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DETERMINATION OF TOTAL PHENOLIC, TOTAL FLAVONOID, ASCORBIC ACID CONTENTS AND ANTIOXIDANT ACTIVITY OF PUMPKIN FLESH, PEEL AND SEEDS

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ABSTRACT. Pumpkins are increasingly regarded as functional foods since they contain valuable nutrients and bioactive substances that have positive health effects. The number of bioactive components depends on the origins and parts of the pumpkin, which define its antioxidant capacity. This study evaluated the total phenolic, total flavonoid, and ascorbic acid content and antioxidant activities of three parts of pumpkin from four different sample origins and investigated the correlation between them. The content of bioactive components in pumpkin from Dukem, Ethiopia, was highest in the flesh, peel, and seeds compared to the other sample areas. The Pearson correlation result indicated a strong relationship between the total phenolic, flavonoid, and ascorbic acid contents of pumpkin and their respective antioxidant activities. This study found that the total phenolic, total flavonoid, and ascorbic acid contents and antioxidant activity varied across the pumpkin sample sources and pumpkin parts.

KEY WORDS: Pumpkin, Flavonoid, Phenolic, Ascorbic acid, Antioxidant activity, Ethiopia

INTRODUCTION

Researchers in food science and technology have recently been highly interested in the study of bioactive compounds. Various studies have noted their antioxidant properties, availability in diets, and potential role in avoiding fatal diseases such as cancer, neurological disorders, and cardiovascular problems [1, 2]. Every day, a wide range of foreign substances are introduced to our bodies. The majority of them are produced by humans, and because we are unable to adequately metabolize them, free radical generation negatively impacts our health [3]. Food consumption directly affects how well we feel, how happy we are, and how much hunger, we experience [2]. Maintaining a healthy diet requires paying close attention to the composition and content of plant-based foods. Nowadays consumers are adopting a more environmentally friendly lifestyle by opting to the plant-based foods [2, 4].

Our health is dependent on the antioxidant-containing substances we consume on a daily basis [5]. Antioxidants reduce the risks of many diseases associated with affluence, including osteoporosis, diabetes, cancer, and neurodegenerative illnesses like Alzheimer's and Parkinson's disease. Cardiovascular diseases, such as atherosclerosis, hypertension, heart attacks, and stroke, are other diseases associated with affluence [5]. Natural antioxidants present in many vegetables prevent the negative effects of oxidative stress. Ascorbic acid, flavonoids, and phenolic compounds found in these vegetables reduce oxidative stress and serve as free radical scavengers. They can also be used to treat a number of dangerous human disorders [4].

Pumpkin is considered a useful vegetable crop due to its nutritional value (containing bioactive substances, vitamins, essential fatty acids and essential minerals), which has a positive impact on the body [4-7]. Pumpkin can be consumed fresh or cooked and added to juices, soups, and smoothies. Bread, cakes, cookies, chocolates, and candies are all made from pumpkin flesh [2].

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Pumpkins' different parts are a good source of biologically active compounds like volatile compounds, vitamins, phenolics, flavonoids, and carotenoids [2, 4, 6-9]. The physiologically active form of vitamin C is L-ascorbic acid. Because of its anti-inflammatory and healing qualities, it is a valuable food ingredient [6]. Simple phenols, phenolic acids, flavonoids, lignins, and tannins are phenolic compounds found in plant tissues as secondary metabolites. Color, bitterness, acidic taste, flavor, smell, and antioxidant capacities have been associated with these compounds [1, 10]. It is believed that bioactive compounds in pumpkin have a protective role against many diseases, including hypertension, diabetes, cancer, and coronary heart disease [6]. This is especially significant in poor nations like Ethiopia, where the health care is prohibitively expensive [11]. Therefore, the determination of the bioactive compound content in pumpkin is very important for dietary guidance and the assessment of pumpkin quality.

Different researchers have determined the content of total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid (AA), and antioxidant capabilities of pumpkin. Rakass *et al*. [4] have investigated a comparative evaluation of total phenolic content, total flavonoid content, and antioxidant activity in peel and flesh extracts of *Cucurbita maxima*. The phytochemicals in pumpkin variants from the *Cucurbita moschata* and *Cucurbita pepo* species have been evaluated for their antioxidant potential [2]. The total potential phenolic, flavonoid, and antiradical content of the pumpkin extracts and fractionated leaves was identified by Zubaydah *et al*. [9]. Researchers have evaluated the total amount of polyphenols and antioxidant activity in pumpkin peel and flesh, which were produced as processing waste [12]. Antioxidant properties of two types of pumpkin extracts (*Cucurbita maxima* and *Cucurbita moschata*) were also compared [5]. According to Mokhtar *et al*. [13], phenolic acids and flavonoids were identified in pumpkin (*Cucurbita moschata*) at different ripening stages (young, mature, and ripened) to determine its antioxidant and antimicrobial activities. According to the results, phenolic acids and flavonoids were dependent on the maturity stage. The mature fruits contain the highest total phenolic and flavonoid contents. The polyphenol extract of the mature fruits showed the highest antioxidant activity. Numerous studies have demonstrated that several factors, such as plant genetics, parts of the plant, growing conditions (temperature, light, water, soil type, and mineral nutrients), physiological development, and extraction solvent, can affect the TPC, TFC, AA content, and antioxidant properties of pumpkin [5, 9, 12, 13]. In a previous study [6], samples from a local market in Addis Ababa, Ethiopia, were used to determine the ascorbic acid content. Nevertheless, the pumpkin sample used in the present study was collected from four different sample origins.

However, to the best of our knowledge, there is no reported data on the content of phenolic and flavonoid, and the antioxidant activity of pumpkin (flesh, peel, and seeds) from different locations of Ethiopia. Therefore, the objective of this study is to determine the phenolic, flavonoid, and ascorbic acid content and antioxidant activity of aqueous extracts of flesh, peel, and seed parts of pumpkin collected from different sample origins (Dukem (DM), Debire Birhan (DB), Metehara (MT), and Bulga (BG), Ethiopia), as well as the level of correlation between phenolic, flavonoid, and ascorbic acid content and antioxidant activity.

EXPERIMENTAL

Chemicals and reagents

Folin-Ciocalteu phenol, gallic acid, quercetin, L-ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were all purchased from Sigma-Aldrich, Germany. Sodium carbonate, aluminum chloride, potassium acetate, and ethanol were purchased from BDH Poole, England. All the chemicals used were of analytical grade. Distilled water was used throughout the experiment.

Instruments and apparatus

An electronic balance (ARA520, Ohaus Corp., China) was used to weigh the standard and samples. Dried samples of pumpkin were ground using a mortar and pestle. The mixtures were stirred using a magnetic stirrer (04803-02, USA). The samples were centrifuged (centrifuge machine, 800D, China). Total phenolic content, total flavonoid content, ascorbic acid, and antioxidant activity were measured using a double-beam spectrophotometer with a 1 cm path length quartz cell and a 2 nm resolution (Lambda 950-UV-Vis-NIR, Perkin Elmer, UK).

Sample area description and collection

The pumpkin fruits (*Cucurbita maxima*) were collected from four different sample origins of Ethiopia (Dukem, Debre Birhan, Metehara, and Bulga), in February 2022. Dukem (DM) is a town in central Oromia Region, Ethiopia, and its elevation is 1950 masl. It is located 37 km southeast of Addis Ababa. The settlement of Debre Birhan, which is 120 km to the north-east of Addis Ababa in the North Shewa Zone of the Amhara Region, is situated at an altitude of 2,840 masl. With an elevation of 947 masl, Metehara is situated in the East Shewa Zone of Oromia. It is 187 km from Addis Ababa. Bulga is in the central part of Shewa province. It is a historical region of Ethiopia. With an elevation of 3,006 masl, it now includes the districts of Hagere Mariamna Kesem, Asagirt, and Berehet. To the north of Addis Ababa, it is 373.4 km away.

The pumpkin plant was identified by Dr. Melaku Wondaferash of the National Herbarium of Addis Ababa University. As *Cucurbita maxima Duchesne* ex L, family name *Cucurbitaceae*. Voucher specimen number MH001 has been deposited in the National Herbarium of Addis Ababa University.

Sample preparation and extraction

The preparation of powdered samples of flesh, peel, and seed parts of pumpkin was carried out according to the method described by Hagos *et al*. [6]. Finally, extraction was carried out on powdered samples.

Total phenol, total flavonoids, and ascorbic acid were extracted in an aqueous solution from powdered samples. 1 g of powdered pumpkin peel, flesh, and seed from each of the four sample origins was extracted in 50 mL of distilled water, using a magnetic stirrer. The mixture was constantly swirled for 2 h at room temperature. After being filtered, the solution was centrifuged using the method described by Hagos *et al*. [6]. The residue was thrown away, and the supernatant was collected in vials.

Determination of total phenolic content from pumpkin flesh, peel, and seed

The total phenolic content (TPC) of each extract of the peel, flesh, and seed parts of pumpkins collected from four different parts of Ethiopia were determined using the Folin-Ciocalteu method as described by Mala and Kurian [12]. The absorbance was measured using UV-VIS spectrophotometer at 650 nm against a blank. The content of total phenolic components in the extract was determined using a linear equation based on the calibration curve.

Determination of total flavonoid content from pumpkin flesh, peel, and seed

The total flavonoid contents of sample were determined using the method described by Zubaydah *et al*. [9]. The absorbance was measured by UV-VIS spectrophotometer at 430 nm against a blank. Total flavonoid contents in the extract was calculated as milligrams of quercetin equivalent (QE) per 100 g of sample (mg QE/100 g).

Ascorbic acid determination from pumpkin flesh, peel, and seed

The sample preparation of standard ascorbic acid were made using the method described by Hagos *et al*. [6]. In this study, the content of ascorbic acid in aqueous extracts of pumpkins was determined. The absorption spectral data were collected at 265 nm, which is their typical absorption peak maximum according to the method developed by Hagos *et al*. [6]. The ascorbic acid content was calculated using the calibration curve, prepared from L-ascorbic acid.

DPPH radical-scavenging activity of pumpkin flesh, peel, and seed

The method described by Zubaydah *et al*. [9] was followed to determine the DPPH radical scavenging activity of each extracts of peel, flesh, and seeds of pumpkin. The absorbance was measured at 517 nm using the UV-VIS spectrophotometer method.

Statistical analysis

All statistical analyses were undertaken using Origin 6.0 (Microcal Software, Inc., Northampton, USA). The differences between the means were evaluated by subjecting the data to a one-way analysis of variance (ANOVA). A Pearson correlation analysis between antioxidant activity and total phenolic, total flavonoid, and ascorbic acid content was also performed.

RESULTS AND DISCUSSION

Determination of total phenolic content (TPC), total flavonoid content (TFC), and ascorbic acid content (AAC) of pumpkin

The total phenolic contents of aqueous extracts of flesh, peel, and seed of pumpkins collected from four different sample origins of Ethiopia (Dukem, Metehara, Debire Birhan, and Bulga) were determined by the Folin–Ciocalteu method using gallic acid as the standard. The pumpkin extracts (flesh, peel, and seed) and gallic acid UV-VIS spectra are displayed (Figure 1A). To demonstrate the presence of gallic acid in the pumpkin extracts, the spectra of gallic acid and sample extracts were overlaid (Figure 1A). This was consistent with the reported wavelength (650 nm) utilized by Sankhalkar and Vernekar [14] to determine the TPC.

In another research, Mala and Kurian [12] used a spectrophotometer to determine TPC at 675 nm. According to Zubaydah *et al*. [9], TPC was also determined at 750 nm. The wavelength variance between the present study and previously reported studies may be influenced by the sample preparation as well as the extraction solvent.

The total flavonoid content of pumpkin peel, flesh, and seed extracts from Dukem, Metehara, Debire Birhan, and Bulga was determined using a colorimetric aluminum chloride method. UV-VIS spectra of quercetin and pumpkin (flesh, peel, and seed) extracts were scanned from 300 to 550 nm, with the maximum absorption occurring at 430 nm (Figure 1B). This was consistent with the reported wavelength (430 nm) used by Zubaydah *et al*. [9] to determine TFC. The overlay of spectra (Figure 1B) demonstrated the presence of quercetin in the pumpkin extracts. The differing chemical conditions between the sample extracts and the reference solution may be the cause of the change in maximum absorption and curve shape.

Samples of pumpkin flesh, peel, and seeds were collected from different sample sources, and the ascorbic acid level in each was determined using the UV-VIS method, which was established by Hagos *et al*. [6], and the maximum absorption was seen at 265 nm in the UV-VIS spectrum.

The regression equation of the calibration curve ($y = 0.01004x-0.0029$) in Figure 2 (A, B) was used to calculate the total phenolic content of the extracts, which was then represented as mg of gallic acid equivalents (GAE) per 100 g of sample in dry weight (mg/100 g). The linearity of the

method was evaluated over the range of 1 to 50 mg/L, and the results were good, with a regression coefficient of $R^2 = 0.9998$.

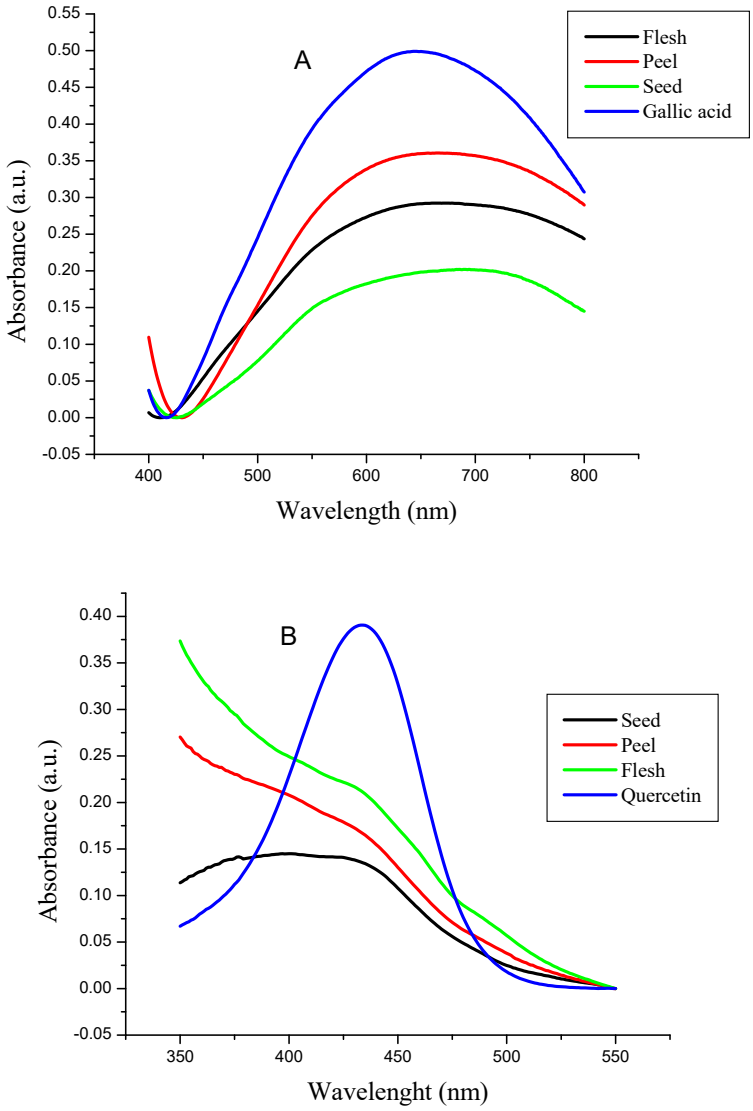


Figure 1. UV-VIS spectra of A) standard gallic acid and extracts of the flesh, peel, and seed parts of pumpkin and B) standard quercetin and extracts of the flesh, peel, and seed parts of pumpkin.

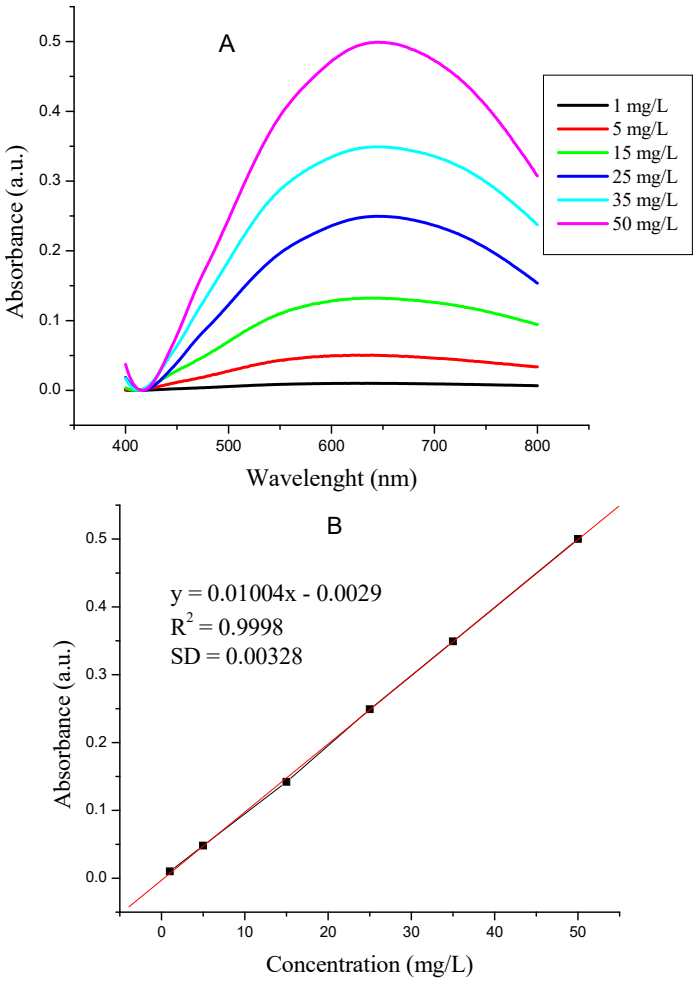


Figure 2. Standard gallic acid in water at various concentrations (1 to 50 mg/L): UV-VIS spectra (A) and calibration curve (B).

The hydroxyl groups in phenolic compounds facilitate free radical scavenging. They are significant plant ingredients with redox characteristics that also impart antioxidant action. They are thought to be responsible for a significant amount of the antioxidant capacity in many plants [15]. The total phenolic content of pumpkin peel, flesh, and seed samples ranged from 354±1.4 to 385±2.1 mg GAE/100 g, 288±0.8 to 369±1.8 mg GAE/100 g, and 80.6±0.4 to 102±1.2 mg GAE/100 g, respectively (Table 1). According to the findings of the presence study, each sample extract of peel had the highest TPC value, followed by its flesh, and its seeds had the lowest value. The TPC value in pumpkin peel and flesh samples collected from Dukem had the highest total phenolic content, followed by Metehara, Debire Birhan, and Bulga. One-way ANOVA statistical analysis revealed a significant difference ($p < 0.05$) in the phenolic contents of the flesh, peel, and seed parts of pumpkin samples collected from the four locations. These variations might be related to geographic and environmental factors as well as different parts of the pumpkin.

The total phenolic content of pumpkin peel, flesh, and seed extracts obtained in the present study was higher than the value reported for pumpkin flesh by Kulczyski *et al*. [2], pumpkin peel, and flesh by Ali and Naz [16], but was lower than pumpkin peel and pulp extracts reported by Mala and Kurian [12]. The TPC reported by Mala and Kurian [12] supported our research work by confirming that pumpkin peel contains more total phenolic content than flesh. However, Hussain *et al*. [8] reported higher TPC in pumpkin seed, followed by flesh and peel. The variety of plant parts, growth circumstances, environment, processing, and analytical methods could all be considered in explaining the considerable variances in the phenolic contents of distinct medicinal plants [15].

The total flavonoid content of each extract was determined using the calibration curve regression equation (y = 0.00688x-0.0054) and reported as mg quercetin equivalents (QE) per 100 g of sample in dry weight (mg QE/100 g). The linearity of the method was evaluated in the range of 5 to 100 mg/L, and the results were good, with a regression coefficient of $R^2 = 0.9995$ in Figure 3 (A, B).

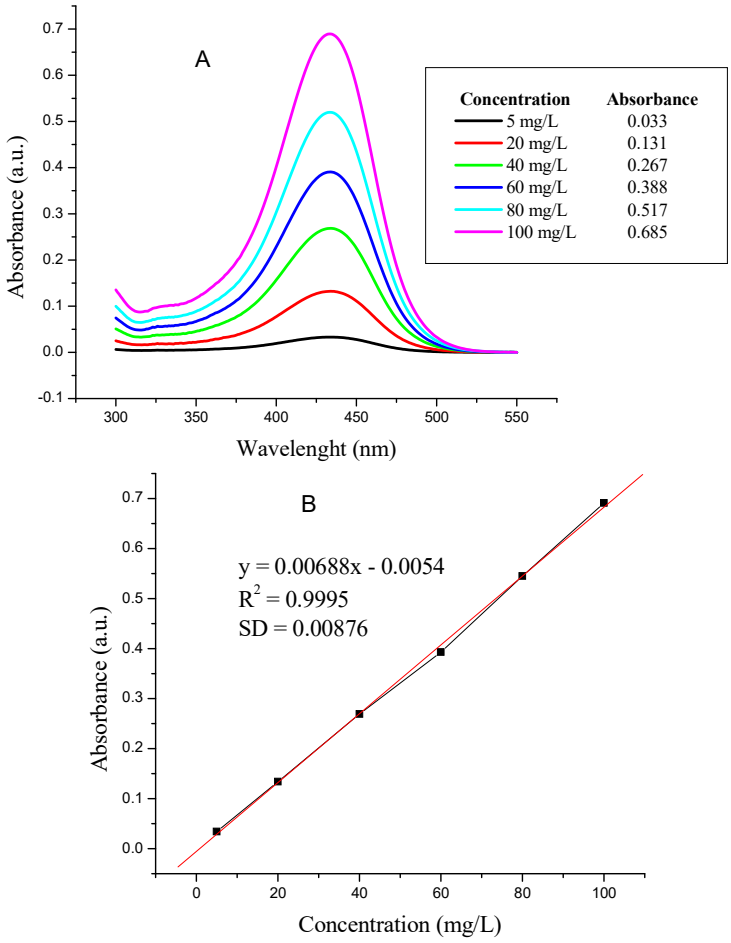


Figure 3. Standard quercetin in water at various concentrations (5 to 100 mg/L): UV-VIS spectra (A) and calibration curve (B).

One of the main classes of phenolic compounds are flavonoids, which have a variety of chemical and biological functions, especially those that scavenge free radicals [15]. The total flavonoid contents in this study followed the same general pattern as the total phenolic values. The concentrations of total flavonoids in the pumpkin peel, flesh, and seed extracts of each sample ranged from 130±1.7 to 153±1.1 mg QE/100 g, and 103.±1.3 to 118±0.9 mg QE/100 g and 51.1±0.5 to 67.4±0.2 mg QE/100 g, respectively (Table 1). Among the collected pumpkin samples, the highest concentration of total flavonoids was found in the peel extract from Dukem, whereas the lowest was found in seed extracts from Metehara sample. One-way ANOVA statistical analysis revealed a significant difference ($p < 0.05$) in the flavonoid contents of the flesh, peel, and seed parts of pumpkin samples collected from four locations. These variances could be attributed to the pumpkin's different sections as well as regional and environmental influences.

Jang *et al*. [17] examined the total flavonoid concentrations in the peel, flesh, and fiber of freeze-dried pumpkin powder, finding values of 81.5, 38.7, and 67.9 mg/100 g powder, respectively. These findings confirmed that pumpkin peel had a higher total flavonoid concentration than flesh, which supported our investigation. The quantity of total flavonoids in the pumpkin peel, flesh, and seed was quantified by Hussain *et al*. [8] and reported the total flavonoid content of pumpkin peel, flesh, and seed to be 45.0, 77.1, and 139 mg QE/100 g, respectively. Results of the present study are different from those reported by Hussain *et al*. [8]. This variation may be due to the type of pumpkin used, the extraction agents used, the climatic and agricultural conditions of the fruits, and the storage of the fruits after harvest.

The ascorbic acid contents of each extract were calculated from the regression equation of the calibration curve ($y = 0.06609x–0.00777$) according to the method developed by Hagos *et al*. [6] and expressed as mg of ascorbic acid equivalents (AAE) per gram of sample in dry weight (mg/100 g).

The pumpkin peel and flesh from Dukem (37.6±1.7 and 34.4±2.2 mg/100 g) and Metehara (35.8±0.8 and 34.4±1.5 mg/100 g), as well as the seeds from Debire Birhan (36.4±0.9 mg/100 g) and Bulga (34.4±1.3 mg/100 g), had the highest ascorbic acid values. The lower ascorbic acid value was obtained in the pumpkin seed from Metehara (21.2±0.5 mg/100 g) (Table 1). A one-way ANOVA revealed statistically significant differences ($p < 0.05$) in the ascorbic acid contents of pumpkin flesh, peel, and seeds collected from four different locations.

Amin *et al*. [18] used the HPLC method to assess the ascorbic acid concentration in samples of pumpkin peel, flesh, and seed from native and hybrid kinds. The pumpkin seed had the highest ascorbic acid content, followed by the flesh and peel of the native pumpkin. However, the ascorbic acid content of the flesh of the hybrid pumpkin cultivar was higher than that of the pumpkin's seeds and peel. In a previous study [6], samples from a local market in Addis Ababa, Ethiopia, were used to determine the ascorbic acid content, and it was found that pumpkin seeds had the highest content, followed by the flesh and peel. The maturity, environmental, and agricultural circumstances of the fruits as well as how they are stored after harvest may have an effect on this variety.

DPPH radical scavenging activity of aqueous extracts of pumpkin flesh, peel and seed

The existence of bioactive chemicals in plants is largely responsible for their antioxidant activity. The DPPH assay, which is widely used for its efficiency and speed, was employed to assess the radical scavenging activity of fruit and vegetable extracts [15]. A DPPH radical scavenging method was used in this study to evaluate the antioxidant properties of pumpkin flesh, peel, and seed aqueous extracts obtained from various sample origins. All the extracts showed a concentration-dependent increase in radical scavenging activity.

Table 1. Total phenolic content (TPC) (mg GAE/100 g), total flavonoid content (TFC) (mg QE/100 g), and ascorbic acid content (AAC) (mg AA/100 g) of aqueous extracts of different parts of pumpkin from different sample areas.

Sample area	Content of the bioactive compounds in different parts of pumpkin								
	TPC			TFC			AAC		
	Flesh	Peel	Seed	Flesh	Peel	Seed	Flesh	Peel	Seed
DM	369±1.8	385±2.1	96.5±1.1	106±0.9	153±1.1	58.5±0.7	37.6±1.7	34.4±2.2	30.2±1.5
DB	319±1.5	354±1.4	102±1.2	103±1.3	130±1.7	67.4±0.2	24.2±0.7	25.2±0.5	35.4±0.9
MT	362±1.3	371±1.5	80.6±0.7	118±0.9	149±1.5	51.1±0.5	35.8±0.8	34.4±1.5	21.2±0.5
BG	288±0.8	360±1.4	95.6±0.4	113±0.7	152±1.4	56.3±1.1	27.2±0.8	32.8±2.1	34.4±1.3

Dukem (DM), Debre Birhan (DB), Metehara (MT), and Bulga (BG).

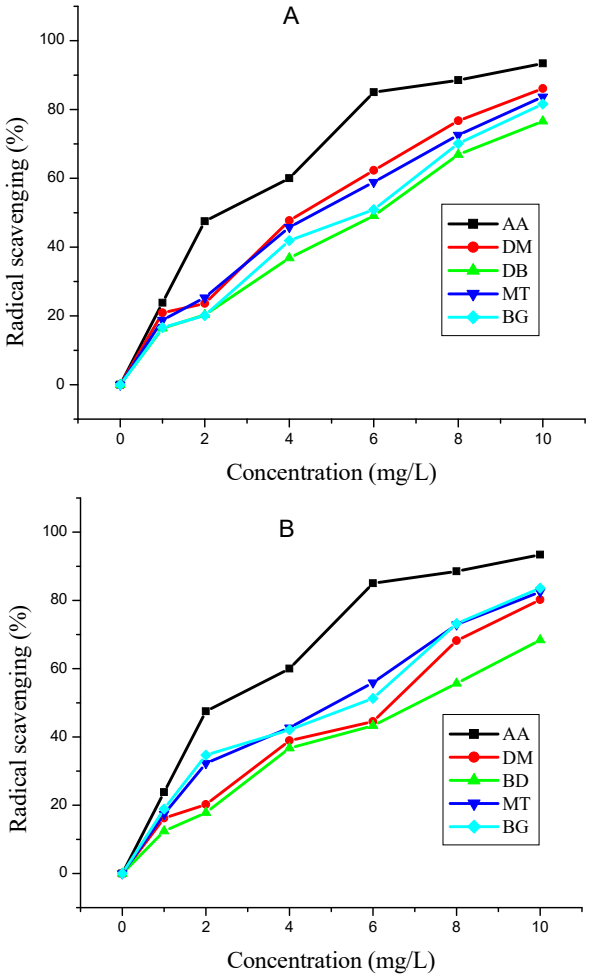
Figure 4 (A, B, and C) depicts the percentage inhibition at various extract concentrations of the flesh, peel, and seed parts of pumpkin from four sample origins. The reducing power of these extracts was calculated based on the concentrations that provided 50% inhibition (IC_{50}), or the quantity needed to scavenge 50% of DPPH free radicals. The value of antioxidant strength (IC_{50}) was calculated based on the linear regression equation between % inhibitions and the concentration of the sample. The regression equation ($y = ax + b$) for each sample extract is reported in (Table 2). Then, the y value was replaced by 50, where IC_{50} was defined as the concentration of the sample needed to inhibit 50% of DPPH radicals [9]. The higher IC_{50} value indicates lower radical scavenging activity or lower antioxidant potential.

In the present study, the IC_{50} values of the pumpkin flesh, peel, and seed extracts from four sample origins were found to be in the range of 4.98 to 6.05 mg/L, 5.28 to 6.95 mg/L, and 5.39 to 9.32 mg/L, respectively (Table 2). The standard ascorbic acid IC_{50} value was 3.66 mg/L. The Dukem sample from pumpkin flesh components exhibited the highest ability to scavenge DPPH radicals (IC_{50} = 4.98 mg/L) compared to ascorbic acid. Compared to the three parts of pumpkin and four sample origins, the lowest antioxidant potential was found in the seeds of the Metehara sample origin (IC_{50} = 9.32 mg/L). A one-way ANOVA revealed that there were statistically significant differences ($p < 0.05$) in the DPPH radical scavenging activity of the flesh, peel, and seed parts, respectively, of the pumpkins collected from four different locations.

Table 2.Regression equation, R^2 and IC_{50} (mg/L) of aqueous extracts of different parts of pumpkin from different sample areas and standard ascorbic acid.

Sample area	Different parts of pumpkin	Regression equation	R^2	IC_{50} (mg/L)
Dukem (DM)	Flesh	y = 8.19 + 8.39x	0.98546	4.98
	Peel	y = 4.51 + 7.63x	0.99061	5.96
	Seed	y = 6.43 + 6.13x	0.98354	7.11
Debire Birhan (DB)	Flesh	y = 5.13 + 7.42x	0.99369	6.05
	Peel	y = 4.56 + 6.53x	0.99138	6.95
	Seed	y = 7.14 + 7.94x	0.98982	5.39
Metehara (MT)	Flesh	y = 8.00 + 8.03x	0.98782	5.23
	Peel	y = 8.92 + 7.78x	0.98372	5.28
	Seed	y = 7.96 + 4.51x	0.9657	9.32
Bulga (BG)	Flesh	y = 5.19 + 7.89x	0.99256	5.68
	Peel	y = 9.51 + 7.65x	0.9779	5.29
	Seed	y = 8.64 + 5.72x	0.96773	7.23
Standard	Ascorbic acid	y = 17.3+ 8.92x	0.9399	3.66

Dar *et al*. [19] analyzed the effect of the solvent used on the antioxidant activity of extracts from *Cucurbita pepo* L. leaves. They observed that extracts of ethyl acetate (79.4%), n-butanol (68.9%), and water acetate (59.9%) showed the highest ability to inhibit the activity of DPPH radicals. The weakest antiradical properties were found for chloroform (47.5%) and n-hexane extracts (40.5%). Telesiski *et al*. [20] analyzed the antioxidant properties of the flesh of four pumpkin cultivars belonging to *Cucurbita moschata*: "Kurinishiki," "Butternut Rugosa," "Muscade de Provence," and "Muscatna." The highest ability to scavenge DPPH radicals was characteristic of the Kurinishiki cultivar (31.4% inhibition). The lowest antioxidant potential was found in the "Muscatna" cultivar (17.4%). The findings of the present study are in agreement with those of Saavedra *et al*. [21], who observed that about 2–3 times higher antioxidant activity was characteristic for extracts prepared from pumpkin peel than from seeds.



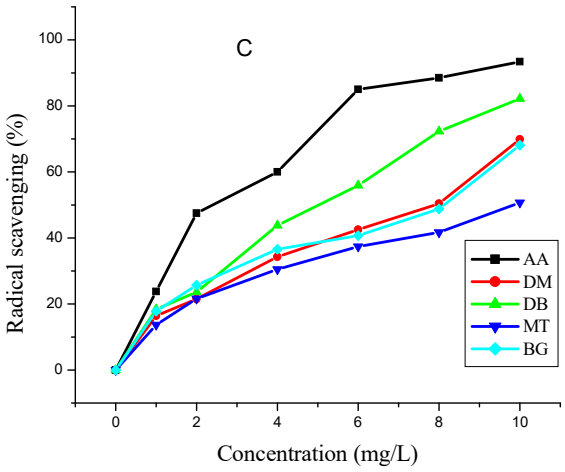
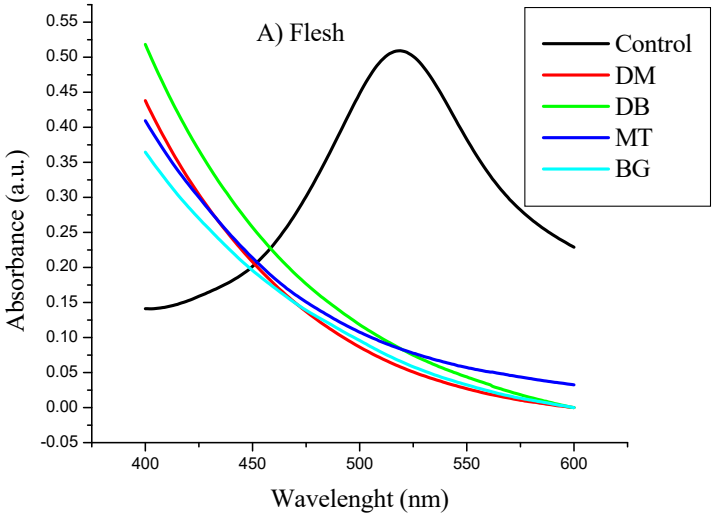


Figure 4. Percent radical scavenging of different concentrations of each pumpkin extract and standard ascorbic acid in the A) flesh, B) peel, and C) seed. AA = ascorbic acid, DM = Dukem, DB = Debire Birhan, MT = Metehara, BG = Bulga.

Comparisons of antioxidant activities of pumpkin flesh, peel, and seed extracts from different sample origins at a concentration of 10 mg/L

The antioxidant activities of pumpkin flesh, peel, and seed extracts of Dukem, Metehara, Debire Birhan, and Bulga sample origins were compared at the same concentration (10 mg/L) in Figure 5 (A, B, and C).



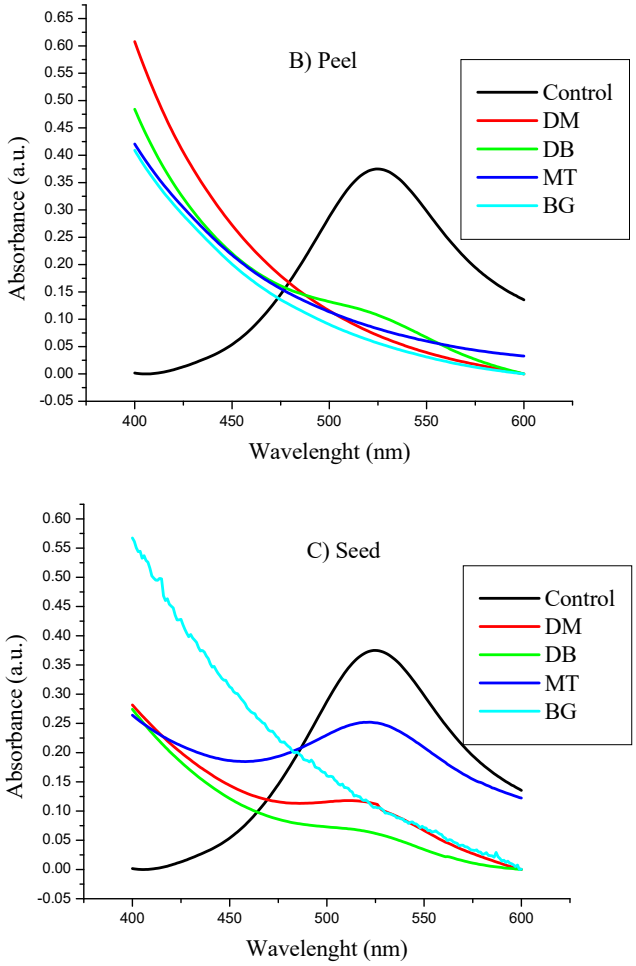


Figure 5. Comparisons of radical scavenging activity of pumpkin flesh, peel, and seed extracts in each sample area at (10 mg/L) A) peel, B) flesh, and C) seed. (DM = Dukem, DB = Debire Birhan, MT = Metehara, BG = Bulga).

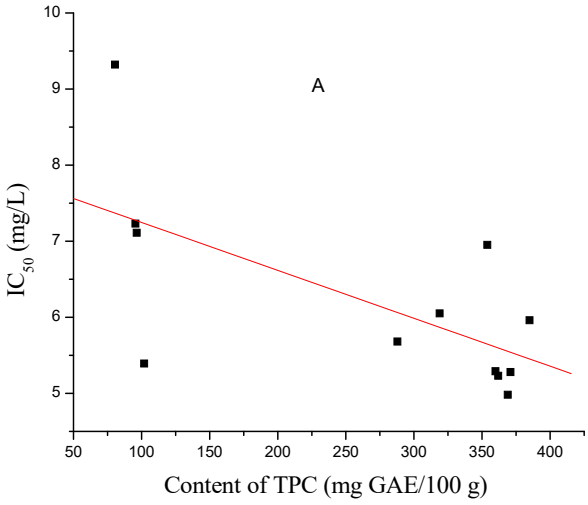
From the given concentration (1 to 10 mg/L), 10 mg/L was selected for comparison of the antioxidant activities of pumpkin peel, flesh, and seed from four sample origins. The sample extract with higher antioxidants indicates lower absorbance compared to the control absorbance (DPPH absorbance). No absorbance means all DPPH is consumed by the sample extracts, which have higher antioxidant activity. The flesh of the pumpkin sample from Dukem indicated high antioxidant activity, followed by Bulga (Figure 5A). The pumpkin peel sample from Bulga seed have the highest antioxidant activity, followed by Dukem (Figure 5B). The antioxidant activity of pumpkin seed from Debire Birhan was found to be higher than the other sample origins (Figure 5C). Carotenoids, polyphenolic compounds, vitamins, and mineral components are all abundant in the flesh, peel, and seed parts of pumpkins. The pumpkin cultivar, the extraction methods employed, the climatic and agricultural circumstances of the fruits, as well as how the fruits are

stored after harvest, all have an impact on the content of bioactive compounds, which affect the fruits' antioxidant qualities [2, 8, 9]. The findings of this study supported the wide range of origins of pumpkins in terms of their antioxidant activity. Additionally, it was discovered that the various pumpkin components have a greater impact on the antioxidant activity.

The results of present study were compared to information that had already been published [19, 20]. As a result, the determination of the antioxidant potential of various pumpkin sections obtained from various sample locations in this study was more effective than earlier studies.

Correlation of total phenolic, flavonoid and ascorbic acid of pumpkin flesh, peel and seed with their IC_{50} of DPPH scavenging activities

Based on the findings, a correlation analysis was carried out to see whether there was any correlation between the antioxidant properties of the various pumpkin sections collected from four different sample locations. The graph of the IC_{50} of the DPPH-scavenging activities of pumpkin flesh, peel, and seed versus total phenolic, flavonoid, and ascorbic acid is presented in (Figure 6). The total phenolic, flavonoid, and ascorbic acid contents of pumpkin were correlated with their IC_{50} DPPH scavenging activities using Pearson's correlation analysis at $r^2 = 0.333$ to 0.972, $r^2 = 0.351$ to 0.969, and $r^2 = 0.867$ to 0.985, respectively (Table 3). In all pumpkin parts, there was a very strong negative correlation between ascorbic acid and IC_{50}. The link between TPC and IC_{50} in the pumpkin's flesh and seeds showed a very high negative correlation, while the peel showed only a mild negative correlation. When TFC in pumpkin peel and seed parts was correlated to IC_{50}, the results showed a very significant negative association, whereas the results for the flesh showed a somewhat negative correlation. The conclusion from this experiment is that higher antioxidant properties were indicated by a lower IC_{50} value. When the amount of TPC, TFC, and AA increased, the value of IC_{50} decreased. Pumpkin extracts from the peel, flesh, and seeds are rich in phenolics, flavonoids, and ascorbic acid, which may considerably contribute to their antioxidant benefits. These qualities may have encouraged the use of this pumpkin in certain traditional herbal medicines.



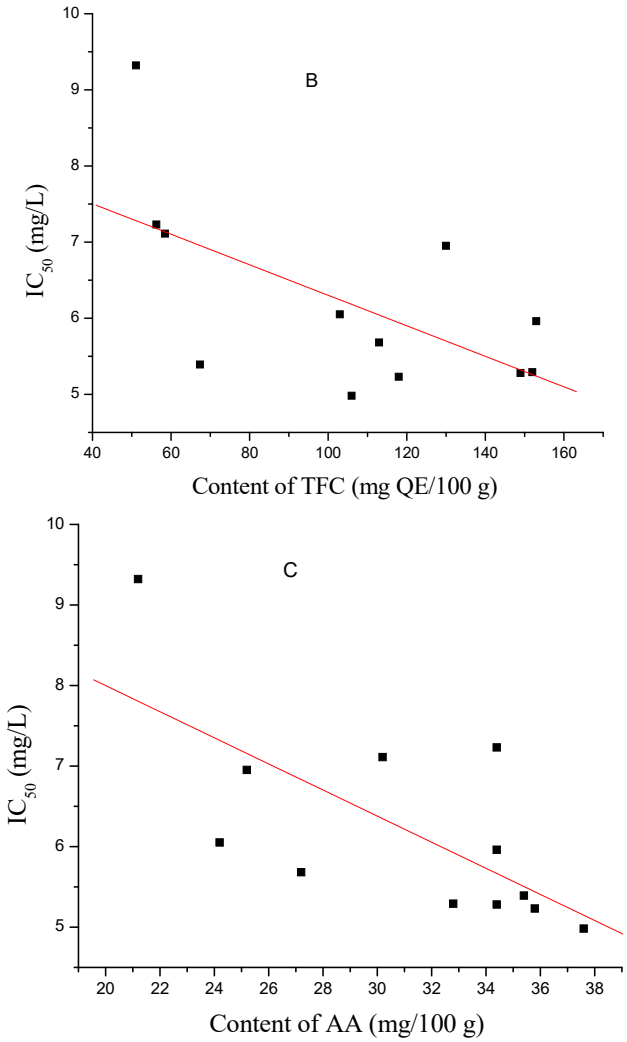


Figure 6. Correlation of IC_{50} of DPPH scavenging activities with A) total phenolic content, B) total flavonoid content, and C) ascorbic acid content in pumpkin flesh, peel, and seed.

Table 3. Correlation of total phenolic, flavonoid, and ascorbic acid with their IC_{50} of DPPH scavenging activities in pumpkin flesh, peel, and seed samples.

IC_{50} (mg/L)	TPC			TFC			AA		
	Flesh	Peel	Seed	Flesh	Peel	Seed	Flesh	Peel	Seed
Correlation (r^2)	0.779	0.333	0.972	0.351	0.858	0.969	0.985	0.867	0.909

TPC = Total phenolic content, TFC = total flavonoid content, AA = ascorbic acid.

CONCLUSION

Pumpkins are one of the widely produced and consumed vegetables in the world. The health benefits of pumpkins are mainly attributed to their phenolic, flavonoid, and ascorbic acid contents. The results of this study support the idea that the pumpkins collected from four different locations are promising sources of natural antioxidants. The total phenolic, total flavonoid, and ascorbic acid content and antioxidant properties of the flesh, peel, and seed extracts of pumpkin differ significantly among all sample areas. The sample from Dukem has the highest total phenolic, total flavonoid, and ascorbic acid content. This sample exhibited the strongest DPPH radical scavenging and total antioxidant capacity. Differences in the TPC, TFC, and AA content and antioxidant properties of different habitats may plausibly be due to geographical variations in chemical constituents. The concentrations of ascorbic acid, total phenols, and total flavonoids in pumpkin flesh, peel, and seeds correlate significantly with antioxidant activity. The results of the present study suggest that the flesh, peel, and seed parts of pumpkin could be a potent source of natural antioxidants because of their phenolic, flavonoid, and ascorbic acid content and their remarkable scavenging effects on DPPH. Therefore, pumpkin fruits are increasingly regarded as "functional foods," since they contain valuable nutrients and bioactive substances that have a number of positive health effects. Consumption of pumpkin is linked, through mechanisms including antioxidant and anti-inflammatory capabilities, with the onset of many diseases or their symptoms.

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