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NUTRITIONAL POTENTIAL, MINERAL COMPOSITION AND ANTIOXIDANT ACTIVITY SQUASH (*CUCURBITA PEPO L.)* FRUITS GROWN IN EGYPT.

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ABSTRACT
The nutritional potential of fruits, fruit pulp and seeds of Squash (*Cucurbita pepo L.*) collected from Giza, Egypt was evaluated for their proximate values and mineral composition. In addition to; the total phenolic and flavonoid contents and antioxidant activities by using (DPPH, ABTS and TAC) of the defatted methanolic extract of fruits, and seeds of (*C. pepo*) were determined. The results indicated that the fruit pulp and fruits have a high content of moisture and crude fibres whereas, the seeds have high protein, fat and ash contents. The fresh fruit pulp and fruits had a high content of β- carotene. Although fruits and fruit pulp contained high amounts of magnesium, potassium, sodium, phosphorus, manganese and calcium but the seeds have highest values. Parallel to the results revealed the seeds have a high content of phenolic and flavonoid and also higher antioxidant activity. The results also showed a clear relationship between total phenolic and flavonoid contents with the antioxidant activity of the seeds and fruits. From the obtained results, the fruits of *(C. pepo*) are good alternative source of nutritional content and essential minerals which required to human for a healthy and active life.

KEYWORDS: antioxidant activity, *Cucurbita pepo*, flavonoid, mineral composition, nutritional potential, Squash.

INTRODUCTION
Consumption of fruits and vegetables has been increased rapidly by people due to awareness regarding their health benefits. So many efforts have been carried out to cultivate of these plants as a food source all over the world. A diet rich in vegetables and fruits can lower blood pressure and the risk of eye and digestive problems as well as reduce the risk of heart and cancer diseases because of it contains a good amount of vitamins and minerals. Also, it protects the human body from oxidative stress and increases the immunity of our bodies due to it has antioxidant properties.[1,2] Requirements of man for vitamin A are not less than of a milligram per day. The major of this quantity come from the green and yellow vegetables such as lettuce, spinach, sweet potatoes, pumpkins and carrots which are rich in carotenes.[1, 2, 3, 4]

The genus Cucurbita is a member of the Cucurbitaceae plant family which includes five major species (*Cucurbita moschata, Cucurbita pepo, Cucurbita argyrosperma, Cucurbita maxima and Cucurbita ficifolia*). Many hundreds of varieties and cultivars of these are grown around the world and are major agricultural commodities. The most important Cucurbit genus members include; gourd, melon, cucumber, squash and .pumpkin are used as food and industrial crops.[5,6,7] Several studies revealed that the fruits of cucurbit plants contain β-carotene. This is important in mitigating the problem of vitamin A deficiency, which is the second most deficient micronutrient after iron that affects the vulnerable group that includes women and children. The fruits and the seeds of these plants also contain substantial amounts of minerals which are important for enzymatic activity and normal physiological function in the human body.[5]

Squash (*Cucurbita pepo L.*) is one of the most popular vegetable crops in Egypt. It is cultivated in Egypt all over the year, outdoor in summer and indoor either in green houses or in tunnels in winter.[8] Squash cultivate for human consumption (cooked flesh). It supplies the man with carbohydrates, soluble fibres, pro-vitamin A and protein.[9,10] Squash plants have health benefits such as anti-diabetic antifungal, antibacterial and anti-inflammation.[9, 10, 11, 12]

The objectives of this study were to determine the proximate composition, β-carotene content, and mineral composition of fruits, fruit pulp and seeds of squash (*Cucurbita pepo*). And also, determination of the phenolic and flavonoid contents as well as the antioxidant properties of the defatted methanolic extract of fruits and seeds of the plant were carried out.

MATERIAL AND METHODS

Plant Materials

The whole of Squash (*Cucurbita pepo L.*) was collected from farmers and markets in Giza, Egypt from March to May 2014. The whole plant was identified by Asst. Prof. Rim Samir Hamdy Professor of Plant Taxonomy, Faculty of Science, Cairo University. The voucher specimens of the plant were stored in Medicinal Chemistry Department, Theodor Bilharz Research Institute. The mature fruits of the plant were washed with distilled water and cut into small species. The seeds were separated and cleaned by removing the pulp using filter paper and air-dried at room temperature. Each of fruits and fruit pulp was ground and stored in a freezer until the time of analysis.

Reagents and standards

DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Sigma -Aldrich Co. (USA), ammonium molybdate, Folin- Ciocalteu's reagent, Ascorbic acid, gallic acid, rutin, aluminum chloride, sodium nitrite, sodium hydroxide. All reagents including solvents were purchased from the scientific company (Sigma; Aldrich) and from other common sources.

Proximate Analysis

Proximate analysis of each of fruits, fruit pulp and seeds of Squash (*C. pepo*) for moisture, crude fat, protein, ash, fibers contents were carried out using standard methods as outlined by Association of Official Analytical Chemists.[13,14] For determination of the percentage carbohydrates the following formula was used:

% Carbohydrates = 100 – (% moisture + % proteins + % fats+ % ash + % fibers).[15, 16]

Beta Carotene

β- carotene of fresh fruits and fruit pulp of Squash (*C. pepo*) were determined using Standard Official Methods of Analysis.[13]

Mineral Composition

The mineral composition of magnesium (Mg), zinc (Zn), iron (Fe), calcium (Ca), copper (Cu) and manganese (Mn) of each fruits, fruit pulp and seeds of Squash (Cucurbita *pepo*) were estimated using inductively coupled plasma atomic emission spectroscopy while sodium (Na) and potassium (K) were determined using emission flame photometer and colorimetric method was used to determine of phosphorus (P). All procedures were carried out using standard methods.[17, 18]

Preparation of plant extract

Each fruit and seed of Squash (Cucurbita *pepo*) was extracted several times with 85% methanol. The methanol extract of each plant part was filtered and concentrated by using rotatory evaporator until drying then each extract was kept in the desiccators until the time for analysis.

Total phenolic content

The phenolic content of the extracts of fruits, and seeds of Squash (C. *pepo*) was determined using spectrophotometric method.[19] The reaction mixture was prepared mixing 0.5ml of sample (methanolic solution of extracts) (250mg/ml); 2.5ml of Folin-Ciocalteu reagent (10%) dissolved in water and 2.5ml $NaHCO_3$ (7.5%). Blank was prepared to contain 0.5ml methanol, 2.5ml of Folin-Ciocalteus reagent (10%) dissolved in water and 2.5ml $NaHCO_3$ (7.5%). The mixture was shaken and incubated at 45°C for 45 min. The absorbance was measured at 765 nm against samples, blank and gallic acid as standard. Estimation of phenolic content was carried out in triplicate. The content of phenolic in extracts was expressed in terms of gallic acid equivalent (GAE) per gram dry weight extract (mg GAE eq. /g of extract).

Total flavonoid content

The content of flavonoids of fruits and seeds of Squash (C. *pepo*) was determined using a colorimetric assay.[20] 0.5 ml of extract was mixed with 2 ml distilled water and 150 µl of $NaNO_2$ (5%) for 6 min, then 150 µl of $AlCl_3$ (10%) was added and allow to stand 5 min then 2ml of NaOH (4%) and adjusted to 5ml with 200 µl distilled water. The mixture was incubated at room temperature for 15 min. the absorbance was measured at 510nm against samples, blank and rutin as standard. Estimation of flavonoid content was carried out in triplicate. The total flavonoid content was estimated as mg rutin equivalents (RE) per gram extract (mg RE eq. /g extract).

Antioxidant Assays

-DPPH radical scavenging activity

The antioxidant activity of fruits and seeds of Squash (C. *pepo*) was evaluated using DPPH (2, 2'-diphenyl-1-picrylhydrazyl) free radical scavenging method.[21] 2 ml of each extract concentration solution was mixed with 2 mL of DPPH in methanol (0.1 m mol/L). The control contained solvent and DPPH except for the extract. The mixtures were shaken well and kept in dark for 30 min at 37˚C. The absorbance was measured at 517nm. Ascorbic acid and vitamin E were used as standards and the experiment was carried out in triplicate. The DPPH scavenging activity of the extracts was calculated as SC_{50} (Concentration of sample required to scavenge 50% of DPPH radicals compared with that of ascorbic acid). The lower SC_{50} value is an indication of higher scavenging activity or higher antioxidant activity of plant extracts.

Scavenging activity % =
[(Blank absorbance - sample absorbance) / (Blank absorbance)] × 100

ABTS radical scavenging Activity

ABTS radical scavenging Activity of fruits and seeds of Squash (*C. pepo*) was evaluated. The method was carried out according to procedure using ABTS (2, 2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid)[22], which acts on the reduction of ABTS radicals by antioxidants of plant extract samples. The concentrated reagent solution was prepared by dissolving 9.6 mg ABTS in 2.5mL water and then adding 110µl of a solution made by dissolving 37.5 mg of potassium persulphate, $K_2S_2O_8$, in 1 mL of water to produce ABTS radical cation. The stock solution was kept in the dark room for 12-16 hours before use; for study the ABTS solution was diluted to an absorbance value between 0.7 and 0.8 at wavelength 734 nm. Subsequently, 100µl of aqueous or alcoholic plant extract (according to solubility) was added to 1 ml of work solution and it was measured exactly after 2 min and 30 sec. Also, an appropriate solvent blank was measured. Calibration curve for ABTS was obtained using (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) Trolox as standard. The experiment was carried out in triplicates. Results were expressed in terms of mm Trolox® equivalent per 100 g dry weight of plant extract.

Total antioxidant activity (TAC)

The total antioxidant capacity of the fruits and seeds of Squash (*C. pepo*) was determined by phosphomolybdate method using ascorbic acid as a standard.[23,24] An aliquot of 0.1ml of sample (200 µg/ml) solution was mixed with 1ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Blank was prepared to contain 1ml of the reagent solution and the appropriate volume of the same solvent used for the samples. The tubes were capped and incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against the blank. The experiment was repeated for 3 times. The antioxidant activity of the extracts was expressed as the number of equivalents (AAE) of mg ascorbic acid per gram extract (mg AAE/ g extract).

RESULTS AND DISCUSSION

Proximate analysis

In the present study the carbohydrates, proteins, moisture, crude fibres, ash and fat of fruits, fruit pulp and seeds of Squash (*Cucurbita pepo)* were determined and the results are presented in **Table (1)**. These results revealed that the fruits, fruit pulp and seeds have a high content of carbohydrates (14.51, 9.22 and 10.93 % respectively). Also, the fruits and fruit pulp have high moisture content (70.25 and 75.59 %) respectively but the seeds have lowest value (5.63 %). On the other hand, the seeds had the the highest content of fat and proteins (33.46 and 39.50 %) respectively. These results reflected that Squash is a very good source of energy due to they have a high content of carbohydrate.[25,26,27, 28] Because of the low content of the crude fat and protein of the fruits and fruit pulp, we could recommended the fruits of Squash as a diet to the fat patients whereas the seeds of Squash can recommend as a protein source for the cases of protein deficiency.[16,18, 29]

It has been reported that although, fibre containing food are known to expand the inside walls of the colon, easing the passage of waste, thus making it an effective anti-constipation, it lowers cholesterol level in the blood and reduce the risk of various cancers. But emphasis has been placed on the importance of keeping fibre intake low in the nutrition of infants and weaning children because high fiber levels in weaning diet can lead to irritation of the gut mucosa.[18,29] In this respect, the crude fibre content of the fruit pulp and fruits (3.65 and 3.17%), which is desirable and could lead to lower digestibility and an overall decrease in nutrient utilization since the fibre slows down the rate at which nutrients enter the blood stream. Also, this value of the fibre could also help to smooth out the ups and downs of blood sugar level and provides more constituent energy throughout the day.[16]

Elinge *et al.*, 2012 reported that the percentage ash of the sample gave an idea of the inorganic content of the samples from where the mineral content could be obtained.[29] In this study as shown in **Table (1)** the ash content of the three tested parts, fruit and fruits pulp and seeds (5.97, 5.29 and 8.89%) respectively. From these results, the seeds have a high content of the ash therefore the ash content of the seeds is expected to have a high concentration of various mineral elements which are expected to speed up metabolic process, improve growth.[25, 26, 29, 30,31] Due to the high content of the moisture of fruits and fruit pulp of Squash (*Cucurbita pepo*), as shown in **Table (1)** (70.25, 75.59 and 5.63%) respectively, therefore the rapid spoilage of fruits or fruit pulp of Squash (*Cucurbita pepo*) can observe. So we need to store the fruit of Squash in cool condition if they are to be kept for a long period without spoilage.[25, 26, 32]

Table (1): Proximate composition of fruits, fruit pulp and seeds of Squash (*Cucurbita pepo*):

Composition(%)	Fruits	Fruit pulp	Seeds
Moisture	70.25	75.59	5.63
Fat	1.65	1.50	33.46
Protein	4.45	4.75	39.50
Ash	5.97	5.29	8.89
Crude fiber	3.17	3.65	1.59
Carbohydrate	14.51	9.22	10.93

The high contents of carbohydrates, moisture, ash and crude fibre, in Squash fruits, have beneficial nutritional potential and furnish the energy. These results are in agreement with several previous studies which revealed that *Cucurbita pepo* species are the most important food because of they have higher content of moisture, carbohydrates, and fat, fiber and energy values as compared to other vegetable species.[25, 26, 32]

Beta-Carotene

Carotenoids are natural components more than 500 types. The most prominent one is β-carotene.[33,34] Carotenoids are a major source of vitamin A which is necessary for normal eyesight, growth, and embryonic development. Vitamin A deficiency is a common cause of blindness and infant mortality.[25, 35] In the present study, the β-carotene content of fruits and fruit pulps of Squash was appeared that the β-carotene content in fruit pulps and fruits of Squash are high (37.83 and 34.75 µg/g) respectively. So, the fruits of Squash are good sources of pro-vitamin A and have antioxidant activity. These results are in full agreement with the reported results[35], which showed that fruit pulps and the fruits of *Cucurbita maxima* have high β-carotene contents. Also, these results agree with Muntean[34] that proved that *Cucurbita pepo* fruit juice is beneficial as it is rich in carotenoids which possess promising antioxidant activity. However, several previous studies showed that the plant carotene content (one of the most important classes of plant pigments which responsible for many plant colors) is very important to partially or completely protect intact cells (e.g. human liver cell line HepG2) against oxidant-induced lipid peroxidation and the protective effect of this plant fruits is independent of pro-vitamin A and vitamin C content. Therefore, the Squash (*Cucurbita pepo*) fruits diet have a major role in nutrition due to the presence of high content of pro-vitamin A and vitamin C.[36, 37, 38, 39]

Mineral content

Several reports stated that many minerals are considered to be essential in human nutrition and they are important constituents of bones, teeth, tissues, muscles, blood and nerve cells.[40,41] Generally, the minerals help in the maintenance of acid-base balance, the response of nerves to physiological stimulation and blood clotting.[42]

In the present study, the mineral contents of fruits, fruit pulp and seeds of Squash (*Cucurbita pepo*) were determined and were showed in **Table (2).** This results indicated that although the fruits, fruit pulp and seeds contain a higher level of the analysed mineral elements K, Na, Mg, P, Fe, Ca and Mn but the seeds have the highest value (223.57, 155.06, 60.45, 33.65, 12.39, 6.65 and 4.55 mg /100gm) respectively. These results are in full agreement with the high content of ash in the seeds of Squash as shown in **Table (1).** It has been reported that Potassium ions aid in maintaining the water balance in the body and the blood composition. And also, the Na/K ratio in the body is of great importance for the prevention of high blood pressure.[29, 30, 31] This study revealed that the fruits, seeds and fruit pulps of Squash (*Cucurbita pepo*) have high contents of K and Na Therefore, the Squash fruits are very important in the human diet for prevention of high blood pressure.[31] Iron performs several functions in the body, it helps in the formation of blood and it helps in the transfer of oxygen and carbon dioxide from one tissue to another.Iron deficiency results in an anaemia which impairs muscles metabolism, its deficiency in children result in an impaired learning ability and behavioural problems.[43] Calcium stimulates muscles and it is essential in calcium metabolism in bones, in addition to its coenzyme activity and regulates the passage of nutrients through cell walls.[25, 44] Phosphorus is needed for bone growth, kidney function and cell growth. It also plays a role in maintaining the body's acid-alkaline balance [41]. Also, the magnesium initiates the relaxation process and its deficiency results uncontrollable twisting of muscles hence leading to convulsion and tetanus.[25,43,44,45] It was reported that magnesium content is a component of chlorophyll and it is an important macro-mineral element in connection with ischemic heart disease.[25] Magnesium is important in tissue respiration specifically in oxidative phosphorylation that leading to the biosynthesis of Adenosine triphosphate. Although it was found in the least content in the present study but this element plays an important role in all mental functions and aids in the transfer of oxygen from lungs to cells.[25, 29, 43, 46] Also the level of copper in the fruits, fruit pulp and seeds in this study (0.49-0.85 mg/ gm) is within the acceptable range set by WHO of (2–5 mg) intake per day.[47,48] Therefore the present study showed that Squash (*Cucurbita pepo*) is a good human diet and it contains essential elements for a healthy and active life.

Table (2): Mineral content of Fruits, fruit pulp and seeds of Squash (*Cucurbita pepo*) (mg/100 gm)

Plant part	Elements (mg/100 gm)								
	Mg	Ca	Na	K	Zn	Fe	Mn	Cu	P
Fruits	25.05	5.50	76.50	189.30	0.65	6.48	1.35	0.55	27.47
Fruit pulp	32.61	5.32	68.89	152.50	0.05	4.95	1.25	0.49	23.15
seeds	60.45	6.65	155,06	223.57	0.85	12.39	4.55	0.85	33.65

Phenolic and flavonoid contents

It has been reported that the phenolic compounds have a significant action on the stabilisation of lipid oxidation and they have been associated with antioxidant activity as well as they have an inhibitory effect on mutagenesis and carcinogenesis.[23] In the present work, the phenolic and flavonoid contents of the methanolic extract of the fruits and seeds of Squash (*Cucurbita pepo)* were determined. The results in **Table (3)** exhibited that the methanolic extract of seeds have high phenolic and flavonoid contents (56.41±0.46 mg GAE/g extract and 30.60±0.46 mg RE /g extract) respectively. The phenolic compounds have the ability to scavenge free radicals due to the presence of hydroxyl groups.[49] Also, flavonoids are a group of polyphenol compounds that act as effective scavengers to the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants.[50] These results are supported by other previous studies which stated that the different *Cucurbita* species extracts have a considerable phenolic and flavonoid contents.[51, 52, 53]

Table (3): Total phenolic and flavonoid contents of the methanolic extract of fruits, and seeds of Squash (*Cucurbita pepo*)

Methanolic extract of	Total phenols (mg GA eq. /g extract)	Total flavonoids (mg RE eq. /g extract)
fruits	49.80±0.44	27.30±1.22
seeds	56.41±0.46	30.60±0.96

Antioxidant activity

Antioxidant activity of vegetables or leafy vegetables is very important for the determination of the nutritional and health values of the tested plants. In the present study, the antioxidant activities of the methanolic extract of fruits and seeds of Squash (*Cucurbita pepo*) were determined using three methods as DPPH free radical assay, Total antioxidant (Phosphomolybdate assay) and ABTS radical scavenging activity.

DPPH radical-scavenging activity

The DPPH method is widely used to test the ability of plant extracts or compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The parameter SC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour).[18, 54, 55] In this study, SC_{50} values **Table (4**) of methanolic extract of fruits and seeds of the tested plant were (643.09± 6.31 and 576.19± 5.35 µg/ml) respectively. Also, the results revealed that the antioxidant activity of the fruits and seeds of the plant is almost correlated with the phenolic and flavonoid contents of these parts.

ABTS radical - scavenging activity

ABTS radical scavenging activity is fast and effective scavengers. Also, ABTS is a protonated radical, has characteristic absorbance maxima at wavelength 734 nm which decreases with the scavenging of the proton radicals. The scavenging of the $ABTS^{+}$ radical by the extracts was found to be much higher than that of DPPH radical[18,54,55] In this study, the ABTS radical-scavenging activity of the methanol extracts of fruits and seeds of Cucurbita pepo were comparable to that of Trolox. The results in **Table (4**) exhibited that the scavenging activity of two extracts is (23.01±0.30 and 52.40±1.18 m mol Trolox eq/ Kg extract) respectively.

Total antioxidant capacity

The total antioxidant capacity was based on the reduction of Mo (VI) to Mo (V) by the extract by the formation of green phosphate /Mo (V) complex at acid medium. It evaluates both water-soluble and fat-soluble antioxidants.[56] The results in **Table (4**) indicated the high total antioxidant capacity of fruits and seeds are (201.37±1.37 and 222.1±1.40 mg AAE/ g extract) respectively. It was observed that the extract of the *Cucurbita pepo* possesses the significant total antioxidant capacity of the two parts.These results are in full agreement with many previous studies which reported that there are a correlation between total phenolic content and antioxidant activity of vegetables and this relation may be attributed to their ability to scavenge free radicals.[57,58,59] Also, there was a positive correlation between total flavonoid content and antioxidant capacity of the two tested plant parts. It has been reported that the flavonoids are also a kind of natural antioxidant substances capable of scavenging free superoxide radicals.[60] Finally, this study showed the capability of the methanolic extract of the fruits of Squash as antioxidant properties. So, they may be useful as therapeutic agents for treating of any radical-related pathological damage.

Table (4): DPPH, ABTS and total antioxidant capacity of the methanolic extracts of fruits and seeds of Squash (*Cucurbita pepo*)

Methanolic extract of	DPPH free radical scavenging activity SC_{50} (µg/ ml)	Total antioxidant capacity (mg eq. AAE/g extract)	ABTS (m mol Trolox eq/ Kg extract)
Fruits	643.09±6.31	201.37±1.30	23.01±0. 30
Seeds	576.19±5.35	222.1±1.40	52.40±1.18

CONCLUSION

The present study revealed that the fresh Squash fruits, which consumed in Egypt as popular diet have a major amount of carbohydrates, moisture, total ash, crude fibre as well as β-carotene. So, it has beneficial nutritional potential. In addition to of high levels of essential minerals; magnesium, potassium, sodium, phosphorus and which have very important advantageous to human. Moreover, the antioxidant activity of Squash fruits may be useful as therapeutic agents for treating of any radical-related pathological damage in many diseases.

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