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Antioxidant Activity, Total Phenolic and Total Flavonoid Content of 22 Sabah Traditional Rice Varieties

(Aktiviti Antioksidan, Jumlah Fenol dan Jumlah Kandungan Flavonoid 22 Varieti Nasi Tradisi Sabah)

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ABSTRACT

Studies have demonstrated that the traditional rice cultivated in Sabah, East Malaysia, exhibits substantial genetic diversity and is renowned for its outstanding nutritional content. However, there are limited studies to ascertain the antioxidant activity and composition of biochemical substances in it. The present study evaluates the antioxidant properties of 22 Sabah Traditional Rice varieties, which are classified into pigmented and non-pigmented types. An analysis was performed on 22 Sabah Traditional Rice varieties to determine their Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH assay. In addition, a metabolic profiling analysis was performed on biochemical substances extracted from a specific rice variety, *Beras Perang*, which is known for its high levels of TPC, TFC, and antioxidant activity. It was observed that pigmented rice possesses high phenolic content, namely *Beras Perang* and *Baragang Merah* (semi-polished), with values ranging from 1.300±0.004 to 1.967±0.019 mg GAE/g. For TFC results, *Beras Perang, Masuri Hitam* and *Tadong Biasa* (unpolished) showed higher abundance (1.000±0.001, 0.888±0.001 and 0.793±0.003 mg QE/g), respectively. The antioxidant assay results indicate that three varieties, namely *Baragang Merah* (92.832± 0.651%), *Beras Perang* (91.916± 0.331%), and *Rongguol* (91.965± 0.515%) have antioxidant levels exceeding 90%. *Beras Perang* has the greatest levels of TPC, TFC, and antioxidants. In metabolic profile analysis, *Beras Perang* contains a specific form of linoleic acid (12-oxo-10Z-octadecenoic acid) which is not present in the negative control. In conclusion, Sabah Traditional Rice exhibits a highly promising antioxidant activity, over 90%, making it a potential candidate for further development as an alternative superfood.

Keywords: Antioxidant activity; nutritional value; Sabah traditional rice; total flavonoid content (TFC); total phenolic content (TPC)

ABSTRAK

Kajian terdahulu menunjukkan padi tradisi yang ditanam di Sabah, Malaysia Timur, menunjukkan kepelbagaian genetik yang tinggi dan terkenal dengan kandungan nutrisi yang luar biasa. Walau bagaimanapun, kajian aktiviti antioksidan dan komposisi bahan biokimia di dalamnya adalah terhad. Kajian ini menilai sifat antioksidan 22 jenis Beras Tradisi Sabah, yang mana dikelaskan kepada jenis berpigmen dan tidak berpigmen. Analisis ke atas 22 varieti Beras Tradisi Sabah dijalankan bagi menentukan Jumlah Kandungan Fenol (TPC), Jumlah Kandungan Flavonoid (TFC) dan ujian DPPH. Di samping itu, analisis pemprofilan metabolik dilakukan ke atas bahan biokimia yang diekstrak daripada varieti beras tertentu yang dipanggil Beras Perang, yang terkenal dengan tahap TPC, TFC dan aktiviti antioksidan yang tinggi. Adalah diperhatikan bahawa beras berpigmen, iaitu Beras Perang dan Baragang Merah (separuh giling), mempunyai kandungan fenol yang tinggi dengan nilai antara 1.300±0.004 hingga 1.967±0.019 mg GAE/g. Bagi keputusan TFC, Beras Perang, Masuri Hitam dan Tadong Biasa (tidak digiling) menunjukkan kelimpahan yang lebih tinggi (1.000±0.001, 0.888±0.001 dan 0.793±0.003 mg QE/g). Keputusan ujian antioksidan pula menunjukkan tiga varieti iaitu Baragang Merah (92.832± 0.651%), Beras Perang (91.916± 0.331%) dan Rongguol (91.965± 0.515%) mempunyai tahap antioksidan melebihi 90%.

Beras Perang mempunyai tahap TPC, TFC dan antioksidan yang paling tinggi. Dalam analisis profil metabolik, Beras Perang mengandungi bentuk asid linoleik khusus (asid 12-oxo-10Z-oktadesenoik) yang tidak terdapat dalam kawalan negatif. Kesimpulannya, Beras Tradisi Sabah menunjukkan aktiviti antioksidan yang sangat tinggi dengan nilai melebihi 90%, menjadikan ia berpotensi untuk dibangunkan sebagai makanan super alternatif.

Kata kunci: Aktiviti antioksidan; beras tradisi Sabah; jumlah kandungan fenol (TPC); jumlah kandungan flavonoid (TFC); nilai pemakanan

INTRODUCTION

Rice, scientifically known as *Oryza sativa* L., is a fundamental food crop that is eaten by approximately 50% of the global population. The plant is classified under the Poaceae (Graminae) family and has over 40,000 distinct types either non-pigmented or pigmented (Rathna Priya et al. 2019). Nowadays, the consumption of pigmented rice is common due to its functional features, such as its ability to prevent cancer, provide antioxidant activity, and alleviate illnesses like cardiovascular diseases and symptoms of type 2 diabetes mellitus (Ma et al. 2022). For example, brown rice manifests an improvement of glycemia in diabetic rats after 7 weeks besides the decrease of triglycerides and lipid peroxides (Imam et al. 2012). An additional study from Japan also showed reduced levels of glycated HbA1c after consumption of brown rice for 31 weeks by Otsuka Long-Evans Tokushima Fatty (OLEFT) rats, a model for type 2 diabetes in rats (Imam et al. 2012).

This is due to the abundance of compounds such as phytic acid, y-oryzanol and ferulic acid in rice, which bind to calcium that catalyse amylase activity, and inhibit α-glucosidase, respectively, to reduce the blood glucose (Pereira et al. 2021). Findings from preclinical data mirror some of those from clinical trial data. A randomized control study conducted in Japan has demonstrated consumption of brown rice did improve glycemic control in T2DM patients (Nakayama et al. 2017) after 2 months. Another study of consumption of black grain wheat resulted in a significant decrease of glycated albumin level in T2DM patients after a 5-week intervention (Liu et al. 2018). In light of the aforementioned, pigmented rice did possess a high potential of antidiabetic properties.

Besides, pigmented rice is also known for its antioxidant effect with the presence of compounds such as anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, phytic acid, phenolic and flavonoid contents (Mbanjo et al. 2020). These compounds, particularly phenolic and flavonoid contents, are responsible for the brown, red, and violet colors of pigmented rice. All of these compounds are known to possess protective effect against reactive oxygen species (ROS), which have established association with mitigating the risk of diseases such as cardiovascular diseases and type 2 diabetes mellitus. Research has shown that traditional rice from Sabah in East Malaysia has a significant amount of genetic variation and is known for its exceptional nutritional value (Sarena et al. 2022).

Nevertheless, there has been little research conducted on the Sabah traditional rice, specifically with a lack of in-depth analysis of the variations in the distribution of bioactive chemicals across different types of red, violet, and white Sabah traditional rice varieties. Therefore, the objective of this study was to determine and examine the antioxidant characteristics of 22 different types of Sabah traditional rice obtained from two specific places. In addition, our objective was to determine the levels of flavonoids and phenolics in different varieties of rice by examining the relationship between the concentrations of these active components and the antioxidant activity. We anticipate that our results will serve as corroborating evidence for the advancement of functional rice or other supplements, hence facilitating the introduction of these neglected rice types.

MATERIALS AND METHODS

COLLECTION OF SABAH TRADITIONAL RICE VARIETIES

A total of 22 traditional rice varieties from Sabah were collected from the paddy fields in Sabah, namely from Tenghilan and Tambunan regions (Figure 1). The types of rice included *Japonica* tropical and *Indica* available in white, red, and violet colors. The rice was collected in March 2023. Basmati, a variety of fragrant *Indica* rice, was purchased from the local market and utilised as a positive control. white commercial rice, on the other hand, was also obtained from the local market and served as the negative control.

Samples Extraction

The extraction process was conducted following the methodology outlined by Soliman, Abdelhameed and Metwally (2023) with modification. The rice grain was pulverized into a fine powder using a blender. Subsequently, a precise measurement of 1 gram of rice powder was obtained and added into a volumetric flask with a capacity of 50 mL. A volume of approximately 7.5 mL of ethanol with a concentration of 99.5% (v/v) was used to rinse the rice powder in a volumetric flask. Afterwards, the volumetric flask was gently shaken to enhance the mixing process. The volumetric flask was thereafter placed in a shaking water bath incubator at a temperature of 37 °C and a speed of 120 rpm for a duration of 2 h. Subsequently, the concoction was transferred into a 15 mL centrifuge

FIGURE 1. Twenty-two Sabah Traditional Rice varieties obtained from the Tenghilan and Tambunan districts in Sabah were divided into non-pigmented and pigmented (red and violet)

tube and subjected to centrifugation at a speed of 7000 rpm at ambient temperature for 10 min. The mixture was subsequently filtered using filter paper and then proceeded to the subsequent experiments.

TOTAL FLAVONOID CONTENT

Total Flavonoid Content (TFC) was measured using the aluminium chloride assay with quercetin as standard based on the method by Aryal et al. (2019). A volume of 1 mL of sample or quercetin was pipetted into a test tube by adding 0.2 mL of 1M potassium acetate (CH_3COOK) solution, and 0.2 mL of 10% $AlCl_3$. The solution was then added with 5.6 mL of distilled water and was incubated for 30 min at room temperature, before measuring the absorbance at 415 nm wavelength. The outcome data was expressed as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of dry extract in triplicates. TFC was determined by the formula as follows.

$$TFC = C_1 \times \left(\frac{v}{m}\right)$$

where C_1 is the concentration of extracts (mg/mL); v is the volume of extract/solvent (mL); and m is the weight of sample (g).

TOTAL PHENOLIC CONTENT

Total Phenolic Content (TPC) was determined according to the spectrophotometric Folin-Ciocalteu method, with slight modifications (Aryal et al. 2019). Sample or standard (gallic acid) solutions of 0.5 mL were added to a test tube, followed by 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of distilled water. Subsequently, the mixture was incubated for 5 min at room temperature in the dark, before 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. Mixtures were then incubated for 15 min at room temperature and measured the absorbance at 765 nm.

The outcome data was expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract in triplicates. TPC was determined by the formula below.

$$TPC = C_1 \times \left(\frac{v}{m}\right)$$

where C_1 is the concentration of extracts (mg/mL); v is the volume of extract/solvent (mL); and m is the weight of sample (g).

DPPH (2,2-DIPHENYL-1-PICRYLHYDRAZYL) RADICAL SCAVENGING ACTIVITY (RSA)

The DPPH Radical Scavenging Activity (RSA) was performed with slight modification (Blois 1958; Canikli et al. 2023) to evaluate the antioxidant activity. Firstly, 1 mL of the sample extract was transferred to a 15 mL centrifuge tube. Approximately, 3 mL of 0.2 mM DPPH solution was added to the sample, followed by 6 mL of 99.5% ethanol to make the final volume of 10 mL. Subsequently, the mixture was shaken gently and incubated in the dark at room temperature for 30 min before absorbance was measured at 517 nm using a Thermo Scientific Genesys 50 UV-Vis spectrophotometer. The percentage (%) of RSA was then determined through the plotting of the standard curve by using ascorbic acid as the standard in triplicates. The % RSA was calculated by using the formula as follows:

$$\%RSA = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100\%$$

METABOLITE PROFILING OF SELECTED SABAH TRADITIONAL RICE VARIETY BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETER-QUADRUPOLE TIME-OF-FLIGHT (LC-MS-QTOF)

The metabolite extraction technique utilized 100 mg of the specified rice variety, based on the favourable results (high contents of TFC, TPC, and antioxidant activity) obtained from sections 3.0-5.0. This was performed with the methodology previously documented by Kang et al. (2019), with slight modifications. Briefly, a 100 (± 1) mg portion of ground rice sample was subjected to extraction using a 600 µL mixture of methanol and water in a ratio of 2:1 (Optima, LC/MS, Fisher). The extraction process involved sonication at 40 kHz of frequency for 20 min. The solution was centrifuged at 14,000 rpm for 10 min before the supernatant was transferred to a 2.0ml autosampler vial. The resulting extract was concentrated to dryness with the Eppendorf Vacufuge Plus Concentrator.

The metabolite profiling of *Beras Perang* including commercial white rice, negative control was conducted with the Agilent 1200 Liquid Chromatography-Mass Spectrometer-Quadrupole Time-of-Flight (LC-MS-QTOF) system, equipped with a binary pump, a vacuum degasser unit, an autosampler and 6520 quadrupole time of flight mass spectrometers with an electrospray ionization (ESI) source. The column used was Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (2.1 × 100 mm) 1.8 µm. The sample was dissolved in 1 mL of methanol. The sample concentrations are approximately in the range of 5,000 ppm to 126,000 ppm. The chromatographic separation was performed at 40 °C using Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (2.1 × 100 mm) 1.8 µm. Two modes of ionization were performed. For positive ionization mode, the used mobile phase is (A) 0.1% formic acid in ddH20 and (B) 0.1% formic acid in acetonitrile. Meanwhile, for the negative ionization mode, the used mobile phase is (A)ddH2O + 0.1% ammonium formate and (B) 100% acetonitrile. The gradient elution program was 0.00 - 18.00 min, 5 - 95%(B); 18 to 23 min; 95% (B); 23.10 min; 5% (B). The total run time was 30 min. The LC condition was re-equilibrated for 2 min before starting the new injection. The sample injection volume was set at 5 µL and the flow rate of the mobile phase was set at 0.25 mL/min. The mass spectrometer was operated in positive and negative electrospray ionization (ESI) mode with the optimum gas temperature at 325 °C, gas flow at 10 L/min and nebulizer at 30 psi, respectively. The Agilent Mass Hunter Qualitative Analysis B.05.00 software (Agilent Technologies, Santa Clara, CA, USA) was used for the data analysis (MS data (.d)). The chromatographic profile was analyzed based on the accurate mass data identified and the predicted compounds were annotated using METLIN and NIST20 database.

STATISTICAL ANALYSIS

The experiment was carried out using three replicates per cycle, totalling three cycles. The TPC and TFC were presented as Mean ± Standard deviation and were analyzed using analysis of variance (ANOVA) at a significance level of 5% using The Statistical Package for Social Science (SPSS) version 29. The Duncan Multiple Range Test (DMRT) was employed to ascertain disparities among the means.

RESULTS AND DISCUSSION

For many years, rice has been the subject of several studies, particularly focusing on pigmented rice, preferred by many researchers. This is because pigmented rice varieties have been demonstrated to contain exceptional pharmacological effects as a result of their high nutritional value. One example of a well-researched type of pigmented rice is brown rice, which has been extensively examined for its phytochemical properties (Colombo et al. 2023). Nevertheless, information is scarce regarding other varieties of pigmented rice, such as red and violet, particularly concerning indigenous or traditional rice found in specific nations. According to a report, traditional rice varieties have superior nutritional content than modern rice types because of their biochemical elements and pigmentation in grains (Ashokkumar et al. 2020). Sabah Traditional Rice is an indigenous rice variety discovered in East Malaysia that possesses significant genetic diversity, resulting in excellent nutritional value and pharmacological effects (Sarena et al. 2022). The high amylose content that we found on Sabah Traditional Rice varieties is comparable to, and even higher than, the well-known Basmati rice which is recognized for its ability to mitigate Type 2 Diabetes Mellitus (Belobrajdic et al. 2019; Chee et al. 2019). Therefore, the objective of this study was to identify the impact of antioxidants and quantify the phenolic and flavonoid contents in 22 varieties of Sabah Traditional Rice sourced from various locations.

The content of bioactive components in 22 Sabah Traditional Rice types was evaluated using Total Phenolic Content (TPC) and Total Flavonoid Content (TFC), as indicated in Table 1. The results of both studies showed that commercial white rice had the lowest TPC of 0.050±0.002 mg GAE/mg and TFC of 0.043±0.003 mg QE/g. This is consistent with its position as a negative control and non-pigmented nature.

The *Rongguol* variety had the highest TPC, followed by *Beras Perang* and *Baragang Merah* (semi polished), with values ranging from 1.302±0.011 to 1.973±0.025 mg GAE/g. The TPC of Sabah Traditional Rice varied between 0.167 and 1.973 mg GAE/g for red variations, 0.151 to

TABLE 1. Total phenolic and total flavonoids contents of 22 Sabah traditional rice varieties with negative control, white commercial rice in triplicates

Type	Color	Rice varieties	TPC (mg GAE/g)	TFC (mg QE/g)
Pigment	Red	*Rongguol*	1.973±0.025^{a}	0.678±0.004^{f}
		Baragang (Polished)	0.227±0.006^{j}	0.238±0.002^{m}
		Baragang Merah (Semi Polished)	1.302±0.011^{c}	0.776±0.003^{d}
		Beras Bukit Wangi	0.378±0.007^{h}	0.312±0.003^{k}
		Beras Perang	1.936±0.011^{b}	1.000±0.001^{a}
	Violet	*Pulut Tadong*	0.514±0.004^{f}	0.625±0.005^{g}
		Pulut Tadong (Unpolished)	0.891±0.004^{d}	0.784±0.005^{cd}
		Tadong Biasa	0.475±0.005^{g}	0.425±0.002^{i}
		Tadong Biasa (Unpolished)	0.782±0.012^{e}	0.793±0.003^{c}
		Tadong	0.887±0.014^{d}	0.744±0.002^{e}
		Tadong (Over Polished)	0.151±0.003^{k}	0.144±0.002^{op}
		Tadong Bukit	0.298±0.003^{i}	0.468±0.003^{h}
		Masuri Hitam	0.799±0.001^{e}	0.888±0.001^{b}
		Beras Baru Keladi	0.167±0.00^{k}	0.361±0.003^{j}
Non-pigment	White	*Beriu Wangi*	0.151±0.004^{k}	0.257±0.004^{l}
		Ajaib	0.096±0.005^{n}	0.143±0.003^{op}
		Lujuh	0.124±0.004^{l}	0.137±0.002^{p}
		Beriu Biasa	0.077±0.004^{no}	0.139±0.003^{p}
		Beras Adan	0.104±0.005^{lm}	0.150±0.005^{o}
		Planta	0.527±0.005^{f}	0.743±0.002^{e}
		Beras Bario	0.108±0.00^{lm}	0.305±0.004^{k}
		Sibor	0.126±0.004^{l}	0.232±0.004^{m}
		White Commercial Rice (Negative Control)	0.050±0.002^{p}	0.043±0.003^{r}

Values are means of 3 replicates ± SD; different letters within the same column indicate significant differences at P < 0.05 by DMRT test

0.891 mg GAE/g for violet varieties, and 0.077 to 0.527 mg GAE/g for white varieties (without take into consider the commercial white rice). We found that darker color such as red varieties have a higher TPC compared to violet kinds (Colombo et al. 2023), which is consistent with previous studies on rice from different parts of the world. However, we should not underestimate the TFC because black and red rice cultivars are renowned for their high levels of flavonoids (Chen et al. 2022).

The white varieties demonstrated lower TPC and TFC in agreement with the previous study due to most of the biochemical compounds such as phenolic abundance in the outer layers of rice and embryo which are removed by the milling process that polished the rice to white color (Mbanjo et al. 2020). All rice cultivars with pigmentation, particularly *Beras Perang*, have significant levels of both TPC and TFC. *Beras Perang* had the greatest TFC, with *Masuri Hitam* and *Tadong Biasa* (unpolished) following closely after. Similar to TPC, we saw that deeper color varieties have greater TFC levels. For instance, we discovered this in darker violet varieties such as *Tadong Biasa* and *Masuri Hitam*. The high TFC of these two deeper color types, measured at 56.512±1.141% and 67.910±0.603% correspondingly contributing to their high antioxidant properties (Chen et al. 2022; Dias et al. 2021).

Following the determination of the TPC and TFC of 22 Sabah Traditional Rice varieties, we performed an antioxidant activity assessment on these varieties using the DPPH assay. This was done since TPC and TFC have been found to have well-established correlation with antioxidant activity due to phenolics and flavonoids have long been recognized for their strong antioxidant properties (Mbanjo et al. 2020). The scatter plot analysis was performed to compare the relationship between the antioxidant compounds (TPC and TFC) and antioxidant capacities (DPPH) (Figure 2).

Linear regression on the scatterplot diagram indicated stronger positive correlations between TPC and antioxidant activity (R^2= 0.8503), compared with the correlations between TFC and antioxidant activity by DPPH (R^2= 0.7124). We investigated the role of phenolic compounds in the overall antioxidant activity and found that phenolic compounds exhibit a higher association (r = 0.907; p < 0.001) with antioxidants compared to flavonoids. There is a direct correlation between the level of TPC (total phenolic compounds) and the antioxidant activity that safeguards individuals against diabetes and cardiovascular diseases (Ashokkumar et al. 2020).

According to Figure 3, three rice types of pigmented varieties showed a considerably high capacity to scavenge free radicals, exceeding 90%. The three types of rice are *Baragang Merah* (semi polished) with 92.832± 0.651%, *Beras Perang* with 91.916± 0.331%, and *Rongguol* with 91.965± 0.515%. Our discovery of TPC, TFC, and antioxidant percentages aligns with the comparison we made between milled rice kinds (*Pulut Tadong, Tadong Biasa*, and *Tadong*). In our investigation, we observed that the milling process of violet cultivars leads to a decrease in their antioxidant activity. Specifically, we found that the over milling of *Tadong* resulted in a loss of its antioxidant

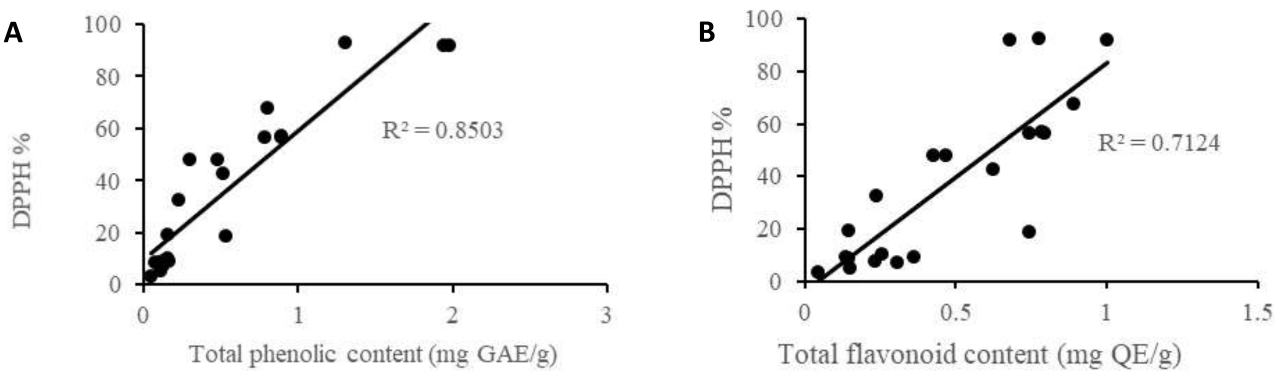

FIGURE 2. Scatter plot diagram of TPC and TFC of Sabah traditional rice extracts and their antioxidant capacity by DPPH assay. (A) DPPH and TPC; (B) DPPH and TFC

activity from 56% to 19%. The antioxidant activity can be significantly reduced, up to 80%, in red cultivars due to variations in the degree of milling (Finocchiaro et al. 2007; Liu et al. 2022).

Based on the results of high TPC and TFC and potent antioxidant from 22 varieties, *Beras Perang* is the varieties that have 3 characteristics; high TPC (1.936 mg GAE/g), high TFC (1 mg QE/g) and potent antioxidant to 91% compared to other varieties. Hence *Beras Perang* was selected to undergoes metabolic profiling using LC-MS-QTOF. From the detected compounds, a total of 1476 and 1126 compounds were detected in positive mode for *Beras Perang* and White Rice, respectively. Meanwhile, negative mode detected less compounds for *Beras Perang* (642) and White Rice (369) based on molecular features (m/z). Upon comparing both kinds based on Table 2, it was discovered that *Beras Perang* has 2 bioactive compounds in positive mode and 1 compound in negative mode that are not present in White rice. The substances identified are monoacylglycerols, specifically 1-Linoleoyl Glycerol and PC(13:0/0:0), detected in positive mode. Additionally, a type of lineoleic acid, 12-oxo-10Z-octadecenoic acid was detected in negative mode.

1-Linoleoyl Glycerol is a type of substance classified as a monoacylglycerol, which is present in *Beras Perang*. This compound is also present in a small number of medicinal plants, such as *Stenochlaena palustris* and *Macadamia integrifolia*, which contribute to their antioxidant properties (Fatmawati et al. 2022; Shuai et al. 2021). A recent discovery showed that it possesses antidiabetic effect in East Java red rice bran (Fauziah & Bakhtra 2023). Another compound is 12-oxo-10Z-octadecenoic acid, a form of linoleic acid. It is frequently present in rice bran and is obtained as rice bran oil at a concentration of 34% from the total unsaturated fats amounting to 75%, is well-known for its ability to lower cholesterol levels (Fox et al. 2022; Hariri et al. 2023). Furthermore, substantial research has been conducted to determine the effectiveness of α-linoleic acid (ALA) in reducing the risk of coronary heart disease (CHD) and cardiovascular events. The findings of these studies suggest that a recommended daily intake of 2-3 grams of ALA can help prevent CHD (Bertoni et al. 2023; Fleming & Kris-Etherton 2014; Naghshi et al. 2021). Moreover, it is hypothesized that there is a direct influence on anti-inflammatory eicosanoids in order to modify the antiarrhythmic impact (Bertoni et al. 2023). These findings indicate that it has significant promise as both a staple food and a 'superfood' for reducing the risk of chronic diseases such CHD and diabetes.

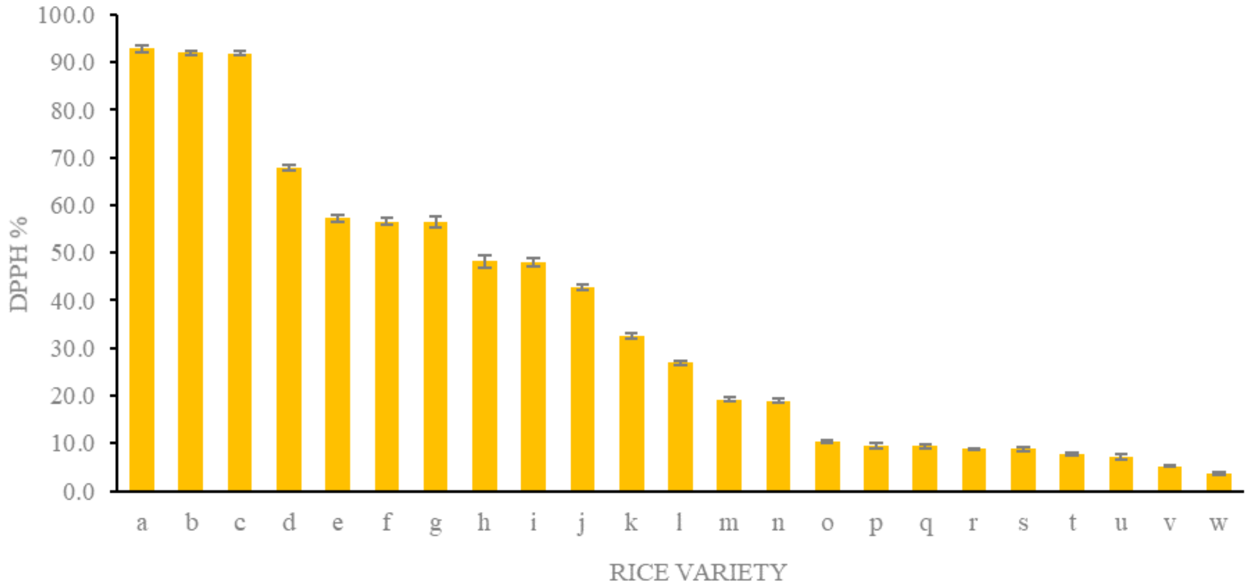

FIGURE 3. DPPH radical scavenging activity of Sabah traditional rice varieties. (a) Baragang merah (semi polished); (b) Rongguol; (c) Beras perang; (d) Masuri hitam; (e) Pulut tadong (unpolished); (f) Tadong; (g) Tadong biasa (unpolished); (h) Tadong bukit; (i) Tadong biasa; (j) Pulut tadong; (k) Baragang (polished); (l) Beras bukit wangi (mixed); (m) Tadong (overpolished); (n) Planta; (o) Beriu wangi; (p) Beras baru keladi; (q) Lujuh; (r) Ajaib; (s) Beriu biasa; (t) Sibor; (u) Beras barrio; (v) Beras Adan; (w) White commercial rice (negative control)

TABLE 2. Comparison of the metabolite profile putatively identified compounds for *Beras Perang* and white rice in positive and negative ionization mode based on molecular features (m/z) by METLIN and NIST20 databases

Ionization modes	Bioactive compounds	Formula	m/z $[M+H]^+$	mass	*Beras Perang*	White Rice
	Unknown	-	679.5111	678.5042	✓	-
	Unknown	-	396.8013	791.5876	✓	-
	PE(17:0/0:0)	C22 H46 N O7 P	468.308	467.3007	✓	✓
	Unknown	-	520.3387	519.3314	✓	✓
	LysoPE(0:0/18:2(9Z,12Z))	C23 H44 N O7 P	478.2926	477.2852	✓	✓
	Unknown	-	520.3391	519.3318	✓	✓
	PE(19:0/0:0)	C24 H50 N O7 P	496.339	495.3316	✓	✓
	PC(13:0/0:0)	**C21 H45 N O7 P**	**454.2918**	**454.2923**	✓	-
	PE(16:0/0:0)	C21 H44 N O7 P	454.2921	453.2848	-	✓
	PE(19:0/0:0)	C24 H50 N O7 P	496.3391	495.3318	✓	✓
	9Z,12Z,15E-octadecatrienoic acid	C18 H30 O2	279.231	278.2236	✓	✓
Positive	Unknown	-	522.3545	521.3472	✓	-
	Unknown	-	522.3549	521.3476	-	✓
	1-Linoleoyl Glycerol	**C21 H38 O4**	**355.2835**	**354.2763**	✓	-
	Unknown	-	319.2839	318.2766	-	✓
	1-Monopalmitin	C19 H38 O4	331.2838	330.2766	✓	✓
	6E,9E-octadecadienoic acid	C18 H32 O2	281.2468	280.2396	✓	✓
	Unknown	-	363.292	362.2848	✓	✓
	Unknown	-	347.3153	346.308	✓	-
	3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	C24 H38 O4	391.2834	390.2762	✓	✓
	Docosanedioic acid	C22 H42 O4	371.315	370.3079	✓	✓
	N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	338.3412	337.3339	✓	✓
	Docosahexaenoyl Serotonin	C32 H42 N2 O2	504.359	486.3252	✓	✓

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Negative	PE(17:0/0:0)	C22 H46 N O7 P	512.3015	467.3029	-	✓
	Unknown	-	476.2797	477.2865	-	✓
	Unknown	-	564.3332	565.3405	✓	✓
	12-oxo-10Z-octadecenoic acid	**C18 H32 O3**	**295.2289**	**296.236**	✓	**-**
	(25R)-3alpha,7alpha-dihydroxy-5beta-cholestan-27-oyl taurine	C29 H51 N O6 S	540.3332	541.3405	✓	✓
	Unknown	-	1035.6643	1036.6716	-	✓
	PS(21:0/0:0)	C27 H54 N O9 P	566.3483	567.3552	-	✓
	9Z,12Z,15E-octadecatrienoic acid	C18 H30 O2	277.2184	278.2256	-	✓
	6E,9E-octadecadienoic acid	C18 H32 O2	279.2343	280.2416	✓	✓
	MG(0:0/18:2(9Z,12Z)/0:0)	C21 H38 O4	399.2769	354.2785	-	✓
	1-Monopalmitin	C19 H38 O4	375.2765	330.278	✓	✓
	2-hexyl-decanoic acid	C16 H32 O2	255.2339	256.2412	✓	✓
	16Z-octadecenoic acid	C18 H34 O2	281.2501	282.2573	✓	✓
	Unknown	-	403.3084	404.3156	-	✓

CONCLUSION

To preserve the nutritional properties, it was necessary to thoroughly analyze the pharmacological effects of traditional varieties in order to uncover their underutilized potential. This study is the first to offer comprehensive data on the Sabah Traditional Rice's total phenolic content and total flavonoid content, as well as its antioxidant effect, in comparison to modern rice available in the market. Characterizing various Sabah Traditional Rice variations, both pigmented and non-pigmented, could provide valuable insights for future research on the pharmacological properties of these rice variants. Further research might prioritize the examination of additional popular Sabah Traditional Rice varieties, such as *Tadong* and *Beras Baru Keladi*. These distinctive rice varieties possess a distinct texture and yam fragrance, respectively, and it would be beneficial to determine their medicinal properties. This information is essential for the development of the novel rice industry. Systematic clinical studies on Sabah Traditional Rice would enhance our comprehension of its therapeutic properties and broaden its potential applications in treating various ailments.

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REFERENCES

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. & Koirala, N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants* 8(4): 96. https://doi.org/10.3390/plants8040096

Ashokkumar, K., Govindaraj, M., Vellaikumar, S., Shobhana, V.G., Karthikeyan, A., Akilan, M. & Sathishkumar, J. 2020. Comparative profiling of volatile compounds in popular South Indian traditional and modern rice varieties by gas chromatography–mass spectrometry analysis. *Frontiers in Nutrition* https://doi.org/10.3389/fnut.2020.599119

Belobrajdic, D.P., Regina, A., Klingner, B., Zajac, I., Chapron, S., Berbezy, P. & Bird, A.R. 2019. High-amylose wheat lowers the postprandial glycemic response to bread in healthy adults: A randomized controlled crossover trial. *Journal of Nutrition* 149(8): 1335-1345. https://doi.org/10.1093/jn/nxz067

Bertoni, C., Abodi, M., D'Oria, V., Milani, G.P., Agostoni, C. & Mazzocchi, A. 2023. Alpha-linolenic acid and cardiovascular events: A narrative review. *International Journal of Molecular Sciences* 24(18): 14319. https://doi.org/10.3390/ijms241814319

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 181: 1199-1200.

Canikli, A., Yıldırım, A., Erdem, H. & Genç, N. 2023. Nutritional composition, antioxidant activity and gossypol level of Nazilli glandless cottonseed, cottonseed kernel and their cold-pressed meal. *Beni-Suef University Journal of Basic and Applied Sciences* 12: 59. https://doi.org/10.1186/s43088-023-00398-1

Chee, F., Lem, F., Latip, M. & Kumar, V. 2019. Diabetic hidden gem: High amylose content in Sabah traditional rice germplasm. *Malaysian Journal of Medicine and Health Sciences* 15: 394-401.

Chen, X., Yang, Y., Yang, X., Zhu, G., Lu, X., Jia, F., Diao, B., Yu, S., Ali, A., Zhang, H., Xu, P., Liao, Y., Sun, C., Zhou, H., Liu, Y., Wang, Y., Zhu, J., Xiang, Q. & Wu, X. 2022. Investigation of flavonoid components and their associated antioxidant capacity in different pigmented rice varieties. *Food Research International* 161: 111726. https://doi.org/10.1016/j.foodres.2022.111726

Colombo, F., Cappa, C., Bani, C., Magni, M., Biella, S., Restani, P. & Di Lorenzo, C. 2023. Characterization of color, phenolic profile, and antioxidant activity of Italian pigmented rice varieties after different technological treatments. *Food Bioscience* 53: 102674. https://doi.org/10.1016/j.fbio.2023.102674

Dias, M.C., Pinto, D.C.G.A. & Silva, A.M.S. 2021. Plant flavonoids: Chemical characteristics and biological activity. *Molecules* 26(17): 5377. https://doi.org/10.3390/molecules26175377

Fatmawati, Fauzana, N.A., Aisiah, S., Rini, R.K., Olga, Tanod, W.A. & Riyadi, P.H. 2022. Chemicals profile of kelakai leaves extracts (*Stenochlaena palustris*) with antioxidant and antibacterial activity against *Aeromonas hydrophila*. *Sains Malaysiana* 51(8): 2531-2546. https://doi.org/10.17576/jsm-2022-5108-14

Fauziah, F. & Bakhtra, D.D.A. 2023. LC-HRMS profile of chemical compounds in *Penicillium citrinum* XT6 extract. *International Journal of Pharmaceutical Sciences and Medicine* 8(6): 80-102. https://doi.org/10.47760/ijpsm.2023.v08i06.007

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

Fleming, J.A. & Kris-Etherton, P.M. 2014. The evidence for α-linolenic acid and cardiovascular disease benefits: Comparisons with eicosapentaenoic acid and docosahexaenoic acid. *Advances in Nutrition* 5(6): 863S-876S. https://doi.org/10.3945/an.114.005850

Fox, M., Newcomb, K., Oliveira, C., Shakiba, E. & Nawarathne, I.N. 2022. Facile analysis of rice bran oil to compare free unsaturated fatty acid compositions of parental and hybrid rice lines. *Journal of the American Oil Chemists' Society* 99(12): 1103-1111. https://doi.org/10.1002/aocs.12631

Hariri, Z., Afzalzade, F., Sohrab, G., Saadati, S. & Yari, Z. 2023. The effects of rice bran supplementation for management of blood lipids: A GRADE-assessed systematic review, dose–response meta-analysis, and meta-regression of randomized controlled trials. *Systematic Reviews* 12: 65. https://doi.org/10.1186/s13643-023-02228-y

Imam, M.U., Azmi, N.H., Bhanger, M.I., Ismail, N. & Ismail, M. 2012. Antidiabetic properties of germinated brown rice : A systematic review. *Evid. Based Complement. Alternat. Med.* 2012: 816501. https://doi.org/10.1155/2012/816501

Kang, K., Yue, L., Xia, X., Liu, K. & Zhang, W. 2019. Comparative metabolomics analysis of different resistant rice varieties in response to the brown planthopper *Nilaparvata lugens* Hemiptera: Delphacidae. *Metabolomics* 15(4): 62. https://doi.org/10.1007/s11306-019-1523-4

Liu, Y., Liu, Y., Zhang, J. & Hou, H. 2022. Effects of degree of milling on phenolics and antioxidant activity of cooked rice during *in vitro* digestion. *Cereal Chemistry* 99(2): 393-404. https://doi.org/10.1002/cche.10501

Liu, Y., Qiu, J., Yue, Y., Li, K. & Ren, G. 2018. Dietary black-grained wheat intake improves glycemic control and inflammatory profile in patients with type 2 diabetes: A randomized controlled trial. *Ther. Clin. Risk Manag.* 14: 247-256.

Ma, Y., Zhang, S., Rong, L., Wu, Z. & Sun, W. 2022. Polyphenol composition and antioxidant activity of *Japonica* rice cultivars and intake status. *Foods* 11(23): 3788. https://doi.org/10.3390/foods11233788

Mbanjo, E.G.N., Kretzschmar, T., Jones, H., Ereful, N., Blanchard, C., Boyd, L.A. & Sreenivasulu, N. 2020. The genetic basis and nutritional benefits of pigmented rice grain. *Frontiers in Genetics* 11: 229. https://doi.org/10.3389/fgene.2020.00229

Naghshi, S., Aune, D., Beyene, J., Mobarak, S., Asadi, M. & Sadeghi, O. 2021. Dietary intake and biomarkers of alpha linolenic acid and risk of all cause, cardiovascular, and cancer mortality: Systematic review and dose-response meta-analysis of cohort studies. *The BMJ* 375: n2213. https://doi.org/10.1136/bmj.n2213

Nakayama, T., Nagai, Y., Uehara, Y., Nakamura, Y., Ishii, S., Kato, H. & Tanaka, Y. 2017. Eating glutinous brown rice twice a day for 8 weeks improves glycemic control in Japanese patients with diabetes mellitus. *Nutr. Diabetes* 7(5): e273. https://doi.org/10.1038/nutd.2017.26

Pereira, C., Lourenço, V.M., Menezes, R. & Brites, C. 2021. Rice compounds with impact on diabetic control. *Foods* 10(9): 1992. https://doi.org/10.3390/foods10091992

Rathna Priya, T.S., Eliazer 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. https://doi.org/10.1186/s42779-019-0017-3

Sarena Che Omar, Nik Syafiah Anis Nik Sharifulden, Dr Teoh Ai Ni, Khoo Wei Yang. 2022. *The Paddy and Rice Industry of Sabah and Sarawak*: *Status and Potential.* Kuala Lumpur: Khazanah Research Institute. https://www.krinstitute.org/Publications-@-The_Paddy_and_Rice_Industry_of_Sabah_and_Sarawak-;_Status_and_Potential.aspx

Shuai, X., Dai, T., Chen, M., Liang, R., Du, L., Chen, J. & Liu, C. 2021. Comparative study of chemical compositions and antioxidant capacities of oils obtained from 15 macadamia (*Macadamia integrifolia*) cultivars in China. *Foods* 10(5): 1031.

Soliman, S.A., Abdelhameed, R.E. & Metwally, R.A. 2023. *In vivo* and *in vitro* evaluation of the antifungal activity of the PGPR *Bacillus amyloliquefaciens* RaSh1 (MZ945930) against *Alternaria alternata* with growth promotion influences on *Capsicum annuum* L. plants. *Microbial Cell Factories* 22: 70. https://doi.org/10.1186/s12934-023-02080-8

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