhance muscle growth and tissue regeneration (Tangjaidee, 2023). Remarkably, it was observed that the phytochemical composition varies among different rice cultivars. Previous reports suggested that the environment, precipitation, soil nutrients, pests, harvest time, and rice seed storage can affect the levels of phenolic compounds, flavonoids, anthocyanins, terpenoids, total protein, and β-carotene among rice cultivars (Goufo & Trindade, 2014; Khampa et al., 2023; Khatun & Mollah, 2024).

2. Antioxidant activity of four black glutinous rice cultivars

The antioxidant activity of the 4 rice cultivars was measured using DPPH, ABTS, and FRAP assays and the results are shown in Table 3. The DPPH assay revealed substantial differences in antioxidant capability among four black glutinous rice cultivars ($P < 0.05$). It was observed that Leum Pua rice had the highest free radical scavenging activity, with an average IC_{50} of 48.62±2.41 µL/mL, followed by Rachini rice, Niaw Dam Chaw Mai Phai 49 rice, and Kum Doi Saked rice, respectively. Ascorbic acid, a standard drug showed the IC_{50} value of 11.57±0.11 µg/mL. In the ABTS assay, Leum Pua rice showed the highest antioxidant activity, with an average IC_{50} of 17.97±2.12 µL/mL, followed by Niaw Dam Chaw Mai Phai 49 rice, Rachini rice, and Kum Doi Saked rice, respectively. The IC_{50} for ascorbic acid in the ABTS assay was 0.19±0.01 µg/mL. The FRAP assay revealed that Leum Pua rice and Niaw Dam Chaw Mai Phai 49 rice exhibited significantly greater reducing power than the other rice cultivars ($P < 0.05$), with average values of 2.11±1.01 and 1.08±0.26 µM/mL, respectively. The FRAP value of ascorbic acid was 2.12±0.53 µM/mL.

Table 3 Antioxidant activity of four black glutinous rice cultivars

Rice cultivars	DPPH assay IC_{50} (µL/mL)	ABTS assay IC_{50} (µL/mL)	FRAP assay (µM Fe(II)/mL)
Leum Pua	48.62±2.41^{c}	17.97±2.12^{c}	2.11±0.01^{a}
Rachini	75.37±3.34^{b}	128.00±10.62^{b}	0.85±0.14^{c}
Kum Doi Saked	487.50±15.51^{a}	235.54±23.21^{a}	0.40±0.37^{c}
Niaw Dam Chaw Mai Phai 49	77.37±4.55^{b}	126.65±33.72^{b}	1.08±0.26^{b}
Ascorbic acid (µg/mL)	11.57±0.11	0.19±0.01	2.12±0.53

Remark: Results are represented as mean ± standard error of the mean (SEM). Different superscripts ($^{a-c}$) within the same column indicate a significant difference among rice cultivars ($P < 0.05$; $n = 3$).

Prior research demonstrated that oxidative stress is caused by the body's metabolic activities, which produce unstable reactive oxygen species that damage cells and tissues and contribute to inflammation, atherosclerosis, and aging (Pietta, 2000; Valko et al., 2007). Regular intake of antioxidants helps prevent diseases and decreases some chronic conditions (He & Giusti, 2010). The current data indicated that Leum Pua rice exhibited superior antioxidant capabilities in all assays when compared to other rice cultivars. In addition, the IC_{50} values for ascorbic acid in DPPH and ABTS tests were 11.57±011 and 0.19±0.01 µg/mL, respectively. In the FRAP test, ascorbic acid had a reducing powder of 2.12±0.53 µM Fe(II)/mL, while rice extracts had reducing power values ranging from 0.40 to 2.11 µM Fe(II)/mL. Previous reports have indicated that ten rice cultivars exhibited antioxidant activity, as determined by the DPPH assay, with values ranging from 2.71 to 34.77 mM Trolox equivalents/100 g dry weight (Kammapana, 2023). In addition, 4 glutinous rice cultivars (Kor Khor 6, Niao Daeng, Niaodum Kumbaikeaw, and Niaodum Kumnoiy) showed antioxidant activity measured by both DPPH and FRAP assays, ranging from 30.63 to 56.21 mg Trolox equivalents/100 g dry weight and 172.23 to 974.06 mmol Fe(II)/100 g dry weight, respectively (Teravecharoenchai et al., 2021). The antioxidant potential of different rice cultivars may be attributable to the presence of phytochemicals such as phenolic compounds, flavonoids, anthocyanins, terpenoids, and β-carotene, which contribute through several mechanisms: (1) direct donation of hydrogen atoms or electrons to free radicals (Heim et al., 2002; Wisetkomolmat et al., 2023); (2) inhibition of singlet oxygen activity (Dangles, 2012); and (3) termination of free radical chain reactions (Maisuthisakul et al., 2007). Interestingly, Leum Pua rice contains lower levels of anthocyanins, terpenoids, β-carotene, phenolic compounds, and flavonoids than other rice cultivars, yet exhibits stronger antioxidant activity. This phenomenon may be explained by factors such as (Das et al., 2023; Dewan et al., 2023; Maisuthisakul & Changchub, 2014; Zheng et al., 2022): (1) the diversity of phenols, flavonoids, anthocyanins, or terpenoids, which affect antioxidant activity variably; (2) the structural differences among these compounds; (3) the reaction mechanisms of each assay; and (4) the presence of additional antioxidant constituents in the rice, such as vitamin E, pH, or other inhibitors. Therefore, further research should aim to isolate the key components in rice extracts and elucidate their specific antioxidant mechanisms.

3. Anti-inflammatory activity of four black glutinous rice cultivars

The anti-inflammatory efficacy of the 4 rice cultivars was evaluated and the results are presented in Table 4. Anti-inflammatory activities of the rice cultivars, as indicated by IC_{50} values ranging from 16.34 to 19.53 µL/mL, did not show significant differences ($P > 0.05$) among the samples. Diclofenac sodium exhibited an average IC_{50} of 10.88±0.95 µg/mL.

Table 4 Anti-inflammatory activity of four black glutinous rice cultivars.

Rice cultivars	Anti-inflammatory activity (IC_{50}, µL/mL)^{ns}
Leum Pua	16.34±1.02
Rachini	17.20±2.74
Kum Doi Saked	19.53±0.80
Niaw Dam Chaw Mai Phai 49	17.23±3.19
Diclofenac sodium (µg/mL)	10.88±0.95

Remark: Results are represented as mean ± standard error of the mean (SEM), Superscript ^{ns} exhibits no statistical difference ($P > 0.05$; $n = 3$).

Protein denaturation is the loss of a protein's functional structure due to external stimuli or aging (Samson et al., 2016). Structural alterations impair biological functions and can promote inflammation and various pathological conditions (Dharmadeva et al., 2018). Heat treatment is commonly used to simulate the inflammatory process by disrupting hydrogen and hydrophobic interactions, leading to protein misfolding and aggregation (Mizushima & Kobayashi, 1968; Samson et al., 2016).

In the present study, anti-inflammatory activity was evaluated in 4 rice cultivars using the albumin denaturation method. No significant differences were observed among the cultivars. The rice extracts exhibited IC_{50} values ranging from 16.34 to 19.53 µL/mL, while diclofenac sodium, used as a reference drug, showed an IC_{50} of 10.88 ± 0.95 µg/mL. The inhibitory effects of rice extracts on protein denaturation may be attributed to: (1) bioactive compounds that enhance protein–hydrogen bond stability, thereby maintaining structural integrity (Zhang et al., 2024); (2) phytochemicals that bind to protein structures via hydrogen bonds or hydrophobic interactions, reducing heat-induced degradation (Shallangwa et al., 2013; Zhang et al., 2024); and (3) flavonoids and anthocyanins that mitigate oxidative reactions within protein structures (Feng et al., 2023; Valko et al., 2007). These findings suggest that all four rice cultivars possess potential as natural anti-inflammatory agents.

4. α-Glucosidase and α-amylase inhibitory activity of four black glutinous rice cultivars

The results of α-Glucosidase and α-amylase inhibitory activity of 4 black glutinous rice cultivars are exhibited in Table 5. The IC_{50} values of 4 rice cultivars demonstrated no significant differences in α-glucosidase inhibitory action ($P > 0.05$). Acarbose, serving as the reference drug, had an IC_{50} value of 35.65±1.63 µg/mL. Study on the α-amylase inhibitory activity indicated that Leum Pua rice had the highest inhibition potential ($P < 0.05$), with an IC_{50} value of 21.76±1.68 µL/mL, followed by Rachini rice, Niaw Dam Chaw Mai Phai 49 rice, and Kum Doi Saked rice, respectively. In addition, acarbose showed α-amylase inhibitory action with an IC_{50} value of 35.65±1.37 µg/mL.

The inhibition of α-glucosidase and α-amylase activities by active substances is a crucial strategy for regulating blood glucose levels in diabetes (Kashtoh & Baek, 2023; Sales et al., 2012; Tadera et al., 2006). α-Glucosidase is typically located in the microvilli of intestinal epithelial cells, where it hydrolyzes disaccharides into monosaccharides (Ye et al., 2022). Conversely, α-amylase is synthesized by the salivary glands and pancreas and plays a key role in the hydrolysis of α-1,4-glycosidic bonds in starch, producing maltotriose, maltose, and glucose, which are subsequently absorbed into the bloodstream (Kashtoh & Baek, 2023). The use of synthetic drugs to inhibit α-glucosidase and α-amylase activities may cause side effects in diabetic patients, including weight gain, headaches, and bloating (Chaudhury et al., 2018). This concern has encouraged researchers to explore natural bioactive compounds capable of suppressing these enzymatic activities (Phanthurat & Thatsanasuwan, 2023; Ye et al., 2022). The present findings indicated no significant differences in α-glucosidase inhibitory activity among the 4 rice cultivars. In contrast, Leum Pua rice exhibited the lowest IC_{50} value for α-amylase inhibition, suggesting stronger activity compared to the other cultivars. Acarbose produced lower IC_{50} values for α-glucosidase and α-amylase inhibitory assays (10.19 ± 0.02 and 35.65 ± 1.37 µg/mL, respectively) compared to rice extracts. These findings are consistent with previous studies. Khampa et al. (2023) reported that extracts of 4 local rice cultivars (Selabporn, Kusuma, Phetratri, and Vessantara) exhibited potent α-amylase inhibitory activity, with IC_{50} values ranging from 4.78 to 5.77 µg/mL. Similarly, Suwannawong and Waratchareeyakul (2020) found that methanolic extracts of Lon Yung rice and Mae Phaya Tong Dum rice exhibited α-amylase inhibitory activity, with IC_{50} values of 0.108 and 0.289 mg/mL, respectively. The inhibitory effects of rice extracts on α-glucosidase and α-amylase activities are associated with several mechanisms: (1) phytochemicals with structural similarity to enzyme substrates enable competitive binding at active sites, thereby reducing enzyme activity (Dirir et al., 2021; Sales et al., 2012); (2) phenolic compounds form hydrogen bonds with

Table 5 α-Glucosidase and α-amylase inhibitory activity of four black glutinous rice cultivars.

Rice cultivars	α-glucosidase inhibitory assay (IC_{50}, µL/mL)^{ns}	α-amylase inhibitory assay (IC_{50}, µL/mL)
Leum Pua	15.74±0.74	21.76±1.68^{d}
Rachini	14.82±0.83	100.14±1.62^{c}
Kum Doi Saked	16.60±0.55	275.71±10.45^{a}
Niaw Dam Chaw Mai Phai 49	15.16±0.55	163.72±10.34^{b}
Acarbose (µg/mL)	10.19±0.02	35.65±1.37

Remark: Values are mean ± SEM ($n = 3$). Superscript ^{ns} indicates no significant difference ($P > 0.05$); different superscripts ($^{a–d}$) within a column indicate significant differences ($P < 0.05$).

amino acid residues at the active site or interact via hydrophobic forces, inducing conformational changes that diminish enzyme function (Zhang et al., 2015); (3) certain bioactive compounds, such as flavonoids, act as allosteric inhibitors by binding to alternative sites, altering the active site conformation (Tadera et al., 2006); and (4) anthocyanins interact with the enzyme–substrate complex, thereby hindering enzymatic activity (Oliveira et al., 2020).

Conclusion

Research on the phytochemical composition and biological activities of four rice cultivars revealed that Niaw Dam Chaw Mai Phai 49 rice and Rachini rice contained the highest levels of total phenolic compounds. The highest total flavonoid content was detected in Niaw Dam Chaw Mai Phai 49 rice. Rachini rice exhibited the highest contents of anthocyanins, terpenoids, and β-carotene, while Doi Saket rice demonstrated the highest total protein content. Leum Pua rice showed the greatest antioxidant activity in the DPPH, ABTS, and FRAP assays compared to the other cultivars. Anti-inflammatory and α-glucosidase inhibitory effects did not differ significantly among the four rice cultivars. Notably, Leum Pua rice exhibited the most potent α-amylase inhibitory activity compared to the other cultivars. These findings suggest that Leum Pua rice and Niaw Dam Chaw Mai Phai 49 rice have strong potential for use in the development of nutritional supplements, health beverages, and cosmetic products. To ensure the safe application of rice grain extracts in the development of functional foods and nutraceutical products, future studies should assess their cytotoxicity to determine the appropriate dosage for human consumption.

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