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Phytochemicals and antioxidant activities of Sarawak Bario rice varieties

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ABSTRACT

Rice serves as a primary food source for almost half of the world's population and is available in numerous varieties, ranging from pigmented to non-pigmented types. Compounds such as phenolic and flavonoids are well known for their antioxidant capacity and beneficial effects on health. This study investigated the TPC, TFC, and antioxidant activity, assessed through the DPPH assay, in Bario rice varieties, an indigenous crop from Sarawak, Malaysia. Results showed that pigmented varieties (BC, BT, and BMS) exhibited significantly higher TPC compared to non-pigmented varieties (TQR and BAH). At 25 mg/ml, TPC values were 8.31, 4.69, and 4.0 mg GAE/ g dry weight for BC, BT, and BMS, respectively, whereas TQR and BAH recorded 2.48 and 2.41 mg/GAE/g dry weight. A similar trend was observed in TFC, with BMS showing the highest value (3.06 mg QE/g) at 3.5 mg/ml. The DPPH assay further confirmed stronger antioxidant potential in pigmented rice, particularly BC, which achieved 59.61% inhibition at 70 mg/ml. The enhanced antioxidant capacity was attributed to the retention of rice bran, rich in phytochemicals. Overall, the findings highlight the nutritional and functional potential of Bario rice varieties, suggesting their relevance in promoting human health.

Keywords: antioxidant, bario rice, DPPH, scavenging assay, TPC, TFC



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1. Introduction

Rice (*Oryza sativa* L.) is the second most widely consumed cereal after wheat and serves as a primary staple food for nearly half of the global population. In many Asian countries, rice accounts for about 80% of daily caloric intake, as it is predominantly consumed as cooked whole grains [1], [2]. The main rice producers, China, India, Indonesia, Bangladesh, and Vietnam, are also its largest consumers. Meanwhile, countries such as Indonesia, Malaysia, and the Philippines are classified as net importers [3]. Rice is cultivated under diverse ecological conditions, including irrigated lowlands, rainfed lowlands, flood-prone areas, and upland systems where crops are grown on dry, aerated soils without standing water [4]. Globally, Asia accounts for nearly 90% of rice production, with more than half of it derived from intensive irrigated farming practices.

Bario rice is an indigenous variety native to the highlands of Sarawak, Malaysia, and is well known for its distinctive aroma and exceptional eating quality when cooked [5]. It is characterized by its small grain size, soft texture, and unique flavour, setting it apart from other common rice types. This rice is traditionally cultivated in the Bario Highlands at an elevation of about 1,200 meters above sea level, where the cool climate and clean mountain streams provide ideal growing conditions. The farming system is labor-intensive, relying on traditional methods of manual planting and harvesting, with minimal use of chemical fertilisers [6]. Four main types of Bario rice have been identified: Bario Adan Halus (BAH), Bario Tuan (BT), Bario Celum (BC), and Bario Merah Sederhana (BMS). Among them, BAH is generally recognized as white rice, BT appears slightly brownish, while BC and BMS are pigmented varieties. Bario rice has been highlighted as a potential ingredient for gluten-free product development [7], [8]. Furthermore, studies have shown that pigmented varieties contain higher levels of total phenolic compounds compared to their non-pigmented counterparts [9].

Phenolic compounds are recognized as major contributors to antioxidant potential, making it important to understand the mechanisms by which they neutralize free radicals. Antioxidant activity is generally classified into two main categories depending on the underlying reaction mechanism: hydrogen atom transfer (HAT) and single electron transfer (SET) [10]. In HAT-based methods, the antioxidant donates a hydrogen atom to stabilise free radicals, whereas SET-based assays rely on the ability of antioxidants to donate an electron and thereby reduce target molecules or compounds [11]. Phytochemicals, which are non-nutritive but biologically active compounds present in plant-derived foods such as fruits, cereals, and vegetables, are well known for their antioxidant potential and have been linked to lowering the risk of chronic diseases [12]. Rice is particularly rich in phytochemicals, including phenolic compounds, γ -oryzanol, sterols, tocopherols, and tocotrienols, which are primarily concentrated in the outer grain layers, especially the pericarp and aleurone [13], [14]. Typically, pigmented rice varieties exhibit higher levels of these phytochemicals, which contribute to stronger antioxidant capacity compared with non-pigmented or white rice [13], [15].

Phanthurat and Thatsanasuwan [16] investigated the phytochemical profiles and antioxidant activities of both white and black glutinous rice. The studies demonstrated that black glutinous rice shows strong antioxidant capacity, as a relatively small quantity is sufficient to neutralize free radicals, whereas white glutinous rice requires a greater amount to achieve similar inhibition of oxidation. A clear positive relationship was identified between bioactive compounds and antioxidant performance, indicating that higher polyphenol concentrations significantly boost antioxidant potential. Supporting this, Chen et al. [17] reported that purple rice bran is particularly rich in anthocyanins, phenolics, and flavonoids. Purple rice bran extract has been reported to demonstrate notable antioxidant capacity and the ability to support immune function by improving immune organ indexes, limiting oxidative stress, and elevating inflammatory markers. In addition, Colombo et al. [18] evaluated two newly identified pigmented rice varieties to determine their phenolic composition and antioxidant potential. The study revealed that black rice varieties (0.53 to 1.65 mg GAE/g) had the highest antioxidant capacity, red cultivars showed moderate levels (0.33 and 0.06 mg GAE/g), and the non-pigmented Carnaroli variety recorded the lowest (0.06 – 0.14 mg GAE/g). A significant correlation was observed between polyphenol concentration and antioxidant activity, as well as between anthocyanin content and antioxidant activity, indicating that these compounds could serve as reliable predictors of antioxidant potential.

Nevertheless, information on the antioxidant and phytochemical profiles of Bario rice cultivars remains scarce. To address this gap, the present study examines the antioxidant and phytochemical properties of four Bario rice types, namely BAH, BT, BC, and BMS. Such an investigation is expected to not only expand the current body of knowledge but also underscore the potential of these cultivars for use in developing functional food products.

2. Methods

2.1. Raw materials

Bario rice samples were sourced from Kuching, Sarawak, Malaysia. TQR rice obtained from the commercial market 99 Speedmart (Kota Kinabalu, Sabah, Malaysia) was used as the control sample.

2.2. Rice powder preparation

Rice samples were dry-ground using a laboratory blender, and the resulting flour was sieved to 250 μ m using an automatic shaker. The flour was sealed in airtight bags and stored at 4 °C until analysis.

2.3. Sample extraction

Rice flour (4.0 ± 0.02 g) was extracted twice with 20 ml methanol (15 minutes each), centrifuged at 5000 g for 10 minutes, and the combined supernatants were concentrated at 37°C under vacuum, adjusted to 25 ml with methanol, and filtered (Whatman No. 1).

2.4. Total phenolic content (TPC)

The TPC of Bario rice extracts was determined using the Folin-Ciocalteu colorimetric method as described by Phuyal et al. [19], with minor modifications. The gallic acid standard (1 mg/ml) was mixed with 5 ml of 10% Na_2CO_3 , making up the solution to a total volume of 10 ml. After incubation at 40°C for 30 minutes in a water bath (SSB-45 Wisebath, Sci Lab Sdn. Bhd., Malaysia), absorbance was recorded at 760 nm using a UV-vis spectrophotometer (Lambda 25, Perkin Elmer Inc., Massachusetts, USA). Bario extracts were prepared in the same manner as the standard. Results were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW). The TPC was calculated using Equation (1):

$$\text{Total phenolic content, } C = cV/m \quad (1)$$

2.5. Total flavonoid content (TFC)

The TFC of Bario rice extracts was determined following the aluminium chloride colorimetric method described by Phuyal et al. [19], with slight modifications. Quercetin was used as a standard to prepare a standard curve, and the extracts were prepared in a similar manner. Briefly, 1 ml of standard extract solution was mixed with 4 ml of distilled water, followed by the addition of 0.3 ml of 5% NaNO_2 . After 5 min, 0.3 ml of 10% AlCl_3 was added, and 6 min later, 2 ml of 1 M NaOH was introduced. The final volume was adjusted to 10 ml with distilled water. Absorbance was measured at 510 nm using a UV-vis spectrophotometer Lambda 25, Perkin Elmer Inc., Massachusetts, USA. TFC was expressed as mg quercetin equivalents per gram of dry extract (mg QE/g) based on the standard curve. The TFC in all samples was calculated using Equation (2):

$$\text{Total flavonoid content, } C = cV/m \quad (2)$$

2.6. DPPH radical scavenging assay

The DPPH assay was performed following Phuyal et al. [19] with minor modifications. A 0.1 mM DPPH solution was prepared by 3.94 mg of DPPH in methanol and adjusting the volume to 100 ml; the solution was stored at -20°C until further analysis. Extracts and standard (ascorbic acid) solutions were prepared in methanol. To react, 2 mL DPPH solution was added to the standard and rice extract solution at various concentrations and incubated for 30 min at room temperature in the dark. According to Jun et al. [20], the strength of antioxidant activity is determined by IC_{50} values, where compounds with values below 50 $\mu\text{g/ml}$ are considered highly active, those between 50 – 100 $\mu\text{g/ml}$ are active, 101 – 250 $\mu\text{g/ml}$ are moderate, 250 – 500 $\mu\text{g/ml}$ are weak, and values exceeding 500 $\mu\text{g/ml}$ are regarded as inactive. The percentage of inhibition was calculated using Equation (3):

$$\text{DPPH, } I\% = (AC - AO)/AC \times 100 \quad (3)$$

2.7. Statistical analysis

Data were analysed using SPSS version 26.0 with a randomised complete block design. Results are presented as mean $\pm$ standard deviation, and differences were tested by one-way ANOVA with Tukey's HSD ($p < 0.05$).

3. Results and Discussion

3.1. Total phenolic content (TPC)

Phenolic compounds are well-recognised secondary metabolites that protect biological macromolecules by scavenging free radicals and mitigating oxidative stress through their reducing properties, which stabilise reactive species [21], [22]. In this study, the TPC of TQR and Bario rice varieties increased with rising concentrations (Figure 1). Among the samples, BC recorded the highest TPC (8.31 to 23.87 mg GAE/g dry weight; 25 – 100 mg/ml, $p < 0.05$), while TQR (2.48 – 3.88 mg GAE/g dry weight) and BAH (2.41 – 3.68 mg GAE/g dry weight) showed the lowest values, with no significant difference ($p > 0.05$). Likewise, BT (4.69 – 10.09 mg GAE/g dry weight) and BMS (4.0 – 9.18 mg GAE/g dry weight) were not significantly different ($p > 0.05$). Overall, pigmented varieties (BT, BC, BMS) exhibited higher TPC than the non-pigmented TQR and BAH (Figure 1).

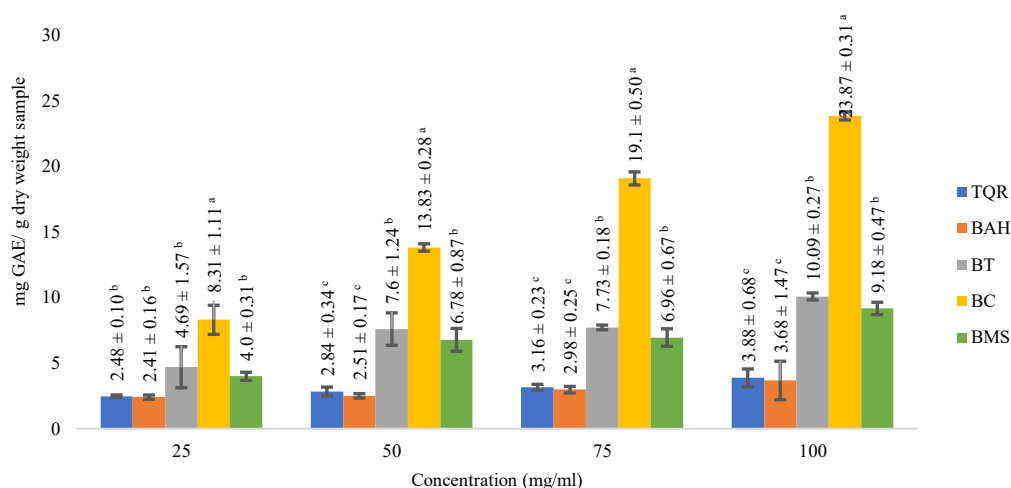


Figure 1. Total phenolic content of rice varieties at different concentrations

Similar trends have been documented by Peanparkdee et al. [23] and Arribas et al. [24], indicating that TPC is typically higher in pigmented rice than in non-pigmented types, aligning with the present findings. This has been attributed to the presence of a greater proportion of highly polar compounds, contributing to the superior phenolic composition of pigmented rice, which generally follows the order of black > red > brown. For example, Ghasemzadeh et al. [25] observed that among sixteen Malaysian pigmented rice varieties, black rice exhibited the highest TPC, followed by red and then brown rice. Similarly, Devi and Badwaik [26] found higher TPC in black rice (Chakhao Amubi, 12.70 mg GAE/g) compared with red rice (Chakhao Angangba, 6.58 mg GAE/g). Aalim and Luo [27] also observed significantly ($p < 0.05$) higher phenolic content in raw red rice (133.07 mg/100 g) compared with raw brown rice (96.70 mg/100 g). In contrast, the present study revealed that the brown variety (BT) exhibited a more pronounced TPC compared with the red pigmented variety (BMS). This suggests that TPC is strongly influenced by genetic differences among rice subspecies and cultivars [28]. Supporting this notion, Dutta et al. [29] reported that Bangladeshi brown rice typically contains 0.14 – 0.25 mg GAE/g, while Yu et al. [32] documented TPC values of 1.42–5.3 mg GAE/g dry matter in wild Chinese rice.

The elevated TPC in pigmented rice is largely attributed to the bran layer surrounding the kernels. This outer layer is rich in both macro- and micronutrients [31], [32]. Beyond its nutrient profile, rice bran is also recognised as a valuable source of bioactive compounds that are linked to protective effects against chronic diseases like diabetes, cancer, and cardiovascular disorders [17]. Phenolic compounds, such as anthocyanins and phenolic acids, are abundantly concentrated in the rice bran layer [33]. However, during conventional processing, rice typically undergoes milling and polishing to remove the husk and bran. From an industrial perspective, bran removal enhances the grain's physical appearance, sensory attributes, and shelf-life stability, but this process also eliminates a significant proportion of the rice kernel's nutrients, particularly phytochemicals [34]. These findings align with the present study, where pigmented rice varieties exhibited greater TPC compared with non-pigmented types. The lower TPC is mainly associated with the removal of rice bran, the main source of phenolic compounds. Supporting this, Ghasemzadeh et al. [25] reported that phenolic and flavonoid compounds in rice bran are predominantly present in free forms.

3.2. Total flavonoid content (TFC)

Flavonoids are among the major classes of phenolic compounds and are well known for their antioxidant properties, particularly through their ability to scavenge free radicals and reduce their accumulation in biological systems [35]. Beyond their antioxidant role, flavonoids have also been associated with various health benefits, including reducing blood glucose and lipid levels, lowering the risk of cardiovascular disease, and inhibiting cholesterol absorption in the human body [36]. In the present study, TFC in rice extracts increased proportionally with sample concentration (mg/ml). The BMS variety showed the greatest TFC, ranging between 1.76 and 3.06 mg QE/g. this was followed by BC: 1.55 – 2.75 mg QE/g), and BT (1.29 – 2.86 mg QE/g). At the highest tested concentration, 3.5 mg/ml, the differences among these varieties were not statistically significant ($p > 0.05$) (Figure 2). Consistent with the TPC results, pigmented varieties generally contained more flavonoids than their non-pigmented counterparts. These findings are in agreement with Chen et al. [17] and Devi and Badwaik [26], who also reported superior TFC in pigmented rice relative to white rice.

Interestingly, the present study revealed that BMS contained higher TFC than BC. This contrasts with previous evidence indicating that black rice generally possesses greater anthocyanin levels than red rice [37], and is often expected to exhibit higher TFC.

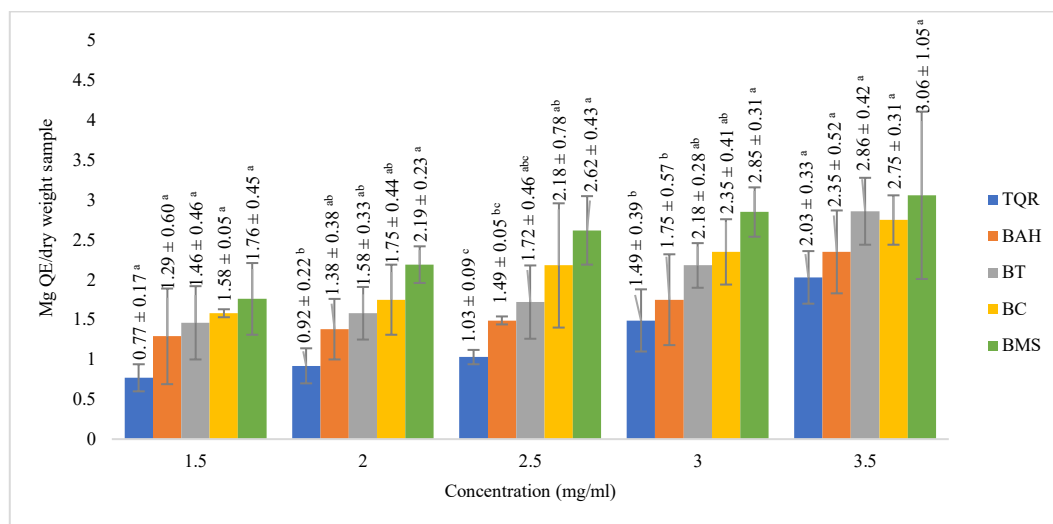


Figure 2. Total flavonoid content of rice varieties at different concentrations

Anthocyanin pigments are responsible for the red to purple coloration in rice, with higher concentrations resulting in a black appearance. Anthocyanidins, the aglycone forms of anthocyanins, are commonly bound to glycosides such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. Structurally, anthocyanins are characterised by a distinctive B-ring with hydroxyl or methoxyl substitutions attached to a 2-phenylbenzopyrylium or flavylium ion [38]. The reactivity of the B-ring, together with the positive charge on the oxygen atom of the C-ring (oxonium ion), confers strong antioxidant capacity to anthocyanins [39]. Moreover, an increased number of hydroxyl groups on the B-ring tends to enhance the blue coloration, a hallmark feature of anthocyanins. In contrast, pelargonidin is unique among anthocyanidins, producing an orange-red pigment in its natural state [40].

Chen et al. [17] investigated the flavonoid composition and antioxidant potential of pigmented rice in China and suggested that the higher TFC observed in red rice compared with black rice may be attributed to its greater catechin content, which was identified as the dominant flavonoid. The authors further emphasise that pigmented rice cultivars exhibit considerable genetic diversity, leading to distinct variations in the type and abundance of flavonoid and phenolic compounds. This genetic variability ultimately determined the unique phytochemical profiles of each pigmented rice variety. Consequently, the higher TFC observed in BMS may be attributed to elevated catechin levels.

3.3. DPPH radical scavenging assay

The radical scavenging activity of the rice varieties showed a concentration-dependent increase, reflecting their intrinsic antioxidant potential (Figure 3). As a reference standard, AA exhibited the highest inhibition percentage (I%) and was significantly superior ($p < 0.05$) to all rice extracts. Among the rice varieties, BC demonstrated the second-highest antioxidant activity, with inhibition values between $59.61 \pm 3.71\%$ and $83.18 \pm 0.11\%$, followed by BMS ($13.57 \pm 1.25\% - 71.35 \pm 0.65\%$), BT ($8.86 \pm 1.2\% - 55.7 \pm 0.47\%$), TQR ($1.93 \pm 0.13\% - 11.6 \pm 0.64\%$), and BAH ($0.41\% - 5.63\%$). In general, a higher I% indicates stronger antioxidant capacity.

The IC_{50} values, defined as the concentration required to inhibit 50% of radicals, were used to further assess reducing power. A lower IC_{50} value reflects stronger radical scavenging ability and thus higher antioxidant potential [41], [42]. AA exhibited an IC_{50} of 7.98 mg/ml, confirming its classification as a highly active antioxidant. Among the rice varieties, BC recorded the lowest IC_{50} (47.85 mg/ml), indicating strong antioxidant activity. In contrast, BT and BMS showed IC_{50} values of 515.08 mg/ml and 331.95 mg/ml, respectively, corresponding to the classification of inactive and weak antioxidants. For TQR and BAH, IC_{50} values could not be determined even at the highest tested concentration, likely due to the removal of the bran layer during milling, which depletes phenolic compounds and other antioxidants in polished (non-pigmented) rice.

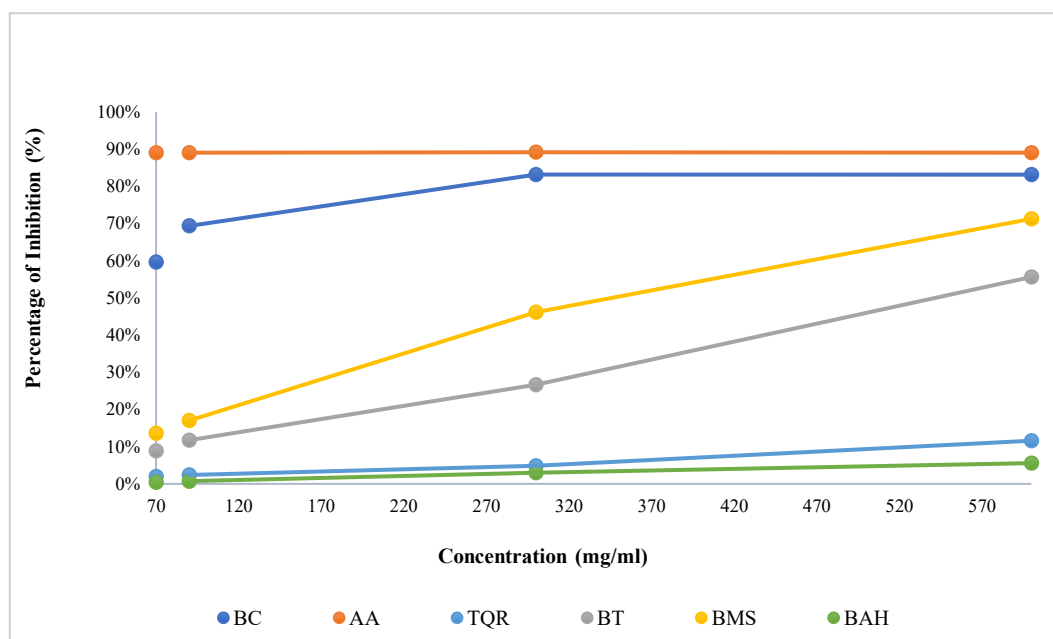


Figure 3. Percentage of inhibition (%) of rice varieties (BC, BMS, BT, BAH, and TOR) compare with AA (standard) across concentrations ranging from 70 to 600 mg/ mL

In general, the milling process substantially reduces nutrients and phytochemical compounds in rice [43]. Incorporating the aleurone layer of unpolished rice into food products is beneficial, as it provides essential nutrients and bioactive compounds, including γ -oryzanol, tocotrienols, and polyphenols [44]. Furthermore, a positive correlation has been observed between DPPH radical scavenging activity and the presence of anthocyanins and proanthocyanidins, where higher concentrations of these compounds enhance antioxidant activity [45]. Priyanthi and Sivakanesan [46] reported IC_{50} values for Indian red rice varieties, Attakkari (26.07 mg/ml), Bg2907 (32.66 mg/ml), and Bg406 (53.66 mg/ml), which were lower than the values obtained for BMS in the present study. According to Munarko et al. [47], the Indonesian brown rice Inpari 17 achieved the highest antioxidant activity together with the lowest IC_{50} value. In contrast, the IPB3S variety showed weaker antioxidant activity and recorded the highest IC_{50} values. The findings suggest that Indonesian brown rice cultivars display stronger antioxidant activity than the Bario varieties, with the exception of BC, which showed a comparable performance based on its IC_{50} value (47.85 ± 0.53 mg/ml). This highlights that differences in antioxidant capacity are closely linked to varietal characteristics. Overall, the DPPH assay confirmed BC as the most potent variety, followed by BMS and BT.

4. Conclusion

Overall, this study emphasised the phytochemical content and antioxidant potential of various Bario rice varieties. Findings revealed that pigmented types (BC, BMS, and BT) possessed higher levels of phytochemicals and antioxidant activity compared with the non-pigmented varieties (TQR and BAH). Among them, black rice (BC) showed the highest TPC, while red rice (BMS) exhibited the highest TFC. Furthermore, the DPPH radical scavenging assay confirmed that black rice (BC) possessed strong antioxidant properties, consistent with its elevated phenolic content. These findings indicate that pigmented rice varieties have superior antioxidant potential, largely attributed to their unpolished state, which retains the rice bran and preserved bioactive compounds. Therefore, pigmented Bario rice varieties may represent a valuable dietary source with potential health benefits, particularly in mitigating oxidative stress-related conditions. Future research, especially in vivo investigations, is recommended to further evaluate their health-promoting effects in humans.

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