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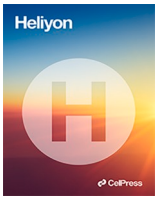

Research article

Nutritional characterization and antioxidant properties of various edible portions of *Cucurbita maxima*: A potential source of nutraceuticals

Farhana Jahan [a,*], Md. Badrul Islam [a], Shyama Prosad Moulick [b,**], Mahci Al Bashera [a], Md. Sabbir Hasan [a], Nishat Tasnim [c], Trissa Saha [d], Farhana Boby [a], Md. Waliullah [a], Anik Kumar Saha [a], Amin Hossain [a], Lailatul Ferdousi [a], Md. Mahmudur Rahman [a], Barun Kanti Saha [a], Md Nurul Huda Bhuiyan [a,***]

[a] *BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh*
[b] *BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh*
[c] *Institute of Technology Transfer and Innovation, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh*
[d] *Institute of Fuel Research and Development, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh*

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ABSTRACT

Pumpkin (*Cucurbita maxima*) is a widely grown vegetable in Bangladesh and is known as the sole supplier of various nutrients. Many studies evidence the nutritional value of flesh and seed while peel, flower, and leaves were reported scarcely with limited information. Therefore, the study aimed to investigate the nutritional composition and antioxidant properties of flesh, peel, seed, leaves, and flowers of *Cucurbita maxima*. The seed had a remarkable composition of nutrients and amino acids. Flowers and leaves possessed higher content of minerals, phenols, flavonoids, carotenes, and total antioxidant activity. The order of IC_{50} value (peel > seed > leaves > flesh > flower) indicates higher DPPH radicals scavenging activity of the flower. Moreover, a significant positive relationship was observed among these phytochemical constituents (TPC, TFC, TCC, TAA) and DPPH radicals scavenging activity. It could be concluded that these five parts of the pumpkin plant have an intense potency to be an exigent component of functional food or medicinal herbs.

1. Introduction

Pumpkin from the Cucurbitaceae family is extensively cultivated and consumed for its multistory nutritional constituents [1]. Different species namely *Cucurbita pepo, Cucurbita moschata, Cucurbita mixta,* and *Cucurbita maxima* of pumpkin are commonly grown

* Corresponding author. BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Bangladesh.
** Corresponding author. BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Bangladesh.
*** Corresponding author. BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Bangladesh.
E-mail addresses: farhana.jahan@bcsir.gov.bd (F. Jahan), moulik.shyama@bcsir.gov.bd (S.P. Moulick), nhbmb@bcsir.gov.bd (M.N. Huda Bhuiyan).

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all over the world since the earliest event of history [1,2]. Furthermore, the cultivation of pumpkin is popular in Asia for domestic purposes as well as commercial [2]. *Cucurbita maxima*, one of the economically important vegetables, is cultivated in most regions of Bangladesh under a broad range of climatic conditions [3]. Many countries including China, India, Korea, Mexico, and Brazil utilize pumpkins for traditional medicine purposes [4,5]. Various parts like fresh leaves, seeds, flowers, and fruits (immature and mature) of the pumpkin plant are consumed as vegetables throughout the world [6]. The pumpkin contains nutrients like carbohydrates, minerals, and dietary fibers as well as an inexpensive source of vitamins, particularly carotenes [7]. Hypoglycemic, in-vitro antioxidant, cholinesterase and tyrosinase inhibition, and hypolipidemic effects of pumpkin flesh have been reported [7–9]. The peel is an agro-byproduct of pumpkin that is produced at the time of flesh processing. Researchers investigated the peel of pumpkins and revealed that it is rich in nutrients and bioactive compounds (like pectin) having the potential to be an ingredient in food products [10,11]. Pumpkin seeds consumed in some countries as snacks are a wonderful source of proteins, lipids, minerals, and phytonutrients that establish their medicinal and nutraceutical importance [12]. Due to the presence of these bioactive nutrients, it is assumed to be used as a remedy for lowering blood cholesterol or depression [13,14]. Besides that, anti-diabetic, antiulcer, wound healing, antioxidant, and hepatoprotective properties of the seed of pumpkin have also been investigated [15–17]. The fresh leaves and flowers also harbor important bioactive compounds with pharmacological activities such as antimicrobial, antifungal, anti-mutagenic, antioxidant, hepato-protection, anti-hyperglycemic, antidepressant, and anti-inflammation [9,18–20].

It is proved that natural antioxidants are more effective as well as possess no harmful effect on human health in comparison with synthetic ones [16]. Natural antioxidant compounds including carotenoids, tocopherols, flavonoids, phenolic acids, vitamin C, and some minerals are mostly found in vegetables and fruits [6,21]. Phenols and flavonoids are a broad group of phytochemical compounds that are produced in plants during metabolisms as secondary metabolites relating to plant physiological activities such as reducing oxidative radicals and modulating the growth of plants. The mechanism behind this antioxidant activity is that the presence of functional hydroxyl groups in phenols and flavonoids neutralizes reactive oxygen species produced in plant tissues [22]. Thus, researchers are now looking for natural antioxidants that are preserved in vegetables and fruits.

Flour of pumpkin flesh, peel, and seed have been used in the formulation of bakery, snacks, and confectioneries in numerous countries such as Korea, Poland, Iraq, China, Russia, and India [23–27]. Though pumpkin is a highly available vegetable all year round, the only pumpkin fruit flesh is most popularly consumed in Bangladesh. Moreover, commercial cultivation of pumpkin has increased in the north-western part of this country due to its simple way of farming, elevated production, extended storage properties (longer than five months), profitable market price, and excellent alimentary features establishing it as a valuable crop. But there is no such functional or value-added food product in the market of this country that is made from pumpkin. The peel, seed, leaves, and flowers of pumpkin are being treated as agro-wastes and not used for industrial purposes. Few studies have reported the nutritional profiling of flesh, peel, and the seed of pumpkin available in Bangladesh with limited information for the production of functional food [3,12]. Moreover, no information about leaves and flowers are recorded yet in Bangladesh. Besides that, there are no hearty tidings about the nutritive and antioxidant properties of these whole sections of the pumpkin plant produced in this country. Since the nutritional constituents and phytochemical compositions of pumpkins vary depending on their emergence, climatic and cultivation conditions [5], it is necessary to reveal their characteristics which are grown in Bangladesh. Therefore, the study was designed to explore the nutritional composition and antioxidant properties of various parts (flesh, peel, seed, leaves, and flower) of *Cucurbita maxima* which may assist in the fruitful utilization of these parts as a lucrative component of functional food or medicinal purpose.

2. Method and materials

2.1. Sample collection and preparation

Pumpkin (*Cucurbita maxima* Duchesne) seeds were collected from Binodpur, a local market of Rajshahi (north-western part) in Bangladesh. These seeds were sowed in the field (24°43'59.9" N latitude and 88°10'59.9" E longitude) nearer to BCSIR, Rajshahi laboratories, Bangladesh. The soil of the research site was loamy soil with a pH value of 8.40–8.60. No additional fertilizers were used in the field. The temperature was about 25 °C–35 °C and the relative humidity was 74.33%. The resulting plant was grown around February to June (during the summer season) (Supplement Fig. S1). The specimen was submitted to the Department of Botany, the University of Jahangirnagar, Dhaka, Bangladesh for identification (Supplement Fig. S4). The flowers (male) and leaves (mature and middle-aged) from the plants as well as the fruit (freshly harvested from cultivated land) were taken (Supplement Fig. S2). Several fruits were stored for a few months (about 6 weeks, at temperature around 25 °C–30 °C, and relative humidity 65 ± 5%) and used for total carotene content analysis. Then the flesh peels and seeds were collected from the fruit. These five parts (leaves, flower, flesh, peel, and seed) were then cleaned, sliced, sun-dried, and ground to a fine powder (blander- Eta Mira 011) (Supplement Fig. S3). 25 g of each powder was subjected to Soxhlet extraction at 50 °C for 6 h in methanol. The solvent from crude extracts was removed using a rotary evaporator (Basis Hei-VAP ML, 562-00000-00-0, Heidolph Instruments GmbH & Co.KG, Germany). All the dried extract was stored at 4 °C in a refrigerator for future experiments. The investigations were performed about three times with replicates to ensure the quality of the experiment.

2.2. Chemicals and reagents

The standard for Iron, 1,1-diphenyl-2picrylhydrazyl radical (DPPH), Gallic acid, Quercetin dehydrate, and 2,6-Di-*tert*-butyl-p-cresol or Butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich (St, Louis, MO, USA). L-ascorbic acid, Folin-Ciocalteu's phenol reagent, and ammonium molybdate were purchased from Merck CO. (Darmstadt, Germany). All other chemicals and reagents

used in this work were of analytical grade. Shimadzu UV-3600i plus Spectrophotometer (Tokyo, Japan) was used for colorimetric analysis.

2.3. Proximate analysis

The proximate analysis of dry powder of flesh, peel, seed, leaves, and the flower was accomplished using AOAC standard methods [28] (AOAC Volume-II, 1990). The parameters crude protein, fat, moisture, carbohydrate, ash, and fiber were estimated and expressed in percentages (g/100 g).

The moisture content was estimated by using the method of weight loss from evaporation. The moisture content of each of the samples was recorded after drying in an oven at 105 °C (AOAC 930.15).

For determination of ash content, about 3.0 g of each dried sample was taken into crucibles and incinerated in a muffle furnace at 550 °C (AOAC 942.05).

The crude protein was determined from crude nitrogen content using the Kjeldahl method (AOAC 2001.11). The protein content of each dried sample was calculated using the formula as follows:

$$\%\ Protein = N \times 6.25 \tag{1}$$

Where N is the nitrogen content and 6.25 is the protein conversion factor (R). Crude protein of each sample was estimated through multiple steps using apparatus namely the Kjeldahl digestion unit (DLK 42/26, automatic digestion, VELP Scientifica, Italy) for acid digestion, distillation chamber (UDK 129, distillation unit, VELP Scientifica, Italy) for distillation, and finally titration with acid to determine the nitrogen content.

The crude fiber content of the sample was determined by following steps: boiling in acid and then base, drying of fiber, and finally incineration of fiber at 550 °C (AOAC 978.10).

The fat content of each dried sample was determined in the Soxhlet apparatus using *n*-Hexane as an extraction solvent (AOAC 2003.05).

At first, the sum of the percentages of crude protein, moisture, ash, crude fiber, and crude fat was taken. This value was subtracted to 100 and expressed as a percentage (g/100 g) of carbohydrate content. All analyses were conducted in triplicate and results were reported on a dry and wet basis.

2.4. Estimation of mineral (Na, K, Ca) content

Mineral (sodium, potassium, calcium) contents in the powder of leaves, flower, flesh, peel, and seed of pumpkin were estimated by the method Viera et al. with slide modification [29]. A definite amount of sample powder was taken for dry ash. The ash of each sample was mixed with hydrochloric acid and these solutions of ashes were used for the measurement of mineral contents by flame photometry (LX406FP, LABDEX, UK). The mineral contents were expressed as mg of each mineral per 100 g of sample dry powder.

2.5. Estimation of iron content

The Iron content of leaves, flowers, flesh, peel, and seeds of pumpkin was determined by 1, 10 Phenanthroline (0.1%) [30]. Different concentrations of standard Iron solutions were made for the calibration curve. 2.0 mL of each sample/standard solution was subject to sequentially mixed with 1.0 mL of hydroxylamine hydrochloride (10%), 2.0 mL of 1,10-phenanthroline (0.1%), and 5.0 mL of ammonium acetate buffer. The solutions were made up to 25.0 mL and the absorbance was taken at a wavelength of 510 nm. The content of iron was expressed as mg of iron per 100 g of sample dry powder.

2.6. Amino acid composition

The amino acid composition of the leaves, flowers, flesh, peel and seed of pumpkin was estimated through an amino acid analyzer (S433D, Sykam Co. Ltd, Germany). The dried samples were weighted and hydrolyzed with 6.0 N HCl in a sealed glass tube. Then these were kept in an oven at 110 °C for 24 h [3]. After that, the samples were filtered using Whatman filter paper (Whatman #1444 150, Maidstone, England) and made up to 100 mL with distilled water. These were then subjected to dilution and then diluted samples were filtered with a 0.21 m membrane for quantifying amino acids through an amino acid analyzer. The data for amino acids were presented as mg per g of dry sample.

2.7. Estimation of total phenolic content

The Folin-Ciocalteu method was used for the determination of the total phenolic content of these extracts (leaves, flower, flesh, peel, and seed) [31]. Here, Gallic acid was used as a standard and made various concentrations of standard solution from the stock (500 ppm). The concentration of each stock solution was 1000 ppm for all extracts. Exactly 0.5 mL of each sample/standard solution was mixed with a mixture of Folin-Ciocalteu reagent (10-fold diluted) and sodium carbonate (7.5%). These mixtures were then kept in dark condition for 30 min. The absorbance of each solution was measured using a Spectrophotometer (Shimadzu, Tokyo, Japan, UV-3600i plus) at 760 nm. The value for total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per g of

extract (mg GAE/g extract).

2.8. Estimation of total flavonoid content

A well-known aluminum chloride colorimetric assay using Quercetin as standard was used for the estimation of total flavonoid content (TFC) [32]. A series of solutions having different concentrations of standard and 1000 ppm stock solution for each of the extracts (leaves, flower, flesh, peel, and seed) were prepared for this experiment. At first, 2.5 mL of distilled water and 0.15 mL of 5% sodium nitrate were mixed with 0.5 mL of each sample/standard solution. After 5 min, 0.3 mL of 10% aluminum chloride was added and left for 5 min. Then, 1 mL of 1 mM sodium hydroxide and subsequently 0.55 mL of distilled water were added. Then the mixture was centrifuged and the supernatant was taken for measurement of absorbance at 415 nm against blank. The TFC was expressed as milligrams of quercetin equivalents (QE) per gram of extract (mg QE/g extract).

2.9. Estimation of total antioxidant activity

The phosphomolybdenum method described by Prieto et al. was used for the estimation of the total antioxidant activity (TAA) of these extracts [33]. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex under acidic conditions. Ascorbic acid was used as a standard for this analysis. Different concentrations of working standards were made for the calibration curve. Exactly 0.5 mL of sample/standard solutions were added to a reagent mixture of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 1% ammonium molybdate. The solution was kept for incubation at 95 °C for 90 min. At room temperature, the absorbance was taken at 695 nm against blank. The total antioxidant activities were expressed as mg of ascorbic acid equivalents (AAE) per g of extract (mg AAE/g extract).

2.10. DPPH radical scavenging capacity

The inhibition activity of methanolic extracts of leaves, flowers, flesh, peel, and the seed of pumpkin was measured by scavenging of 1,1-diphenyl-2picrylhydrazyl radicals (DPPH) following the method narrated by Braca et al. with some modifications [34]. Several concentrations of BHT and extracts of all samples were prepared for the experiment using 80% methanol as solvent. An equal volume of sample/standard and 0.004% DPPH (in 80% methanol) were mixed. After 30 min, the absorbance of the mixtures was taken at 517 nm. The DPPH inhibition percentage was calculated using the following equation:

$$\%\ \text{of DPPH Inhibition} = \left(\frac{Mc - Ms}{Mc}\right) \times 100 \tag{2}$$

where Mc is the absorbance of the control, and Ms is the absorbance of the sample/standard. DPPH scavenging activity is expressed as IC_{50}, the concentration of a sample required to reduce DPPH activity by 50%. The IC_{50} value was determined using the equation of line made by plotting a graph of concentration (ppm) versus % inhibition.

2.11. Estimation of total carotene content

The determination of the total carotene (TCC) content of leaves, flowers, flesh, peel, and the seed was performed according to Panpraneecharoen et al. [35]. Exactly 0.2 g of dry powders of each sample were weighed and then subjected to extract using *n*-hexane as solvent. These were filtered through Whatman No.1 filter paper (Whatman #1444 150, Maidstone, England) and the absorbance of the filtered solutions were recorded at 446 nm through a Spectrophotometer (Shimadzu, Tokyo, Japan, UV-3600i plus). The results were calculated from the following equation:

$$TCC = 25 \times 383\ (As - Ab) / 100W \tag{3}$$

where As is the absorbance of sample, A_b is the absorbance of blank, 25 is the volume of *n*-hexane (mL), 383 is the diffusion coefficient, and W is the weight of dry sample in grams. The data was presented as mg of carotene per kg of dry powder of samples (leaves, flower, flesh, peel, and seed).

2.12. Statistical analysis

Data are expressed as mean ± standard deviation (SD) using three replicates (n = 3) for proximate composition and six replicates (n = 6) for antioxidant properties. Descriptive, variation among determinants using one-way analysis of variance (ANOVA), significant differences at the level of 5% ($p < 0.05$) using Tukey's HSD test, and correlations between TPC, TFC, TAA, TCC, and DPPH results were determined using Pearson Correlation Analysis. All of these analyses were performed using Statistical Package for Social Science (SPSS) (Version 25).

3. Results

3.1. Nutritional composition

The data for the nutritive value of flesh, peel, seed, leaves, and flower of *Cucurbita maxima* is depicted in Table 1 and these were reported based on wet and dry matter. The following results of proximate analysis have been described on dry matter samples. The highest content of moisture was found in the seed whereas no significant difference in moisture content between flower and peel as well as between peel and flesh. On the other hand, leaves had the lowest moisture content. Data for ash content was shown to be significantly ($p < 0.05$) higher in flowers but there was no statistically significant difference between flower and leaves as well as between seed and flesh. The seed had the highest content of crude protein among all other parts of the pumpkin plant. However, the crude protein in leaves and flowers was not statistically different; peel was found to have a considerable amount of crude protein than flesh which had the lowest value. The seed was found to be rich in terms of fat content among other parts of the pumpkin plant. The fat content in flowers and leaves was found to have no significant difference and a similar result was found between leaves and peel. The flesh was found to contain a lower percentage of fat than other parts of the plant. The greatest content of fiber was observed in the seed whereas flesh had the lowest. There was no statistically significant difference in fiber content between the peel and leaves but the flower had a notable amount of fiber. The carbohydrate content was found to be higher in flesh and the least percentage of carbohydrate was found in the seed.

3.2. Mineral contents

Table 2 represents sodium, potassium, calcium, and iron in various parts (flesh, peel, seed, leaves, and flower) of *Cucurbita maxima*. Sodium content in leaves, peel, and flowers showed no significant differences. The flesh contained significantly ($p < 0.05$) the lowest value for sodium. Values for potassium content were found to be significantly ($p < 0.05$) higher in the flower while the seed contained a significantly ($p < 0.05$) lower value. The potassium content in peel and flesh had no significant difference. Leaves of pumpkin plants had the greatest content of calcium whereas no significant difference in calcium content was found among the seed, flesh, and peel. The iron content was significantly ($p < 0.05$) higher in flowers than in other parts of the pumpkin plant and no significant difference in iron content between flesh and seed.

3.3. Amino acid composition

The amino acid composition of these samples based on the dry matter is summarized in Table 3. About seventeen out of twenty types of amino acids in these pumpkin plant parts were recorded. Among these seventeen, there were 9 essential and 8 nonessential amino acids respectively. Altogether, glutamic acid (nonessential amino acid) was the major amino acid found in high concentrations in pumpkin flesh, seed, leaves, and flower. On the other hand, pumpkin peel had the highest value for lysine which is an essential amino acid as compared to other amino acids. A significantly highest value for glutamic acid was recorded in the seed and the lowest value was found in flesh. Among essential amino acids, flesh, peel, and seed contained the highest amount of valine, lysine, and arginine respectively whereas leucine was high both in leaves and flowers. Similarly, other essential amino acids in these samples were present in a noticeable amount. Among nonessential amino acids, aspartic acid was the second major amino acid followed by glutamic acid found in all pumpkin plant parts.

Table 1
The nutritional composition (g/100 g) of various edible parts of the *Cucurbita maxima* plant.

Sample	Moisture		Ash		Protein		Fat		Fiber		Carbohydrate	
	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis
Flesh	86.78 ± 0.73^{a}	7.54 ± 0.26^{c}	0.31 ± 0.00^{d}	3.60 ± 0.03^{c}	0.50 ± 0.05^{d}	5.76 ± 0.52^{d}	0.41 ± 0.01^{d}	4.67 ± 0.6^{d}	0.38 ± 0.01^{e}	4.43 ± 0.09^{d}	6.42 ± 0.07^{s}	74.01 ± 0.77^{a}
Peel	78.34 ± 1.01^{c}	7.95 ± 0.44^{bc}	0.45 ± 0.01^{c}	4.47 ± 0.05^{b}	1.57 ± 0.07^{c}	15.45 ± 0.69^{c}	0.59 ± 0.01^{c}	5.80 ± 0.14^{c}	1.04 ± 0.01^{b}	10.24 ± 0.08^{b}	5.70 ± 0.03^{b}	56.09 ± 0.33^{b}
Seed	63.07 ± 0.84^{e}	11.66 ± 0.24^{a}	0.70 ± 0.01^{b}	3.77 ± 0.06^{c}	4.57 ± 0.39^{a}	24.69 ± 2.13^{a}	6.51 ± 0.20^{a}	35.23 ± 1.08^{a}	2.20 ± 0.01^{a}	11.90 ± 0.05^{a}	2.36 ± 0.35^{d}	12.75 ± 1.87^{e}
Leaves	74.83 ± 0.20^{d}	5.73 ± 0.19^{d}	0.87 ± 0.04^{a}	11.32 ± 0.47^{a}	1.59 ± 0.02^{c}	20.76 ± 0.22^{b}	0.49 ± 0.03^{cd}	6.35 ± 0.43^{bc}	0.78 ± 0.01^{d}	10.18 ± 0.10^{b}	3.50 ± 0.04^{d}	45.66 ± 0.49^{c}
Flower	83.62 ± 0.57^{b}	8.40 ± 0.28^{b}	1.18 ± 0.11^{a}	11.76 ± 1.11^{a}	2.02 ± 0.02^{b}	20.09 ± 0.23^{b}	0.78 ± 0.03^{b}	7.78 ± 0.34^{b}	0.96 ± 0.03^{c}	9.60 ± 0.31^{c}	4.26 ± 0.15^{c}	42.36 ± 1.49^{d}

* Values are presented as mean ± SD (n = 3) which are statistically analyzed by ANOVA to evaluate the property of the sample significantly different at the level of 5% ($p < 0.05$).
Different uppercase letters in the same column indicate that mean values differ significantly.

Table 2
Mineral contents (mg/100 g) of various parts of *Cucurbita maxima*.

Sample	Sodium	Potassium	Calcium	Iron
Flesh	3.71 ± 0.43^{c}	373.86 ± 5.04^{c}	7.09 ± 0.40^{c}	0.17 ± 0.01^{d}
Peel	33.92 ± 0.65^{a}	378.81 ± 3.29^{c}	6.87 ± 0.15^{c}	0.24 ± 0.04^{c}
Seed	10.55 ± 0.78^{b}	138.36 ± 2.43^{d}	7.58 ± 0.49^{c}	0.14 ± 0.00^{d}
Leaves	34.01 ± 1.11^{a}	999.66 ± 4.81^{b}	19.42 ± 0.68^{a}	0.38 ± 0.00^{b}
Flower	32.81 ± 1.89^{a}	1108.13 ± 5.87^{a}	16.22 ± 0.80^{b}	0.64 ± 0.04^{a}

Mineral contents are presented as mean ± SD (n = 3); statistically analyzed by ANOVA to evaluate significant differences at the level of 5% ($p < 0.05$). Different uppercase letters in the same column indicate that mean values differ significantly.

Table 3
Amino acid composition (mg/g) of various parts of *Cucurbita maxima*.

Amino acid	Flesh	Peel	Seed	Leaves	Flower
Aspartic Acid	4.79 ± 0.31^{e}	8.49 ± 0.14^{d}	21.81 ± 0.17^{a}	17.77 ± 0.23^{b}	12.33 ± 0.45^{c}
Threonine	1.86 ± 0.07^{d}	3.44 ± 0.11^{c}	5.86 ± 0.10^{b}	7.11 ± 0.09^{a}	4.23 ± 0.32^{c}
Serine	2.58 ± 0.13^{e}	4.73 ± 0.12^{d}	11.69 ± 0.16^{a}	7.74 ± 0.10^{b}	6.32 ± 0.07^{c}
Glutamic acid	8.54 ± 0.11^{e}	11.48 ± 0.09^{d}	44.79 ± 0.21^{a}	22.69 ± 0.30^{c}	25.78 ± 0.34^{b}
Glycine	2.70 ± 0.13^{e}	4.85 ± 0.11^{d}	19.64 ± 0.18^{a}	9.53 ± 0.28^{b}	6.17 ± 0.21^{c}
Alanine	4.74 ± 0.07^{e}	6.39 ± 0.17^{d}	10.64 ± 0.08^{b}	14.49 ± 0.24^{a}	7.48 ± 0.27^{c}
Cysteine	1.16 ± 0.04^{c}	1.77 ± 0.03^{a}	1.54 ± 0.03^{b}	1.83 ± 0.02^{a}	1.13 ± 0.01^{c}
Valine	5.44 ± 0.23^{e}	7.16 ± 0.29^{c}	10.38 ± 0.15^{b}	11.71 ± 0.17^{a}	6.21 ± 0.42^{d}
Methionine	0.55 ± 0.04^{d}	2.88 ± 0.08^{b}	2.13 ± 0.10^{c}	4.52 ± 0.09^{a}	1.49 ± 0.51^{bcd}
Isoleucine	1.81 ± 0.13^{d}	3.62 ± 0.13^{c}	6.79 ± 0.05^{b}	7.66 ± 0.17^{a}	4.03 ± 0.27^{c}
Leucine	3.66 ± 0.07^{d}	6.99 ± 0.19^{c}	14.78 ± 0.08^{a}	15.26 ± 0.33^{a}	8.05 ± 0.30^{b}
Tyrosine	1.87 ± 0.06^{e}	3.95 ± 0.24^{c}	16.50 ± 0.10^{a}	9.44 ± 0.11^{b}	3.51 ± 0.07^{d}
Phenylalanine	2.32 ± 0.11^{e}	4.64 ± 0.09^{d}	12.04 ± 0.08^{a}	11.54 ± 0.26^{b}	5.39 ± 0.17^{c}
Histidine	2.53 ± 0.29^{e}	5.65 ± 0.20^{c}	7.96 ± 0.06^{a}	6.43 ± 0.21^{b}	4.81 ± 0.10^{d}
Lysine	3.77 ± 0.10^{e}	15.74 ± 0.26^{a}	11.16 ± 0.17^{c}	13.68 ± 0.11^{b}	6.84 ± 0.20^{d}
Arginine	2.94 ± 0.06^{e}	4.65 ± 0.09^{d}	30.87 ± 0.24^{a}	10.26 ± 0.29^{b}	5.32 ± 0.04^{c}
Proline	2.29 ± 0.21^{d}	3.64 ± 0.10^{c}	8.80 ± 0.17^{a}	7.48 ± 0.14^{b}	4.77 ± 0.67^{bcd}

Values are presented as mean ± SD (n = 3); statistically analyzed by ANOVA to evaluate significant differences at the level of 5% ($p < 0.05$). Different uppercase letters in the same row indicate that mean values differ significantly.

3.4. Antioxidant properties

Dried powder of flesh, peel, seed, leaves, and flower of pumpkin plant was used for methanolic extraction and their yield percentages in methanol were 12.02%, 11.73%, 7.12%, 18.64%, and 13.85% respectively. The antioxidant properties of these methanolic extracts were determined. The total phenolic content, total flavonoid content, and total antioxidant activity of methanolic extracts of various parts of *Cucurbita maxima* were shown in Fig. 1.

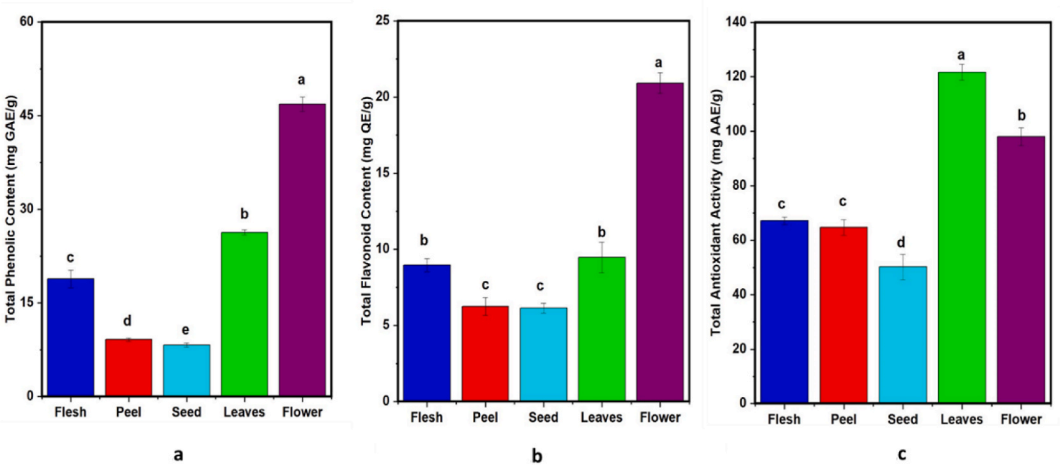

Fig. 1. The total phenolic content (a), total flavonoid content (b), and total antioxidant activity (c) of various parts (methanolic extracts of flesh, peel, seed, leaves, and flower) of *Cucurbita maxima*.

3.4.1. Total phenolic content (TPC)

The total phenolic content (Fig. 1a) was found to be significantly different in each type of methanolic extract. The highest amount of TPC was observed in the pumpkin flower (46.81 ± 1.17 mg GAE/g) while the lowest was in the seed (8.27 ± 0.34 mg GAE/g). The methanolic extracts of leaves (26.31 ± 0.37 mg GAE/g), flesh (18.83 ± 1.42 mg GAE/g), and the peel (9.13 ± 0.26 mg GAE/g) contained a considerable amount of TPC.

3.4.2. Total flavonoid content (TFC)

The highest content of total flavonoid (Fig. 1b) was observed in the methanolic extract of pumpkin flower (20.91 ± 0.67 mg QE/g) which had a significant ($p < 0.05$) difference from others, while the least value was determined in seed (6.14 ± 0.32 mg QE/g) though there was no significant difference in TFC between the seed (6.14 ± 0.32 mg QE/g) and peel (6.25 ± 0.59 mg QE/g). Similarly, TFC in leaves (9.47 ± 1.00 mg QE/g) and flesh (8.98 ± 0.43 mg QE/g) was not statistically different.

3.4.3. Total antioxidant activity (TAA)

The total antioxidant activity (Fig. 1c) was found to be significantly ($p < 0.05$) higher in the methanolic extract of leaves (121.69 ± 2.95 mg AAE/g) as compared to others whereas the lowest amount of TAA was found in seed (50.19 ± 4.64 mg AAE/g). Pumpkin flower extracts (98.08 ± 3.27 mg AAE/g) had an appreciable content of TAA but no significant difference in TAA was found in the flesh (67.17 ± 1.39 mg AAE/g) and peel (64.72 ± 2.82 mg AAE/g).

3.4.4. DPPH radicals scavenging capacity

Antioxidant activity of these extracts analyzed by the DPPH radicals scavenging assay is presented in Fig. 2. From this figure, it is evidenced that DPPH radicals scavenging capacity is increased with the increase in concentration. DPPH radicals scavenging activity of flesh, peel, seed, leaves, and flower extract were expressed as IC_{50} value (concentration of a sample required to reduce DPPH radicals at 50%). Fig. 3 shows the IC_{50} value of BHT, flesh, peel, seed, leaves, and flowers extracts which are as follows: 3.08 ppm, 302.94 ppm, 1337.87 ppm, 1090.07 ppm, 321.22 ppm, and 152.02 ppm. The IC_{50} of pumpkin plant materials suggested that the methanolic extract of flowers showed the highest antioxidant activity against DPPH radicals as compared to others.

3.4.5. Total carotene content (TCC)

The results of the total carotene content of the flesh, peel, seed, leaves, and flower of pumpkin have been shown in Fig. 4. Significantly, a different value for the TCC of each dried powder was observed. The highest value for TCC was found in pumpkin flowers (39.95 ± 3.00 mg/100 g) and the lowest value was recorded in flesh (3.02 ± 0.48 mg/100 g). Pumpkin leaves (32.33 ± 1.62 mg/100 g), seed (5.82 ± 0.51 mg/100 g), and peel (4.54 ± 0.34 mg/100 g) had a noticeable amount of TCC. When flesh, peel, and the seed of mature pumpkin (which were stored for a few months) were analyzed, TCC was increased by 157.62% in the flesh, 589.65% in the peel, and 38.49% in the seed (Table 4).

3.4.6. Correlation analysis

Table 5 represents the Pearson correlation coefficient between TPC, TFC, TCC, DPPH, and TAA of pumpkin flesh, peel, seed, leaves, and flower parts. There was a strong positive correlation ($r = 0.963$) between TPC and TFC with a significant value ($p < 0.01$) and it implies that both have the potential to influence each other. DPPH showed a moderate correlation with TAA ($r = 0.693$), although having a substantial positive correlation with TCC ($r = 0.779$), TFC ($r = 0.921$), and TPC ($r = 0.964$). Both TPC and TFC were also moderately correlated with TAA. Furthermore, the strong positive correlation ($r = 0.856$) between TAA and TCC reported in the study

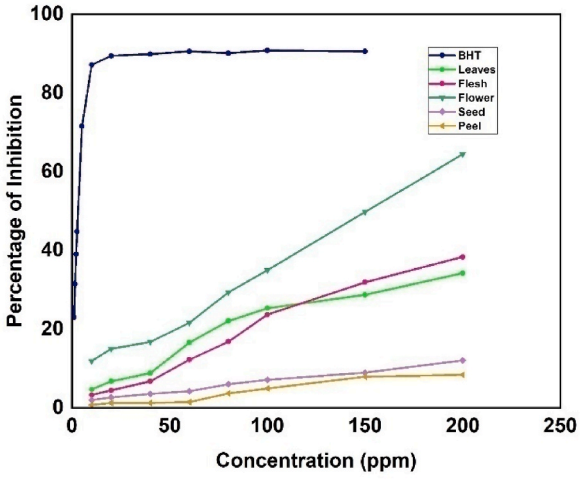

Fig. 2. Percent inhibition of DPPH radicals by BHT and methanolic extracts of flesh, peel, seed, leaves, and flower with concentration.

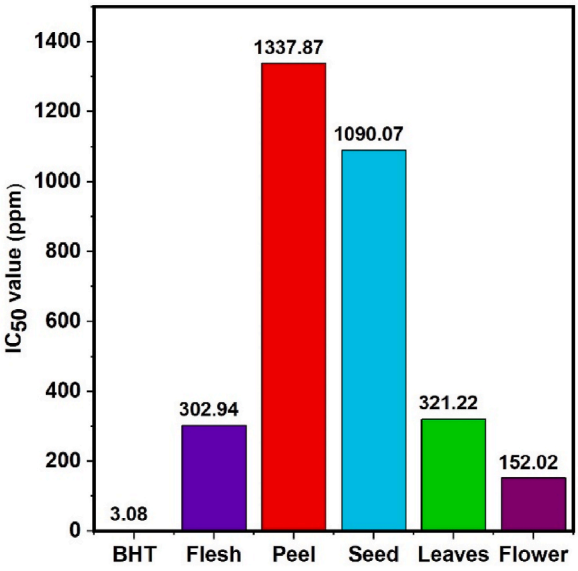

Fig. 3. The IC_{50} value for DPPH radicals scavenging activity of BHT and various parts (methanolic extracts) of *Cucurbita maxima*.

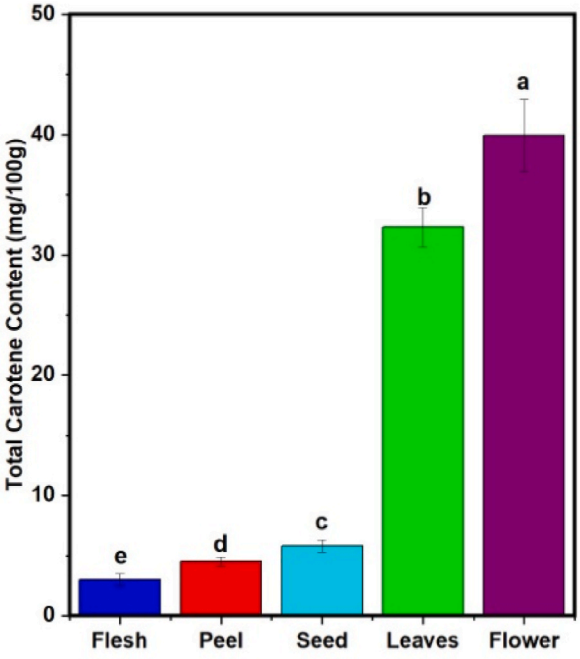

Fig. 4. The total carotene content of various parts of *Cucurbita maxima*.

was also significant ($p < 0.01$). Finally, this result indicates that the antioxidant activities of pumpkin plant parts are directly influenced by the concentration of total phenols, total flavonoids, and total carotenes.

4. Discussion

Both commercially and domestically, pumpkin flesh is more popular for consumption than other parts due to the unavailability of information about them. Bangladesh is a developing country and its geographical condition helps to grow a wide variety of vegetables that cover most dietary processes. Therefore, this study was conducted to explore the nutritional and antioxidant properties of various parts of pumpkin plant materials. Analysis of nutritional composition is important as it is necessary to understand the quality and the health-beneficiary effects of food or food product materials. This study revealed the nutritional composition including proximate

Table 4
The total carotene content of various parts of fruit (flesh, peel, and seed) after storage for a few months.

Sample name	Total Carotene Content (mg/100 g dried powder)
Flesh	7.78 ± 0.45^{b}
Peel	31.31 ± 0.88^{a}
Seed	8.06 ± 0.09^{b}

Values are presented as mean ± SD (n = 6); statistically analyzed by ANOVA to evaluate significant differences at the level of 5% ($p < 0.05$). Different uppercase letters indicate that mean values differ significantly.

Table 5
Pearson correlation coefficient (r) between TPC, TFC, TCC, DPPH, and TAA.

	Correlation Coefficient (r)	*p*-value*
TAA vs TPC	0.693	0.000
TAA vs TFC	0.506	0.004
TFC vs TPC	0.963	0.000
TFC vs DPPH	0.921	0.000
TPC vs DPPH	0.964	0.000
TAA vs DPPH	0.623	0.000
TCC VS DPPH	0.779	0.000
TAA vs TCC	0.856	0.000

Correlation is significant at the (*p*-value*) 0.01 level.

analysis (ash, moisture, protein, fat, fiber, and carbohydrate), mineral contents, and amino acid composition of flesh, peel, seeds, leaves, and flowers parts of the pumpkin (*Cucurbita maxima*) plant. These edible parts of pumpkin might help an individual to meet the daily recommended intake of micronutrients [36]. The investigation found that the seed had the most abundant content of protein, fat, and fiber which shows an accordance with the report of previous studies [12,37]. Similarly, pumpkin leaves, flowers, and peel had a more noticeable profile of nutrients than the most consumed portion of the pumpkin (flesh) plant. The results of the nutritional composition of flesh, peel, and the seed of *Cucurbita maxima* observed in this study are comparable with the results reported by Mohaammed et al. and Kim et al. [5,38]. This investigation observed higher nutritional values for pumpkin leaves than was studied in South Africa and Nigeria [39,40]. The nutrient contents (wet basis) of flowers are comparable with previous studies conducted in India and Mexico [41,42]. Mineral contents are essential in trace amounts for human nutrition and this study found potassium as the most abundant element which was higher in pumpkin flowers. So pumpkin flowers and leaves are rich in minerals as compared to other parts and each part could be able to meet the requirement of daily mineral contents. The reported results of mineral contents show variation from several previous studies [3,4,41,43,44]. The proximate and mineral contents are highly variable due to differences in region, varieties among species, climate conditions, and agricultural practice [3]. Amino acids and other important organic compounds involving many physiological activities in the living system were also investigated in these targeted plant materials. The results of the amino acid composition of pumpkin flesh, peel, seed, and leaves are comparable with some previous studies [3,5,45,46]. It also revealed that the seed had a remarkable profile of amino acids particularly arginine, glutamic acid, aspartic acid, glycine, leucine, cysteine, methionine, phenylalanine, tyrosine, and valine which could be helpful for particular health benefits. Other parts including leaves, flowers, and peel had an appreciable composition of amino acids and these were found to be excellent in comparison with the flesh. Essential amino acid profile of several edible parts (seeds and leaves) might provide the required daily requirements of EEA (by FAO/WHO) for healthy individuals [47]. Furthermore, pumpkin seed, leaves, flowers, and the peel along with the flesh part can be utilized as a protein-rich diet to avert protein-calorie malnutrition commonly apparent in developing countries as well as for complete vegetarians.

A phytochemical study including TPC, TFC, TAA, and DPPH radicals scavenging capacity of these plant materials was also evaluated. This investigation observed that the flower and leaves had a relatively higher amount of TPC, TFC, and TAA. The TPC of pumpkin peel, seed, and leaves are approximately 69%, 74%, and 26% higher than those reported by Dissanayake et al. [48]. Similarly, the TPC of the flesh, peel, and seed is 14-fold, 9-fold, and 3-fold greater than those reported previously [4]. The phenolic content in the methanolic extract of pumpkin flowers was 17.39 μg GAE/ml reported by Ghosh and Rana [41]. All the targeted pumpkin plant parts had a notable concentration of TFC. The TFC of the seed, leaves, peel, and flesh reported in this study shows at least a 2-fold higher value than those cultivated in Sri Lanka and Pakistan [4,48]. The flavonoid content in the aqueous extract of pumpkin flowers was 17.134 μg QE/ml reported by Ghosh and Rana [41]. The leaves had the highest concentration of TAA though other parts had a considerable amount of TAA. The study did not find a good article for justifying the values for TAA as well as the phytochemical compounds in flowers. The content of phenols and flavonoids in plants are affected by some aspects such as differences in plant varieties, their growth, season, weather pattern, light, level of ripeness, food processing, and preparation [49].

Similarly, it is shown that all pumpkin plant parts can scavenge DPPH radicals, and the decreasing order of IC_{50} was peel > seed > leaves > flesh > flower. Previously reported IC_{50} values of the flesh and peel ranged as follows: 1.55–4.01 mg/mL and 1.12–67.64 mg/mL [50]. IC_{50} values for seed and leaves are 1.25% lower and 39% higher than those reported earlier [19,51]. The DPPH radical

scavenging activity of the methanolic extract of pumpkin flowers was 51.65% reported by Ghosh and Rana [41].

Thus, pumpkin flowers and leaves could be a good source of natural antioxidants and pigment. A study reported that phenols and flavonoids are widely distributed in leaves, flowers, stems, and barks [52]. These compounds are also associated with attracting insects for pollination and seed dispersion, giving protection against microbial attacks and regulating hormones [53].

Moreover, analysis of TCC shows that pumpkin flowers and leaves are the major reservoirs of carotenes. An earlier study reported 10.7% higher TCC in fresh *Cucurbita maxima* flowers [41]. The high amount of carotene content is one of the major factors responsible for the bright orange-yellow color of the pumpkin flower [41]. Seroczynska et al. recorded a range of values for total carotenoids content in pumpkin flower and fruit flesh as 1.23–18.79 mg/100 g and 0.07–8.92 mg/100 g respectively; also conclude that there was a strong positive correlation between total carotenoids content and pumpkin flower color [54]. The pumpkin flesh, seed, and peel had a relatively lower amount of TCC. After a few months of storage, the TCC in the flesh, peel, and seed was increased greatly. These data are partially matched with a previous report [4]. Degree of ripeness, maturity, fruit size, and composition of nutrients and minerals are the major responsible factors that influence the carotene contents of fruits [55].

Lastly, Pearson Correlation Analysis was performed to find out the relationship among TPC, TFC, TCC, DPPH, and TAA. The study found that there was a strong positive correlation among these parameters. Although there was a dispute about the consistency of the correlation of TAA with other antioxidant assay methods this result is also in good agreement with the study of Saavedra et al. [56,57]. These findings could be suggested that the presence of phytochemicals and other bioactive compounds in these parts of *Cucurbita maxima* is responsible for antioxidant properties.

Several studies showed that the consumption of pumpkin seeds reduces the risk of cardiovascular diseases, diabetes, and cancers (breast, gastric, lung, and colorectal cancers) [58]. Therefore, various edible parts including flesh, peel, seed, leaves, and flowers of *Cucurbita maxima* could be a rich source of nutrients having the ability to meet the daily dietary requirement as well as can be used as food supplements (reducing oxidative stress) and finally helps in the management of agro-waste utilization.

5. Conclusion

The study revealed a comparative investigation of nutritional values and antioxidant properties of flesh, peel, seed, leaves, and flower of pumpkin and showed that all these parts have an appreciable amount of valuable nutrients and bioactive compounds. So, along with the flesh, other parts of pumpkin (peel, seed, leaves, and flower) have a huge potential to utilize as a health beneficiary food product. Likely, seeds can be used as a protein and fat-rich food supplement; on the other hand flowers and leaves can be used as minerals and antioxidant-rich dietary food products capable of reducing oxidative stress. Finally, the information of this study will help to inspire the cultivar, nutritional practitioner, and mass people to proper utilization of this agro-waste, and industries to formulate functional foods or nutraceuticals.

Author contribution statement

Farhana Jahan : Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Md. Badrul Islama: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Shyama Prosad Moulick, Md Nurul Huda Bhuiyan: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mahci Al Bashera, Md. Sabbir Hasan, Trissa Saha, Farhana Boby, Md. Waliullahm, Anik Kumar Saha, Amin Hossain, Lailatul Ferdousi, Md. Mahmudur Rahman: Performed the experiment.

Nishat Tasnim, Barun Kanti Saha: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

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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 study and also do not have any conflict with any other research work.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16628.

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