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Developmental and Species-specific Variation in Physicochemical, Phytonutrients, and Antioxidant Properties of Selected *Vigna* spp.

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***Vigna* spp. are fast-growing, affordable legumes that offer valuable nutrients; however, their optimal nutritional value across developmental stages remains underexplored. This study investigated the physicochemical properties, phytonutrient contents, and antioxidant activity of three *Vigna* spp.: mung bean (MB, *V. radiata*), black gram (BG, *V. mungo*), and adzuki bean (AB, *V. angularis*), at three developmental stages: seed, sprout, and microgreen. Fresh and dry weights, as well as plant length, were recorded. Comparative analyses were performed on seed, top (TOP), and middle (MID) parts to assess phytonutrient levels, antioxidant activity, and enzymatic antioxidants. The results revealed that AB microgreens exhibited the highest biomass and total phenolic content, while MB microgreens had the longest plant length, titratable acidity, ascorbic acid, and FRAP-based antioxidant activity. BG microgreens excelled in terms of total flavonoid content and catalase activity. Overall, microgreens demonstrated consistently higher phytonutrient and antioxidant capacity than sprouts and seeds, particularly within TOP. Conversely, seeds consistently exhibited higher protein and soluble solid contents than their sprout and microgreen counterparts. Distinct species- and part-specific variations were evident across developmental stages. This study highlights the importance of species selection and harvest timing in optimizing the nutraceutical value of legume-based sprouts and microgreens for functional food applications.**

Key Words: antioxidant, legumes, microgreens, phytonutrients, sprouts.

Introduction

The growing interest in functional foods has underscored the nutritional potential of edible plants harvested at early stages, particularly sprouts and microgreens. These tissues often contain elevated levels of phenolics, flavonoids, and other nutritional compounds compared to mature plants, resulting in higher antioxidant capacity and associated health benefits (Ebert, 2022; Kowitcharoen et al., 2021). While Brassicaceae and culinary herbs such as basil, fenugreek, and spinach have been extensively studied (Ghoora et al., 2020; Kyriacou et al., 2016), legumes remain comparatively underexplored despite their importance in human diets.

In legumes, several studies have demonstrated that the sprouting and microgreen stages have greater nutritional qualities in soybean, chickpea, and lentil, with consistent increases in phenolic content, flavonoids, and antioxidant capacity relative to seeds (Saha et al., 2025; Wojdyło et al., 2020). A study showed that there were no differences in total antioxidants, total phenolics, or dietary fiber contents between lentil and mung bean (MB, *Vigna radiata*) microgreens (Dhaka et al., 2023). Additionally, germination was found to enhance the total antioxidant activity of adzuki bean (AB, *V. angularis*) to a level comparable to that of germinated soybean, despite its lower content in the mature seed (Lin and Lai, 2006). Nonetheless, comparable data among *Vigna* spp., including MB, black gram (BG, *V. mungo*), and AB are limited. Most studies emphasize mature seeds (Luo et al., 2016; Wang et al., 2021), sprouts (Chen

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et al., 2019; Guo et al., 2012), and microgreens (Dhaka et al., 2023; Saha et al., 2025) with limited comparative analyses across developmental stages of these *Vigna* spp. Wojdyło et al. (2020) compared sprouts and microgreens of various plant families and species; however, the heterogeneous growth environments (as the grown plant materials were acquired from a market rather than being cultivated) may have contributed to the differences observed in their nutritional profiles. Therefore, direct comparisons of seeds, sprouts, and microgreens of the same species are particularly scarce, leaving uncertainties about the optimal harvest stage for maximizing nutritional and functional attributes. This gap is especially evident for the aforementioned three *Vigna* spp., which are among the legumes most widely cultivated and consumed in Asia.

The tissue-specific distribution of phytochemicals and antioxidants is another underexplored aspect. The top portions (TOP; cotyledons and developing leaves) and middle sections (MID; hypocotyl or epicotyl) constitute the main edible portions of sprouts and microgreens. Conversely, the lower region, which comprises roots and seed remnants, is generally discarded due to its tougher texture and limited culinary use. Focusing on TOP and MID is therefore more relevant for both nutritional assessment and consumer application, as these portions represent those most likely to be consumed. Despite the nutritional relevance of TOP and MID, systematic comparisons across these tissues in *Vigna* spp. are lacking. Integrated analyses that combine developmental stages (seed, sprout, and microgreen) with tissue-level differences (TOP and MID) are rare for legumes. Addressing these gaps, the present study aimed to provide new and valuable insights into optimizing the nutritional quality and functional food applications of *Vigna*-based sprouts and microgreens. Hence, the objective of this study was to assess the transformations in the physicochemical and biochemical parameters of three distinct *Vigna* spp. during the various growth stages of the seed, sprout, and microgreen. Particular attention was given to the distribution of these compounds between TOP and MID tissues and the implications for nutritional quality.

Materials and Methods

Plant samples and cultivations

High-quality seeds of three *Vigna* spp. of MB, BG, and AB were procured locally from Modern Scientific Sdn. Bhd., Malaysia. MB was of the local variety BINA moog5, BG of T9 was from India and AB of Jingnong #5 was from China. The seeds were selected for uniformity of size, shape, and color. Mechanically damaged seeds were removed. The seeds were then weighed and rinsed three times with tap water to remove debris and dirt. Subsequently, the seeds were sanitized using 200 $mg \cdot L^{-1}$ sodium hypochlorite for 30 min at ambient temperature. The sanitized seeds were rinsed with running tap water for 30 s. Thereafter, the seeds were soaked in tap water overnight at a ratio of 1:10 (g/v) at 35°C, following farmer practices. The seeds were divided into two main groups: one for sprouts and another for microgreen production.

The rehydrated seeds were sprouted in a double-layered polypropylene sowing tray (34 cm × 26 cm × 4.5 cm), with the first layer perforated with 310 holes (0.4 cm per hole), at 28 ± 2°C, with relative humidity (RH) of 80–90%, in complete darkness by covering the tray with a sheet of aluminium. The sprouts were harvested four days after sowing (DAS) for MB and BG, and 6 DAS for AB, with sprouts lengths of 7–9 cm (MB), 8–11 cm (BG), and 8–10 cm (AB), following commercial production practices (Kowitcharoen et al., 2021). For microgreens, sprouts were cultivated hydroponically (using only tap water as the growth medium) in the same sowing tray used for sprouting and exposed to artificial light. They were harvested when the first two primary leaves fully opened at 8 DAS for MB and BG, and 10 DAS for AB with length of 15–17 cm (MB), 22–25 cm (BG), and 19–22 cm (AB), following commercial production guidelines. Light intensity was maintained at 80–90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (12-hour photoperiod) at 28 ± 2°C, with relative humidity at 80–90%. The plants were watered daily with an exact volume of 150 mL, both morning and evening. In addition, the water in the tray was changed every day. The sprouts and microgreens were divided into three parts (Fig. 1). The TOP consisted of primary leaves (for all species) and cotyledons (for MG and BG), while the MID primarily included the hypocotyl or epicotyl. The bottom part mainly comprised roots (for all species) or the root-cotyledon (for AB). The separated TOP and middle MID parts of sprouts and microgreens were frozen with liquid nitrogen for further physicochemical and biochemical analysis, whereas the physical analysis used all of the three attached parts. For physical analysis, individual samples were used (100 seeds or plants per replication), but pools of seeds or plants per tray (per replication) were employed for physicochemical and biochemical analysis.

Determination of physical characteristics

A total of 100 plants per replication of sprouts and microgreens of each *Vigna* spp. were harvested to determine plant length, fresh weight (FW), and dry weight (DW). TOP and MID plant lengths were measured with a ruler. FW was measured by removing excess water and weighing the samples using an electronic balance. For DW, the plants were dried in an oven at 60°C until a constant weight was achieved. Both FW and DW were obtained from whole sprouts and microgreens (all three parts without separation).

Physicochemical properties assessment

The physicochemical qualities, including soluble solids

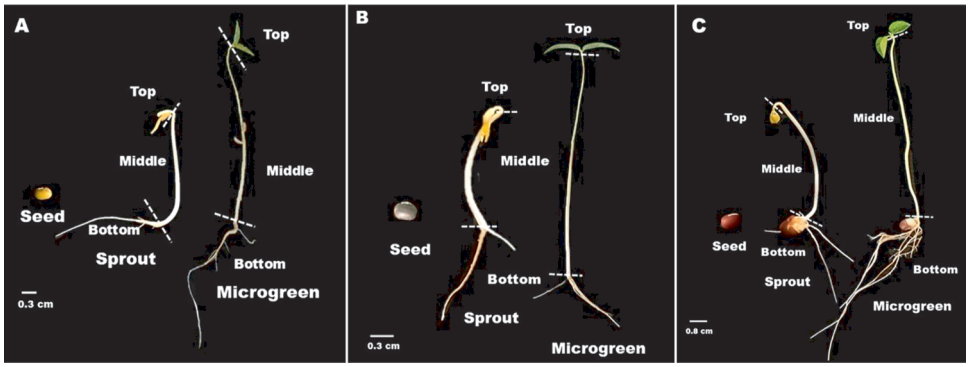

Fig. 1. Photographs of seed, sprout and microgreens of (A) mung bean, (B) black gram and (C) adzuki beans, including the sampling boundaries of the top, middle, and bottom parts of sprouts and microgreens.

concentration (SSC), titratable acidity (TA), and pH of the seeds, sprouts, and microgreens of the three *Vigna* spp., were assessed using water extraction. Samples weighing 20 g were homogenized with distilled water and filtered. SSC was determined by placing 2–3 drops of the filtered solution on the glass prism of a digital refractometer (Model PAL-1; Atago, Tokyo, Japan). TA was estimated following Choon et al. (2010). The filtrate, containing 1–2 drops of 1% phenolphthalein, was titrated with 0.1 N NaOH until a pink hue was observed. The results were expressed as% citric acid. The remainder of the filtrate was used to determine pH, which was measured using a pH meter (HI2002-02 edge® Dedicated pH/ORP meter; Hanna Instruments Sdn. Bhd., Selangor, Malaysia).

Total phenolic and flavonoid content (TPC and TFC)

To quantify TPC and TFC, a 1 g sample was homogenized using 10 mL of 80% methanol, then centrifuged at 12,000 × g for 20 min at 4°C. The extract was utilised for both quantifications. TPC of *Vigna* spp. was estimated using the Folin-Ciocalteu assay, based on the work of Zamakshshari et al. (2019), with some modifications. About 2.7 mL of Na_2CO_3 was mixed with 0.5 mL of the extract, followed by addition of 2.5 mL of Folin-Ciocalteu reagent. The mixture was incubated in the dark at room temperature (RT) for 1 h before being measured at 760 nm against a blank; the values were obtained using a gallic acid (GA) standard curve. The findings were presented as grams of gallic acid equivalents (GAE) per kilogram of fresh weight sample (g GAE·kg^{-1} FW). TFC was measured by mixing 1.5 mL of the extract with 1.5 mL of a 2% $AlCl_3$, and 2 mL of methanol, following the method of Rizaldy et al. (2022) with some modifications. After 1 h of storage in darkness at ambient temperature, the absorbance at 430 nm was measured, and the values were obtained by comparing with a quercetin (QE) standard curve. The results were expressed as gram of quercetin equivalents (QE) per kilogram of fresh weight (g QE·kg^{-1} FW).

Ascorbic acid content

The method of Kowitcharoen et al. (2021) was employed to determine ascorbic acid content with some modifications. A 2.5 g sample was homogenised with 10 mL of 5% metaphosphoric acid then filtered through muslin cloth. The filtrate was collected and mixed with 0.2 mL of 0.02% 2,6 DCPIP, 0.4 mL of thiourea, and 0.2 mL of 2,4 DNP. The mixture was incubated for 1 h at 50°C. After that, 1 mL of 85% sulfuric acid was added. At 540 nm, the absorbance was measured and recorded. The result was reported as g·kg^{-1} FW.

Total antioxidant activity (TAA) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assay

Each sample, weighing 1.5 g, was extracted with 10 mL of 80% ethanol and used to estimate TAA for both assays. The scavenging activity of DPPH was measured according to the method of Phornvillay et al. (2024). An aliquot of DPPH working solution was mixed with 150 µL of extract. The mixture was then kept in the dark at RT for 30 min, and absorbance was measured at 515 nm. The findings were presented as a percentage of the DPPH radical inhibition. For the FRAP assay, the method of Benzie and Strain (1996) was employed to estimate the TAA of the samples, comparing them with a Trolox standard curve. The results were expressed as g·kg^{-1} FW of Trolox equivalent (TE).

Antioxidant enzyme determination

A 1 g sample was extracted using 100 mM sodium phosphate buffer at pH 7.0, which contained 1 mM EDTA, 0.50 mM PEG, and 1% PVPP. The homogenate underwent centrifugation at 12,000 × g and 4°C for 15 min. The obtained supernatant was used to determine peroxidase (POD) and catalase (CAT) activity. POD activity was assessed using the method of Phornvillay et al. (2019). One unit of enzyme activity was defined as the change in absorbance at 470 nm·min^{-1}·mg^{-1} protein. CAT activity was measured

by adopting Aebi's (1984) method with some adjustments, monitoring the decrease in absorbance at 240 nm. The reaction mixture comprised 1 mL of 50 mM phosphate buffer (pH 7.0), 0.8 mL of 40 mM hydrogen peroxide, and 0.7 mL of enzyme extract. The activity was calculated as mmol $H_2O_2 \cdot min^{-1} \cdot mg^{-1}$ protein (Phornvillay et al., 2019).

Total protein content

Total protein content was quantified using the Bradford (1976) method. The content was determined by measuring absorbance at 595 nm, using a bovine serum albumin standard with values from 0 to 100 $mg \cdot L^{-1}$.

Statistical analysis

The experiments were structured using a completely randomized design with five replications of biological samples and three measurements. R software (R and RStudio Ver. 4.3.3) was used to perform analysis of covariate (ANCOVA) on the obtained data. The Duncan Multiple Range test was employed to differentiate the means when the F-test was significant at a significance level of 5%. Further, simple correlation coefficients among the parameters studied were also computed, with the significance level set at 5% (*).

Results

Fresh weight, dry weight, and plant length

The results showed that AB microgreens had the highest FW and DW (Fig. 2A and B), registering 102.78 g and 20.93 g, respectively. AB sprouts also showed significantly higher FW and DW than sprouts and microgreens from MB and BG. The lowest FW and DW were recorded for BG microgreens (20.66 g and 1.56 g, respectively). Nonetheless, sprouts recorded higher FW than microgreens for BG. There were no differences between FW and DW for sprouts and microgreens of MB. Regarding plant length, microgreens consistently exhibited significantly greater heights than sprouts, irrespective of the plant species, with the BG microgreen demonstrating the maximum height, followed by AB and MB microgreens (Fig. 2C).

Soluble solids content, titratable acidity, and pH

As shown in Figures 3A and S1A, the SSC in the seeds was significantly higher than in the sprouts and microgreens, regardless of species. The highest SSC was found in AB seeds, and the lowest in BG seeds. In addition, the SSC was higher in microgreens than in sprouts for MB, but no difference was detected for BG and AB (Fig. S1A). For microgreens, the TOP consistently had higher SSC than the MID, with the highest found in AB, followed by BG and MB. In sprouts, only AB showed a difference between the growth parts, with TOP being higher than MID (Fig. 3A), while the lowest SSC was found in MB sprouts (Fig. S1A).

The trends in TA levels across growth stages and parts varied with plant species (Fig. 3B). For MB and AB, the seeds registered significantly higher TA than sprouts and microgreens (Fig. S1B). On the contrary, for BG, the TA level in the seeds was lower than in sprouts and microgreens. In seeds, MB recorded 18.75% and 22.58% higher TA than BG and AB,

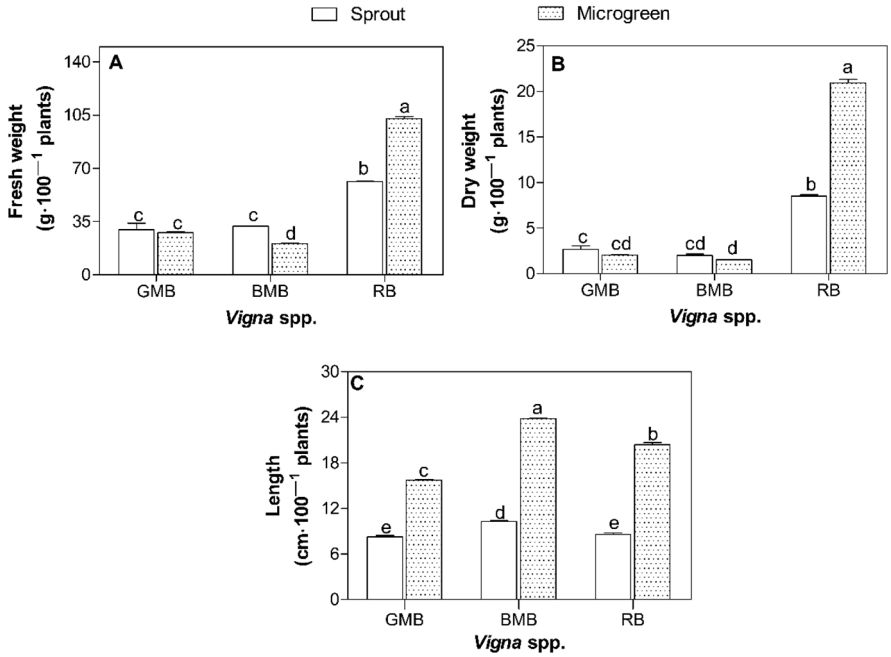

Fig. 2. (A) Fresh weight, (B) dry weight and (C) length of sprouts and microgreens of three different *Vigna* spp. (MB = mung bean; BG = black gram; AB = adzuki bean). Data are means of five replicates ± SE. Means with different letter are significantly different at the 5% level by Duncan's Multiple Range test.

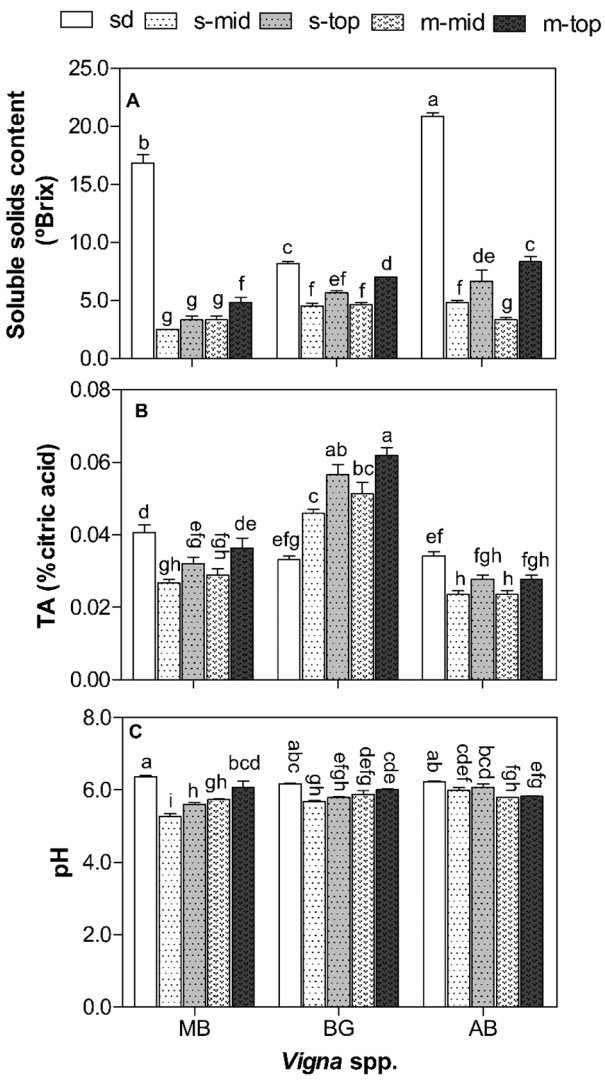

Fig. 3. (A) Soluble solid content, (B) titratable acidity (TA) and (C) pH of different growth stages and parts of three different *Vigna* spp. (MB = mung bean; BG = black gram; AB = adzuki bean). Data are means of five replicates ± SE. Means with different letters are significantly different at the 5% level by Duncan's Multiple Range test. Note: sd = seed; s-top = sprout top part; s-mid = sprout middle part; m-top = microgreen top part; m-mid = microgreen middle part.

respectively. For sprouts and microgreens, BG had the highest TA, followed by MB and AB (Fig. S1B). When comparing sprouts and microgreens, microgreens exhibited higher TA than sprouts for BG, but no differences were found for MB and AB. When comparing the growth parts, TOP had higher TA levels than MID for BG (in both sprouts and microgreens) and MB (in microgreens only); no differences were detected in AB.

Figure 3C shows that the pH measurements of seeds, sprouts, and microgreens in MB, BG, and AB ranged between 5.27 and 6.36. Regardless of plant species, the seeds had a higher pH (6.17–6.36) than sprouts and microgreens (Fig. S1C). Microgreens showed higher pH than sprouts for MB and BG; the opposite trend was observed for AB. Across the species, AB sprouts had the highest pH and MB spouts the lowest; there were no differences in pH for microgreens. The trends for pH in TOP and MID differed by plant species. For instance, the TOP of MB had a higher pH than the MID, while BG and AB showed no differences.

Phytonutrients

As Figure 4A indicates, TPC varied with plant species, growth stages, and parts. A clear trend was that microgreens registered a higher TPC than seeds, regardless of plant species (Fig. S2A). In the seeds and microgreens, the content was highest in AB and lowest in MB. For sprouts, the highest TPC was obtained from BG, and the lowest was from AB. Between sprouts and microgreens, microgreens exhibited a higher TPC in AB, whereas in BG, sprouts had higher TPC than microgreens. For growth parts, the TPC in the TOP was significantly higher than in the MID of sprouts (except BG) and microgreens for all species. Overall, the highest TPC was found in AB microgreens.

For TFC, a pronounced accumulation of TFC was observed in the TOP of the microgreen as compared to the MID (in sprouts and microgreens) and seeds, regardless of plant species (Fig. 4B). The TOP of BG microgreens exhibited the highest content, registering, on average, 35.33% higher than those of MB and AB. The difference in TFC between TOP and MID in sprouts was found only in MB, and not in BG and AB. Therefore, in sprouts, MB showed the highest TFC, followed by AB and BG (Fig. S2B). It was also observed that the TFC was higher in microgreens than sprouts for BG and AB (4.29-fold for BG and 3.32-fold for AB). In seeds, TFC was highest in BG and lowest in AB. Unlike MB and AB, TFC in BG seeds was significantly higher than in sprouts.

Remarkably, ascorbic acid content was highest in the seeds, MID, and TOP (sprout and microgreen) of MB compared to those of BG and AB (Fig. 4C). Therefore, MB scored the highest ascorbic acid content among BG and AB across the growth stages, with the highest content found in microgreens and the lowest in seeds (Fig. S2C). Additionally, the ascorbic acid content was consistently higher in the TOP compared to the MID and seeds, especially in microgreens across all species. Nevertheless, for AB, the MID of the sprout registered higher ascorbic acid content than the TOP and seed, being 2.13 and 2.36 times higher, respectively. AB scored the lowest ascorbic acid across the growth stages. The analysis of ascorbic acid content showed that microgreens had higher levels than both sprouts and seeds for MB and AB. For BG, the content was not different between seed and sprouts, but was lower than in microgreens.

The results for total protein content indicated that the content was more prominent in seeds compared to the TOP and MID of both sprouts and microgreens for all three species studied (Fig. 4D). Among the three

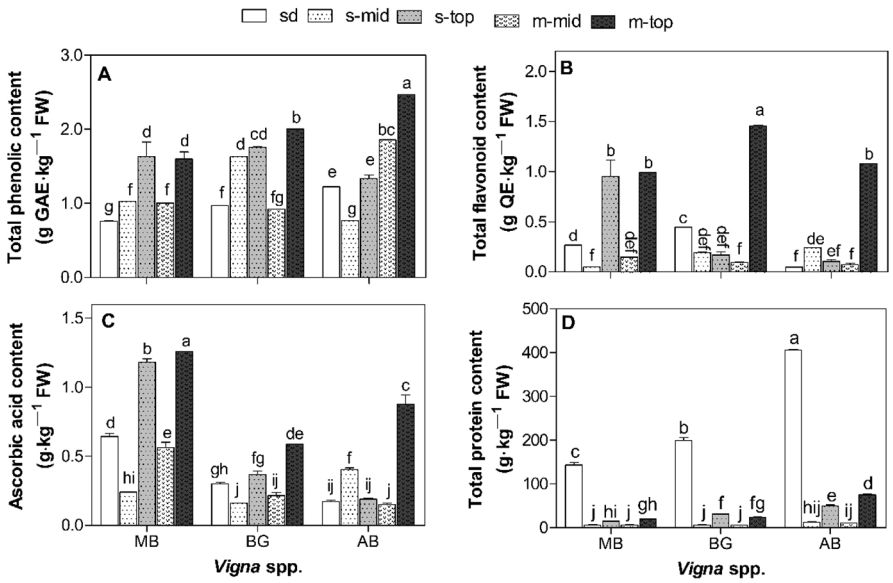

Fig. 4. (A) Total phenolic content, (B) total flavonoid content, (C) ascorbic acid content and (D) total protein content of different growth stages and parts of three different *Vigna* spp. (MB = mung bean; BG = black gram; AB = adzuki bean). Data are means of five replicates ± SE. Means with different letter are significantly different at the 5% level by Duncan's Multiple Range test. Note: sd = seed; s-top = sprout top part; s-mid = sprout middle part; m-top = microgreen top part; m-mid = microgreen middle part.

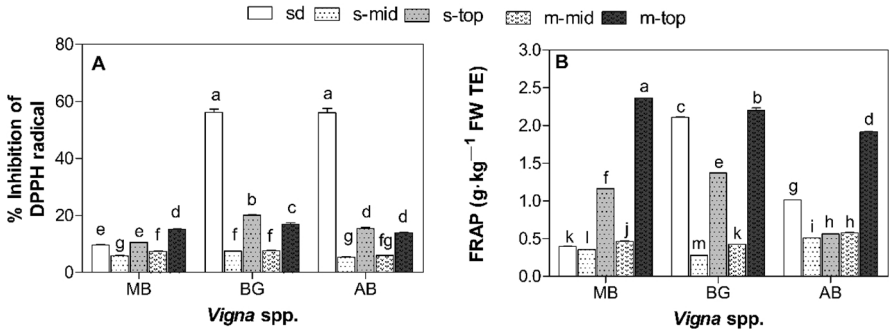

Fig. 5. Total antioxidant activity based on (A) DPPH scavenging activity and (B) a ferric reducing antioxidant power (FRAP) assay of different growth stages and parts of three different *Vigna* spp. (MB = mung bean; BG = black gram; AB = adzuki bean). Data are means of five replicates ± SE. Means with different letter are significantly different at the 5% level by Duncan's Multiple Range test. Note: sd = seed; s-top = sprout top part; s-mid = sprout middle part; m-top = microgreen top part; m-mid = microgreen middle part.

species, AB seeds registered the highest content, with 3-fold and 2-fold higher content than MB and BG, respectively. The results also revealed that the TOP had more total protein content than the MID (in both sprouts and microgreens). The overall protein content in sprouts and microgreens was greatest in AB, followed by BG and MB (Fig. S2D). The difference in protein content between microgreens and sprouts varied by plant species. Microgreens had a higher content than sprouts for AB, while MB and BG showed no difference.

Total antioxidant activity

The trends for TAA in seeds, sprouts, and microgreens, as measured by two methods of DPPH scavenging activity and FRAP assay, were different (Fig. 5). Among the three species, seeds of BG and AB recorded the highest DPPH scavenging activity (Fig. 5A), with the lowest activity found in the MID. Therefore, the TOP consistently exhibited higher DPPH scavenging activity than the MID. For microgreens, BG showed the highest scavenging activity, and no difference was detected between MB and AB (Fig. S3A). For sprouts, BG also showed the highest activity, followed by AB and MB. In contrast, seeds showed superior activity with respect to sprouts and microgreens for BG and AB.

As for the FRAP assay, the results showed that the TAA was always higher in the TOP of microgreens than in sprouts and seeds (Fig. 5B). The assay detected the highest TAA in the TOP of microgreens in MB, and the lowest in AB. TAA was higher in microgreens than sprouts for all the studied species (Fig. S3B). For microgreens, MB exhibited an average TAA that was 34.40% higher than that of BG and AB, with no difference observed between BG and AB. For sprouts, the TAA was highest in the TOP of BG, followed by the

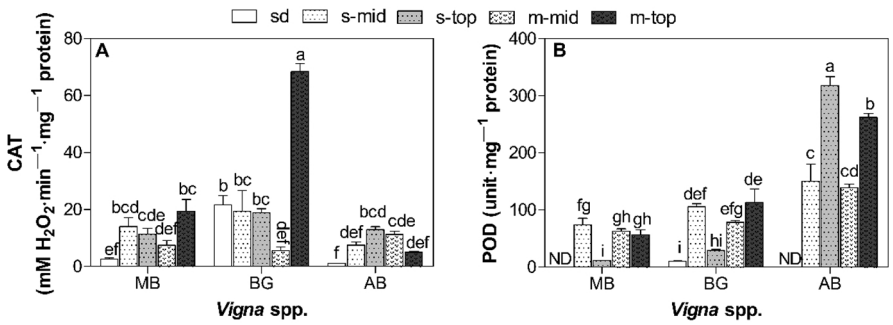

Fig. 6. (A) Catalase (CAT) and (B) peroxidase (POD) activity of different growth stages and parts of three different *Vigna* spp. (MB = mung bean; BG = black gram; AB = adzuki bean). Data are means of five replicates ± SE. Means with different letter are significantly different at the 5% level by Duncan's Multiple Range test. Note: ND = not detected; sd = seed; s-top = sprout top part; s-mid = sprout middle part; m-top = microgreen top part; m-mid = microgreen middle part.

TOP of MB and the TOP of AB. The sprouts of AB recorded the lowest activity on average, while there was no clear difference in TAA between MB and BG. Similarly, the TAA in microgreens was also higher than in seeds. In seeds, BG seeds exhibited a higher TAA than AB and MB (2.08 and 5.57 times higher, respectively).

Antioxidant enzyme activity

The trend for CAT activity revealed variance in *Vigna* spp. (Fig. 6A). For BG, the TOP of microgreens exhibited the highest activity, and the MID the lowest. There were no differences between the seed and sprouts. For MB, the seeds recorded the lowest CAT activity, but no difference in the activity was detected between sprouts and microgreens (Fig. S4A). A similar trend was observed for AB. Across the plant species, BG microgreens and seeds exhibited superior CAT activity to MB and AB.

For POD activity, activity in seeds was detected only in BG seeds (Fig. 6B). For BG, the highest POD activity was detected in microgreens and the lowest in seeds (Fig. S4B). For AB, the TOP showed higher POD activity than the MID for both sprouts and microgreens. For MB and BG, the TOP of the sprouts showed the lowest activity, while the microgreens showed no difference. Across the species, the highest activity was found in AB, followed by BG and MB.

Discussion

The biomass differences observed in *Vigna* microgreens appear to be linked to seed size and initial seed reserves, such as sugars and amino acids, which fuel respiration and metabolic activity during germination (Chen et al., 2019; Nonogaki, 2019). AB microgreens had the highest FW and DW, supported by superior nutrient reserves (as indicated by their larger size, higher SSC, and protein content) and vigorous seedling growth, in which sugars and proteins are catabolised during germination to fuel respiration and growth. This is evidenced by the decrease in SSC and total protein content in AB cotyledons as growth progressed (Fig. S5). In contrast, BG microgreens showed the lowest biomass, as reflected by the lowest SSC in the seeds. As germination and growth progressed, organic acids were broken down during sprouting (Chen et al., 2019), as reflected by the reduction in TA from seeds to microgreens (AB and MB). Conversely, BG exhibited an increase in acidity during sprouting, possibly due to enhanced organic acid biosynthesis or reduced consumption. The general decline in pH from seeds to microgreens is due to organic acid accumulation and cellular respiration, with species-specific variability reflecting different metabolic fluxes (Bushell et al., 2019).

Reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide (H_2O_2) are produced during the metabolic processes of germination and seedling growth (Bailly, 2023). AB, which had vigorous growth, was likely generating more ROS than BG and MB; thus, the elevation of the content and activity of oxidant scavenging systems is essential to maintain cell homeostasis. This phenomenon could explain the prominent TPC and, specifically, POD activity in AB as a means of scavenging ROS. ROS are often reported to act as signalling molecules in phenolics production (Zagoskina et al., 2023). Furthermore, phenolics have been reported to act as secondary scavengers of H_2O_2 in conjunction with POD (Rao et al., 2025). POD activity is also known to be elevated in actively developing leaves associated with auxin production and lignin biosynthesis (Thakur and Vasudevan, 2019). Evidently, the rise in POD activity in the TOP of AB sprouts corresponds to the active development of the primary leaves. Overall, the specific elevation of POD activity in AB is thought to contribute to better nutrient uptake and mobilization, which is directly proportional to its biomass accumulation, as reported by Taghvaei et al. (2025). However, further detailed studies are required to explore this phenomenon more thoroughly.

On the other hand, the BG microgreens showed the highest TFC and CAT activity, underscoring genotype-specific regulation of ROS and secondary metabolite

synthesis, favoring flavonoid biosynthesis. The findings suggest that CAT could play a role in the hypocotyl elongation of BG by preventing rising levels of H_2O_2 (Rajashekar and Baek, 2014) and altering the generation of plant hormones (Taghvaei et al., 2025), thus explaining BG producing the tallest plants. Also, the pronounced elevation of CAT activity in the TOP of BG microgreens, accompanied by high chlorophyll content (data not shown), possibly reflect a direct response to increased metabolic activity and ROS production during chloroplast development (Li and Kim, 2022). Similar species- and tissue-specific responses have been reported in germinating peas, cucumbers, and wheat, where the changes in phenolics and flavonoids depend on the alterations in their metabolites, such as organic acids (Szablińska-Piernik and Lahuta, 2023). Additionally, the TPC and TFC levels increased substantially in the TOP of microgreens, corroborating the role of light in activating phenylpropanoid biosynthesis pathways (Ferreyra et al., 2012). It is also clear that the high TAA in the TOP of BG and AB microgreens is possibly due to the accumulation of TPC and TFC. The interspecies variability in the accumulation of phenolics, flavonoids, and enzymatic responses underscores the unique antioxidant defence strategies encoded in each *Vigna* spp.

The metabolites accumulation mechanism for MB is dominated by the accumulation of ascorbic acid during germination and further under light exposure, with the highest content detected in the TOP of microgreens, confirming previous reports that MB is a rich source of vitamin C during early growth (Guo et al., 2012). During exposure to physical or physiological stress, free ascorbic acid is oxidized to dehydroascorbic acid, functioning as an electron donor in redox reactions. This transition enables the ascorbate–dehydroascorbate system to neutralize ROS, thereby mitigating oxidative injury in plant cells (Dhaka et al., 2023). Moreover, the accumulation of ascorbic acid water-soluble antioxidants also correlated with TAA as measured by a FRAP assay in the TOP of MB microgreens ($r = 0.85^*$). Nonetheless, the unexpectedly high ascorbic acid content in the MID of AB sprouts may indicate a localized oxidative stress response during early germination, an adaptive trait potentially valuable for functional food applications. These findings support the notion that species and developmental stages must be carefully selected to optimize nutritional outputs in sprout or microgreen production systems.

Seeds of BG and AB exhibited high DPPH scavenging activity, which could be closely related to the antioxidants present in the colored seed coats of the beans (Nagao et al., 2023). Additionally, the polysaccharides in AB reportedly contribute to the high antioxidant activity (Wang et al., 2022), as the SSC was highly correlated with DPPH scavenging activity ($r = 0.95^*$). Overall, this study posits that the antioxidant activities of microgreens are primarily due to vitamins and secondary metabolites, with some species exhibiting vitamin-dominant antioxidant activity, whilst others are predominantly influenced by secondary metabolites. This species- and part-specific variation provides deeper insight into the biochemical contributions to antioxidant capacity in microgreens.

In conclusion, this study highlights how different *Vigna* spp. and growth stages offer unique nutritional and antioxidant advantages. AB (*V. angularis*) microgreens TOP stood out with the highest biomass, phenolic content and antioxidant activity, particularly POD activity, making them an excellent option for boosting dietary polyphenols. MB (*V. radiata*) microgreens were rich in ascorbic acid and exhibited strong antioxidant potential, as observed by TAA in the FRAP assay, while BG (*V. mungo*) excelled in flavonoid content and CAT enzymatic antioxidant activity in both sprouts and microgreens. Seeds remained the best source of protein and sugar, with the highest recorded by AB seeds, but sprouts and microgreens developed additional health-promoting compounds, particularly in TOP. Overall, TOP were more nutrient rich than MID across all stages and species, with microgreens outperforming sprouts and seeds. A key limitation, however, is that the typically consumed edible TOP and MID only account for a small portion of the whole plant, meaning the edible yield is relatively low. Growing sprouts and microgreens also require more seeds and frequent production cycles compared to using mature seeds, which can increase costs and limit large-scale adoption. Nevertheless, their short growth period, adaptability to indoor systems, and minimal land requirements make them attractive for urban farming and resource-efficient agriculture. By combining an understanding of nutritional benefits with considerations of yield and cost, this study offers a step towards making *Vigna* sprouts and microgreens a more accessible and sustainable option. With careful cultivation strategies and innovations in production, these nutrient-rich foods could become not only affordable for consumers, but also a valuable component of future functional diets.

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