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EVALUATION OF THE NUTRITIONAL PROPERTIES AND THE ANTIOXIDANT ACTIVITY OF RICE BEAN (*VIGNA UMBELLATA*) SEEDS COLLECTED IN SƠN LA PROVINCE

Le Hong Luyen[1], Nguyen Trang Linh[1], Nguyen Chi Thanh[1], Le Thi Van Anh[1,✉]

[1]*University of Sciences and Technology of Hanoi, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Hanoi, Vietnam*

✉To whom correspondence should be addressed. E-mail: le-thi-van.anh@usth.edu.vn

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ABSTRACT

Vigna umbellata is an underutilized species in Vietnam, and more information about its nutritional values needs to be provided. This study investigated the nutritional composition and antioxidant activity of the seeds of 10 rice bean varieties collected from different districts of Son La province. Moisture content, ash and nutritional composition, including total protein, lipid, and carbohydrate were estimated. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, total phenolic content (TPC), and total flavonoid content (TFC) were carried out to assess the antioxidant activity. The results showed as follows: moisture content ranged from 0.44% to 1.23%, total ash values were obtained in the range between 38.46 and 45.72 mg/g DW, total protein content spread from 106.65 to 156.77 mg/g; total lipid varied from 7.37 to 11.90 mg/g, and total carbohydrate dominated the nutritional profile with 786.10 to 834.73 mg/g. Remarkably, there were significant differences ($p < 0.05$ using ANOVA) among the rice bean species from various districts of Son La province regarding moisture and protein content. Still, similar values were observed for other parameters ($p > 0.05$). The IC_{50} of the DPPH assay ranged from 259.06 µg/mL to 479.99 µg/mL, while the TPC varied from 2.85 to 4.98 mg GAE/g, and TFC values were in the range between 6.46 and 13.73 mg QE/g DW. Both TPC and TFC were significantly negatively correlated to antioxidant activity. This study is the first report on the nutritional compositions and antioxidant activity in the seeds of 10 rice bean varieties collected in Son La province. The findings in this study contribute to valorizing the *V. umbellata* species, which might be a promising plant as a low-priced staple food for ethnic people in mountainous regions.

Keywords: *Vigna umbellata*, rice bean, nutritional compositions, antioxidant activity.

INTRODUCTION

Rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi) belongs to the Fabaceae family of the genus *Vigna*. It is a warm-season annual and small scandent legume characterized by a hairy appearance bearing bright yellow flowers that developed into small edible beans. The pods and seeds are consumed as vegetables. The seed color varies from red to yellow. Although an underutilized legume, rice bean contains a high protein content with essential amino acids like methionine, tryptophan, lysine, tyrosine, and valine (Kaur *et al*., 2023). Recently, the protein content of 50 rice bean accessions was determined to be up to 30 mg/L g of dry seeds, with 60% to 80% of that protein content digestible (Bhagyawant *et al*., 2019). In addition to protein, rice beans possessed a lower overall fat content (around 3%) than other beans, but they contained a very high percentage of unsaturated fatty acids, which ranges from 70% to 80% of total fat (Bhagyawant *et al.,* 2019). Further, the rice bean seeds also demonstrated remarkable antioxidant properties. The study by Yao and colleagues reported the potential antioxidant activity of 13 rice bean accessions from China, with the value ranging from 39.87 to 46.40 µM TE/g (Yao *et al*., 2012).

In Vietnam, rice beans are mainly cultivated in Ha Giang, Lai Chau, Yen Bai, and Son La, and offer protection against soil erosion and weed growth in the orchard. Moreover, dried rice bean seeds with a high protein content can be used as a low-priced staple food for ethnic people in mountainous regions. However, due to the lack of information about the nutritional value and the low yield of rice beans, the area for rice bean cultivation has declined sharply in recent years. This problem leads to the decline of native gene sources, reducing the biodiversity of crop ecosystems (Nguyễn *et al*., 2019; Nguyễn *et al*., 2019).

The present study aimed to investigate the moisture, total ash, and nutritional compositions, including total lipid, protein, and carbohydrate in the dried seed of 10 rice bean varieties harvested in different districts of Son La province. In addition, the total phenolic compounds, the total flavonoid compounds, and the antioxidant activity of these samples were also examined as a criterion of concern for staple food plants in mountainous regions of Vietnam.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu (FC) reagent, gallic acid, quercetin, ascorbic acid, trolox, and DPPH were purchased from Sigma-Aldrich, and Merck, Germany. $NaNO_2$, $AlCl_3$, NaOH, Na_2CO_3, dimethyl sulfoxide (DMSO), methanol, and ethanol were provided by Xilong Scientific Co., Ltd, China.

Plant materials

The seeds of ten different accessions of rice beans were kindly provided by the Northern Mountainous Agriculture & Forestry Science Institute (NOMAFSI). The green seeds of these varieties were previously collected from various districts of Son La province and were dried and stored at NOMAFSI. The seed morphology and other information about 10 accessions are presented in Figure 1 and Table 1, respectively. The seed materials were thoroughly dried at 45 ^oC for 3 days, ground to fine powders, and stored at 4oC at the University of Science and Technology of Hanoi for further analysis.

Table 1. The information of 10 rice bean accessions collected by NOMAFSI.

Accessions	Seed color	Place of sample collecting	Time of sample collecting
Vg1	Red purple	Ngọc Miến, Mường La, Sơn La	June – December, 2019

Vg2	Gray, with scattered dark blue dots	Tòng Lạnh, Thuận Châu, Sơn La	June – December, 2019
Vg3	Gray-yellow, with scattered dark blue dots	Chiềng Bằng, Quỳnh Nhai, Sơn La	June – December, 2018
Vg4	Gray-yellow, with scattered dark blue dots	Mường Lầm, Sông Mã, Sơn La	June – December, 2018
Vg5	Green, with scattered dark blue dots	Chiềng Ngần, Sơn La city, Sơn La	June – December, 2019
Vg6	Some seeds have a dark green color with scattered blue dots; some others have a brown color with scattered black dots	Tông Cọ, Thuận Châu, Sơn La	June – December, 2018
Vg7	Gray-yellow, with scattered dark blue dots	Chiềng Sinh, Sơn La city, Sơn La	June – December, 2019
Vg8	Some seeds have a red color with scattered black dots; some others have a gray color with scattered blue dots	Mường Sang, Mộc Châu, Sơn La	June – December, 2019
Vg9	gray, with scattered dark blue dots	Mương Bú, Mường La, Sơn La	June – December, 2018
Vg10	Dark green with scattered black dots	Chiềng An, Sơn La city, Sơn La	June – December, 2018

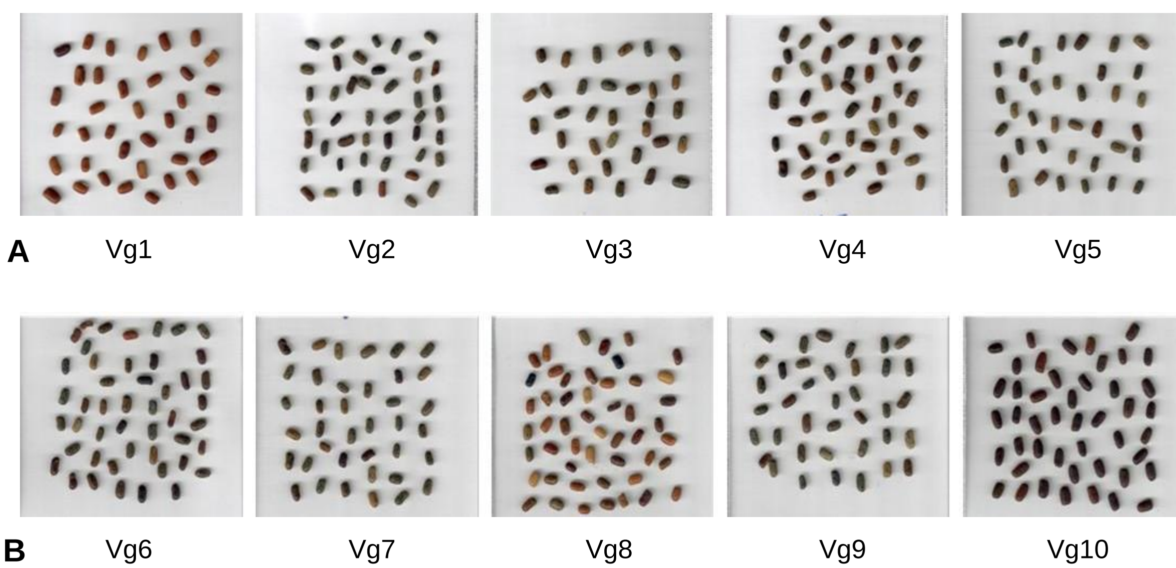

Figure 1. Seed morphology of 10 rice bean varieties from Son La province.

Moisture analysis

The moisture of seed samples was evaluated in 5 replications using a moisture (Model MA37, Sartorius, Germany) at 105 ^{o}C. Around 1 g of seeds was placed into the analyzer for two min and the moisture content was recorded.

Total protein analysis

The total protein analysis of rice bean seeds was conducted twice and was followed by Maehre's method with minor modifications (Maehre *et al.*, 2016). Briefly, 10 mg of powder sample was sonicated in 1 mL of NaOH 0.1M dissolved in 3.5% NaCl for 60 min at a temperature lower than 25 °C. The suspension was then centrifuged at 4000 x g for 30 min at 4 °C. Then the supernatant was collected for analysis. Total protein content was measured using the Bradford method as follows: 5 µL of the supernatant was mixed with 200 µL of Coomassie Blue G-250 reagent, a standard curve was made from bovine serum albumin (BSA), and the absorbance was read at 595 nm.

$$\text{Total protein}\left(\frac{\text{mg}}{\text{g}}\right) = \text{C} \times 100$$

where C (mg/mL) represents protein concentration determined based on the calibration curve of BSA.

Total lipid analysis

The total lipid content was determined twice using the Soxhlet method (Niveditha *et al.*, 2012). A rice bean powder (2 g) packed in filter paper was extracted with 150 mL of n-hexane in a Soxhlet system for 8 h at 70 °C. The hexane-extracted layer was evaporated by a rotary evaporator until the lipid dried. The lipid content was calculated as follows:

$$\text{Total lipid}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{m_{lipid}}{m_{sample}}$$

where m_{lipid} is the weight of total lipid, which is determined by the difference of the rotavapor flask after and before evaporation, m_{sample} is the weight of the rice bean flour.

Total ash analysis

The total ash analysis was evaluated twice. Rice bean powder (1 g) was incinerated by heating for 6 h in a furnace, gradually increasing to 600 °C (Adel Pilerood and Prakash, 2014). The below formula determines the total ash:

$$\text{Total ash}\left(\frac{\text{mg}}{\text{g}}\right) = \frac{m_{ash}}{m_{sample}}$$

in which m_{ash} is the weight of ash after burning and m_{sample} is the weight of the rice bean flour.

Determination of total carbohydrate

The total carbohydrate was determined by subtraction of other parameters, according to the method of BeMiller (2017).

$$\text{Total carbohydrate (mg/g)} = 1000 - (M + L + P + A)$$

where M is the moisture, L is the total lipid, P is the total protein, and A is the total ash. The units of all variables are given in mg/ g of the dried sample.

Antioxidant capacity

Preparation of rice bean seeds crude extract

One (1) g of seed powder was macerated in 50 mL of 70% ethanol for 30 min, and then the extract was filtered. The resultant solution was concentrated by a rotary evaporator. The extraction yields were in the range of 10.29 - 13.26%. The obtained extracts were stored at 4 °C for further use.

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH radical scavenging assay was employed to evaluate the antioxidant ability of rice bean seeds according to a previous method (Le *et al.*, 2022). The rice bean crude extracts and the positive control - ascorbic acid – were dissolved and serially diluted in DMSO to achieve the desired concentration. In each well of a 96-well plate, 10 µL of sample was mixed with 190 µL of DPPH 0.25 mM (in methanol), incubating at 37 °C for 15 min. The absorbance was measured at 517 nm. The percentage of radical inhibition (% I) was calculated according to the formula:

$$\% \text{ I} = \frac{OD_s - OD_c}{OD_s} 100$$

in which OD_s is the average optical density of the sample, and OD_c is the average optical density of the control (without sample). IC_{50} values were determined as the concentration of the extracts that scavenged 50% of the radicals.

Total phenolic content

The TPC was performed using the FC assay (Luyen *et al.*, 2020). Ten µL of the rice bean crude extracts were incubated with 95 µL FC reagent and 95 µL Na_2CO_3 6% at 40 °C for 15 min in a 96-well plate. The TPC is calculated as mg of gallic acid equivalents per gram of dried weight (mg GAE/g DW) by using the formula:

$$\text{TPC}\left(\text{mg}\frac{\text{GAE}}{\text{g}}\text{DW}\right) = 10 \times \frac{C_{GAE} \times 100}{C_0} \times \frac{1}{H}$$

where C_{GAE} (mg/mL) is the concentration equivalent to gallic acid obtained from the calibration curve, C_0 (g/mL) is the final concentration of the dried sample, and H (%) is the crude extraction yield.

Total flavonoid content

The determination of TFC was carried out according to the aluminum chloride colorimetric method (Le *et al.*, 2022). The standard curve was constructed with quercetin in a concentration range of 0.115625 to 1 mg/mL. In each eppendorf tube, 240 µL of the rice bean crude extracts were continuously mixed with 40 µL of $NaNO_2$ 10% and 40 µL of $AlCl_3$ 10%. After each step, the mixture was incubated for 6 min. 400 µL of NaOH 1M and 280 µL of ethanol 30%) were added before agitating the solution for 30 min at room temperature. The final solution was detected at 510 nm. TFC was expressed as quercetin equivalent per gram of dried weight (mg QE/g DW):

$$\text{TFC}\left(\text{mg}\frac{\text{QE}}{\text{g}}\text{DW}\right) = 10 \times \frac{C_{QE} \times 100}{C_0} \times \frac{1}{H}$$

where C_{QE} (mg/mL) represents the concentration of quercetin obtained from the calibration curve, C_0 (g/mL) denotes the final concentration of the dried sample, and H (%) illustrates extraction yield.

Data analysis

The total protein, total lipid, and total ash analyses were repeated twice. DPPH assay, TPC, and TFC analysis were conducted 3 times. The moisture analysis was repeated 5 times. All the results were presented in terms of the mean ± standard deviation. The data was analyzed with a one-way analysis of variance to determine the difference between samples. The Pearson correlation coefficient was calculated to find the association between variables. A p-value < 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

The moisture, ash, and nutritional composition

The moisture, ash, and nutritional compositions in the seeds of 10 rice bean accessions are reported in Table 2.

The moisture

Moisture content is one of the most essential parameters in nutritional evaluation. Therefore, there are numerous methods available to determine moisture content. The mass-loss method by oven drying is highly applicable due to its ease and cost-efficiency, yet the mass loss is caused by water and other volatile components (Isengard, 2001). In addition, drying to a constant mass is difficult because the tightly bound water can resist evaporation, leading to poor data accuracy (Rückold *et al.*, 2000). The application of infrared heat, as conducted in our study, can shorten the heating time, leading to less volatile matter production but more water evaporation, even tightly bound water, and, by extension, a higher moisture content of samples.

The moisture content in the seeds of 10 rice bean varieties ranged from 0.44% (Vg10) to 1.23% (Vg7) (Table 2). There was a significant difference among all accessions (ANOVA, $p < 0.05$), meaning that rice beans' water content differs in various Son La province collecting districts. Several researchers (Kaur and Kapoor, 1992; Saikia *et al.*, 1999; Awasthi *et al.*, 2011) previously found that the moisture content in the rice bean seeds grown in India was around 10%, which is remarkably higher than the values obtained in our study. This indicates that the moisture might vastly vary based on the collecting locations.

Table 2: The moisture, ash, and nutritional composition in the seeds of 10 rice bean accessions.

Accessions	Moisture (%)	Total protein (mg/g)	Total lipid (mg/g)	Total ash (mg/g)	Total carbohydrate (mg/g)
Vg1	0.75 ± 0.09^{bc}	106.65 ± 2.40^{b}	11.32 ± 1.37	39.82 ± 3.98	834.73
Vg2	0.81 ± 0.17^{abc}	116.99 ± 4.42^{b}	10.30 ± 2.20	38.46 ± 0.01	826.19
Vg3	0.58 ± 0.08^{c}	141.34 ± 7.13^{ab}	10.40 ± 2.10	41.56 ± 2.33	800.88
Vg4	1.23 ± 0.04^{a}	135.25 ± 8.76^{ab}	8.35 ± 1.05	45.12 ± 1.32	799.03
Vg5	0.71 ± 0.19^{bc}	133.44 ± 10.43^{ab}	10.55 ± 1.10	41.48 ± 0.90	807.42
Vg6	0.57 ± 0.24^{c}	156.77 ± 23.06^{a}	8.58 ± 0.37	42.86 ± 0.01	786.10
Vg7	1.09 ± 0.46^{ab}	133.35 ± 13.80^{ab}	10.77 ± 0.13	39.56 ± 0.52	805.42
Vg8	0.80 ± 0.13^{abc}	135.20 ± 12.81^{ab}	11.90 ± 0.15	41.28 ± 1.34	803.60
Vg9	0.68 ± 0.20^{bc}	141.95 ± 24.52^{ab}	7.37 ± 1.27	45.72 ± 0.89	798.13
Vg10	0.44 ± 0.03^{c}	138.78 ± 4.32^{ab}	9.60 ± 0.70	41.64 ± 1.28	805.56

Results are in the form of mean ± standard deviation. Using Duncan's multiple-range test, different letters in the same column indicate statistical difference ($p < 0.05$).

Total protein content

The protein content in the rice bean seeds spread from 106.65 mg/g in Vg1 to 156.77 mg/g in Vg6. Like the moisture, there was a significant difference among 10 samples ($p < 0.05$) (Table 2). This means that the total protein amount can be used as an indicator parameter to differentiate rice beans collected from various districts of Son La province. The literature reports that the protein content in rice bean seeds ranges from 14% to 26% (Dhillon *et al.*, 2018), which notably higher than the values in the current study (10 to 15%). The Kjeldahl method is recognized as the official method for food protein determination (George, 2016). This approach quantifies the total protein based on nitrogen determination, followed by conversion using a nitrogen-to-protein conversion factor of 6.25. The Kjeldahl method might cause an overestimation of the protein content in most foods, especially plant foods, because of a wide range of other compounds such as nitrate, ammonia, urea, nucleic acids, free amino acids, and chlorophylls that contain non-protein nitrogen (Maehre *et al.*, 2016). Therefore, in this study, the Bradford method was selected to determine total protein content indirectly. Although the Bradford method has been admitted to being less prone to such interference, it usually results in lower protein content than the Kjeldahl method, as the alkaline amino acids contribute more to the final color of the complex between the sample and Coomassie blue reagent than do other amino acids (Wilson *et al.*, 2010).

Total lipid content

Lipid is one of the major nutrients, and it is a source of nutritional components and bioactive compounds. In the present study, the results revealed that the overall lipid content of rice beans was around 9.92 mg/g. Sample Vg8 contained the highest lipid content with a value of 11.90 ± 1.19 mg/g, while Vg9 possessed the lowest value of 7.38 ± 1.27 mg lipid per 1 g dried sample. However, levels of total lipid in various varieties of rice beans did not differ significantly from one another ($p > 0.05$) (Table 2).

The total lipid content in our study ranges from 0.7 to 1.2%, which is within the published values of 0.3% to 3.4% (Katoch, 2013; Kaur *et al.*, 2013). The average value obtained in our results was also consistent with the other study on 6 rice bean accessions collected in Điện Biên, Sơn La, and Lai Châu (Nguyễn *et al.*, 2020). Interestingly, the amount of lipid in rice bean seeds found in our study was approximately equal to the average value found in 10 Vietnamese mung bean varieties, considered a highly nutritious bean in Vietnam (Nguyễn *et al.*, 2012). Therefore, rice bean seeds are nutritionally beneficial, so their production and consumption as a low-priced staple food should be promoted.

Total ash content

Ash content was measured by the mineralization of inorganic noncombustible sample material. In rice bean seeds, after burning for 6 hours at 600 °C, the ash content was around 40 mg/g. The lowest value belonged to sample Vg1 (38.46 ± 0.05 mg/g), and the highest amount was obtained in Vg9 (45.72 ± 1.59 mg/g) (Table 2). Ash's content in this research is similar to the previous studies of Saikia *et al.* (1999) on four cultivars of rice bean seeds in India, with the ash level at around 42 - 44 mg/g. Similar to the lipid content, the ash content did not significantly differ among the 10 varieties of rice bean seeds. This result means that the variation in collecting locations might not be a crucial factor that affects the amount of minerals contained in the seeds.

Total carbohydrate content

Total carbohydrate content was the most accounting parameter for *V. umbellata* seeds, with around 80% of values equaling 800 mg/g (Table 2). The accessions with the fewest carbohydrate were Vg6 (765.56 mg/g), while the maximal value was 843.73 mg/g, which belonged to the Vg1 sample. In the same vein as the lipid content, no statistically significant differences were observed among all the samples. The total carbohydrates in our research are significantly higher than in earlier studies, which ranged from 50% to 70% (Awasthi *et al.*, 2011; Katoch, 2013; Dhillon and Tanwar, 2018).

In general, the average nutritional percentage of 10 rice bean accessions from Son La province was determined as follows: moisture: 0.77 ± 0.23%, lipid: 1.01 ± 0.11%, protein: 13.40 ± 1.37%, ash: 4.18 ± 0.23% and carbohydrate: 80.67 ± 1.40% (Figure 2).

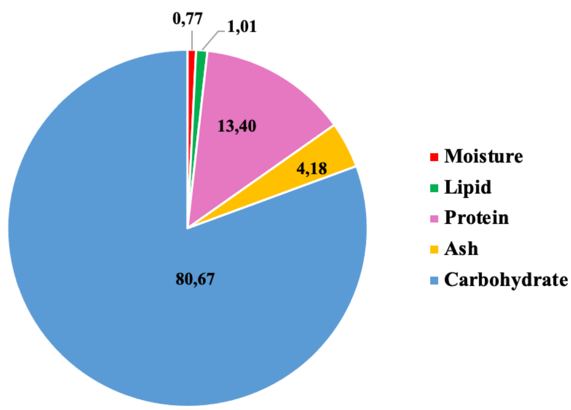

Figure 2. Nutritional composition of rice bean samples.

Antioxidant capacity

In the DPPH assay, positive control ascorbic acid exhibited an IC_{50} (the half-maximal inhibitory concentration) of 12.31 ± 1.50 µg/mL. Table 3 represents the wide range of DPPH scavenging activity of rice bean seeds. The highest IC_{50} value belongs to Vg10 (479.99 ± 17.52 µg/mL), which is approximately twice as high compared to the lowest value of 259.06 ± 6.31 µg/mL recorded for Vg8. In general, the antioxidant activity of rice beans is relatively weak since their IC_{50} is remarkably higher than that of ascorbic acids.

Table 3. Antioxidant activity of rice bean samples.

Samples	Concentration (µg/mL)					IC_{50} (µg/mL)
	31.25	62.5	125	250	500	
Vg1	3.73±0.56	8.22±0.54	16.36±2.01	35.87±3.68	67.87±1.92	365.50±14.94
Vg2	4.70±0.48	7.77±0.63	19.12±1.08	40.28±1.48	74.94±2.06	322.36±14.75

Vg3	3.71±0.58	8.16±1.00	17.94±1.08	35.65±0.56	72.85±1.97	358.87±17.72
Vg4	4.67±1.32	8.47±0.54	20.42±0.45	39.88±0.14	79.43±3.49	321.59±14.42
Vg5	6.03±1.12	10.97±0.10	18.56±0.89	35.27±1.97	74.77±1.72	345.61±12.71
Vg6	4.29±0.78	6.45±1.07	20.27±0.15	38.05±0.25	76.17±3.23	329.83±12.25
Vg7	8.56±0.21	13.38±0.34	22.04±0.20	40.90±2.85	80.30±2.46	314.77±14.53
Vg8	16.54±2.65	20.56±1.57	31.92±0.72	51.44±2.23	86.10±0.79	259.06±7.73
Vg9	13.42±2.59	17.44±0.54	28.50±0.85	56.90±1.27	82.69±0.64	281.11±2.20
Vg10	9.93±0.76	17.23±0.88	22.49±5.56	32.24±0.65	58.78±1.06	479.99±21.46

In Figure 3, the total phenolic content of all samples gave an average value of 4.29 mg GAE/g. Among 10 accessions, Vg8 (4.98 ± 0.26 mg GAE/g DW) and Vg7 (4.93 ± 0.79 mg GAE/g DW) contained the highest TPC value. Vg10 accompanies the minimum value of TPC at 2.85 ± 0.06 mg GAE/g DW. Compared with the results of Yao *et al*. (2012), the total phenolic content in rice beans ranges from 3.27 to 6.43 mg GAE/g, which is relatively similar to the results in our study. Similarly, the highest TFC value was obtained in the sample Vg7 at 13.73 ± 1.23 mg QE/g DW, while the smallest value was found in the sample Vg10 at 6.46 ± 0.37 mg QE/g DW. An average TFC of 11.46 mg QE/g was measured for all samples. The observation of 40.26 mg QE/100 g for TFC expressed a lower level than the data from our research, whose average values were 11.32 mg QE/g of TFC (Yao *et al*., 2012). In addition, the TPC and TFC of *Vigna umbellata* seeds are similar to those values tested from the seeds of some legume plants, such as *Vigna unguiculata* subsp. *sesquipedalis* (L.), *Phaseolus vulgaris* (Tungmunnithum *et al*., 2021), but these data seem to be significantly smaller than the content of aerial parts of *Vigna* genus (Vergun *et al*., 2022).

There was a negative linear relationship between antioxidant activity (expressed by IC_{50} values) and either TPC (R^2 = -0.41, $p < 0.05$) or TFC (R^2 = -0.74, $p < 0.05$) using Pearson correlation analysis. This indicates that the more potent the antioxidant activity of the samples, the higher the TPC and TFC they contain. However, both correlation coefficient values were much higher than -1, which leads to the fact that there might be other compounds responding to the antioxidant capacity of rice beans. This hypothesis coincided with Katoch *et al*. (2013) and Kaur & Kapoor (1992) investigations that evaluated the phytic acids (1.8% - 8.2%) and saponins (1.2% - 2.5%) in rice bean seeds. Phytic acids, as demonstrated, can form a unique iron chelate, suppress iron-catalyzed oxidative reactions, and then serve a potent antioxidant function in the seeds (Bhagyawant *et al*., 2019). Along with phytic acids, saponins also possess intense antioxidant activity based on promoting antioxidant enzymes (Kang *et al*., 2016).

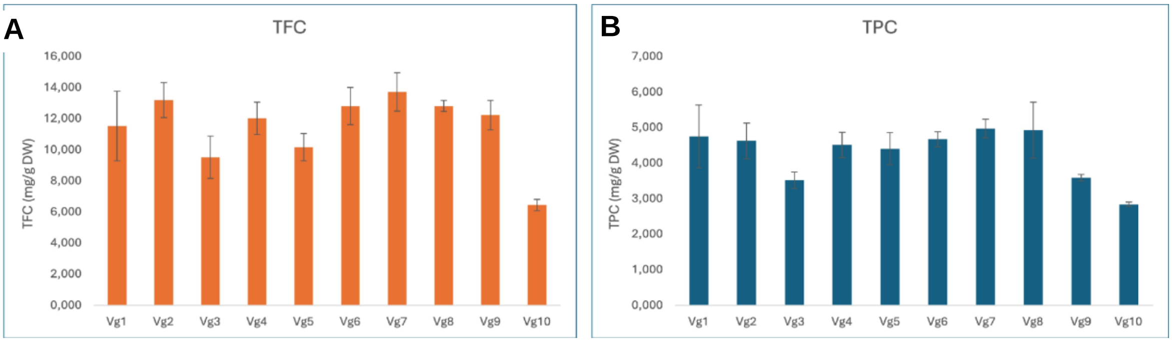

Figure 3. Total phenolic content (TPC) and total flavonoid content (TFC) of rice bean seeds.

CONCLUSION

This study investigated the nutritional composition of the seeds of 10 rice bean varieties from Son La province. Among all samples tested, Vg6 contained the highest protein content; Vg8 contained the highest amount of lipid, while the highest carbohydrate content was found in the Vg1 sample. In addition, Vg8 exhibited excellent antioxidant activity and high TPC and TFC. Rice beans are nutritionally beneficial, so their production and consumption as a low-priced staple food should be promoted. They can be used for agricultural cultivation in other provinces to green up barren hills and enhance the productivity and economy of ethnic people in mountainous areas.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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