Nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species

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Six selected weedy *Amaranthus* genotypes (three accessions from each species of *A. viridis* and *A. spinosus*) were evaluated in terms of nutrients, minerals, antioxidant constituents and antioxidant activity for the possibilities of weedy species as a vegetable cultivar in a randomized complete block design with three replications. As leafy vegetable, weedy *Amaranthus* has remarkable protein, dietary fiber, carbohydrates, Ca, K, Mg, P, S, Fe, Mn, Cu, Zn, Na, Mo, B, chlorophylls, β-cyanins, β-xanthins, betalains, β-carotene, vitamin C, TPC, TFC, and TAC (DPPH and ABTS) compared to any cultivated species. The *A. viridis* genotype WAV7 and *A. spinosus* genotype WAS13 had the highest nutrients, pigments, vitamins, phenolics, flavonoids, and antioxidant. Hence, these two weedy accessions could be used as an antioxidant profile enriched cultivar with high nutritional and antioxidant activity. Pigments, β-carotene, vitamin C, phenolics, and flavonoids had strong antioxidant activity and played a vital role in the antioxidant activity of weedy *Amaranthus* genotypes. Weedy species are an excellent source of phenolics, flavonoids, and antioxidants that have many pharmacological and medicinal effects of their traditional applications and detoxify ROS and offered huge prospects for feeding the antioxidant-deficient community to cope with the hidden hunger and attaining nutritional and antioxidant sufficiency.

The family Amaranthaceae consists of 70 *Amaranthus* species of which 17 produce edible leaves and 3 produce food grains1. *Amaranthus* species are C4 plants with rapidly grown vegetables, ornamental, and grains plants. It is widely distributed and cultivated in Asia, Africa, America, Australia, and Europe. Leaves and succulent stems of *Amaranthus* are inexpensive and excellent sources of protein with essential amino acids lysine and methionine, carotenoids, ascorbic acid, dietary fiber, and essential minerals, such as calcium, magnesium, potassium, phosphorus, iron, zinc, copper, and manganese2–8. Some genera of this family are widely used as traditional medicinal plants for remedy of viral diseases, malarial, diabetic, bacterial, helminthic infections and as snake antidote9–11. Besides these, it is also an excellent and unique source of antioxidant leaf pigments, such as β-cyanin, β-xanthin, betalain, and a source of other pigments, such as carotenoids, anthocyanin, and chlorophylls12,13, and antioxidant phytochemicals, such as β-carotene, vitamin C, phenolics, and flavonoids14. Most of these compounds are natural antioxidants and detoxify ROS in the human body, hence, it had great importance for the food industry15,16, β-Cyanin, β-xanthin, betalain, carotenoids, and amaranthine pigments have important free radical-scavenging activity17. It has wide adaptability to different abiotic stresses like drought18–21 and salinity22–24 and versatile uses.

Weedy amaranth (*A. spinosus* and *A. viridis*) originates probably from lowland of South and Central America. At present, it is wide spreads over the tropical and subtropical regions, including tropical Africa, South East Asia, Americas as well as temperate Europe. In tropical Africa and elsewhere weedy amaranth leaves and young plants are collected for sale on markets for home consumption as a cooked, steamed or fried vegetable, especially during

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Table 1. Proximate compositions (g 100 g\(^{-1}\) fresh weight) and dietary fiber (g 100 g\(^{-1}\) FW) of six selected genotypes of A. viridis and A. spinosus weedy species. CV, Coefficient of variation; n = 3; In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test (P < 0.01).

| Genotypes | Moisture (g 100 g\(^{-1}\)) | Protein (g 100 g\(^{-1}\)) | Fat (g 100 g\(^{-1}\)) | Carbohydrates (g 100 g\(^{-1}\)) | Energy (Kcal) | Ash (g 100 g\(^{-1}\)) | Dietary fiber (g 100 g\(^{-1}\) FW) |
|-----------|------------------|-----------------|-----------------|------------------|-------------|-----------------|------------------|
| A. viridis |                  |                 |                 |                  |             |                 |                  |
| WAV4      | 80.35 ± 1.14f    | 4.12 ± 0.05b    | 0.35 ± 0.01c    | 8.67 ± 0.07b     | 35.29 ± 0.33a | 8.86 ± 0.02a   | 9.38 ± 0.35b     |
| WAV7      | 82.28 ± 1.26d    | 4.52 ± 0.04b    | 0.42 ± 0.03d    | 6.31 ± 0.06c     | 36.72 ± 0.37a | 7.75 ± 0.03a   | 9.17 ± 0.37b     |
| WAV9      | 81.54 ± 1.18e    | 4.26 ± 0.06b    | 0.28 ± 0.04f    | 9.03 ± 0.05a     | 34.98 ± 0.62b | 5.43 ± 0.04b   | 9.26 ± 0.45b     |
| A. spinosus |                |                 |                 |                  |             |                 |                  |
| WAS11     | 86.26 ± 1.82a    | 5.54 ± 0.03a    | 0.51 ± 0.01b    | 2.33 ± 0.06c     | 28.45 ± 0.44c | 5.62 ± 0.02b   | 10.65 ± 0.65a    |
| WAS13     | 84.47 ± 1.34c    | 5.78 ± 0.06a    | 0.63 ± 0.02a    | 4.41 ± 0.03d     | 27.89 ± 0.46d | 5.18 ± 0.04b   | 11.24 ± 0.72a    |
| WAS15     | 85.42 ± 1.55b    | 5.28 ± 0.05a    | 0.47 ± 0.03c    | 4.16 ± 0.05d     | 29.56 ± 0.52c | 5.09 ± 0.03b   | 10.58 ± 0.58a    |

Table 2. Mineral compositions (Macroelements mg g\(^{-1}\) FW) of six selected genotypes of A. viridis and A. spinosus weedy species. CV, Coefficient of variation; K, Potassium; Ca, Calcium; Mg, Magnesium; P, Phosphorus; S, Sulphur; n = 3; In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test (P < 0.01).

| Genotypes | Macroelements (mg g\(^{-1}\) FW) | K     | Ca     | Mg     | P     | S     |
|-----------|----------------------------------|-------|--------|--------|-------|-------|
| A. viridis |                                  |       |        |        |       |       |
| WAV4      | 6.61 ± 0.02ab                    | 2.56 ± 0.06ab | 3.53 ± 0.03a | 0.86 ± 0.001b | 1.42 ± 0.06c |
| WAV7      | 7.22 ± 0.05a                     | 2.46 ± 0.05ab | 3.65 ± 0.07a | 0.94 ± 0.002a | 1.52 ± 0.05b |
| WAV9      | 6.98 ± 0.04a                     | 2.84 ± 0.03a | 3.78 ± 0.05a | 0.79 ± 0.002b | 1.66 ± 0.05a |
| A. spinosus |                                |       |        |        |       |       |
| WAS11     | 6.45 ± 0.06ab                    | 2.68 ± 0.04a | 2.88 ± 0.03ab | 0.72 ± 0.003b | 1.34 ± 0.03d |
| WAS13     | 6.48 ± 0.04ab                    | 2.44 ± 0.05ab | 3.02 ± 0.05ab | 0.68 ± 0.005b | 1.25 ± 0.04c |
| WAS15     | 6.82 ± 0.06a                     | 2.71 ± 0.06a | 2.97 ± 0.04ab | 0.75 ± 0.003b | 1.18 ± 0.02e |

Results and Discussion

The analysis of variance revealed that all the studied traits differed significantly in terms of the genotypes (Tables 1, 2, 3, 4, 5). %CV and Mean performance of proximate, mineral compositions, antioxidant leaf pigments, vitamins, TPC, TFC, TAC (DPHH) and TAC (ABTS\(^{+}\)) in selected six A. viridis and A. spinosus genotypes are presented in Tables 1, 2, 3, 4, 5.

Proximate compositions. Proximate compositions of six A. viridis and A. spinosus genotypes are presented in Table 1. The moisture content of six selected genotypes of two weedy Amaranthus species ranged from 81.54
### Table 3. Mineral compositions (Microelements μg g\(^{-1}\) FW) of six selected genotypes of *A. viridis* and *A. spinosus* weedy species. CV, Coefficient of variation; Fe, Iron; Mn, Manganese; Cu, Copper; Zn, Zinc, Na, Sodium; Mo, Molybdenum; B, Boron; n = 3; In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test (P < 0.01).

| Genotypes | Fe          | Mn          | Cu          | Zn          | Na          | B           | Mo          |
|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| *A. viridis* |             |             |             |             |             |             |             |
| WAV4       | 21.98 ± 0.09a | 8.66 ± 0.06b | 2.65 ± 0.03b | 12.96 ± 0.08b | 28.76 ± 0.10c | 11.25 ± 0.02b | 0.34 ± 0.05c |
| WAV7       | 21.94 ± 0.12a | 8.72 ± 0.05b | 2.84 ± 0.07b | 13.55 ± 0.12b | 29.38 ± 0.13b | 12.42 ± 0.05a | 0.38 ± 0.03b |
| WAV9       | 22.18 ± 0.11a | 8.99 ± 0.07b | 3.02 ± 0.02a | 14.72 ± 0.13a | 30.26 ± 0.08a | 12.74 ± 0.03a | 0.43 ± 0.04a |
| *A. spinosus* |             |             |             |             |             |             |             |
| WAS11      | 14.86 ± 0.08b | 9.74 ± 0.05a | 1.37 ± 0.03d | 11.35 ± 0.11c | 24.56 ± 0.04e | 6.35 ± 0.08d | 0.35 ± 0.04c |
| WAS13      | 15.34 ± 0.09b | 10.23 ± 0.06a | 2.04 ± 0.07c | 10.99 ± 0.13c | 25.73 ± 0.14d | 7.25 ± 0.06c | 0.32 ± 0.02d |
| WAS15      | 14.78 ± 0.08b | 9.86 ± 0.06a | 1.68 ± 0.04d | 11.64 ± 0.12c | 25.66 ± 0.12d | 6.96 ± 0.05c | 0.35 ± 0.05c |
| F values   | ***         | ***         | ***         | ***         | ***         | ***         | ***         |
| CV%        | 0.25        | 0.22        | 0.08        | 0.12        | 0.21        | 0.07        | 0.04        |

### Table 4. Mean performance of antioxidant leaf pigments of six selected genotypes of *A. viridis* and *A. spinosus* weedy species. CV, Coefficient of variation; n = 3; In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test (P < 0.01).

| Genotypes | Chlorophyll a (μg g\(^{-1}\) FW) | Chlorophyll b (μg g\(^{-1}\) FW) | Chlorophyll ab (μg g\(^{-1}\) FW) | β-cyanins (ng g\(^{-1}\) FW) | β-xanthins (ng g\(^{-1}\) FW) | Betalains (ng g\(^{-1}\) FW) | Carotenoids (mg 100 g\(^{-1}\) DW) |
|------------|----------------------------------|----------------------------------|----------------------------------|----------------------------|----------------------------|--------------------------|-------------------------------|
| *A. viridis* |                                   |                                  |                                  |                            |                            |                          |                                |
| WAV4       | 288.33 ± 3.15c                   | 135.26 ± 1.78e                   | 423.59 ± 2.36d                   | 276.34 ± 1.67d             | 255.78 ± 1.96d             | 532.12 ± 1.85c            | 86.48 ± 1.24c               |
| WAV7       | 302.56 ± 1.23a                   | 142.66 ± 1.61c                   | 445.22 ± 1.22a                   | 285.33 ± 1.84a             | 256.87 ± 1.54a             | 532.24 ± 1.64a            | 82.87 ± 1.33a               |
| WAV9       | 295.47 ± 3.63b                   | 138.55 ± 1.77d                   | 434.02 ± 3.28b                   | 287.56 ± 1.36a             | 252.37 ± 1.48d             | 539.93 ± 1.82d            | 88.29 ± 1.45b               |
| F values   | ***                              | **                               | ***                              | **                         | ***                         | **                        |                                |
| CV%        | 0.96                             | 1.32                             | 1.58                             | 2.26                       | 2.43                       | 1.17                      | 1.25                          |

### Table 5. Mean performance of β-Carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC (ABTS\(^+\)) of six selected genotypes of *A. viridis* and *A. spinosus* weedy species. CV, Coefficient of variation; TAC = Total antioxidant capacity; TPC = Total polyphenol content; TFC = Total flavonoid content; n = 3; In a column, mean values with different letters are differed significantly by Duncan Multiple Range Test (P < 0.01).

| Genotypes | β-Carotene (mg 100 g\(^{-1}\) FW) | Vitamin C (mg 100 g\(^{-1}\) FW) | TPC (GAE μg g\(^{-1}\) FW) | TFC (RE μg g\(^{-1}\) DW) | TAC (DPPH) (TEAC μg g\(^{-1}\) DW) | TAC (ABTS\(^+\)) (TEAC μg g\(^{-1}\) DW) |
|------------|---------------------------------|---------------------------------|---------------------------|---------------------------|----------------------------------|----------------------------------|
| *A. viridis* |                                 |                                 |                           |                           |                                  |                                  |
| WAV4       | 62.56 ± 0.62b                   | 104.55 ± 0.19b                  | 40.26 ± 0.27c             | 174.58 ± 0.36c            | 23.78 ± 0.08e                    | 45.84 ± 0.41c                   |
| WAV7       | 64.22 ± 0.56a                   | 106.64 ± 0.22a                  | 43.24 ± 0.32b             | 175.64 ± 0.29c            | 21.96 ± 0.14f                    | 48.23 ± 0.31b                   |
| WAV9       | 61.87 ± 0.75b                   | 107.45 ± 0.18a                  | 46.72 ± 0.22a             | 182.46 ± 0.26a            | 24.65 ± 0.12d                    | 51.23 ± 0.24a                   |
| *A. spinosus* |                                 |                                 |                           |                           |                                  |                                  |
| WAS11      | 46.76 ± 0.26c                   | 44.62 ± 0.12e                   | 25.45 ± 0.42d             | 176.46 ± 0.25c            | 26.45 ± 0.15b                    | 49.67 ± 0.11b                   |
| WAS13      | 48.28 ± 0.28d                   | 48.72 ± 0.08c                   | 24.98 ± 0.34e             | 182.36 ± 0.23a            | 27.56 ± 0.14a                    | 52.35 ± 0.24a                   |
| WAS15      | 52.16 ± 0.88c                   | 46.98 ± 0.09d                   | 26.72 ± 0.32d             | 178.34 ± 0.16b            | 25.87 ± 0.11c                    | 47.87 ± 0.27bc                  |
| F values   | ***                              | ***                             | ***                        | ***                       | ***                              | ***                             |
| CV%        | 1.12                             | 1.75                             | 1.69                       | 1.41                      | 0.35                             | 0.38                             |

To 86.26 g 100 g\(^{-1}\) FW. The highest moisture content was noticed in *A. spinosus* genotype WAS11 (86.26 g 100 g\(^{-1}\) FW) followed by *A. spinosus* genotype WAS15 (85.42 g 100 g\(^{-1}\) FW) and WAS13 (84.47 g 100 g\(^{-1}\) FW). In contrast, the lowest moisture content was recorded in *A. viridis* genotype WAV4 (80.35 g 100 g\(^{-1}\) FW). All the genotypes of *A. viridis* such as WAV4, WAV7, and WAV9 exhibited around 18–20% dry matter could be a promising source of dry matter as higher dry matter ensured with lower moisture contents of leaves. The maturity of the two species...
could have a vital role in the moisture content of leaves. The moisture contents obtained in our study were fully agreed with the reports of Sun et al.\textsuperscript{23} in sweet potato leaves.

As leafy vegetables, leaves of \textit{A. viridis} and \textit{A. spinosus} had high protein content with fewer variations which ranged from 4.12 to 5.78 g 100 g\textsuperscript{−1} FW. The highest protein content was observed in \textit{A. spinosus} genotype WAS13 (5.78 g 100 g\textsuperscript{−1} FW) which was statistically similar to \textit{A. spinosus} genotype WAS11 and WAS15. Conversely, the lowest protein content was exhibited in \textit{A. viridis} genotype WAV4. Weedy amaranth (\textit{A. viridis} and \textit{A. spinosus}) genotypes are the sources of protein for vegetarian and poor people of the third world countries. The protein content of \textit{A. viridis} and \textit{A. spinosus} were much higher as compared to amaranth in our earlier study\textsuperscript{3}. In this investigation, \textit{A. viridis} and \textit{A. spinosus} genotypes showed low-fat content as a leafy vegetable and could be used as a cholesterol free food. \textit{A. spinosus} genotype WAS13 showed the highest fat content (0.63 g 100 g\textsuperscript{−1} FW) followed by \textit{A. spinosus} genotype WAS11. Whereas, \textit{A. viridis} genotype WAV9 exhibited the lowest fat content (0.28 g 100 g\textsuperscript{−1} FW) with a range of 0.28 to 0.63 g 100 g\textsuperscript{−1} FW. Fats help in the digestion, absorption, and transport of fat-soluble vitamins A, D, E, K and source of omega-3 and omega-6 fatty acids. Sun et al.\textsuperscript{23} reported similar results in sweet potato leaves. They revealed that fat involved in the insulation of body organs and the maintenance of body temperature and cell function.

\textit{A. viridis} genotypes had higher carbohydrates content compared to the genotype of \textit{A. spinosus}. Remarkable variations were observed in the carbohydrate content of \textit{A. viridis} and \textit{A. spinosus} genotypes which ranged from 2.33 to 9.30 g 100 g\textsuperscript{−1} FW. \textit{A. viridis} genotype WAV9 exhibited the highest carbohydrates content (9.30 g 100 g\textsuperscript{−1} FW) followed by \textit{A. viridis} genotype WAV4, while \textit{A. spinosus} genotype WAS11 had the lowest carbohydrates content (2.33 g 100 g\textsuperscript{−1} FW). \textit{A. viridis} genotypes had higher energy compared to the genotype of \textit{A. spinosus}. The \textit{A. viridis} genotype WAV4 and WAV7 had the highest energy (36.72, 35.29 Kcal 100 g\textsuperscript{−1} FW) followed by \textit{A. viridis} genotype WAV9. On the other hand, \textit{A. spinosus} genotypes WAS13 exerted the lowest energy (27.89 Kcal 100 g\textsuperscript{−1} FW). \textit{A. viridis} genotypes had higher ash content compared to the genotype of \textit{A. spinosus}. The highest ash content was observed in the \textit{A. viridis} genotype WAV4 and WAV7 (6.86, 6.75 g 100 g\textsuperscript{−1}), while the lowest ash content was recorded in \textit{A. spinosus} genotype WAS13 (5.18 g 100 g\textsuperscript{−1}) which was statistically similar to \textit{A. spinosus} genotype WAS11, WAS15, and \textit{A. viridis} genotype WAV9.

\textit{A. spinosus} genotypes had higher dietary fiber content compared to the genotype of \textit{A. viridis}. The dietary fiber content of selected six \textit{A. viridis} and \textit{A. spinosus} genotypes ranged from 9.17 to 11.24 g 100 g\textsuperscript{−1} FW. \textit{A. spinosus} genotype WAS13, WAS11, and WAS15 showed the highest dietary fiber content (11.24, 10.65 and 10.58 g 100 g\textsuperscript{−1} FW). Conversely, \textit{A. viridis} genotype WAV7 had the lowest dietary fiber content (9.17 g 100 g\textsuperscript{−1} FW) which was similar to WAV4 and WAV9. The dietary fiber played a substantial role in palatability, digestibility, and remedy of constipation\textsuperscript{4}. Like other leafy vegetables, our study showed that leaves of \textit{A. viridis} and \textit{A. spinosus} genotypes are an excellent source of moisture, protein, dietary fiber and carbohydrates. \textit{A. viridis} genotype had the highest carbohydrates, energy, and ash content, while \textit{A. spinosus} genotype exhibited the highest moisture, protein, fat, and dietary fiber content.

Mineral compositions. \textit{A. viridis} and \textit{A. spinosus} genotypes were presented in Tables 2, 3. In this study, the highest K content was observed in \textit{A. viridis} genotype WAV7 (7.22 mg g\textsuperscript{−1} FW) which was statistically similar to \textit{A. viridis} genotype WAV9 (6.98 mg g\textsuperscript{−1} FW) and \textit{A. spinosus} genotype WAS15 (6.82 mg g\textsuperscript{−1} FW) with a range of 6.45 mg g\textsuperscript{−1} to 7.22 mg g\textsuperscript{−1} FW. Whereas, \textit{A. spinosus} genotype WAS11 and WAS13 exhibited the lowest K content (6.45, 6.48 mg g\textsuperscript{−1} FW) which was statistically similar to \textit{A. viridis} genotype WAV4. \textit{A. viridis} genotypes had higher K content compared to the genotype of \textit{A. spinosus}, albeit the differences in K content between to weedy species were no pronounced. Albeit there were no prominent variations in Ca content between to weedy species, \textit{A. spinosus} genotypes had higher Ca content compared to the genotype of \textit{A. viridis} with a range of 2.44 to 2.84 mg g\textsuperscript{−1} FW. The highest Ca content (2.84 mg g\textsuperscript{−1} FW) was reported in \textit{A. viridis} genotype WAV9 which was similar to \textit{A. spinosus} genotype WAS12 and WAS11. In contrast, the lowest Ca content (2.44 mg g\textsuperscript{−1}) was obtained from \textit{A. spinosus} genotype WAS13. In this investigation, \textit{A. viridis} and \textit{A. spinosus} genotypes had no pronounced variations in terms of Mg content (2.88 to 3.78 mg g\textsuperscript{−1} FW). \textit{A. viridis} genotype WAV4, WAV7, and WAV9 exhibited the highest Mg content (3.78, 3.65, 3.52 mg g\textsuperscript{−1} FW), while, \textit{A. spinosus} genotype WAS11, WAS13, and WAS15 showed the lowest Mg content (2.88, 3.02 and 2.97 mg g\textsuperscript{−1} FW). Similarly, \textit{A. viridis} and \textit{A. spinosus} genotypes had no pronounced variations in terms of P content (0.68 to 0.94 mg g\textsuperscript{−1} FW). \textit{A. viridis} genotype WAV7 exhibited the highest P content (0.94 mg g\textsuperscript{−1} FW), while, \textit{A. spinosus} genotype WAS13 showed the lowest P content (0.68 mg g\textsuperscript{−1} FW) which was statistically similar to \textit{A. spinosus} genotype WAS15, WAS11, and \textit{A. viridis} genotype WAV4 and WAV9. S content had significant variations in six \textit{A. viridis} and \textit{A. spinosus} genotypes which ranged from 1.18 to 1.66 mg g\textsuperscript{−1} FW. \textit{A. viridis} genotypes had higher S content compared to the genotype of \textit{A. spinosus} which ranged from 0.94 to 1.18 mg g\textsuperscript{−1} FW followed by WAV7, while \textit{A. spinosus} genotype WAS15 and WAS13 showed the lowest S content (1.18 and 1.25 mg g\textsuperscript{−1} FW). Our investigation revealed that we found remarkable K (7.22 mg g\textsuperscript{−1}), Ca (2.74 mg g\textsuperscript{−1}), Mg (3.52 mg g\textsuperscript{−1}), and S (2.4 mg g\textsuperscript{−1}) in \textit{A. viridis} genotype (fresh weight basis) Jimenez-Aguiru and Grusak\textsuperscript{33} reported high K, Ca, Mg, P, and S (fresh weight basis) in different A. spp. including \textit{A. viridis} and \textit{A. spinosus}. They also reported that spider flower, black nightshade, spinach, and kale had much lower K, Ca, and Mg content than amaranth. Our studied \textit{A. viridis} and \textit{A. spinosus} genotype had higher K, Ca, Mg, P, and S content than \textit{A. spinosus} genotype.

In this study, iron content showed significant variations in terms of \textit{A. viridis} and \textit{A. spinosus} genotype. The highest Fe content was recorded in \textit{A. viridis} WAV9 (22.18 µg g\textsuperscript{−1} FW) and it was statistically similar to \textit{A. viridis} genotype WAV4 and WAV7. On the other hand, \textit{A. spinosus} genotype WAS15 exhibited the lowest iron content which was statistically similar to \textit{A. spinosus} genotype WAS11 and WAS13. \textit{A. viridis} genotype had higher Fe content compared to \textit{A. spinosus} genotype. Our study revealed that significant variations were observed in Mn.
content of *A. viridis* and *A. spinosus* genotype. *A. spinosus* genotypes exhibited higher Mn content compared to the genotype of *A. viridis*. Manganese content ranged between 8.66 μg g⁻¹ FW and 10.23 μg g⁻¹ FW. Manganese content was the highest in *A. spinosus* genotype WAS13 (10.23 μg g⁻¹ FW), which was statistically similar to WAS11 and WAS15. Whereas the lowest manganese content was observed in *A. viridis* genotype WAV4, WAV7, and WAV9 (8.66, 8.72 and 8.99 μg g⁻¹ FW, respectively). *A. viridis* genotypes exhibited higher copper content compared to the genotype of *A. spinosus*. The copper content had significant notable range of variations in the genotypes of *A. viridis* and *A. spinosus* (1.37 to 3.02 μg g⁻¹ FW), *A. viridis* genotype WAV9 had the highest copper content (3.02 μg g⁻¹ FW) followed by *A. viridis* genotype WAV4 and WAV7, while *A. spinosus* genotype WAS11 showed the lowest Cu content (1.31 μg g⁻¹ FW). The zinc content of *A. viridis* and *A. spinosus* genotypes differed significantly. 10.99 μg g⁻¹ FW in *A. spinosus* genotype WAS13 to 14.72 μg g⁻¹ FW in *A. viridis* genotype WAV9. *A. viridis* genotypes exhibited higher Zn content compared to the genotype of *A. spinosus*. Na content showed significant variations in terms of *A. viridis* and *A. spinosus* genotype. The highest Na content was recorded in *A. viridis* genotype WAV9 (25.56 μg g⁻¹ FW) followed by *A. viridis* genotype WAV7, while *A. spinosus* genotype WAS11 exhibited the lowest Na content. *A. viridis* genotype had higher Na content compared to *A. spinosus* genotype. Our study revealed that significant notable variations were observed in B content of *A. viridis* and *A. spinosus* genotype. *A. viridis* genotypes exhibited higher B content compared to the genotype of *A. spinosus*. Boron content ranged between 6.35 μg g⁻¹ FW and 12.74 μg g⁻¹ FW. Boron content was the highest in *A. viridis* genotype WAV9 and WAV713 (12.74, 12.42 μg g⁻¹ FW), whereas, the lowest B content was observed in *A. spinosus* genotype WAS11, (6.35 μg g⁻¹ FW). *A. viridis* genotypes exhibited higher Mo content compared to the genotype of *A. spinosus*. The Mo content had significant range of variations in the genotypes of *A. viridis* and *A. spinosus* (0.32 to 0.42 μg g⁻¹ FW). *A. viridis* genotype WAV9 had the highest Mo content (0.43 μg g⁻¹ FW) followed by *A. viridis* genotype WAV7, while *A. spinosus* genotype WAS13 showed the lowest Mo content (0.32 μg g⁻¹ FW). Iron, and zinc content was higher in *A. viridis* than the leaves of cassava⁴ and beach pea⁵. In this study, we observed remarkable Fe (22.19 μg g⁻¹), Mn (10.23 μg g⁻¹), Cu (3.02 μg g⁻¹), Zn (14.72 μg g⁻¹) Na (30.26 μg g⁻¹), Mo (12.74 μg g⁻¹), and B (0.43 μg g⁻¹) in *A. viridis* genotype (fresh weight basis). Similarly, Jimenez-Aguir and Grusak⁶ reported high Fe, Mn, Cu, Zn, Na, Mo, and B (fresh weight basis) in different *A. spp.* including *A. viridis* and *A. spinosus*. They also stated that black nightshade, spinach, and kala had lower Zn content thanamaranth; kala exhibited less Fe and Cu content than amaranth. *A. viridis* genotype had the highest Fe, Mn, Cu, Zn, Na, Mo, and B content compared to *A. spinosus* genotype.

### Antioxidant leaf pigments

Antioxidant leaf pigments of *A. viridis* and *A. spinosus* genotypes are presented in Table 4. Prominent variations in chlorophyll a content (267.85 to 302.56 μg g⁻¹ FW) were noted in *A. viridis* and *A. spinosus* genotypes. Comparatively, *A. viridis* genotypes exhibited higher chlorophyll a content than *A. spinosus* genotypes. *A. viridis* genotype WAV7 showed the highest chlorophyll a content (302.56 μg g⁻¹ FW) followed by *A. viridis* genotype WAV9. On the other hand, the lowest chlorophyll a content (267.85 μg g⁻¹ FW) was noted in *A. spinosus* genotype WAS13 and WAS15. Similar to chlorophyll a, significant and noticeable differences were recorded in chlorophyll b content (135.26 to 152.42 μg g⁻¹ FW) of selected six *A. viridis* and *A. spinosus* genotypes. *A. spinosus* genotypes showed higher chlorophyll b content compared to the genotype of *A. viridis*. The highest chlorophyll b content was observed in *A. spinosus* genotype WAS13 (152.42 μg g⁻¹ FW) followed by WAS11 and WAS15. In contrast, *A. viridis* genotype WAV4 had the lowest chlorophyll b content (135.26 μg g⁻¹ FW). The significant variations in chlorophyll ab content were noted in selected six *A. viridis* and *A. spinosus* genotypes. (413.61 to 445.22 μg g⁻¹ FW). *A. viridis* genotypes showed higher chlorophyll ab content compared to the genotype of *A. spinosus*. *A. viridis* genotype WAV7 showed the highest chlorophyll ab content (445.22 μg g⁻¹ FW) followed by *A. viridis* genotype WAV7 and *A. spinosus* genotype WAS13, while *A. spinosus* genotype WAS11 exhibited the lowest chlorophyll ab content (413.61 μg g⁻¹ FW). In this study, we observed notable chlorophyll a (302.56 μg g⁻¹ FW) and chlorophyll ab content (445.22 μg g⁻¹ FW) in *A. viridis* genotype and chlorophyll b (152.42 μg g⁻¹ FW) in *A. spinosus* genotype, whereas, Khanam and Oba⁸ reported comparatively lower chlorophyll content in red and green amaranth. *A. viridis* genotype had the highest chlorophyll a and chlorophyll ab content, while *A. spinosus* genotype exhibited the highest chlorophyll b content.

β-Cyanins content had no prominent variations in selected six *A. spinosus* and *A. viridis* genotypes (185.52 to 538.51 ng g⁻¹ FW) albeit it showed significant variations in terms of genotypes. Comparatively, *A. viridis* genotypes exhibited higher β-cyanins content than *A. spinosus* genotypes, albeit *A. spinosus* genotype WAS15 had the highest β-cyanins content (286.46 ng g⁻¹ FW) along with *A. viridis* genotypes WAV9 and WAV7 (287.56, 285.33 ng g⁻¹ FW). Higher β-cyanins content was noted in *A. spinosus* genotype WAS11 (282.84 ng g⁻¹ FW). On the other hand, *A. viridis* genotype WAV4 showed the lowest β-cyanins content (276.34 ng g⁻¹ FW). The significant variations were observed in β-xanthins content in selected six *A. spinosus* and *A. viridis* genotypes with a range of 246.87 to 275.86 ng g⁻¹ FW. *A. spinosus* genotypes exhibited higher β-xanthins content compared to *A. viridis* genotypes. *A. spinosus* genotype WAS13 exhibited the highest β-xanthins content (275.86 ng g⁻¹ FW) followed by *A. spinosus* genotype WAS15. Conversely, the lowest β-xanthins content was noted in *A. viridis* genotype WAV7 (246.87 ng g⁻¹ FW). The significant variations were recorded for betalains content of selected six *A. spinosus* and *A. viridis* genotypes (532.12 to 561.42 ng g⁻¹ FW). *A. spinosus* genotypes exhibited higher betalains content compared to *A. viridis* genotypes. Betalains content was the highest in *A. spinosus* genotype WAS15 (561.42 ng g⁻¹ FW) followed by *A. spinosus* genotype WAS13, while the lowest betalains content was reported in *A. viridis* genotype WAV4 and WAV7 (532.12, 532.24 ng g⁻¹ FW). Like betalains, carotenoids showed significant variability in selected six *A. viridis* and *A. spinosus* genotypes (68.52 to 92.87 mg 100 g⁻¹ FW). *A. viridis* genotypes exhibited higher carotenoids content compared to *A. spinosus* genotypes. The highest carotenoids content was observed in *A. viridis* genotype WAV7 (92.87 mg 100 g⁻¹ FW) followed by *A. viridis* genotype WAV9. Whereas, *A. spinosus* genotype WAS11 and WAS13 exhibited the lowest carotenoids content (68.52, 69.82 mg 100 g⁻¹ FW). Our study showed notable chlorophyll a (302.56 μg g⁻¹ FW), chlorophyll ab (445.22 μg g⁻¹ FW),...
β-cyanins (287.56 ng g⁻¹ FW), and carotenoids content (92.87 mg 100g⁻¹ FW) in A. viridis genotype, while chlorophyll b (152.42 μg g⁻¹ FW), β-cyanins (286.46 ng g⁻¹ FW), β-xanthins (274.96 ng g⁻¹ FW), and betalains content (561.42 ng g⁻¹ FW) in A. spinosus genotype. Similarly, Khanam and Obas observed similar trend in chlorophyll a, chlorophyll b, chlorophyll ab, β-cyanins, β-xanthins, betalains and carotenoids content of green and red amaranth. A. viridis genotype had the highest chlorophyll a, chlorophyll ab, β-cyanins, and carotenoids content while A. spinosus genotype exhibited the highest chlorophyll b, β-cyanins, β-xanthins, and betalains content.

Antioxidant phytochemicals and antioxidant capacity. β-Carotene, Vitamin C, TPC, TFC and TAC of A. viridis and A. spinosus genotypes are presented in Table 5. Pronounced variations were observed in β-carotene content of selected six A. viridis and A. spinosus genotypes which ranged from 46.76 in A. viridis genotype WAV7 to 64.22 mg 100g⁻¹ FW in A. spinosus genotype WAS11. Both species exhibited high β-carotene as compared to leafy vegetable. A. viridis genotypes exhibited higher β-carotene compared to A. spinosus genotypes. Higher β-carotene content was noticed in A. viridis genotype WAV4 and WAV9. A. viridis and A. spinosus genotypes showed prominent variations in vitamin C content with a range of 44.62 to 107.45 mg 100g⁻¹ FW. Both species exhibited high vitamin C as compared to leafy vegetable. Vitamin C was the highest in A. viridis genotype WAV7 and WAV9 (107.45, 106.64 mg 100g⁻¹ FW) and the lowest in A. spinosus genotype WAS11 (44.62 mg 100g⁻¹ FW). A. viridis genotypes exhibited higher vitamin C content compared to A. spinosus genotypes. Marked and significant variations were noted in total polyphenol content (TPC) of A. viridis and A. spinosus genotypes which ranged from 25.98 GAE μg g⁻¹ FW to 46.72 GAE μg g⁻¹ FW. Genotypes of both species exhibited high phenolics as compared to leafy vegetable. A. viridis genotype WAV9 showed the highest TPC content 46.72 GAE μg g⁻¹ FW followed by A. viridis genotype WAV7. While A. spinosus genotype WAS13 exhibited the lowest TPC (24.98 GAE μg g⁻¹ FW). TFC showed no noticeable variations in terms of six selected A. viridis and A. spinosus genotypes, though genotypes of both species had high flavonoid content (174.58 RE μg g⁻¹ DW to 182.46 RE μg g⁻¹ DW). A. viridis genotype WAV9 and A. spinosus genotype WAS13 had the highest TFC (182.46, 182.36 RE μg g⁻¹ DW) followed by A. spinosus genotype WAS13, whereas A. spinosus genotype WAS11 showed the lowest TFC (174.58, 175.64 and 176.46 RE μg g⁻¹ DW) though both weedy species had high flavonoids. A. spinosus genotypes exhibited higher TFC compared to A. viridis genotypes, albeit differences were very low. A. viridis and A. spinosus genotypes exhibited high TAC (DPPH and ABTS⁺) as a leafy vegetable and there were pronounced variations in terms of TAC (DPPH and ABTS⁺) of A. spinosus genotypes exhibited higher TAC (DPPH and ABTS⁺) compared to A. viridis genotypes. The highest TAC (DPPH and ABTS⁺) was observed in A. spinosus genotype WAS13 (27.56, 52.35 TEAC μg g⁻¹ DW) followed by A. spinosus genotype WAS11 and WAS15. On the other hand, the lowest TAC (DPPH and ABTS⁺) was recorded in A. viridis genotype WAV7 (21.96, 48.23 TEAC μg g⁻¹ DW). A similar trend of TAC (DPPH) and TAC (ABTS⁺) in terms of genotypes validated the measurement of two different methods of antioxidant capacities. The highest β-carotene, vitamin C, TPC, TFC, and TAC (DPPH and ABTS⁺) were obtained from A. viridis genotypes, while A. viridis genotypes had the highest TFC, and TAC (DPPH and ABTS⁺). In the present investigation, A. viridis genotypes exhibited outstanding β-carotene and vitamin C (64.22 and 107.45 mg 100g⁻¹ FW) which was higher than red amaranth of our previous studies. TPC (46.72 GAE μg g⁻¹ FW) obtained in this study was higher than the results of Khanam et al. in red and green amaranth. TFC (182.46 RE μg g⁻¹ DW) TAC (DPPH) (27.56 TEAC μg g⁻¹ DW) and TAC (ABTS⁺) (52.35 TEAC μg g⁻¹ DW) obtained from weedy amaranth in this study, were similar to the results of Khanam et al. in red amaranth whereas, our obtained results were higher than the results of Khanam et al. in green amaranth. The A. viridis genotype WAV7 and A. spinosus genotype WAS13 had high nutrients, pigments, vitamins, phenolics, flavonoids, and antioxidant. These two weedy Amaranthus accessions could be used as antioxidant profile enriched high-yielding varieties with high nutritional and antioxidant activity. The present investigation revealed that weedy Amaranthus is an excellent source of nutritional value, antioxidant phytochemicals, and antioxidant activity offered huge prospects as cultivated vegetable amaranth to feeding the mineral, vitamin, and antioxidant deficient community.

Correlation studies. Correlation of antioxidant leaf pigments, β-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) of A. viridis and A. spinosus genotypes are presented in Table 6. Correlation of antioxidant leaf pigments, β-carotene, vitamin C, TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) of A. viridis and A. spinosus genotypes showed interesting results. Significant positive associations with TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) were observed for all antioxidant leaf pigments. It indicated that the increase in TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) were directly related to the increment of chlorophylls, β-cyanins, β-xanthins, betalains, and carotenoids content or vice versa. It meant all leaf pigments had strong antioxidant activity. Similarly, vitamin C had a significant positive interrelationship with TPC, TFC, and TAC, while it exhibited insignificant negative associations among all antioxidant leaf pigments. Sarker and Obas in their earlier work in amaranth also observed a similar trend. A significant positive association was exhibited among β-carotene, vitamin C, TPC, TFC, TAC (DPPH), and TAC (ABTS⁺). The significant positive interrelationship of β-carotene, vitamin C, TPC, TFC, TAC (DPPH), and TAC (ABTS⁺) signify that β-carotene, vitamin C, TPC, TAC had strong antioxidant activity. The validation of the antioxidant capacity of A. viridis and A. spinosus genotypes by two different methods of antioxidant capacity measurements were confirmed with the significant positive associations between TAC (DPPH) and TAC (ABTS⁺). Antioxidant phytochemicals such as leaf pigments, β-carotene, vitamin C, TPC, and TFC had strong antioxidant activity, as these showed the significant associations with TAC (DPPH) and TAC (ABTS⁺). In the present investigation, all antioxidant leaf pigments, β-carotene, vitamin C, TPC, and TFC played a vital role in the antioxidant activity of A. viridis and A. spinosus genotypes as these compounds had strong antioxidant activity.
Table 6. Correlation coefficient of antioxidant leaf pigments, vitamin C, TPC, TFC, TAC (DPPH) and TAC (ABTS⁺) of six selected genotypes of A. viridis and A. spinosus weedy species. 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 (µg g⁻¹ FW) | Chl ab (µg g⁻¹ FW) | β-cyanins (ng g⁻¹ FW) | β-xanthins (ng g⁻¹ FW) | Carotenoids (mg 100 g⁻¹ FW) | β-Carotene (mg 100 g⁻¹ FW) | Vitamin C (mg 100 g⁻¹ FW) | TPC (GAE µg g⁻¹ FW) | TFC (RE µg g⁻¹ DW) | TAC (DPPH) (TEAC µg g⁻¹ DW) | TAC (ABTS⁺) (TEAC µg g⁻¹ DW) |
|--------|-----------------|-----------------|-----------------|-----------------|----------------------|----------------------|----------------------|------------------|------------------|----------------------|----------------------|
| Chlorophyll a (µg g⁻¹ FW) | 0.87** | 0.96** | 0.86** | 0.88** | 0.88** | −0.82* | −0.71* | −0.017 | 0.88** | 0.87** | 0.89** | 0.95** |
| Chlorophyll b (µg g⁻¹ FW) | 0.93** | 0.72* | 0.86** | 0.85** | 0.76 | −0.64 | −0.028 | 0.74* | 0.74* | 0.88** | 0.89** |
| Chlorophyll ab (µg g⁻¹ FW) | 0.72* | 0.75* | 0.84* | 0.87* | 0.76* | 0.023 | 0.77* | 0.79** | 0.77* | 0.88** |
| β-cyanins (ng g⁻¹ FW) | 0.89** | 0.95** | 0.86* | 0.77* | 0.112 | 0.76* | 0.786** | 0.94** | 0.89** |
| β-xanthins (ng g⁻¹ FW) | 0.98** | 0.88** | 0.87** | 0.132 | 0.72* | 0.73* | 0.76* | 0.96** |
| Betalains (mg g⁻¹ FW) | 0.92** | 0.92** | 0.125 | 0.94** | 0.77* | 0.75** | 0.98** |
| Carotenoids (mg 100 g⁻¹ FW) | 0.86** | 0.232 | 0.95** | 0.89** | 0.96** | 0.95** |
| β-Carotene (mg 100 g⁻¹ FW) | 0.76* | 0.82* | 0.95** | 0.87** | 0.88** |
| Vitamin C (mg 100 g⁻¹ FW) | 0.75* | 0.92** | 0.89** | 0.98** |
| TPC (GAE µg g⁻¹ FW) | 0.88** | 0.88** | 0.97** |
| TFC (RE µg g⁻¹ DW) | 0.86** | 0.96** |
| TAC (DPPH) (TEAC µg g⁻¹ DW) | 0.95** |

In conclusion, the present study has demonstrated that leaves of both weedy *Amaranthus* genotypes exhibited as a good source of potassium, calcium, magnesium, P, S, Fe, Mn, Cu, Zn, Na, B, Mo, protein, dietary fiber, carbohydrates as a leafy vegetable. It is an excellent source of antioxidant leaf pigments, β-carotene, vitamin C, TAC, TPC and TFC and antioxidant that could contribute to human nutrition and health. The *A. viridis* genotype WAV7 and *A. spinosus* genotype WAS13 identified as the best accessions and could be cultivated as like as cultivar as a potential source of nutritional value, antioxidant leaf pigments, β-carotene, vitamin C, phenolics, flavonoids and antioxidants in our daily diet to reduce the hidden hunger and accomplishing nutritional and antioxidant sufficiency. Weedy *Amaranthus* species are the excellent source of phenolics, flavonoids, and antioxidants that have many pharmacological effects of their traditional applications. Finally, the obtained data present a valuable contribution to the scientific evaluation of pharmacologically active principles in weedy species.

Methods

Experiment materials, design, layout, and cultural practices. Department of Genetics and Plant Breeding of Bangabandhu Sheikh Mujibur Rahman Agricultural University collected several accessions (genotypes) of weedy amaranth (*A. spinosus* and *A. viridis*) from different agro-ecological zones of Bangladesh. We selected six genotypes (three accessions from each species) based on different morphological traits and different agroecological zones. We grew the selected genotypes at the experimental field of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh in a randomized complete block design (RCBD) with three replications. The unit plot size of each genotype was one square meter. The spacing of each genotype was two meters between plants of a row. As a necessity, weeding and hoeing were done to remove the weed. To maintain the normal growth of the crop proper irrigations were provided. At 30 days after sowing of seed, leaves samples were collected. All the parameters were measured in three replicates.

Chemicals. Solvent: acetone and methanol. Reagents: H₂SO₄, HNO₃, HClO₃, NaOH, dithiothreitol (DTT), caesium chloride, ascorbic acid, standard compounds of pure Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid), gallic acid, rutin, folin-ciocalteu reagent, DPPH (2, 2-diphenyl-1-picrylhidrazyl), ABTS⁺, aluminium chloride hexahydrate, sodium carbonate, potassium acetate, and potassium persulfate. All solvents and reagents used in this study were high purity laboratory products obtained from Kanto Chemical Co. Inc. (Tokyo, Japan) and Merck (Germany).
Proximate composition. AOAC method was followed\textsuperscript{18} to estimate the ash, moisture, crude fat, fiber, crude protein contents, and gross energy. Micro-Kjeldahl method was followed to determine crude protein with nitrogen to a protein conversion factor of 6.25 (AOAC method 976.05). We subtracted the sum of moisture, ash, crude fat, and crude protein percentage from 100 to measure carbohydrate content (g 100 g\textsuperscript{-1} FW).

Estimation of mineral content. At first, \textit{A. spinosus} and \textit{A. viridis} leaves were dried at 70 \textdegree C in a well-ventilated oven for 24 hours. Dried leaves were grounded finely in a mill. Nitric-perchloric acid digestion method\textsuperscript{18} was followed to determine the macronutrients (Ca, Mg, K, P, and S) and microelements (Fe, Mn, Cu, Zn, Na, Mo, and B) from powdered leaves. For this digestion, 400 ml of nitric acid (65%), 40 ml of perchloric acid (70%) and 10 ml of sulphuric acid (96%) in the presence of carborundum beads were added to 0.5 g dried leaf sample. After digestion, the solution was appropriately diluted in triplicate for measuring P following ascorbic acid method. Yellow-colored complex converted to a blue-colored phosphomolybdenum complex when ascorbic acid and Sb was added to the solution. Sarker and Oba\textsuperscript{18} method was followed to read the absorbance by atomic absorption spectrophotometry (AAS) (Hitachi, Tokyo, Japan) at wavelength of 76.65 nm (K), 422.7 nm (Ca), 285.2 nm (Mg), 880 nm (S), 248.3 nm (Fe), 279.5 nm (Mn), 324.8 nm (Cu), 213.9 nm (Zn), 589 nm (Na), 313.3 nm (Mo), and 430 nm (B).

Determination of chlorophylls and total carotenoids. The fresh \textit{A. spinosus} and \textit{A. viridis} leaves were extracted in 80% methanol containing 50 mM ascorbic acid to measure chlorophyll \textit{a}, chlorophyll \textit{b}, chlorophyll \textit{ab} and total carotenoids following Sarker and Oba\textsuperscript{18} method. A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to read the absorbance at 663, 646 and 470 nm for chlorophyll \textit{a}, chlorophyll \textit{b} and total carotenoids, respectively. Data were expressed as \( \mu g \) chlorophyll per g fresh weight (FW) and mg carotenoids per 100 g FW.

Determination of \( \beta \)-cyanin and \( \beta \)-xanthin content. The fresh \textit{A. spinosus} and \textit{A. viridis} leaves were extracted in 80% methanol containing 50 mM ascorbic acid to measure \( \beta \)-cyanin and \( \beta \)-xanthin following the method of Sarker and Oba\textsuperscript{18,38}. A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to read the absorbance at 540 and 475 nm for \( \beta \)-cyanin and \( \beta \)-xanthin, respectively. The results were expressed as nanogram betanin equivalent to per gram FW for \( \beta \)-cyanin and nanograms indicaxanthin equivalent to per gram FW for \( \beta \)-xanthin.

Estimation of \( \beta \)-carotene. Method of Sarker and Oba\textsuperscript{18,38} was followed to extract and determine \( \beta \)-carotene content. 500 mg of fresh leaf sample was ground in 10 ml of 80% acetone and centrifuged at 10,000 rpm for 3–4 min to carry out the extraction process. The final volume was brought up to 20 ml after removing the supernatant in a volumetric flask. A spectrophotometer (Hitachi, U-1800, Tokyo, Japan) was used to read the absorbance at 510 nm and 480 nm. Data were expressed as mg \( \beta \)-carotene per 100 g fresh weight.

The \( \beta \)-carotene content was calculated using the following formula:

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

Determination of vitamin C. The fresh \textit{A. spinosus} and \textit{A. viridis} leaves were used to measure ascorbic acid (AsA) and dehydroascorbate (DHA) spectrophotometrically. For pre-incubation of the sample and reduction of DHA into AsA Dithiothreitol (DTT) was used. AsA reduced Fe\textsuperscript{3+} (AsA) and dehydroascorbate (DHA) spectrophotometrically. For pre-incubation of the sample and reduction extracted in 80% methanol containing 50 mM ascorbic acid to measure Fe\textsuperscript{2+} complexes with 2, 2-dipyridyl\textsuperscript{18,39}.

Finally, the absorbance of the sample solution was read. Data were recorded as mg ascorbic acid per 100 g fresh weight (FW).

Extraction of samples for TPC, TFC and TAC analysis. At the edible stage (30 Days after sowing), \textit{A. spinosus} and \textit{A. viridis} leaves were harvested. The leaves were air dried in shade for chemical analysis. 40 ml of 90% aqueous methanol was used to extract 1 g of grounded dried leaves from each cultivar in a tightly capped bottle (100 ml). The extract was then placed in a shaking water bath (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan) for 1 h. Then the extract was filtered for further analytical assays of total polyphenol content, total flavonoid content, total antioxidant activity.

Determination of total polyphenol content (TPC). Method of Sarker and Oba\textsuperscript{18,40} was followed to estimate the total phenolic content of \textit{A. spinosus} and \textit{A. viridis} using the folin-ciocalteu reagent with gallic acid as a standard phenolic compound. In a test tube, 1 ml of folin-ciocalteu reagent (previously diluted 1:4, reagent: distilled water) was added to extract 1 g of grounded dried leaves from each cultivar in a tightly capped bottle (100 ml). The extract was then placed in a shaking water bath (Thomastant T-N22S, Thomas Kagaku Co. Ltd., Japan) for 1 h. Then the extract was filtered for further analytical assays of total polyphenol content, total flavonoid content, total antioxidant activity.

Determination of total flavonoid content (TFC). The aluminum chloride colorimetric method\textsuperscript{38,41} was used to estimate the total flavonoid content of \textit{A. spinosus} and \textit{A. viridis} extract. In a test tube, 1.5 ml of methanol was added to 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, 2.8 ml of distilled water and 500 \( \mu l \) of leaf extract for 30 min at room temperature. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to take the absorbance of the reaction mixture at 415 nm. TFC is expressed as \( \mu g \) rutin equivalent (RE) g\textsuperscript{-1} dry weight (DW) using rutin as the standard compound.
Total antioxidant capacity (TAC). Diphenyl-picrylhydrazyl (DPPH) radical degradation method\(^{18,42}\) was used to estimate the antioxidant activity. In a test tube, 1 ml of 250 µM DPPH solution was added to 10 µl of leaf extract solution (triplicate) and 4 ml of distilled water and allowed to stand for 30 min in the dark. A Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to absorb the reading at 517 nm. Method of Sarkar and Oba\(^{43}\) was followed for ABTS\(^+\) assay. 7.4 mM ABTS\(^+\) solution and 2.6 mM potassium persulphate were used in the stock solutions. The two stock solutions were mixed in equal quantities and allowing them to react for 12 h at room temperature in the dark for preparation of the working solution. 2850 µl of ABTS\(^+\) solution (1 ml ABTS\(^+\) solution mixed with 60 ml methanol) was mixed with 150 µl sample of leaf extract and allowed to react for 2 h in the dark. Aa Hitachi U1800 spectrophotometer (Hitachi, Tokyo, Japan) was used to read the absorbance against methanol at 734 nm. The percent of inhibition of DPPH and ABTS\(^+\) relative to the control were used to determine antioxidant activity using the following equation:

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

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. The results were reported as the average of three measurements (n = 3). The data were also statistically analyzed by ANOVA using Statistix 8 software, and the means were compared by the Duncan's Multiple Range Test (DMRT) at 1% and level of probability.

Ethical statement. The lab and field experiment in this study was carried out following guidelines and recommendations of “biosafety guidelines of Bangladesh” published by 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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Author contributions
U.S. initiated the research work and conceived the study; U.S. performed the experiments; 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.

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