Nutrients, minerals, pigments, phytochemicals, and radical scavenging activity in *Amaranthus blitum* leafy vegetables

Umakanta Sarker*1 & Shinya Oba2

*blitum* is good sources of abundant natural antioxidant phytopigments such as anthocyanin, betalain, betaxanthin, and betacyanin and antioxidant phytochemicals of interest in the food industry. The chances of utilizing amaranth pigments and phytochemicals had been evaluated for extracting colorful juice as drink purposes. Hence, the presence of nutrients, phytopigments, phytochemicals, and radical scavenging activity of selected *A. blitum* leafy vegetables were evaluated. Leaves of *A. blitum* have considerable fiber, moisture, protein, and carbohydrates. It has considerable magnesium, calcium, potassium (30.42, 24.74, 10.24 mg g⁻¹), zinc, iron, copper, manganese, (878.98, 1153.83, 26.13, 207.50 µg g⁻¹), phytopigments such as chlorophyll a, chlorophyll ab, chlorophyll b, (63.69, 90.60, 29.32 mg 100 g⁻¹), betalain, betaxanthin, betacyanin (112.01, 58.38, 53.63 µg 100 g⁻¹), vitamin C (1848.15 µg g⁻¹), total carotenoids, (β-carotene (1675.38, 1281.66 µg g⁻¹), TPC, TFC (253.45 GAE and 162.97 RE µg g⁻¹ DW), and TAC (29.46, 55.72 µg g⁻¹ DW in Tolax equivalent DPPH and ABTS radical scavenging capacity) in *A. blitum*. The accessions DS3, DS6, DS8, and DS12 exhibited the highest TAC in Trolox equivalent DPPH and ABTS radical scavenging capacity, flavonoids, and considerable phytopigments. These accessions had excellent antioxidant profiles along with high yielding potentiality. Hence, *A. blitum* provides an excellent source of proximate, phenolics, minerals, flavonoids, vitamins, and phytopigments to address the nutritional and antioxidant deficiency in daily diet.

The genus *Amaranthus* is C₄ leafy vegetables of great diversity and plasticity1 with many culinary purposes. Bangladesh, Africa, south-east Asia, and South America consumed *A. blitum* as famous leafy vegetables. Its popularity is continuously increasing in the Asian continent and elsewhere because of its attractive leaf color, taste, and adequate nutritional value. In Bangladesh, it can be produced throughout the year as well as in the gaps period of leafy vegetables between winter and hot summer2,3. It is very cheap and has adequate protein with essential amino acids, such as methionine and lysine, dietary fiber, minerals, phytopigments, and bioactive compounds, such as betacyanin, chlorophyll, betaxanthin, carotenoids, (β-carotene, vitamin C, phenolic compounds, and flavonoids4–10.

In the world, food insecurity results in a continuous calorie deficit of approximately 795 million malnourished people11. Deficiency of vitamins or minerals results in hidden hunger in over two billion people12. Staple foods are deficient of micronutrients, mainly iron, zinc and iodine, pro-vitamin A, carotenoids, vitamin C, E, albeit these are a source of energy13. Consequently, staple foods in our daily diet result in hidden hunger12. We can ensure a balanced and healthy diet by the consumption of vegetables and fruits as a source of minerals and vitamins accomplished with staple food. Furthermore, we protect human health and decrease the risk of cancer, cardiovascular, and other chronic diseases by consuming vegetables and fruits. Phytochemical compounds, such as phytopigments, vitamin C, phenolics, and flavonoids are thought to contribute to those health benefits14–16.

Recently, researchers and consumers interested in natural antioxidants of vegetables. Phytopigments (betalain, betaxanthin, chlorophyll, and carotenoids), vitamin C, phenolics and flavonoids are available natural antioxidants in *Amaranthus*4,17. These natural antioxidant phytochemicals protect many diseases, such as cancer,
### Results and Discussion

The prominent variations were detected among the studied characters in terms of genotypes.

#### Composition of proximate.

Fat, moisture, carbohydrates, protein, energy, ash, and dietary fiber contents of *A. blitum* are shown in Table 1. The highest moisture content was recorded in DS13 and DS7 (88.46, 88.45 g 100 g FW), while the lowest moisture content was found in DS6 and DS4 (81.43, 81.46 g 100 g FW). The range of moisture content was 81.43 to 88.46 g 100 g FW. As high dry matter of leaf was obtained from lower moisture contents, seven genotypes (15–18% dry matter) exhibited considerable dry matter. The leaf moisture content of *A. blitum* leafy vegetables directly associated with the maturity of the plant. The findings obtained in this study were corroborated with the results of *A. tricolor* and sweet potato leaves by Sarker and Obasa, and Sun et al.

### Table 1. Compositions of proximate (per 100 g FW) and dietary fiber (µg g⁻¹ FW) of 16 *A. blitum* genotypes.

| Genotypes | Moisture (g) | Protein (g) | Fat (g) | Carbohydrates (g) | Energy (kcal) | Ash (g) | Dietary fiber (µg g⁻¹ FW) |
|-----------|-------------|-------------|---------|-------------------|---------------|---------|---------------------------|
| DS1       | 85.46 ± 1.87d | 1.13 ± 0.03k | 0.25 ± 0.03e | 9.71 ± 0.07b | 46.32 ± 0.35g | 3.45 ± 0.03g | 73.40 ± 1.05g |
| DS2       | 87.82 ± 1.21bc | 3.18 ± 0.02h | 0.24 ± 0.02e | 5.88 ± 0.081 | 36.05 ± 0.421 | 2.88 ± 0.02 | 73.83 ± 1.25f |
| DS3       | 82.74 ± 1.35j | 4.26 ± 0.04d | 0.16 ± 0.02f | 7.52 ± 0.07b | 45.93 ± 0.38g | 5.32 ± 0.03b | 77.75 ± 0.98e |
| DS4       | 81.46 ± 1.51g | 6.22 ± 0.04a | 0.18 ± 0.03f | 6.49 ± 0.11j | 55.33 ± 0.75b | 5.65 ± 0.04a | 67.72 ± 0.65j |
| DS5       | 88.05 ± 0.76b | 2.15 ± 0.03j | 0.45 ± 0.02a | 6.13 ± 0.14k | 35.78 ± 0.62b | 3.22 ± 0.03b | 67.16 ± 0.62k |
| DS6       | 81.43 ± 1.17g | 5.45 ± 0.05b | 0.28 ± 0.01d | 7.72 ± 0.16g | 56.07 ± 0.91a | 5.12 ± 0.03d | 77.21 ± 0.85e |
| DS7       | 88.45 ± 1.33a | 4.45 ± 0.03c | 0.28 ± 0.02d | 1.50 ± 0.07n | 26.82 ± 0.72j | 5.28 ± 0.05c | 68.80 ± 0.18h |
| DS8       | 82.76 ± 1.62f | 3.55 ± 0.03f | 0.14 ± 0.03h | 9.00 ± 0.15d | 49.75 ± 0.53d | 4.55 ± 0.01e | 91.94 ± 0.52d |
| DS9       | 86.49 ± 1.24c | 3.38 ± 0.04g | 0.34 ± 0.01c | 6.57 ± 0.12j | 40.27 ± 0.49j | 3.22 ± 0.01h | 59.96 ± 0.75m |
| DS10      | 84.65 ± 1.13e | 2.26 ± 0.04c | 0.29 ± 0.02c | 8.56 ± 0.17c | 43.74 ± 0.62b | 4.24 ± 0.06f | 66.54 ± 0.62c |
| DS11      | 88.07 ± 1.74b | 3.39 ± 0.02g | 0.32 ± 0.02ed | 6.17 ± 0.16k | 39.78 ± 0.43 | 2.05 ± 0.021 | 68.66 ± 0.47c |
| DS12      | 84.73 ± 1.64c | 3.59 ± 0.06f | 0.29 ± 0.01d | 8.33 ± 0.11c | 48.00 ± 0.48 | 3.06 ± 0.02c | 93.65 ± 0.35b |
| DS13      | 88.46 ± 1.87a | 4.26 ± 0.06d | 0.41 ± 0.04b | 4.59 ± 0.13m | 36.93 ± 0.46k | 2.28 ± 0.03k | 97.88 ± 0.62a |
| DS14      | 84.95 ± 1.05e | 2.29 ± 0.05i | 0.33 ± 0.03c | 9.21 ± 0.12c | 47.51 ± 0.65f | 3.22 ± 0.05b | 69.15 ± 0.53h |
| DS15      | 82.54 ± 1.13f | 2.86 ± 0.05j | 0.18 ± 0.01f | 10.17 ± 0.13a | 52.85 ± 0.51a | 4.25 ± 0.06f | 92.35 ± 0.42c |
| DS16      | 86.43 ± 1.62c | 3.66 ± 0.04e | 0.34 ± 0.01c | 6.69 ± 0.11i | 43.95 ± 0.67b | 2.88 ± 0.02 | 77.63 ± 0.48e |
| Mean      | 85.28         | 3.51         | 0.28       | 7.14           | 44.08         | 3.79       | 76.60                     |
| CV%       | 1.060         | 0.132        | 0.135      | 0.135          | 0.226         | 0.564      | 0.419                     |

### atherosclerosis, cataracts, cardiovascular diseases, retinopathy, arthritis, emphysema, and neurodegenerative diseases. This genus is adapted to abiotic stresses, such as salinity and drought. This species has scarce information albeit it adapted to abiotic stress and low-cost leafy vegetables containing considerable antioxidant phytochemicals, minerals, fiber, and protein. In the previous study, we evaluated *A. tricolor* for morphological, proximate, minerals, antioxidant phytopigments, antioxidant phytochemicals. To our knowledge, it is the first report on phenolics, proximate compositions, phytopigments, mineral, flavonoids, and vitamins in *A. blitum* germplasms. Therefore, this investigation was performed to investigate phenolics, proximate compositions, vitamins, mineral, phytopigments, and flavonoids content in 16 *A. blitum* genotypes and to evaluate variations among traits in 16 *A. blitum* genotypes.
observed similar results. Jimenez-Aguiar and Grusak 28 reported a good amount of Mg, K, and Ca in different iron content. The range of manganese content was 132.65 to 356.84 mined based on the dry weight. Chakrabarty Composition of Minerals (Macro and microelements, A. blitum (10.24 mg g −1), calcium (24.74 mg g −1) and magnesium (30.42 mg g −1) in the leaf of A. blitum, albeit we determined based on the dry weight. Chakrabarty et al. 27 in stem amaranth and Sarker and Oba23 in A. tricolor also observed similar results. Jimenez-Aguilar and Grusak24 reported a good amount of Mg, K, and Ca in different species of amaranth. They reported that Mg, Ca, and K content of different species of amaranth was much greater than kale, black nightshade, spider flower, and spinach.

Iron content showed prominent variations in terms of genotypes (195.12 to 2057.02 µg g −1). The highest iron content was observed in the genotypes DS6. In contrast, the lowest iron content was obtained from the genotype DS11, with a mean iron content of 1153.83 µg g −1. Six accessions exhibited higher content of iron than their mean iron content. The range of manganese content was 132.65 to 356.84 µg g −1, with a mean value of 207.50 µg g −1. The accessions DS2, DS1, DS5, DS9, and DS7 had considerable content of manganese, while the lowest manganese content was recorded in the genotype DS16 (3.86 µg g −1). Copper content exhibited considerable variations in terms of accessions (16.09–45.12 µg g −1). The accession DS3 showed the highest copper content (45.12 µg g −1), followed by DS7, DS9, DS10, DS5, and DS2. Seven accessions showed better copper content than the average value (26.13 µg g −1). The accession varied considerably in the content of zinc (680.41, 681.07, 681.38 µg g −1 in DS10, DS13, and DS8, respectively to 1473.54 µg g −1 in DS2). High zinc content was observed in seven genotypes which were higher than the grand mean value (878.98 µg g −1). Three genotypes DS2, DS3, and DS7 exhibited excellent zinc content (1082.09 to 1473.54 µg g −1 DW). Leaves of A. blitum contained higher zinc and iron compared to beach pea29 and the leaves of cassava30. Our study showed that leaves of A. blitum had considerable iron

| Genotypes | Macro elements (mg g −1 DW) | Micro elements (µg g −1 DW) |
|-----------|----------------------------|-----------------------------|
| DS1 | 11.39 ± 0.05 f | 32.82 ± 0.03a | 31.13 ± 0.09c |
| DS2 | 16.28 ± 0.05a | 22.07 ± 0.02 b | 35.43 ± 0.12a |
| DS3 | 8.89 ± 0.07 | 24.02 ± 0.01f | 30.19 ± 0.14e |
| DS4 | 13.86 ± 0.04b | 19.22 ± 0.06d | 29.26 ± 0.17e |
| DS5 | 11.63 ± 0.02e | 27.15 ± 0.02e | 32.05 ± 0.21b |
| DS6 | 10.62 ± 0.07g | 24.02 ± 0.09f | 30.51 ± 0.21d |
| DS7 | 8.94 ± 0.03 | 32.02 ± 0.06e | 30.51 ± 0.14d |
| DS8 | 10.46 ± 0.06b | 16.82 ± 0.06c | 28.63 ± 0.14a |
| DS9 | 6.55 ± 0.07l | 23.22 ± 0.05g | 28.63 ± 0.18b |
| DS10 | 0.95 ± 0.05m | 30.42 ± 0.06d | 30.51 ± 0.17d |
| DS11 | 8.65 ± 0.06k | 22.72 ± 0.07e | 29.88 ± 0.12f |
| DS12 | 12.06 ± 0.05d | 20.02 ± 0.08e | 30.51 ± 0.19d |
| DS13 | 9.83 ± 0.05i | 27.22 ± 0.04e | 30.13 ± 0.12c |
| DS14 | 12.27 ± 0.04c | 31.22 ± 0.08c | 29.57 ± 0.18g |
| DS15 | 11.51 ± 0.03e | 23.22 ± 0.07e | 30.19 ± 0.14e |
| DS16 | 9.88 ± 0.06l | 15.22 ± 0.04i | 28.63 ± 0.12i |
| Mean | 10.24 | 24.74 | 30.42 |
| CV% | 2.075 | 1.280 | 1.329 |

**Table 2.** Composition of Minerals (Macro and microelements, µg g −1 DW and mg g −1 DW, respectively) of 16 A. blitum genotypes. CV, Coefficient of variation; n = 3. **Significant at 1% level, Different letters in each columns are differed significantly by Tukey’s HSD test.**
Genotypes & Genotypes: (1153.83 µg g⁻¹), manganese (207.50 µg g⁻¹), copper (26.13 µg g⁻¹), and zinc (878.98 µg g⁻¹), albeit it was measured based on the dry weight. Jimenez-Aguirar and Grusak²⁸ reported a good amount of iron, manganese, copper, and zinc in the different species of amaranth. They reported that iron, manganese, copper, and zinc content of different species of amaranth were much greater than kale, black nightshade, spider flower, and spinach. The genotype DS2 could be used as potassium, magnesium, iron, manganese, and zinc enrich leafy vegetables. The genotype DS1 could be used as calcium enrich leafy vegetable, while DS3 could be used as copper and DS6 as iron enrich leafy vegetables.

Composition of antioxidant pigments. Table 3 represents the composition of antioxidant pigments of 16 A. blitum genotypes. Chlorophyll a content differed remarkably in A. blitum genotypes (13.17 to 63.69 mg 100 g⁻¹). The highest chlorophyll a was measured from the genotype DS8 (63.69 mg 100 g⁻¹), while the accessions DS3 and DS4 showed the lowest chlorophyll a (13.17 and 13.26 mg 100 g⁻¹). Chlorophyll a content was high in the genotypes DS13, DS4, DS6, and DS15. The chlorophyll a content of six genotypes was higher than the average value. There were prominent variations in chlorophyll b content of 16 A. blitum genotypes (4.97 to 29.32 mg 100 g⁻¹). The highest chlorophyll b content was observed in DS15 (29.32 mg 100 g⁻¹), followed by DS8, DS4, DS12, and DS6. Conversely, DS7 had the lowest chlorophyll b (4.97 mg 100 g⁻¹). Prominent variations were also observed in chlorophyll ab (19.72 to 90.60 mg 100 g⁻¹). DS8, DS4, DS13, DS15, and DS6 showed good content of chlorophyll ab, while DS2 had the lowest chlorophyll ab content (19.72 mg 100 g⁻¹). Eight accessions exhibited higher content of chlorophyll ab than the mean chlorophyll ab content. Our study revealed that stem amaranth genotypes had a considerable amount of chlorophyll ab (90.60 mg 100 g⁻¹), chlorophyll a (63.69 mg 100 g⁻¹), and chlorophyll b (29.32 mg 100 g⁻¹), whereas, chlorophyll b content of A. tricolor reported by Khanam and Oba³¹ were relatively lower.

Betaxanthin ranged from 10.54 to 53.63 µg 100 g⁻¹ with a mean betaxanthin content of 31.26 µg 100 g⁻¹. DS12 exhibited the highest betaxanthin content (53.63 µg 100 g⁻¹), followed by DS4, DS6, DS16, and DS8. In contrast, DS7 exhibited the lowest betaxanthin (10.54 µg 100 g⁻¹). Betaxanthin content showed the significant and notable differences in 16 A. blitum genotypes (18.50 to 83.85 µg 100 g⁻¹). DS12 had the highest betaxanthin content (58.38 µg 100 g⁻¹). High betaxanthin content was recorded in DS4, DS6, DS8, and DS16 with the lowest betaxanthin content of 18.50 µg 100 g⁻¹ in DS2. Five accessions had higher betaxanthin content than the mean value. Pronounced variations were observed in betalain content (20.24 to 112.01 µg 100 g⁻¹). DS2 had the highest betalain (112.01 µg 100 g⁻¹), and DS4, DS6, and DS8 exhibited high betalain content. In contrast, the accession DS7 showed the lowest content of betalain (20.24 µg 100 g⁻¹). Five genotypes had higher betalain content than average value. The range of total carotenoids content was 516.80 µg g⁻¹ in DS4 to 1675.38 µg g⁻¹ in DS14. DS14 showed the highest total carotenoids content (1675.38 µg g⁻¹) and DS7, DS10, DS9, and DS5 showed good total carotenoids content. Seven accessions had higher total carotenoids than average value. In this study, we found considerable betacarotin (53.63 µg 100 g⁻¹), betaxanthin (58.38 µg 100 g⁻¹), betalain (112.01 µg 100 g⁻¹) and total carotenoids (1675.38 µg g⁻¹) in A. blitum, which corroborated with the results of Khanam et al.³² for betacarotin, betaxanthin, betalain, and total carotenoids content of A. tricolor. The genotype DS8 could be used as chlorophylls enrich leafy vegetable. The genotype DS12 could be used as betacarotin, betaxanthin and betalain enrich leafy vegetables, while the genotypes DS14 could be used as total carotenoids enrich leafy vegetables.

Table 3. Performance of antioxidant phytopigments in 16 A. blitum genotypes. CV, Coefficient of variation; n = 3; **Significant at 1% level, Different letters in each columns are differed significantly by Tukey's HSD test.
µequivalent DPPH radical scavenging capacity with mean TAC of 20.90 TEAC TAC in Trolox equivalent DPPH radical scavenging capacity. The accessions DS3, DS13, DS16, and DS4 showed (55.72, 55.65, 55.62, 55.04...color GAE DS6, DS3, DS12, and DS8 exhibited the highest TAC in Trolox equivalent ABTS as DS12, showed the highest TAC (ABTS + radical scavenging capacity was observed in DS2 with an average of 21.93 TEAC in the accessions, DS13, DS16, DS4, and DS7. Conversely, the lowest TAC in Trolox equivalent ABTS + radical scavenging capacity...significant by Tukey's HSD test.

Antioxidant phytochemicals. Table 4 reflects TAC, vitamins, TPC, and TFC of A. blitum genotypes. CV, Coefficient of variation; TAC = Total antioxidant capacity, TPC = Total polyphenol content, TFC = Total flavonoid content, n = 3; **Significant at 1% level, Different letters in each columns are differed significantly by Tukey's HSD test.

| Genotypes | β-carotene (µg g −1 FW) | Vitamin C (µg g −1 FW) | TPC (GAE µ g −1 DW) | TFC (RE µ g −1 DW) | TAC (DPPH) (TEAC µ g −1 DW) | TAC (ABTS) + (TEAC µ g −1 DW) |
|------------|--------------------------|-------------------------|---------------------|-------------------|----------------------------|-------------------------------|
| DS1        | 800.42 ± 1.52 g          | 1722.38 ± 2.13c         | 122.16 ± 0.33m      | 65.23 ± 0.23l     | 15.25 ± 0.10i               | 28.75 ± 0.07j               |
| DS2        | 601.15 ± 1.34l           | 600.65 ± 3.10h          | 92.33 ± 0.25o       | 98.81 ± 0.22t     | 12.27 ± 0.09j               | 21.93 ± 0.08m               |
| DS3        | 516.84 ± 1.52a           | 985.72 ± 2.05e          | 198.75 ± 0.26i      | 162.97 ± 0.15a    | 26.85 ± 0.21b               | 55.65 ± 0.04a               |
| DS4        | 392.24 ± 1.28p           | 1786.76 ± 2.85b         | 253.45 ± 0.36a      | 145.25 ± 0.23c    | 24.31 ± 0.24e               | 46.44 ± 0.06e               |
| DS5        | 844.34 ± 1.18e           | 755.41 ± 2.09y          | 146.87 ± 0.42c      | 102.87 ± 0.12g    | 16.51 ± 0.22g               | 31.86 ± 0.08h               |
| DS6        | 823.46 ± 1.36f           | 616.04 ± 1.56e          | 219.55 ± 0.46e      | 145.25 ± 0.28c    | 29.46 ± 0.24a               | 55.72 ± 0.03a               |
| DS7        | 1208.41 ± 1.65b          | 1848.15 ± 1.69a         | 201.53 ± 0.25h      | 102.27 ± 0.34g    | 20.14 ± 0.33f               | 38.64 ± 0.06f               |
| DS8        | 564.14 ± 1.86m           | 924.29 ± 1.58l          | 246.20 ± 0.12c      | 155.34 ± 0.32b    | 29.46 ± 0.18a               | 55.04 ± 0.08a               |
| DS9        | 901.16 ± 1.33d           | 802.06 ± 2.78h          | 98.74 ± 0.34m       | 97.84 ± 0.15j     | 15.17 ± 0.22j               | 29.35 ± 0.04i               |
| DS10       | 1001.13 ± 2.84c          | 862.35 ± 1.65g          | 92.54 ± 0.42o       | 82.47 ± 0.32k     | 12.83 ± 0.19k               | 22.98 ± 0.04m               |
| DS11       | 691.25 ± 1.52l           | 492.84 ± 1.34i          | 201.90 ± 0.29g      | 100.97 ± 0.16h    | 16.28 ± 0.21h               | 33.40 ± 0.06g               |
| DS12       | 763.34 ± 1.54h           | 121.19 ± 2.36n          | 213.78 ± 0.13f      | 135.54 ± 0.16d    | 29.46 ± 0.19a               | 55.62 ± 0.06a               |
| DS13       | 684.68 ± 1.78k           | 924.26 ± 2.36i          | 246.46 ± 0.15b      | 135.66 ± 0.35d    | 28.31 ± 0.18c               | 50.91 ± 0.08c               |
| DS14       | 1281.66 ± 1.29a          | 431.12 ± 2.46m          | 125.28 ± 0.18i      | 125.64 ± 0.23f    | 14.55 ± 0.18j               | 26.19 ± 0.051               |
| DS15       | 711.70 ± 2.30h           | 1108.46 ± 1.25d         | 128.29 ± 0.62k      | 125.27 ± 0.28f    | 15.26 ± 0.04i               | 28.52 ± 0.08k               |
| DS16       | 441.85 ± 1.56e           | 677.58 ± 3.14e          | 225.42 ± 0.52d      | 129.91 ± 0.28e    | 26.48 ± 0.19d               | 48.49 ± 0.08d               |
| Mean       | 763.61                   | 928.83                  | 175.83              | 119.45            | 20.90                      | 39.30                       |
| CV%        | 4.853                    | 1.427                   | 2.126               | 0.342             | 0.1234                     | 0.2456                      |

Table 4. Performance of TPC, β-carotene, TAC (ABTS + and DPPH), vitamin C, and TFC of 16 A. blitum genotypes. CV, Coefficient of variation; TAC = Total antioxidant capacity, TPC = Total polyphenol content, TFC = Total flavonoid content, n = 3; **Significant at 1% level, Different letters in each columns are differed significantly by Tukey's HSD test.

In this study, we reported considerable β-carotene (1281.66 µg g −1) and vitamin C (1848.15 µg g −1) in A. blitum, which was relatively higher than A. tricolor of our earlier studies. Our obtained TPC (253.45 GAE µ g −1 FW) was higher than the TPC of A. tricolor reported by Khanam et al.32. Our reported TFC (162.97 RE µ g −1 DW) and TAC (ABTS + and DPPH) (55.72 and 29.46 TEAC µ g −1 DW) were corroboration to the results of A. tricolor of Khanam et al.32. The accessions DS14, DS7, and DS4 could be used as beta-carotene, vitamin C, and TPC enrich leafy vegetables, respectively. The accession DS3 showed the highest TAC (ABTS + and DPPH), flavonoids, and copper, as well as DS6, exhibited the highest TAC (ABTS + and DPPH), flavonoids, and iron. Similarly, The accession DS8 contained the highest TAC (ABTS + and DPPH), chlorophylls, flavonoids, and polyphenols, as well as DS12, showed the highest TAC (ABTS + and DPPH), flavonoids, betacyanin, betalain, and betaxanthin. These four accessions had excellent antioxidant profiles along with high yielding potentiality. Hence, A. blitum provides an excellent source of proximate, phenolics, minerals, flavonoids, vitamins, and phytopigments to address the nutritional and antioxidant deficiency in daily diet.
Table 5. Coefficient of correlation for antioxidant phytopigments, β-carotene, TAC (ABTS⁺ and DPPH), vitamin C, TPC, and TFC in 16 A. blitum genotypes. Chl a, Chlorophyll a; Chl ab, Chlorophyll ab; TAC, Total antioxidant capacity; TPC, Total polyphenol content; TFC, Total flavonoid content; *Significant at 5% level; **Significant at 1% level.

| Traits                        | Chl b (mg 100 g⁻¹ FW) | Chl ab (mg 100 g⁻¹ FW) | Beta cyanin (µg 100 g⁻¹ FW) | Beta xanthin (µg 100 g⁻¹ FW) | Total carotenoids (µg 100 g⁻¹ FW) | β-carotene (µg g⁻¹ FW) | Vitamin C (µg g⁻¹ FW) | TPC (GAE µg g⁻¹ DW) | TFC (RE µg g⁻¹ DW) | TAC (TEAC µg g⁻¹ DW) | TAC (ABTS⁺) (TEAC µg g⁻¹ DW) |
|-------------------------------|-----------------------|------------------------|-----------------------------|-----------------------------|-------------------------------|----------------------|----------------------|---------------------|---------------------|---------------------|-----------------------------|
| Chlorophyll a (mg 100 g⁻¹ FW) | 0.88**                | 0.78**                 | 0.62**                      | 0.61**                      | 0.61**                        | −0.53**              | −0.44**              | 0.001               | 0.45**              | 0.44**              | 0.55**                      |
| Chlorophyll b (mg 100 g⁻¹ FW) |                      | 0.83**                 | 0.60**                      | 0.58**                      | 0.59**                        | −0.55**              | −0.49**              | −0.011              | 0.44**              | 0.45**              | 0.53**                      |
| Chlorophyll ab (mg 100 g⁻¹ FW)|                      |                        | 0.64**                      | 0.62**                      | 0.63**                        | −0.62**              | −0.48**              | −0.007              | 0.47**              | 0.47**              | 0.57**                      |
| Betacyanin (µg 100 g⁻¹ FW)    | 0.85**                |                        | 0.87**                      | −0.71**                     | −0.54**                        | −0.10                | 0.53**              | 0.55**              | 0.61**              | 0.68**              |
| Betaxanthin (µg 100 g⁻¹ FW)   | 0.85**                |                        | 0.66**                      | −0.52**                     | −0.12                         | 0.51**              | 0.54**              | 0.60**              | 0.66**              |
| Betalain (µg 100 g⁻¹ FW)      |                      |                        |                            | 0.67**                      | −0.53**                        | −0.11                | 0.52**              | 0.54**              | 0.61**              | 0.75**              |
| Total carotenoids (µg 100 g⁻¹ FW) |                    |                        |                            |                            | 0.84**                        | −0.18                | 0.54**              | 0.48**              | 0.68**              | 0.85**              |
| β-carotene (µg g⁻¹ FW)        |                      |                        |                            |                            | −0.17                         | 0.29**               | 0.44**              | 0.57**              | 0.54**              |
| Vitamin C (µg g⁻¹ FW)         | 0.05                  |                        |                            |                            |                                | 0.02                 | 0.06                | 0.08                |
| TPC (GAE µg g⁻¹ DW)           | 0.74**                |                        |                            |                            |                                | 0.66**               | 0.95**              |
| TFC (RE µg g⁻¹ DW)            | 0.74**                |                        |                            |                            |                                | 0.79**               |
| TAC (DPPH) (TEAC µg g⁻¹ DW)   | 0.98**                |                        |                            |                            |                                |                      |

Correlation studies. The coefficient of correlation of biologically active compounds of A. blitum is shown in Table 5. The coefficient of correlation of biologically active compounds shown in Table 5 had interesting results. We observed a significant positive correlation among TAC (DPPH), chlorophyll ab, betacyanin, chlorophyll a, betaxanthin, betalain, TAC (ABTS⁺), chlorophyll b, and TFC. Shukla et al. also reported positive associations in their earlier work in A. tricolor. Similarly, betacyanin, betaxanthin, and betalain showed positive and significant interrelationships among each of them and with TAC (ABTS⁺), chlorophylls, TFC, TAC (DPPH), and TPC which was corroborated with the results of our earlier studies indicating an increase in any phytopigment was directly related to increment of another phytopigment. The positive and significant interrelationships of TAC (DPPH), all phytopigments, TAC (ABTS⁺), TFC, and TPC indicated that phytopigments, TFC, and TPC exhibited strong antioxidant potential. The significant negative association was observed between phytopigments vs. total carotenoids and phytopigments vs. beta-carotene, while total carotenoids and beta-carotene exhibited a significant positive association with TAC (ABTS⁺), TAC (DPPH), TPC, and TFC which was corroborated with the results of our earlier studies in amaranth. It indicated that the increment of any phytopigment had a direct decrement of total carotenoids and beta-carotene. The positive and significant interrelationship of total carotenoids and beta-carotene with TPC, TAC (ABTS⁺ and DPPH), and TFC signifies that beta-carotene and total carotenoids had excellent antioxidant potentiality. There were positive associations between beta-carotene and total carotenoids. In contrast, the negligible insignificant association was observed between vitamin C and all the characters indicating that vitamin C had no contribution to the antioxidant activity of A. blitum. Jimenez-Aguilar and Grusak reported a negligible insignificant association for ascorbic acid in amaranth. The positive and significant associations were observed among TAC (ABTS⁺), TPC, TAC (DPPH), and TFC as well as all phytopigments, and vitamins indicating the contribution of these compounds in the antioxidant potentiality of A. blitum genotypes. Our reported results revealed that phytopigments, vitamins, phenolics, and total flavonoids played a significant contribution to the antioxidant capacity of A. blitum.

In conclusion, A. blitum leaves were good sources of K, Ca, Mg, iron, manganese, copper, zinc, chlorophyll, vitamin C, betacyanin, betaxanthin, TAC, betalain, carotenoids, β-carotene, dietary fiber, carbohydrates, protein, TPC, and TFC. It could be used as leafy vegetables for potential sources of antioxidant phytopigments, vitamin C, β-carotene, phenolics, minerals and proximate, flavonoids in the human diet to address the nutritional deficiency and gaining antioxidant and nutritional sufficiency. Details studies on animal models and humans are prerequisites to confirm nutrition and pharmacology before promoting the use of the leaves for health purposes.

Methods

Experimental design, layout, materials, and cultural practices. Sixteen A. blitum accessions selected from 75 genotypes were sown in Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, in a randomized complete block design (RCBD) with three replicates. The experimental unit was 1 m x 1 m. The amaranth genotypes were grown maintaining the distance of 20 cm between rows and 5 cm between plants. During
land preparation, total compost (10 ton/ha) was applied. Appropriate fertilizer doses, such as triple superphosphate, urea, gypsum, and muriate of potash at 100, 200, 30, and 150 kg/ha, respectively were maintained. We maintained appropriate spacing between plants of a row through necessary thinning. Weeding and hoeing were done to remove the weeds. Adequate irrigations were applied to ensure the normal growth of amaranth. Leaves were harvested at 30 days old.

**Solvents and reagents.** Solvents: methanol, acetone, and ethanol. Reagents: NaOH, dithiothreitol (DTT), HNO₃, standard compounds of pure Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chromane-2-carboxylic acid), cesium chloride, ascorbic acid, H₂O₂, H₂SO₄, potassium persulfate, ascorbic acid, HClO₃, folin-ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS⁺ (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), rutin, 2,2-dipryidyl, sodium carbonate, aluminum chloride hexahydrate, and potassium acetate. We bought all solvents and reagents from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).

**Measurement of the composition of proximate.** Ash, crude fat, moisture, crude protein contents, fiber, and gross energy were determined following AOAC method36,35. Crude protein was estimated through the Micro-Kjeldahl method multiplying nitrogen by 6.25 (AOAC method 952.05). To estimate carbohydrate (g 100 g⁻¹ FW), the total percentage of protein, ash, fat, and moisture was subtracted from 100.

**Estimation of composition of minerals.** A. blitum leaf samples were dried for 24 hours at 70°C in an oven. We ground the dried leaves in a mill finely. Calcium, potassium, magnesium, iron, manganese, copper, and zinc were determined following nitric-perchloric acid digestion method36. Exactly 0.5 g dried leaf sample was digested with 40 ml HClO₃ (70%), 400 ml HNO₃ (65%), and 10 ml H₂SO₄ (96%) in the presence of carbonium beads. After digestion, the ascorbic acid method was followed to measure P in triplicate from an appropriately diluted solution. Ascorbic acid and Sb were added to the yellow-colored complex solution to convert a blue-colored phosphomolybdenum complex. The method described by Sarker and Oba25,35 was followed to read the absorbance by atomic absorption spectrophotometry (AAS) (Hitachi, Tokyo, Japan) at wavelength of 285.2 nm (magnesium), 76.6.5 nm (potassium), 248.3 nm (iron), 422.7 nm (calcium), 279.5 nm (manganese), 213.9 nm (zinc), 324.8 nm (copper).

**Estimation of carotenoids and chlorophylls.** Method of Sarker and Oba35,37 was followed to estimate chlorophyll a, chlorophyll b, total carotenoids, and chlorophyll a through extracting the fresh leaves of A. blitum in 80% acetone. The absorbance was read at 663 nm for chlorophyll a, 646 nm for chlorophyll b, and 470 nm for total carotenoids, respectively using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Data were expressed as mg chlorophyll per 100 g and μg total carotenoids per g fresh weight.

**Estimation of betacyanin and betaxanthin composition.** Method of Sarker and Oba36,38 was followed to estimate betacyanin and betaxanthin through extracting the leaves of A. blitum in 80% methyl alcohol having 50 mM ascorbate. Betacyanin and betaxanthin were estimated using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) at 540 nm for betacyanin and 475 nm for betaxanthin, respectively. The results were expressed as microgram betanin equivalent per 100 gram fresh weight (FW) for betacyanin and micrograms indicaxanthin equivalent per 100 gram FW for betaxanthin.

**Estimation of β-carotene**

β-carotene content was extracted following the method of Sarker and Oba35. Exactly 500 mg of fresh leaf sample was ground thoroughly in a mortar and pestle with 10 ml of 80% acetone. The absorbance was read at 510 nm and 480 nm using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan). Data were expressed as μg β-carotene per g fresh weight.

The following formula was used to estimate the β-carotene content:

\[
\beta - \text{carotene} = 7.6(\text{Abs. at 480}) - 1.49(\text{Abs. at 510}) \\
\times \frac{\text{Final volume}(1000 \times \text{fresh weight of leaf taken})}{g}
\]

**Vitamin C determination.** A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to estimate dehydroascorbic acid (DHA) and ascorbate (AsA) acid from the fresh A. blitum leaves. Dithiothreitol (DTT) was used for the pre-incubation of the sample and reduction of DHA into AsA. AsA reduced Fe³⁺ to Fe²⁺. AsA was estimated through measuring Fe²⁺ complexes with 2, 2-dipryidyl35,39. Finally, the absorbance of the sample solution was read at 525 nm using a spectrophotometer (Hitachi, U-1800, Tokyo, Japan) and data were expressed as μg vitamin C per g fresh weight. The solution was read at 525 nm and data were expressed as μg vitamin C per g fresh weight.

**Extraction of samples for TAC, TFC, and TPC analysis.** The leaf samples were dried in the air in a shade for chemical analysis. Exactly 1 g of ground dried leaves was extracted in 40 ml of 90% aqueous methanol in a tightly capped bottle (100 ml). We placed the bottles in a shaking water bath (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan) for 1 h. The extract was filtered for measuring total antioxidant capacity, flavonoids, and polyphenols.

**Total polyphenols estimation.** The method described by Sarker and Oba35,40 was followed to estimate the total phenolic content of A. blitum leaf samples. The gallic acid was used as a standard phenolic compound. We diluted the Folin-ciocalteu reagent in the ratio of 1:4, reagent: distilled water. Exactly 1 ml Na₂CO₃ (10%), and 1 ml diluted folin-ciocalteu solution were added to a test tube containing 50 μl extract and mixed thoroughly for 3 min.
The tube was allowed to stand for 1 h in the dark. The absorbance was read at 760 nm using a Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan). A standard gallic acid graph was made to determine the concentration of phenolics in the extracts. The results are expressed as μg gallic acid equivalent (GAE) g⁻¹ DW.

**Estimation of total flavonoids.** The total flavonoid content of *A. blitum* extract was estimated following the AlCl₃ colorimetric method35,41. Exactly 1.5 ml methanol, 0.1 ml 1 M potassium acetate, 0.1 ml 10% aluminium chloride, and 2.8 ml distilled water was added to a test tube containing 500 μl leaf extract and allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was taken at 415 nm using a Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan). TFC is expressed as μg rutin equivalent (RE) g⁻¹ dry weight (DW) using rutin as the standard compound.

**Estimation of total antioxidant capacity (TAC).** The antioxidant capacity was determined through diphenyl-picolrylhydrazyl (DPPH) radical degradation method35. Exactly 1 ml 250 μM DPPH solution was added to a test tube containing 10 μl of leaf extract (in triplicate) with 4 ml distilled water and allowed to stand for 30 min in the dark. The absorbance was read at 517 nm using a Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan). The method described by Sarker and Oba35 was followed to estimate TAC (ABTS⁺) assay. Exactly 2.6 mM potassium persulfate and 7.4 mM ABTS⁺ solution were used in the stock solutions. For the preparation of the working solution, the two stock solutions were mixed in equal quantities and allowed them to react for 12 h at room temperature in the dark. Exactly 150 μl sample of leaf extract was mixed with 2850 μl of ABTS⁺ solution (1 ml ABTS⁺ solution mixed with 60 ml methanol) and allowed to react for 2 h in the dark. The absorbance was read at 734 nm using a Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) against methanol. The percent of inhibition of DPPH and ABTS⁺ relative to the control were used to determine antioxidant activity using the following equation:

\[
\text{Antioxidant activity(%) } = \left( \frac{\text{Abs. blank} - \text{Abs. sample}}{\text{Abs. blank}} \right) \times 100
\]

Where, Abs. blank is the absorbance of the control reaction [10 μl methanol for TAC (DPPH), 150 μl methanol for TAC (ABTS⁺) instead of leaf extract] and Abs. sample is the absorbance of the test compound. Trolox was used as the reference standard, and the results were expressed as μg Trolox equivalent g⁻¹ DW.

**Statistical analysis.** Mineral, phytopigments, chlorophylls, carotenoids, beta-carotene, vitamin C, polyphenols, flavonoids, and antioxidant activity (ABTS⁺ & DPPH) analysis were evaluated in three independent samples per replication (each sample was prepared from a combined sample of leaves from multiple plants) and nine samples per genotype41. Results were expressed as mean value ± standard deviation per genotype. Every mean represents the average of all measurements for the same genotype (Tables 1–4). ANOVA was performed using Statistix 8 software and the means were compared by Tukey’s HSD test at 1% and level of probability.

**Ethical statement.** The lab and field experiments in this study were carried out as per guidelines and recommendations of “Biosafety Guidelines of Bangladesh” published by the Ministry of Environment and Forest, Government of the People’s Republic of Bangladesh (2005).

**Data availability**

Data used in this manuscript will be available to the public.

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**References**

1. Rajan, S. & Markose, B. L. Horticultural Science Series-6. In Peter, K. M. V. (Ed.), Propagation of horticultural crops. New Delhi, India: New India Publishing Agency. (2007).
2. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Variability, heritability and genetic association in vegetable amaranth (*Amaranthus tricolor*). *Span. J. Agric. Res.* 13, 1–8, https://doi.org/10.5424/sjar/2015132-6843 (2015a).
3. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Genotype variability in composition of antioxidant vitamins and minerals in vegetable amaranth. *Genetika*. 47, 85–96 (2015b).
4. Venskonitonas, P. R. & Kraujalis, P. Nutritional components of amaranth seeds and vegetables: A review on composition, properties, and uses. *Comp. Rev. Food Sci. Food Saf.* 12, 381–412 (2013).
5. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Genotypic variability for nutrient, antioxidant, yield and yield contributing traits in vegetable amaranth. *J. Food Agri. Environ.* 12, 168–174 (2014).
6. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Genetic variation and interrelationship among antioxidant, quality and agronomic traits in vegetable amaranth. *Turf. J. Agric. For.* 40, 526–535 (2016).
7. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Genotypic diversity in vegetable amaranth for antioxidant, nutrient and agronomic traits. *Indian J. Genet. Pl. Br.* 77, 173–176 (2017).
8. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Variability in total antioxidant capacity, antioxidant leaf pigments and foliage yield of vegetable amaranth. *J. Integr. Agric.* 17, 1145–1153 (2018a).
9. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Antioxidant leaf pigments and variability in vegetable amaranth. *Genetika*. 50, 209–220 (2018b).
10. Sarker, U., Islam, M. T., Rabbani, M. G. & Oba, S. Phenotypic divergence in vegetable amaranth for total antioxidant capacity, antioxidant profile, dietary fiber, nutritional and agronomic traits. *Acta Agric. Scand. Sect. B–Soil Plant Sci.* 68, 67–76 (2018c).
11. FAO, IFAD, & WFP. The state of food security in the world, 2015. Meeting the 2015 International Hunger Targets: Taking Stock of Uneven Progress Retrieved January 3, 2019, from http://www.fao.org/3/a-i4646e.pdf (2015).
12. Von Grebmer, K., et al. 2014 Global Hunger Index: The Challenge of Hidden Hunger. Welthungerhilfe, International Food Policy Research Institute, and Concern Worldwide, Bonn, Washington, D.C., and Dublin (2014).
13. Afari-Sefa, V., Tenkouano, A., Ojiewo, C. O., Keatinge, J. D. H. & Hughes, J. D. A. Vegetable breeding in Africa: constraints, complexity, and contributions toward achieving food and nutritional security. *Food Security*. 4, 115–127 (2011).
14. Grosso, G. et al. Effects of vitamin C on health: a review of evidence. *Front. Biosci.* 18, 1017–1029 (2013).
15. Isabelle, M. et al. Antioxidant activity and profiles of common fruits in Singapore. Food Chem. 123, 77–84 (2010).
16. Randhawa, M. A., Khan, A. A., Javed, M. S. & Sajid, M. W. Green leafy vegetables: a health-promoting source. In Watson, R. R. (Ed.), *Handbook of Fertility* (pp. 205–220). San Diego, CA, USA: Academic Press (2015).
17. Repo-Carrasco-Valencia, R., Hellstrom, J. K., Pihlava, J. M. & Mattila, P. H. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kaniwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). Food Chem. 120, 128–133 (2010).
18. Dasgupta, N. & De, B. Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chem. 101, 471–474 (2007).
19. Steffen, S. K. et al. Variations in the polyphenol content of seed grown *Amaranthus* genotypes. Food Chem. 129, 131–138 (2011).
20. Sarker, U. & Obá, S. Catalase, superoxide dismutase, and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Sci. Rep. 8, 16496, https://doi.org/10.1038/s41598-018-34944-0 (2018a).
21. Sarker, U. & Obá, S. Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of *Amaranthus* leafy vegetable. BMC Plant Biol. 18, 258, https://doi.org/10.1186/s12870-018-1484-1 (2018b).
22. Sarker, U., Islam, M. T. & Obá, S. Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in *Amaranthus tricolor* leaves. PLOS One. 1–18 https://doi.org/10.1371/journal.pone.0206388 (2018).
23. Sarker, U. & Obá, S. Salinity stress enhances color parameters, bioactive leaf pigments, vitamins, polyphenols, flavonoids and antioxidant activity in selected *Amaranthus* leafy vegetables. J. Sci. Food Agricult. 99, 2275–2284, https://doi.org/10.1002/jsfa.9423 (2019a).
24. Sarker, U. & Obá, S. Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected *Amaranthus tricolor* under salinity stress. Sci. Rep. 8, 12349, https://doi.org/10.1038/s41598-018-30897-6 (2018c).
25. Sarker, U. & Obá, S. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. Food Chem. 252, 72–83 (2018d).
26. Sun, H., Mu, T., Xi, L., Zhang, M. & Chen, J. Sweet potato (*Ipomoea batatas* L.) leaves as nutritional and functional foods. Food Chem. 156, 380–389 (2014).
27. Chakrabarty, T., Sarkar, U., Hasan, M. & Rahman, M. M. Variability in mineral compositions, yield, and yield contributing traits of stem amaranth (*Amaranthus litudinis*). Genetika. 50, 995–1010 (2018).
28. Jimenez-Aguilar, D. M. & Grusak, M. A. Minerals, vitamin C, phenolics, flavonoids and antioxidant activity of *Amaranthus* leafy vegetables. J. Food Compos. Anal. 58, 33–39 (2017).
29. Shahidi, F., Chavan, U. D., Bal, A. K. & McKenzie, D. R. Chemical composition of beach pea (*Lathyrus maritimus* L.) plant parts. Food Chem. 64, 39–44 (1999).
30. Madruga, M. S. & Camara, F. S. The chemical composition of ‘Multimistura’ as a food supplement. Food Chem. 68, 41–44 (2000).
31. Khanam, U. K. S. & Obá, S. Bioactive substances in leaves of two amaranth species, *Amaranthus luidinis* and *A. hypochondriacus*. Can. J. Plant Sci. 93, 47–58 (2013).
32. Khanam, U. K. S., Obá, S., Yanase, E. & Murakami, Y. Phenolic acids, flavonoids, and total antioxidant capacity of selected leafy vegetables. J. Funct. Foods. 4, 979–987 (2012).
33. Shukla, S. et al. Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). Plant. Foods Hum. Nutri. 61, 23–28 (2006).
34. AOAC (Association of Analytical Chemists) (2000). Official methods of analysis (17th ed.). Gaithersburg, MD, USA: AOAC International.
35. Sarker, U. & Obá, S. Protein, dietary fiber, minerals, antioxidant pigments and phytochemicals, and antioxidant activity in selected red morph *Amaranthus* leafy vegetable. PLoS One 14, 0222517, https://doi.org/10.1371/journal.pone.0222517 (2019b).
36. Bader, N. R. Sample preparation for flame atomic absorption spectroscopy: An overview. *Rasayan J. Chem.* 4, 49–55 (2011).
37. Sarker, U. & Obá, S. Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids and antioxidant activity in *Amaranthus tricolor*. Appl. Biochem. Biotechnol. 186, 999–1016, https://doi.org/10.1007/s12010-018-3784-5 (2018e).
38. Sarker, U. & Obá, S. Antioxidant constituents of three selected red and green color *Amaranthus* leafy vegetable. Sci. Rep. 9, 18233, https://doi.org/10.1038/s41598-019-52033-8 (2019c).
39. Sarker, U. & Obá, S. Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species. Sci. Rep. 9, 20413 (2019d). https://doi.org/10.1038/s41598-019-50977-5.
40. Sarker, U. & Obá, S. Nutritional and antioxidant components and antioxidant capacity in green morph *Amaranthus* leafy vegetable. Sci. Rep. 10, 1336, https://doi.org/10.1038/s41598-020-57687-3 (2020a).
41. Sarker, U. & Obá, S. Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. Sci. Rep. (2020b) (accepted). https://doi.org/10.1038/s41598-020-59848-w.

**Author contributions**
U.S. initiated the research work and conceived the study; U.S. performed the experiments; U.S. performed biochemical analysis and statistical analysis; U.S. drafted, edited, interpreted data and prepared the manuscript; S.O. edited the manuscript, provided valuable suggestions during the experiment.

**Competing interests**
The authors declare no competing interests.

**Additional information**
Correspondence and requests for materials should be addressed to U.S.

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