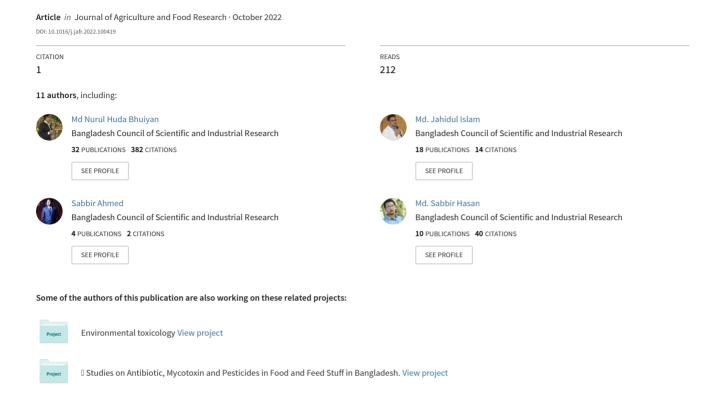
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Amaranthus tricolor (red amaranth), an indigenous source of nutrients, minerals, amino acids, phytochemicals, and assessment of its antibacterial activity

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ABSTRACT

Amaranthus tricolor (red amaranth) is an important member of the Amaranthus species ubiquitously cultivated worldwide for its valuable constituents. In this study, the determination of important nutrients, minerals, amino acids, phytochemicals, carotenes, antiradical activity, and antibacterial activity of the entire part of the red amaranth plant was performed. The analysis of the nutritional composition of the whole part of red amaranth shows that there was an appreciable quantity of protein (26.60 \pm 0.42%), fat (4.49 \pm 0.30%), fiber (6.67 \pm 0.33%), and carbohydrate (39.80 \pm 0.15%). Potassium (1080.02 \pm 32.51 mg/100g dry weight) was found as a major trace element among others (Na. Ca. and Iron). Overall seventeen amino acids were identified and the most abundant amino acid in red amaranth was glutamic acid (23.61 \pm 0.16 mg/g). However, lysine (14.32 \pm 0.20 mg/g) was found to be higher than each of the essential amino acids in red amaranth. The value of total phenolic content, total flavonoid content, and total antioxidant activity in the methanolic extract of red amaranth was 30.27 \pm 1.98 mg gallic acid equivalent/g, 16.75 \pm 0.82 mg quercetin equivalent/g and 62.91 \pm 3.35 mg ascorbic acid equivalent/g extract respectively. The IC_{50} value for DPPH radical scavenging activity was 730.93 $\mu g/mL$. The selected vegetable also contained an appreciable amount of carotenes (15.37 \pm 0.27 mg/100g). Plant extract had a modest extent of bacterial growth inhibition activity. Thus, red amaranth is a wonderful source of nutrients, a natural antiradical, and has other bioactive compounds that should be in a regular dietary item and useful for nutraceuticals.

1. Introduction

Leafy vegetables are one of the major components of a regular diet that provides a combination of necessary nutrients. These are contributors of essential and nonessential organic compounds that have an immense potential for providing health-promoting properties [1]. Due to geographical and economical prospects, leafy vegetables are much more popular as food items in south-east Asia [2]. Amaranths are considered as dominant leafy vegetables in temperate and tropical regions [3]. Amaranthaceae is a family of 70 species where 4 species are cultivated as leafy vegetables in this region. These four species are *Amaranthus blitum* Linn, *Amaranthus tricolor* Linn, *Amaranthus mangostanus* Linn, and *Amaranthus gangeticus* Linn [4]. Among these species,

Amaranthus tricolor Linn is one of the most extensively consumed vegetables in Bangladesh due to its attractive color, nutritious value, and delicious flavor [5,6]. This influenced to understand of the properties of Amaranthus tricolor Linn (red amaranth) rather than the other three species. Apart from that, Amaranthus tricolor Linn is widely recognized as the prime member of amaranths comprising various kinds depending on their leaf colors including red, purple, and green [7]. Red amaranth is a purple-red color leafy vegetable and is well known as an excellent food crop owing to its ability to grow under a wide range of climatic conditions like drought, heat, pests, and diseases [8]. Red amaranth is called Laal Shaak in different parts of India and Bangladesh where it is consumed as fried vegetables and as an ingredient for soup and noodles preparation [8,9]. The leaves of red amaranth is a reasonable source of

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proteins (66.26 g/kg - 11.38 g/kg), dietary fibers (91.94 μ g/g - 59.96μg/g), fat (4.35 g/kg - 1.42 g/kg), carbohydrates (98.54 g/kg - 15.48 g/kg), minerals such as iron (1089.19 μ g/g), calcium (10.13 mg/g), magnesium (30.01 mg/g), potassium (24.96 mg/g), and zinc (986.61 $\mu g/g$) [6]. Other nutrients like vitamins C (955.19 $\mu g/g$), beta carotene (1043.18 $\mu g/g$), betalains (66.40 $\mu g/100g$), betaxanthins (33.09 $\mu g/100g$), and betacyanins (33.30 $\mu g/100g$) are also present in the leaves of red amaranth [6]. Furthermore, it has been proven that leaves of red amaranth contain phytochemicals like alkaloids, glycosides, phenolic acids, flavonoids, amaranthine, tannins, and other pigment compounds [2,8,10]. Phenols, flavonoids, and pigment compounds are ubiquitously presented in vegetables, fruits, and herbs. These compounds can be easily available from these natural sources which could be an alternative way to fight against diseases [3,7,10]. Since its earlier event in history, the amaranth plant has been used in Ayurveda for the treatment of different diseases [8,10]. According to several studies, this leafy vegetable has an extensive capability to a broad range of medicinal properties such as hyperglycemic, hepatoprotective, antiradical, antibacterial, anti-inflammatory, and antidepressant [4,8,10]. These types of leafy vegetables are a wonderful and inexpensive source of nutrients making them an economically important food crop as well as playing a vital role in the health status of a developing nation. Therefore, this study focuses on the analysis of nutritional compositions, antiradical properties, and antibacterial activity of red amaranth for spreading the popularity of leafy vegetables among agricultural practices, consumers, and food industries.

2. Method and materials

2.1. Sample collection and preparation

The plant *Amaranthus tricolor* (red amaranth) was collected from a local market situated beside BCSIR Laboratories, Rajshahi, Bangladesh. The leaves and stems of this plant were separated carefully and cleaned to discard any wasteful material adhering to them. The latter was then dried in an oven at 50 °C and ground to a fine powder using a blender (Eta Mira 011, Czechia) [11]. The dried powder was extracted with methanol using an Ultrasonic water bath (WUC-AO3H, witeg Labortechnik GmbH, Germany) at 40 °C for about 1 h [6]. The extract was dried using a rotary evaporator (BasisHei-VAP ML, 562-00000-00-0, Heidolph Instruments GmbH & Co.KG, Germany). The dried extract was kept in the refrigerator at 4 °C for a future experiment. All these investigations were carried out with replicates at different times.

2.2. Chemicals and reagents

Ammonium molybdate, Folin-Ciocalteu's phenol reagent, and Lascorbic acid standard were purchased from Merck. CO. (Darmstadt, Germany). 1,1-diphenyl-2picrylhydrazyl radical (DPPH), Gallic acid, Quercetin dihydrate, and Butylated hydroxytoluene (BHT), 1,10-phenanthroline were purchased from Sigma Aldrich (St, Louis, MO, USA). All other chemicals in this experiment were analytical grade and used without further purification. Shimadzu UV-3600i plus Spectrophotometer (Tokyo, Japan) was used to measure the absorption of experimental solutions.

2.3. Proximate analysis

The proximate analysis (crude fat, protein, ash, moisture, fiber, and carbohydrate content) of the sample was executed according to the methods of the Association of Official Analytical Chemists [12]. A definite amount of sample was kept in a crucible and was taken into a muffle furnace (L (T)15/12, Nabertherm, Germany) at 600 °C for determination of ash content (AOAC 942.05) [12]. The moisture content was determined at 105 °C (AOAC 930.15) [12]. The crude fat of the sample was measured using n-hexane by the Soxhlet Extraction method

(AOAC 2003.05) [12]. For the estimation of crude fiber, the sample was first boiled in acid, then in base, dried, and then the fiber was incinerated at 550° Celsius (AOAC 925.10) [12]. Utilizing the Kjeldahl method (AOAC 978.04), the crude protein content was calculated [12]. Each sample's protein content was determined using the formula as follows: % *Protein* = $N \times 6.25$, where N is the nitrogen content and 6.25 is the protein conversion factor (R). In order to calculate the crude protein content of the samples, Kjeldahl digestion unit (DLK 42/26, automatic digestion, VELP Scientifica, Italy) for acid digestion and distillation chamber (UDK 129, distillation unit, VELP Scientifica, Italy) for distillation were used. The difference from 100 to the sum of all (crude protein, fat, moisture, ash, and fiber) was the value of total carbohydrates. All the proximate contents were expressed as a percentage (g/100g).

2.4. Minerals content analysis

2.4.1. Determination of sodium, potassium, and calcium

Sodium, Potassium, and Calcium in the powder sample of red amaranth were estimated using flame photometry (LX406FP, LABDEX, UK) reported by the method Vieira et al. with some modifications [13]. The ash of the sample was digested with a mixture of hydrochloric acid, nitric acid, and deionized water. The treated solutions were used for the estimation of minerals (Na, K, and Ca). The content of minerals was expressed as mg per 100 gm of dry mass of sample powder.

2.4.2. Iron content determination

UV–Visible spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan) was used for the determination of iron content by the 1, 10 Phenanthroline method [14]. Different concentrations of standard iron solutions were prepared for making the calibration curve. Exactly 2.0 mL each of standard/sample solutions were mixed with a definite volume of 1, 10-phenanthroline (0.1%), hydroxylamine hydrochloride (10%), and ammonium acetate buffer. The solutions were diluted and absorbance was taken at 510 nm. The value of iron content was expressed as mg per 100 gm of dry sample powder.

2.5. Amino acid composition

The compositions of the essential and non-essential amino acids were quantified by an amino acid analyzer (S433D, Sykam Co. Ltd, Germany). The samples were hydrolyzed in a sealed glass tube with $6.0\,\mathrm{N}$ HCl and kept in an oven at $110\,^\circ\mathrm{C}$ for $24\,\mathrm{h}$ [15]. The samples were filtered (Whatman #1444 150, Maidstone, England) and made up to the mark in a $100\,\mathrm{mL}$ volumetric flask. The sample was further diluted $10\,\mathrm{times}$ and subjected to the amino acid analyzer. The Sykam Standard Type H (mixture of $17\,\mathrm{amino}$ acids) was used in this analysis. The data of amino acids was expressed as mg of each amino acid per gram of dry powder.

2.6. DPPH radical scavenging capacity

The Scavenging Capacity of extract of red amaranth was investigated according to the method detailed by Braca et al. [16] with few modifications using 1,1-diphenyl-2picrylhydrazyl radicals (DPPH). Butylated hydroxytoluene (BHT) was used as standard. 1000 ppm stock solution of standard/samples and 0.004% DPPH were prepared using 80% methanol. From there the standard/samples were made to different concentrations using 80% methanol. An exactly equal volume of each of the standards/samples was mixed with 2.5 mL of 0.004% DPPH. After 30 min of incubation in a dark place; the absorbance of mixtures was taken at 517 nm using a Spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan). The percentage of DPPH inhibition was obtained from the following equation:

% of DPPH Inhibition =
$$\left(\frac{Ac - As}{Ac}\right) \times 100$$

Where A_c and A_s are the absorbance of the control and sample/standard respectively. DPPH scavenging capacity is expressed as IC_{50} , the concentration of a sample required to reduce DPPH activity by 50%. The IC_{50} value was determined from the equation of line made by plotting a graph of concentration (µg/mL) versus % of inhibition.

2.7. Determination of total antioxidant activity

The total antioxidant activity (TAA) of methanolic extract of red amaranth was determined using the phosphomolybdenum method as described by Prieto et al. [17]. This method is designed from the formation of a green complex of phosphate molybdate (V) by the sample/standard after the reduction of Mo (VI) - Mo (V) under an acidic medium. Ascorbic acid was used as standard. The concentrations of stock solution of standard and sample were 350 ppm and 1000 ppm respectively. From the stock solution of ascorbic acid different concentrations of standard solution were prepared. Exactly 0.5 mL of sample/standard solutions was added into a mixture of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 1% ammonium molybdate. The solutions were incubated at 95 $^{\circ}\text{C}$ for 90 min. The absorbance of the solutions was taken at 695 nm after cooling to room temperature followed by centrifugation using a Spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan) against blank (methanol). The TAA was expressed as mg of ascorbic acid equivalents (AAE) per gram of extract (mg AAE/g sample).

2.8. Determination of total phenolic content

Total phenolic content (TPC) was measured by the Folin-Ciocalteu method [18] using Gallic acid as standard. A series of different concentrations of the standard were made from a 500 ppm stock solution of gallic acid for the calibration curve. The concentration of a stock solution of the extract of red amaranth was 1000 ppm. Exactly 0.5 mL each of sample/standard solutions was mixed with a mixture of sodium carbonate (7.5%) and Folin-Ciocalteu reagent (10-fold diluted). The absorbance of all the solutions was taken individually at 760 nm using a Spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan) after a 30 min incubation period in a dark place. The value of TPC was expressed as mg of Gallic acid equivalent per gm of extract (mg GAE/g extract).

2.9. Determination of total flavonoid content

Aluminum chloride colorimetric assay described by Phuyal et al. was used for the determination of total flavonoid content (TFC) using Quercetin as standard [19]. For this analysis 500 ppm and 1000 ppm stock solution of standard and extract of the sample were prepared respectively. From there solutions having various concentrations of the standard were made for the formation of the calibration curve. Exactly 0.5 mL of each sample/standard solution was taken into a mixture of 2.5 mL of distilled water and 0.15 mL of 5% sodium nitrate. After that 0.3 mL of 10% aluminum chloride was poured into these mixtures followed by the addition of 1 mL of 1 mM sodium hydroxide and 0.55 mL of distilled water. These were centrifuged at 4000 rpm for 10 min and the absorbance of the supernatant was measured at 415 nm using a Spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan) against blank. The value of TFC was expressed as mg of quercetin equivalent per gm of extract (mg QE/g extract).

2.10. Determination of total carotene content

The Total Carotene Content (TCC) of powder of red amaranth was measured using the method reported by Panpraneecharoen with some modifications [20]. In this case, 0.2 gm of the dry sample was taken for n-hexane extraction using an ultrasonic water bath (WUC-AO3H, witeg

Labortechnik GmbH, Germany). The extracted solutions were filtered (Whatman #1444 150, Maidstone, England) and made it up to the mark with 25 mL by Hexane. After that, the absorbance of the solutions was taken at 446 nm using a Spectrophotometer (Shimadzu UV-3600i plus Spectrophotometer, Tokyo, Japan). The total carotene content was estimated using the formula, TCC = $25\times383(A_s-A_b)/100W$, Where A_s is the absorbance of sample, A_b are the absorbance of blank and W is the weight of sample in grams. The value of TCC was expressed as mg/kg of dry powder.

2.11. FTIR analysis

The dried powder and dried methanolic extract of *Amaranthus tricolor* were used for FTIR analysis. The data was taken with a scan range of $4000~\rm cm^{-1}$ to $500~\rm cm^{-1}$ using FTIR with ATR (Spectrum, PerkinElmer, USA).

2.12. Antibacterial activity analysis

The assessment of microbial susceptibility to red amaranth plant was performed according to the disc diffusion method [21]. In this study, one Gram-positive named Listeria monocytogenes (ATCC 10708) and one Gram-negative bacteria named Salmonella typhi (ATCC 13311) were used as bacterial cultures. Red amaranth extract (50 $\mu g/\text{disc})$ was infused with the sterile disc (6 mm, Whatman, Maidstone, England) and azithromycin (15 $\mu g)$ was used as a positive control. The selected bacterial organisms were inoculated in a nutrient medium (Mueller-Hinton broth) with the help of a sterile swab. Then, the positive control and plant extract impregnated discs were placed into the incubated plates in an aseptic condition and these plates were incubated at 37 °C for 24 h. After 24 h of incubation, the plates were analyzed for determination of the inhibition zone.

2.13. Statistical Analysis

Results of all the investigations were expressed as mean \pm standard deviation (SD) using six replicates for antiradical activities (TAA, TPC, and TFC) and three replicates for proximate analysis, minerals, TCC, and amino acid composition analysis. Student's t-test method was performed using Statistical Package for Social Science (SPSS) (Version 25) and p < 0.05 was set for the level of significance.

3. Results and discussion

3.1. Nutritional composition

This study uncovered the proximate value of the whole plant of red amaranth both on a dry and wet basis. Table 1 shows the nutritional composition of red amaranth. The content of ash, moisture, protein, fat, fiber, and carbohydrate in the dried powder of red amaranth were 15.68%, 6.76%, 26.60%, 4.49%, 6.67% and 39.80% wherein fresh raw sample were 1.27%, 83.20%, 2.16%, 0.36%, 0.54% and 3.23% respectively. Ferdaus et al. revealed the range of the value of ash (1.51 \pm 0.06% to 1.68 \pm 0.96%), protein (4.24 \pm 0.19% to 4.41 \pm 0.05%), fat (0.14 \pm 0.04% to 0.21 \pm 0.06%), crude fiber (6.78 \pm 0.05% to 6.83 \pm 0.02%), carbohydrate (1.45 \pm 2.25% to 2.97 \pm 0.47%) in the fresh raw sample of red amaranth collected from four different villages in Jessore, Bangladesh [22]. The results of protein, fat, fiber, carbohydrate, ash, and moisture in this study show in accordance with a reported range of a study of 25 red morph amaranths genotypes [6]. The result of protein content is also similar to the study of Singh et al. [23] where about 26.0 \pm 0.05% of protein in Amaranthus tricolor had been reported. The exploration showed that the powder of the whole plant contained a significant amount of protein which is next to the value of carbohydrates. The ash, moisture, fiber, protein, and fat content in red amaranth were found to be present in an appreciable amount necessary for a

Table 1Nutritional composition (g/100g) of *Amaranthus tricolor* plant.

Red amaranth	% In dry powder	% In the raw sample	
Ash	15.68 ± 0.25	1.27 ± 0.02	
Moisture	6.76 ± 0.38	83.20 ± 1.55	
Protein	26.60 ± 0.42	2.16 ± 0.03	
Fat	4.49 ± 0.30	0.36 ± 0.24	
Fiber	6.67 ± 0.33	0.54 ± 0.03	
Carbohydrate	39.80 ± 0.15	3.23 ± 0.01	
Protein 26.60 ± 0.42 Moisture 6.76 ± 0.38	Carbohydrate 39.80 ± 0.15	Moisture 83.20 ± 1.55 Protein Fat Fiber Carbohydrate	
% of Proximate Conten	t based on dry basis	% of Proximate Content based on wet basis	

Values are presented as mean \pm SD (n = 3).

healthy diet. The presence of high saturated fat, cholesterol, and sulfur-containing protein during the consumption of animal proteins has a risk of cardiovascular, bone health, and other physiological system diseases [24]. So, protein from vegetables like red amaranth might be an alternative source for a daily diet chart. Differences in nutritional composition happen mainly due to variation in agricultural practice, soil conditions, climatic conditions, and seasonal variation [15].

3.2. Mineral contents

Trace elements normally considered as micronutrients are engaged in metabolism, blood oxygen transportation, and many other indispensable functions in the bone and tissue of the human body [25]. Table 2 illustrates the value of several mineral contents (Na, K, Ca, and Fe) in red amaranth. Amaranthus tricolor contained a significantly higher amount of potassium (1080.02 mg/100g) than other elements. Potassium plays an important role to minimize hypertension, stroke, and cardiovascular disease [26]. It also contained 52.46 mg/100g and 39.76 mg/100g of sodium and calcium respectively. But the content of iron (0.57 mg/100g) was relatively low. This study showed a higher value of potassium but a lower value of calcium and iron than the reported results by Shukla et al. [27]. Sarker and Oba reported a range of mineral contents (K, Ca, and Iron) in 25 red morph amaranth plants, and these are presented as the content of K varied from 16.28 mg/g to 6.55 mg/g DW, the content of Ca varied from 34.83 mg/g to 16.02 mg/g DW, and content of iron varied from 2057.02 µg/g to 195.12 µg/g respectively [6].

3.3. Amino acid compositions

Table 3 shows the amino acid compositions contained in the dried plant of *Amaranthus tricolor*. Out of twenty about seventeen amino acids were determined using the amino acid analyzer. Eight of them were nonessential and all the essential amino acids except tryptophan were

Table 2 Mineral contents (mg/100g) of *Amaranthus tricolor*.

Minerals	mg/100g in dry weight	
Sodium (Na)	52.46 ± 3.14	
Potassium (K)	1080.02 ± 32.51	
Calcium (Ca)	39.76 ± 1.80	
Iron (Fe)	0.57 ± 0.02	

Mineral content is presented as mean \pm SD (n = 3).

Table 3
Amino acid compositions (mg/g) of red amaranth.

Non-Essential Amino Acids		Essential Amino Acids	
Name	Amount	Name	Amount
Glutamic acid	23.61 ± 0.16	Lysine	14.32 ± 0.20
Aspartic Acid	12.83 ± 0.16	Leucine	11.10 ± 0.11
Alanine	9.40 ± 0.15	Valine	9.90 ± 0.51
Arginine	7.66 ± 0.11	Glycine	8.63 ± 0.18
Serine	6.92 ± 0.05	Phenylalanine	8.36 ± 0.11
Tyrosine	6.92 ± 0.18	Threonine	5.76 ± 0.10
Proline	6.21 ± 0.22	Histidine	5.50 ± 0.13
Cystine	1.16 ± 0.01	Isoleucine	4.75 ± 0.22
·		Methionine	2.74 ± 0.21

Values are presented as mean \pm SD (n = 3).

recorded in this analysis. Glutamic acid (23.61 mg/g) which is a nonessential amino acid had the highest value among all. The vegetable holds the maximum value for lysine (14.32 mg/g) among essential amino acids. A remarkable amount of aspartic acid (12.83 mg/g) and leucine (11.10 mg/g) was found in this investigation. There also exists a considerable amount of essential and non-essential amino acids having the decreasing order of amount valine > glycine > Phenylalanine > threonine > histidine > isoleucine > methionine and alanine > arginine > serine > tyrosine > proline > cysteine respectively. Gins et al. reported the amino acid composition of Amaranthus tricolor variety's early splendor which is quite different from this studied plant [28].

3.4. Antiradical properties

Antiradicals have the ability to squeeze free radicals such as lipid peroxyl, hydroperoxide, and peroxide and minimize degenerative diseases by interdicting oxidative mechanisms [29]. Leafy vegetables are a well-known source of natural antiradicals. In this study, the value of TPC, TFC, TAA, TCC, and IC $_{50}$ by DPPH radical assay were estimated to rummage the antiradical properties of red amaranth.

3.4.1. TPC, TFC, TAA, and TCC of Amaranthus tricolor

The content of TPC TFC, and TAA of methanolic extracts of the *Amaranthus tricolor* plant, are represented in Table 4. The value of TAA (62.91 mg AAE/g extract) was higher than the value of TPC (30.27 mg GAE/g extract), and TFC (16.75 mg QE/g extract). This data shows that the reported TFC value seems to be lower than the TPC value. Khanam et al. showed the value of TPC, TFC, and TAC (total antioxidant capacity) were 133.4 ± 7.6 to 146.1 ± 11.6 µg GAE/g FFW, 53.6 ± 4.5 to 70.4

Table 4Total phenolic content, total flavonoid content, and total antioxidant activity of methanolic extracts of *Amaranthus tricolor*.

Parameter name	Value	Unit
Total Phenolic Content (TPC)	30.27 ± 1.98	mg GAE/g extract
Total Flavonoid Content (TFC)	16.75 ± 0.82	mg QE/g extract
Total Antioxidant Activity (TAA)	62.91 ± 3.35	mg AAE/g extract
Total Carotene Content (TCC)	15.37 ± 0.27	mg/100g of dry powder

The content is presented as mean \pm SD (n = 6).

 \pm 3.7 µg QE/g FFW and 23.7 \pm 2.6 to 30.2 \pm 4.6 µg TEAC/g FFW respectively among four cultivars of Amaranthus tricolor in Bangladesh [3]. TPC ranged from 1.74 to 2.29 mg GAE/100g dry powder among different cultivars of red amaranth in the study by Khandaker et al. [7]. Another previous study reported 162.39 µg FAE/g DW of TPC in red amaranth using the FC method and 125.97 μg RE/g of TFC in red amaranth [3]. The variation of results in terms of phenolic and flavonoids contents in leafy vegetables is due to their production in plants affected by the duration of sunlight exposure, agro-climatic conditions, harvesting of plants, solvents used for extraction, and other experimental conditions [11,30]. The powder of the whole plant also contained an observable amount of total carotene (15.37 mg/100g). A range of total carotenoid contents in 20 leafy amaranth genotypes was recorded as 32.77 mg/100g to 105.08 mg/100g by a previous study [31]. Another study by Sarker and Oba reported a relatively notable range of total carotenoids (65.85-87.25 mg/100g) in 4 genotypes of red amaranth [32].

3.4.2. Antiradical capacity

Fig. 1 shows the increasing rate of radical scavenging with the concentration of BHT and extract of red amaranth. The value of IC50 (the concentration of a sample required to reduce DPPH absorbance by 50%) is also represented in Fig. 1. The IC₅₀ values of BHT and Red amaranth were 3.14 μg/mL and 730.93 μg/mL respectively. Therefore, BHT exhibits a strong radical scavenger while extract Amaranthus tricolor exhibits a poor radical scavenger as the value of IC₅₀ more than 200 $\mu g/mL$ is considered as weak antiradical [33]. But with increasing the concentrations of extract the inhibition activity increases. Pulipati et al. showed the value of IC50 of different extracts of Amaranthus tricolor varies from 290 μ g/mL to 830 μ g/mL [10]. Another study showed a range of IC₅₀ value (206 $\mu g/mL$ -1748 $\mu g/mL$) for DPPH radicals scavenged by different solvent extracts of Amaranthus tricolor [34]. The variations in IC₅₀ value for DPPH radicals often shows as a result of using different solvent systems, different concentration of radicals, and conditions of experiments [35] (see Fig. 1).

It has been extensively studied and reported that amaranthine, betanin, betaxanthin, betalains, betacyanins, other polyphenols, and

carotenoids are present in a notable amount in the red amaranth plant [3,8,32]. These compounds act as natural antiradicals to remove detrimental free radicals resulting from regular cellular functions and circumambient stressors and finally help to prevent degenerative diseases as well as robust the immune system [10,36].

3.5. FTIR analysis

Fig. 2 shows the FTIR spectrum of dry powder and dried methanolic extract of red amaranth. Both the spectrum shows a similar peak position, mainly in the functional group region (>1400 cm $^{-1}$). The sharp peak at 3290.88 cm $^{-1}$ clearly indicates the O–H stretching [37] of the –OH group. The two peaks at 2926.26 and 2849.01 cm $^{-1}$ denote the C–H stretching [37] of SP 3 hybridized carbon. The single peak at 1621.34 cm $^{-1}$ due to the C=O bond [37] and a series of zigzag small peaks around 2000-2100 cm $^{-1}$ indicate aromatic overtone [38] due to the aromatic ring. The peaks near 1384, 1320.94, and 1033.94 cm $^{-1}$ in the finger print region are also for bending modes of -CH2, -CH3, and stretching of C–O respectively [37].

3.6. Antibacterial activity

Antibacterial activity was estimated by determining the zone of inhibition from the examined plates of bacterial culture. It was presented as the zone of inhibition in millimeters (mm) (Fig. 3). The methanolic extract of red amaranth was found to have a modest inhibition effect on bacterial growth when it was compared with the positive control (p < 0.05). The Gram-negative bacteria *Salmonella typhis* showed greater

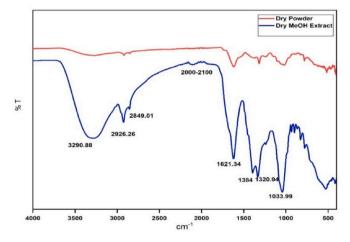


Fig. 2. FTIR spectra Amaranthus tricolor (dry powder and methanolic extract).

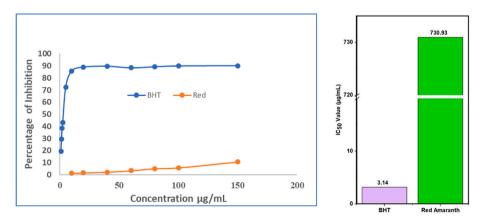


Fig. 1. DPPH radical scavenging capacity and IC50 value of BHT and methanolic extract of Amaranthus tricolor.

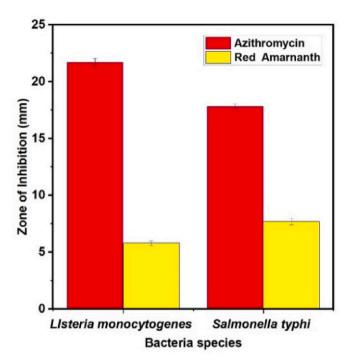


Fig. 3. Antibacterial activity of red amaranth plant extract. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

sensitivity to the studied plant extract than Gram-positive bacteria *Listeria monocytogenes*. This result was comparable with the study of Al-Mamun et al. [39].

Thus, the constituent components of the red amaranth plant have not only antiradicals but also antibacterial activity. Therefore, the whole plant of red amaranth could be a favorable source of natural antiradicals and necessary nutrients for maintaining human health.

4. Conclusion

The study investigated the nutritional compositions and antiradical properties of red amaranth which is widely cultivated and consumed as a vegetable in Bangladesh. This investigation found that there was an appreciable quantity of protein, fat, fiber, carbohydrate, and minerals such as K, Ca, Na and Iron. There was a profile of amino acids of red amaranth where both indispensable and dispensable amino acids were recorded. The presence of carotenes and polyphenols in the selected vegetable substantiates the antiradical properties as well as antibacterial activity. Thus, red amaranth could be recognized as a potent source of nutrients, a natural antiradical, and an important bioactive compound in food industries and pharmaceuticals. This comprehension may also be able to inspire cultivars, nutritional practitioners, and industries.

CRediT authorship contribution statement

Farhana Jahan: Investigation, Formal analysis, Statistical Analysis, Data curation, Writing – original draft, Supervision. Md Nurul Huda Bhuiyan: Writing – review & editing, Supervision. Md. Jahidul Islam: Investigation, Formal analysis. Sabbir Ahmed: Resources, Investigation, Formal analysis. Md. Sabbir Hasan: Visualization. Mahci Al Bashera: Methodology, Formal analysis. Md. Waliullah: Investigation. Arfatun Nahar Chowdhury: Investigation. Md. Badrul Islam: Resources, Methodology, Supervision. Barun Kanti Saha: Supervision, Funding acquisition. Shyama Prosad Moulick: Conceptualization, Methodology, Data curation, Investigation, Writing – original draft, Formal analysis, Writing – review & editing, Visualization, Software,

Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships which can influence the results of this work and also do not have any conflict with any other research work.

Data availability

Data will be made available on request.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2022.100419.

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