Chemical Composition, Bioactive and Phenolic Compounds in three Varieties of Annona Fruit Grown in Egypt

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ABSTRACT

The present study is an attempt to measure the morphological parameters of the three famous Annona varieties grown in Egypt, and study the nutritional value, bioactive compounds and phenolic compounds of the Annona fruits. Concerning the morphological parameters Abdel Razek variety had the highest fruit weight (621.43 g) and fruit peel weight (121.94 g), while Balady variety fruits have the highest ratio of fruit peel to the total fruit weight (0.289) and the number of seeds in one fruit (55). Hindi variety recorded the highest weight values of 100 seed (11.74 g). It was found that the highest levels of fats, fiber, carbohydrates, most quantitative sugar and TSS were recorded in the Abd El-Razik variety. The highest contents of moisture, ash and protein were recorded in the Hindy variety. While the acidity content was nearly the same in the three varieties. Abdel Razek variety was the highest compared with the other varieties in all the estimated mineral elements (P, K, Fe, Ca, Zn and Mg). Abde Razik verity was high in bioactive compounds as ascorbic acid, flavonoid, and antioxidant activity, while Hindy variety was the highest in phenolics and DPPH.

Keywords: Annona fruit, chemical composition, bioactive compounds, phenolic compounds.

INTRODUCTION

Plants synthesize is a wide variety of natural medicinal compounds and this way attracted extensive interest as the principal source for a lot of bioactive metabolites (Yan *et al.*, 2016; Waley *et al.*, 2020). Plant organs like fruits have potential human health benefits and therefore, they have become progressively significant in human nutrition. The nutritional and health values of fruits are ascribed to their high substance of phytochemicals and bioavailable nutrients (Albuquerque *et al*., 2016).

It has been supported that a diet rich in vegetables and fruits can prompt a longer and healthier life (Melo *et al.*, 2008). The incorporation of fruit and its nutritional derivatives in dietary recommendations requires that they provide appreciable quantities of minerals, vitamins, and many other nutrients for health benefits (Nicklas *et al.*, 2011).

Annona is one of the main important delicious fruits belonging to the *Annonacea*e family and had a unique aroma and flavor (Olagunju and Sandewa, 2018). Annona is a little tree that is local to South America, and India. It arrives at 6-8 meters tall. It is cultivated around the tropical world. The Egyptian production of Annona fruits is about 1100 tons annually. The three most important varieties for fruit production are Cherimoya (*Annona cherimola*; Hindy cv.), Soursop (*Annona muricata;* Balady cv.), and Sweetsop or sugar apple Annona (*Annona squamosa*; Abd El-Razik hybrid) (Ibrahim and Mohamed, 2016). It is a nutritional healthy food, made up of protein, sugars carbohydrates, vitamins, minerals, very little fat content, and energy. Consumption of fresh fruit extracts or beverages is growing worldwide, due to the belief that they contribute to a balanced diet and are a good source of vitamins (A, C, B6, and B2), antioxidants and minerals (Ca, K, P, Fe, and Mg). It also has anti-inflammatory, anti-cancer and anti-tumor capabilities and the potential to minimize the risk of many diseases attributed to its biologically active compounds such as polyphenols. The plant is customarily utilized for the treatment of dysentery, epilepsy, worm infestation, cardiac problems, constipation, antibacterial infection, haemorrhage and antiulcer. It additionally has antitumor and antifertility properties (Virgen-Ceceña *et al*., 2019).

The fruits are eaten fresh and are likewise utilized in the preparation of juice and jelly (Bala *et al.*, 2018) and jams (Emelike and Akusu, 2019). The significance of the fruit is due to its sweet pulp which has medicinal applications. It likewise serves as a good source of proteins (1.6%), carbohydrates (23.5%), and minerals (0.9%) *(Bressy et al.,* 2013). Alongside these nutritional constituents, the fruit contains huge amounts of vitamins such as ascorbic acid and folic acid as well as minerals such as P, Ca, and Fe (Dembitsky *et al.*, 2011). In addition, Annona fruit is a good source of natural antioxidant compounds, and all the plant parts are utilized in folk medicine worldwide. Ronowicz *et al.* (2014) reported on the antioxidant activities of fruit extracts, as well as the role of Annona fruit in preventing oxidative damage. Annona fruits contain a considerable number of polyphenolic compounds with antimicrobial, antiviral, and anti-inflammatory activities

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along with their antioxidant properties (Ignat *et al*., 2011).

The present study is an attempt to measure the morphological parameters, nutritional value, and bioactive compounds of the most three famous Annona varieties grown in Egypt.

MATERIALS AND METHODS

Plant materials

Three Annona varieties: Abdelrazik of (*Annona Cherimola x Annona squamosal* Hybrid), Balady (*Annona squamosal* L.) and Hindi (*Annona cheirmola* M.) were obtained from trees grown in the orchards of Osiris Modern Agriculture Company (Latitude: 30.26°, Longitude: 30.19) in Wadi El-natron, El-Beheira Governorate, Egypt, during the harvest season of the year of 2020 (September–October). A sample of 25 fruits per variety was hand-picked when not fully mature, and the color changed from green to yellow. On arrival at the lab., fruits were packed in carton boxes and held at 20 °C in controlled temperature rooms with 90-95% relative humidity and left to ripen under storage conditions.

Methods:

The botanical description of Annona fruits: Plant parameters of Annona fruit including color, length, diameter and weight of fruit, peel weight, Peel/fruit weight, the weight of 100 seeds, and the number of seeds per fruit were measured.

Prepration of samples: The three mature fruit verities were washed with tap water to remove adhered particles. The fruit pulp was manually separated from seeds and peel. An aliquot of the plant parts was used as fresh material and the other part was dried in the oven at 70°C and ground to powder using ceramic mortar and pestle. The powder was sieved with 20 mesh sieves and stored in air-tight polyethylene bags in a desiccator, then used for the following determination.

Chemical composition

Samples of Annona fruits were oven-dried at 70 °C until the weight was stable, and from it, the moisture percentage was calculated, and then the other chemical assessments were performed including protein (N × 5.75), total lipids, ash content, crude fiber, and carbohydrates according to the methods described in **AOAC (2010)**.

Determination of minerals:

Total phosphorus was determined spectrophotometrically by Milten Roy Spectronic 120 at wavelength 725 nm using stannous chloride reduced molybdosulphoric blue color method in the sulphuric system as described by Peters *et al.* (2003). Total potassium was estimated flame photometrically using Jenway Flame photometer, Model corning 400 according to the modified method of the same auther. Calcium (Ca), Magnesium (Mg), Zinc (Zn) and Iron (Fe) were determined using atomic absorption according to the method of Kumpulainen *et al*. (1983).

Titratable acidity:

Titratable acidity (%) in fruit juice was expressed as citric acid using NaOH at 0.1 N and phenolphthalein as an indicator according to the AOAC (2012).

Glucose, fructose, and sucrose determination:

Sugars were dried in vacuo at 65°C for 24 hours, and a 100 mg/ml standard solution of fructose, glucose, and sucrose was produced. Lactose standard solution (50 mg/ml) was utilised as an internal standard. The solutions were either freshly made or kept at -18°C; there was no discernible difference between the two. In a homogenizer, the fruit pulp was blended, then aliquots were precisely weighed (60-70g) and brought to 200 ml with purified water. A 50 mL aliquot was collected and centrifuged for 15 minutes at 2800 g. The supernatant was collected after the sediments were rinsed three times with distilled water (20ml). The samples were then concentrated to 5 ml in a vacuum at room temperature. The samples were filtered through Millipore filters (0.45 m) after passing through a Sep-pak C18 (Water Associates), and 1 ml of lactose solution, used as the internal standard, was added to 2 ml of the samples thus prepared. The amount of sample injected into the HPLC system ranged from 5-10µl.

By separating the fruit pulp into two equal sections, one with no added sugar and the other with sugar added at the appropriate level, the recovery of added fructose, glucose, and sucrose at a concentration of 10 mg/g of sample was assessed. These examples were made in the same way as the previous ones. The recoveries were calculated by subtracting the total amounts determined in the sample with and without sugar from the total amounts determined in the sample without sugar. The ratio peak area of internal standard:peak area of monosaccharide was plotted versus monosaccharide concentration in standard curves. These standard curves were then used to calculate the amounts of glucose, fructose, and sucrose in the samples.

HPLC analysis was carried out according to Salvo *et al*. (1984) to the determination of glucose, fructose, and sucrose. The column used was 250×4.6mm ID Supelcosil Lc-NH4, mobile phase: acetonitrile: water (75: 25). The recorder was 10 mm/ min, 10 mv. The flow rate was 2ml/ min, the detector was a RI-401 differential refractometer. The results were expressed as g/100g sample.

Ascorbic acid content:

Ascorbic acid of Annona fruits was determined according to the method described by Mazumdar and Majumder (2003) using titrimetric estimation with 2, 6 dichlorophenol indophenol reagent.

Determination of total phenolics content:

Total phenolics in Annona fruits were determined using a 4054 UV/ visible Spectrophotometer LKB, Biochrom, USA, according to Amerine and Ough (1980). The results were expressed as mg gallic acid/ 100 gm (dry weight).

Determination of flavonoids content:

The total flavonoids content of Annona fruits extracts was determined by a colorimetric method as described by Zhishen *et al.* (1999). The sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15 %). After 6 minutes, 0.15 ml of aluminium chloride ($AlCl_3$) solution (10 %) was added and allowed to stand for 6 minutes, then 2 ml of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was then determined at 510 nm versus prepared water blank.

Determination of total antioxidant activity:

The total antioxidant activity of the three fruits extracts was determined using the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999). Each sample solution (0.1 mL, 0.5 mg/mL) was combined with 0.3 mL of reagent solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank using a spectrophotometer UVD-3500. The antioxidant activity was expressed as (µmol/100g) of ascorbic acid. Ascorbic acid (0.5 mg/mL) was used as reference compounds.

Determination of radical DPPH scavenging activity (µM TE/ 100g DW):

The free radical scavenging capacity of extracts was determined using the free radical generator (DPPH) assay based on Hwang and Do Thi (2014). The final concentration was 200 µM for DPPH and the final reaction volume was 3.0 ml. The absorbance, using a spectrophotometer, was measured at 517 nm against a blank of methanol after 60 min of incubation in a dark condition without DPPH. The inhibition percentage of the DPPH free radical was calculated by the following equation:

$$\text{Inhibition (\%)} = 100 \times [(A_{control} - A_{sample})/A_{control}]$$

Where: $A_{control}$ is the absorbance of the control reaction and A_{sample} is an absorbance with the test compound

Determination of phenolic compounds:

An amount of 2.5 g of annona fruit powder was weighed, added to 30 mL of ethanol (70%, v/v), and extracted in a Sonorex Digital 10 P ultrasonic bath for 20 minutes at 40°C. The extract obtained was filtered through a paper filter in a 50 mL flask. The extract was filtered through a membrane filter with a pore size of 0.22 µm. The volume of the extract being investigated was 10 µL. The HPLC system was equipped with an auto-sampler (G1329B), a quaternary pump and a diode array detector. The measurements were integrated by ChemStation chromatographic software Computer Program. The analytical column was the ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA) (De Brum *et al.*, 2013).

Estimation of the activity of the antioxidant enzymes:

The estimation of catalase activity was determined by slightly modified procedures (Sinha, 1972).The reaction mixture contained 1 cm3 of 0.01 M phosphate buffer (pH 7.0), 0.4 cm3 of 0.5 M H_2O_2 and 0.5 cm3 of the plant extract. The absorbance was measured at 610 nm. One enzyme unit is defined as mmol H_2O_2/min/g fresh weight.

The estimation of peroxidase activity was done at pH 6 by the increase in absorbance at 420 nm due to the formation of purpurogallin, according to the modified method of Devi (2000).

The estimation of polyphenol oxidase activity was assayed at pH 7 by the increase in absorbance at 420 nm due to the formation of purpurogallin following the modified method of Devi (2000).

RESULTS AND DISCUSSION

The Botanical description of Annona fruits:

Table (1) shows the data of botanical description variations that could be noticed visually for the three Annona varieties under study taken through the growing season of 2020, botanical traits could be illustrated as follow:

Concerning the weight of fruits, data illustrated that the fruit weight of the Abdel Razek variety had the highest fruit weight (621.43 g), while the Balady variety had the lowest fruit weight (256.16 g). As for, fruits of the Abdel Razek variety were the highest in length and diameter followed by the Hindy variety and finally the Balady cultivar.

Also, fruit peel weight was recorded, Abdel Razek variety had the highest fruit peel weight with 121.94 g followed by Balady with 74.03 g and finally Hindi with 56.38 g.

Fruit peel to the total fruit weight (P/W) ratio was also calculated, the reslts showed that Balady variety fruits have the highest ratio (0.289) followed by Abdel Razek (0.196) and finally Hindy (0.177). This ratio was found to express the importunacy of the fruit value better than the fruit weight only. It expresses the percentage of the most useful part of the fruit.

Moreover, some comparisons were made between the seeds of the three studied varieties, including the weight of the seed, and the number of seeds in the one fruit. Hindi variety recorded the highest value of seed weight in the three varieties, followed by Abdel Razek and Balady, but for the number of the seeds in the one fruit, Balady comes in the first with 55 seeds, and in the end, Abdul Razek with 24 seeds. Accordingly, from these results, Abdel Razik variety have higher edible weight (497.65) compared with the other Annona varities under this study.

From obtained these results were in agreement with Mohammed *et al.* (2016); Abdelkawy and El-Nawam (2017) who studied the morphological parameters of some Egyptian Annona varieties. Mariguele and Silva (2010) reported positive correlations between the weight of seeds and their number, and between the number of fruits and yield. The greatest direct effects were those gained for the weight of pulp on fruit weight and fruits mean weight and number of on fruit yield. The most significant indirect impacts were obtained for seeds number and weight of pericarp, gained via weight of pulp, on the weight of fruit, and for the length of fruit and width, obtained via mean fruit weight, on fruit yield.

Proximate composition of Annona fruits:

The results of proximate analysis of three varieties of Annona grown in Egypt were presented in Table (2). The results show that Hindy fruits recorded the highest value of moisture content (76.43%), while Abdel Razik fruits recorded the lowest value (71.16%). The moisture content of fruits gives an indicator of the available dry matter and plays an important role in determining the propensity of the food to spoil (Appiah *et al*., 2011). By this content of moisture, the fruit may therefore be tapped in the commercial production of juices, jams, and jellies.

Figure 1. Typical fruits of the three varieties

Table 1. Botanical description of *Annona* varities.

Parameters	Balady	Hindy	Abdel Razik
Fruit color	Light green	Dark green	Yellowness green
Seeds color	Green	Green	Dark brown
Fruit weight (g)	256.16±1.54c	318.21±2.11b	621.43±1.78a
Fruit length (cm)	6.73±0.12c	7.84±0.22b	11.18±0.08a
Fruit diameter (cm)	6.21±0.11c	7.46±0.14b	10.86±0.15a
Peel weight (g)	74.03±0.29b	56.38±0.26c	121.94±0.38a
Peel/fruit weight ratio	0.289±0.003a	0.177±0.002c	0.196±0.000b
Weight of 100 seeds (g)	5.86±0.09c	11.74±0.07a	7.68±0.12b
The number of seeds in one fruit	55±2.00a	32±3.00b	24±4.00c
Edible weight of pulp (g)	180.72±1.852c	259.012±2.353b	497.65±1.429a

Values (Mean ± standard deviation, three replicates). Different letters in each row show that there are significant differences between varieties at $P<0.05$

Table 2. Proximate composition of three Annona varieties.

Parameters	Hindy	Balady	Abdel Razik
Moisture %	76.43±0.890a	73.18±1.080b	71.16±0.590c
Protein %	2.36±0.110a	2.79±0.950a	2.76±0.120a
Total lipids %	0.38±0.040a	0.29±0.040b	0.41±0.050a
Ash %	1.19±0.040a	0.79±0.040b	1.13±0.030a
Crude fiber %	1.84±0.030c	2.18±0.050b	2.28±0.050a
Total carbohydrates %*	18.99±1.340c	20.95±0.840b	22.34±1.390a

*Total carbohydrates were calculated by difference.

Values (Mean ± standard deviation, three replicates). Different letters in each row show that there are significant differences between varieties at P<0.05

The results showed that there were no significant differences between the three varieties in protein content. In general, protein content ranged from 2.36% (in Hindi fruits) to 2.79% (in Balady fruits).

As obtained from the results of this study, Annona fruits contain a very low content of fat, which ranged between 0.29% in Balady variety and 0.41% in Abdel Razik variety.

The ash content in the three varieties were 1.19, 0.79 and 1.13% for Hindi, Balady, and Abdel Razik fruits, respectively. Ash gives an approximate measure of the total mineral composition of food. Thus, the obtained relativity high ash content suggests considerable mineral content of these underused fruits and the potential for the studied fruits to contribute to the total health of consumers.These minerals act as inorganic co-factors in metabolic processes which mean in the absence of these inorganic co-factors, there could be impaired metabolism (Iheanacho and Udebuani, 2009).

The results (Table 2) show that Annona fruit contains appreciable amounts of crude fiber, which significantly varied among the three studied varieties, where the lowest value was recorded in the Hindi variety (1.84%), while the highest value was recorded in the Abdel Razik variety (2.28%).

Fruits are known to contain high fiber which can be beneficial in the human diet on the muscles of the large and small intestine (Lanza and Butrum, 1986).

The carbohydrates content in Annona fruits is given in Table 2. The results indicated that the content of the carbohydrates in the three Annona varieties ranged from 18.99% (in Hindi variety) to 22.34% (in Abdel Razik variety). These results agree with El-Aidie *et al.* (2020) analyzed the nutritional parameters of *Annona squamosa* L. and who found that chemical composition was 74.6% moisture, 208% crude protein, 0.39% oil content, 1.05% ash and 3.1% fiber.

Data presented in Table (3), showed sugars content in Annona varieties. The three varieties significantly differed (P<0.05) in their content of glucose, fructose, sucrose and total sugars, the highest variety of the total sugars was the Abdel Razek variety and the lowest was the Hindy variety. In the three varieties, total sugar expressed about the sugars content, ranged from 12.71 to 15.43%. El-Aidie *et al.* (2020) who found that *Annona squamosa* L. contains 20.90% total sugar.

While assessing the fruit for consumer acceptance, a producer isn't worried about soluble solids alone but with perceived sweetness, which is determined largely by the relative levels of total soluble solids and acids in the fruits. The total soluble solids are composed of all the soluble solids which are present in the fruits. In desert fruit like Annona fruits, the fruits wonderful perfect sugar-acid mix are liked by consumers. Various types of organic acids and the degree of their concentration play a significant part in the flavor of fruit. Generally, high acidity gives a better mix and flavor (Priyanka *et al*., 2019).

Table 3. Sugars of three Annona varieties.

Parameters	Hindy	Balady	Abdel Razik
Glucose %	5.36±0.030c	6.02±0.060b	6.64±0.050a
Fructose %	6.48±0.070c	7.02±0.040b	7.36±0.060a
Sucrose %	0.87±0.050c	1.21±0.010b	1.43±0.090a
Toal sugars %	12.71±0.070c	14.25±0.040b	15.43±0.280a

Values (Mean ± standard deviation, three replicates). Different letters in each row show that there are significant differences between varieties at P<0.05

Table (4) presented the content of TSS% for different varieties. Abel Razik variety recorded the highest value of TSS% which was 19.26%, followed by Balady variety (17.81%), and Hindy variety (14.18%). For acidity, the three studied varieties scored nearly values as 0.22, 0.25 and 0.29% for Abdel Razik, Balady and Hindy, respectively. Santos *et al.* (2016) resulted that atemoya recorded 5.50 mg/100 g acidity and 32.50 Brix soluble solids. Martínez-Girón *et al*. (2019) studied the physicochemical tests in the pulp of two Annon vartities which scored 0.97% acidity and 8.20 Brix for total soluble solids. El-Aidie *et al.* (2020) who found that *Annona squamosa* L. contains 26.0% total soluble solids, 20.90% total sugar.

The mineral content of Annona fruits:

The minerals content of the three famous Annona grown in Egypt were summarized in Table (5). Overall, significant differences (P<0.05) were observed in the content of the mineral elements that were determind in the three varieties. Abdel Razek's variety was the highest compared with the other varieties of all the determind mineral elements. Phosphorus content of Annona fruits ranged between 39 to 55 mg/100 g dry weight. Potassium was the most abundant mineral element in Annona fruits (Table 3), it ranged from 450 to 730 mg/ 100g.

The Annona fruit becomes useful since its regular consumption might ensure an adequate supply of iron level into the body. the same table found that the fruits of Abdel Razik variety had a rich content which recorded 1.36 mg/100g, while Balady recorded the lowest value (0.56 mg/100g). Concerning the values of calcium in Annona varities, it ranged from 32.22 mg/100g in Hindy to 41.18 mg/100g in Abdel Razik.

Zinc is among the required elements for humans and their daily requirements for adults. The concentration of zinc in table (5), ranged between 0.25 to 0.32 mg/100g in Hindy and Abdel Razik, respectively. Magnesium is a component of chlorophyll, and it is a significant mineral element in connection with calcium metabolism in bones and ischemic heart disease (Ishida *et al.*, 2000). Mg ranged from 44.16 to 53.42 mg/100 g in Hindy and Abdel Razik, respectively.

Similar results were reported by Nair and Agrawal (2017) reported that mineral content (mg/100g) of the *Annona* fruit contains Ca (60), Fe (105), Mg (53), P (80), K (618) and Zn (250 mcg/100g). Additionally, Princewill-Ogbonna *et al*. (2019) reported that. *A. muricata* contains high content of minerals (%) as; Ca (4.20), Mg (1.70), K (0.49), and P (0.23). Also, El-Aidie *et al.* (2020) analyzed the nutritional parameters of *Annona squamosa* L. and who found that it contains minerals such as 64.25, 428, 54.5, and 2.8 mg/ 100 g for Ca, K, Mg, and Fe, respectively.

Bioactive compounds:

Ascorbic acid, phenolics, flavonoids and the other antioxidant compounds are free radical scavengers that prevent cell damage and have strong anticancer activities and thus limit the adverse effects thereof on the body (Pourmorad *et al*., 2006; Ugwu *et al.*, 2013) and they might induce mechanism that impact cancer cells and inhibit tumor invasion (Rafat e*t al*., 2008). These activities are due to their ability to neutralize free radicals (Omale and Okafor, 2008 ; Ugwu *et al.*, 2013).

Table 4. TSS and titratable acidity of three Annona varieties.

Parameters	Hindy	Balady	Abdel Razik
Total soluble solids (TSS %)	14.18±0.080c	17.81±0.070b	19.26±0.080a
Titratable acidity (%)	0.29±0.020a	0.25±0.030ab	0.22±0.040b

Values (Mean ± standard deviation, three replicates). Different letters in each row show that there are significant differences between varieties at P<0.05

Table 5. Mineral contents of *Annona sp.* (mg/ 100 gm dry weight).

Element	Hindy	Balady	Abdel Razik
P	46.00±1.650b	39.00±2.320c	55.00±4.310a
K	680.00±0.050b	450.00±0.050c	730.00±0.040a
Fe	1.18±0.07 b	0.56±0.09c	1.36±0.124a
Ca	32.22±0.040c	38.11±0.040b	41.18±0.020a
Zn	0.25±0.050b	0.27±0.110ab	0.32±0.110a
Mg	44.16±0.080c	48.12±0.070b	53.42±0.080a

Values as Mean ± standard deviation, three replicates. Different letters in each row show that there are significant differences between varieties at P<0.05.

Table (6) presents ascorbic acid, total phenols, flavonoids contents, DPPH, and the antioxidant activity in three varieties of Annona fruits.

There were variations between varieties in ascorbic acid which are mainly due to genetic factors, so it ranged between 55.26 to 81.13 mg/100 g. It is known that ascorbic acid as an antioxidant helps protect cells from oxidative harm caused by free radicals, thus, decreasing the rate of ageing and development of cancer heart disease, and arthritis as well as optimizing the body's immune function and promoting wound healing (Iqbal *et al*., 2004). Boakye *et al*. (2014) obtained 20.33 and 63.67 mg/100g ascorbic acid of sweetsop and soursop fruits, respectively. The high values of ascorbic acid in Annona signify the potential use of the fruit as a good source of ascorbic acid. The recommended daily intake (RDI) of ascorbic acid is about 30 mg/day for adults and 17 mg/day for children (Krinsky *et al*., 2000). Therefore, these fruits could be considered good sources of ascorbic acid for purposes of human nutrition.

Plant phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activity, in addition, phenolic can influence the flavor determining fruit astringency and bitterness (Anuragi *et al*., 2017). The total phenolic content of different species of Annona fruits as gallic acid was presented in the same table, the result indicated that phenolic ranged from 8.41 mg/100g in Abdel Razik to 13.03 mg/100g in Hindy.

Flavonoids are a group of compounds contributing to the total antioxidant capacity of fruits and vegetables. As for total flavonoid found that the highest value scored with Abdel Razik, while the lowest one with Hindy and Balady was in the middle.

The free radical scavenging activity of Anonna fruits was assayed using the DPPH method. in the same table, it revealed that DPPH ranged from 14.64 to 17.24 µM TE/ 100g. This result is consistent with those recorded by Akomolafe and Ajayi (2015) who found high values of DPPH radical of *Annona muricata* L.

The antioxidant activity is an essential biological property of great interest because it reduces the toxic effects of oxidants and thus prevents cellular damage caused by free radicals. From the result, antioxidant activity ranged from 628.57 µmol/g in Hindy to 644.38µmol/g in Abdel Razik.

A study by Priyanka *et al.* (2019) found that the total phenol content ranged between (73.06 to 125.74 and 80.72 to 85.17 mg GAE/100g), total flavonoid (20.43 to 26.89 and 20.84 to 29.03 mg Catechin equivalent/100g) and antioxidant capacity (81.14 to 125.25 and 91.82 to 95.72 mg AEAC/100g) in *Annona* species. Variety Abdel Razek may be considered the poorest source of the total phenolics compared with the other species. While the highest flavonoid content was found in leaves of *A. cherimola* (4.6 mg/g) and the lowest one was from *A. squamosal* fruit and seeds (0.1 and 0.1 mg/g, respectively).

Phenolic compounds profile

Phenolic compounds accumulate in fruits as a defensive response against cold injury, cellular stress, or ethylene exposure. They may have a role in plant resistance to microbial diseases (Rozan, 2017).

Table (7) shows the phenolics profile of Annona fruits. Under the conditions of our research, it was possible to detect eight phenolic compounds in the Hindy variety, and seven compounds in each of Balady and Abdel Razek varieties.

Among the seven detected compounds namely; gallic acid, protocatechuic acid, neochlorogenic acid, 4-hydroxybenzoic acid, chlorogenic acid, syringic acid and cinnamic acid were indicated in both Balady and AbdelRAzik, while coumaric acid was detected under analytical conditions only in the Hindy variety. syringic acid,4-hydroxybenzoic acid, neochlorogenic acid, protocatchuc acid and gallic acid recorded the highest value in Hindy variety with a significant difference than the other two varieties, while cinnamic acid, neochlorogenic acid, and chlorogenic acid recorded the highest value in Balady variety, on the other hand, most of phenolic compounds detected low values in AbdelRazik.

Table 6. Bioactive compounds of *Annona* varities.

Parameters	Hindy	Balady	Abdel Razik
Ascorbic acid (mg/ 100 g DW)	78.17±0.050b	55.26±0.074c	81.13±0.090a
Total phenols (mg gallic acid/g DW)	29.36±1.32a	29.72±1.1a	26.83±1.54b
Total flavonoids (mg CE/g dry matter)	2.8±0.040c	5.9±0.040b	8.1±0.030a
DPPH (µM TE/ 100g DW)	17.24±0.23a	14.64±0.34c	15.94±0.29b
Antioxidant activity µmol/100 g	628.57±4.220b	636.65±3.390a	644.38±4.030a

Values as Mean ± standard deviation, three replicates. Different letters in each row show that there are significant differences between varieties at $P<0.05$.

Table 7. Phenolic profile of Annona fruits

Compound (µg/g DW)	Hindy	Balady	Abdel Razik
Gallic Acid	111.7±0.91a	88.6±0.74b	54.1±0.53c
Protocatechuic acid	171.4±1.23a	113.6±1.50c	138.2±0.44b
Neochlorogenic acid	228.6±1.76b	252.7±1.33a	178.0±1.04c
4-Hydroxybenzoic acid	254.9±1.08a	227.5±1.24b	201.7±0.98c
Chlorogenic acid	31.1±0.45b	57±0.72a	30.8±0.31b
Syringic acid	956.7±1.89a	893.9±2.05b	831.7±1.69c
Coumaric acid	5.2±0.11	ND	ND
Cinnamic acid	365.7±1.28b	391.4±1.68a	321.9±1.17c

Values as Mean ± standard deviation, three replicates. Different letters in each row show that there are significant differences between varieties at P<0.05.

Table 8. Enzyme activity µmol/min/ FW of *Annona* varities.

Parameters	Hindy	Balady	Abdel Razik
Catalase	13.01±0.100b	18.11±0.090a	10.83±0.130c
Peroxidase	7.38±0.070b	5.84±0.080c	32.16±0.120a
Polyphenol oxidase	15.18±0.040b	11.16±0.160c	18.11±0.080a

Values as µmol/min/gm fresh weight (Mean ± standard deviation, three replicates). Different letters in each row show that there are significant differences between varieties at P<0.05.

In general, the largest compounds discovered in Annona fruits were syringic acid (831.7- 956.7 µg/g), followed by cinnamic acid (321.9- 391.4 µg/g), and 4-hydroxybenzoic acid (201.7- 254.9 µg/g) as well as neochlorogenic acid (178- 252.7 µg/g), while the lowest compound was coumaric acid (0.52 µg/g), which was only detected in Hindy variety.

Enzyme activity of *Annona* varities:

Data presented in Table (8) indicated enzymes activity of catalase, peroxidase, and polyphenol oxidase in Annona fruits grown in Egypt (Hindy, Balady and Abde Razik varieties).

Catalase activity ranged from 10.83 µmol/min FW in Abde Razik fruits to 18.11 µmol/min FW in Balady fruits, while Hindy fruits were in the middle with 13.01 µmol/min FW. Catalase serves as a natural antioxidant that protects tissues from the damage induced by reactive oxygen species (Spanou *et al*., 2012).

As for the peroxidase activity, this work indicated that Abdel Razik fruits recorded the highest value (32.16 µmol/min FW) compared with other varities. Peroxidase catalyzes the oxidation of a wide variety of electron donors with the help of H_2O_2 and thereby scavenges the endogenous H_2O_2 (Chamundeeswari *et al.*, 2003).

It has been reported that polyphenol oxidase is actively involved in scavenging oxidant radicals. Polyphenol oxidase and peroxidases are among the most studied enzymes in fruits and vegetables due to their role in antioxidation (Kuijpers e*t al.*, 2014 ; Muthu and Durairaj, 2015)

Polyphenol oxidase activity recorded the highest value in Abde Razik fruits were 18.11 µmol/min/ FW followed by Hindy, then Balady which scored 15.18 and 11.16 µmol/min/ FW, respectively.

CONCLUSIONS

The three studied Annona varieties are well endowed with essential nutrients for humans. The presence of high phytochemical concentrations in the fruits give a new insight into their potential utility as a medicinal plant and it also allows the possibility to be used in many food manufacturers, jams, and juices. The present study reveals that the Abd El Razik variety was the most varied rich in the most studied parameters, especially it is rich in carbohydrates, minerals and most of the bioactive compounds followed by Hindy variety. Therefore, this variety is recommended for cultivation on a large scale in Egypt and using it as a base for the development and crossbreeding of new varieties.

REFERENCES

Abdelkawy, M.M.A.S and M. A.El-Nawam. 2017. Identification of three egyptian annona cultivars morphologically and biochemically using rapid analysis. Alex. J. Agric. Sci. 62: 415-421.

Akomolafe, S and O. Ajayi. 2015. A comparative study on antioxidant properties, proximate and mineral compositions of the peel and pulp of ripe *Annona muricata* (L.) fruit. Inter. Food Res. J. 22: 2381-2388.

Albuquerque, T. G., F. Santos, A. Sanches-Silva, M. B. Oliveira, A. C. Bento and H.S. Costa. 2016. Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefits. Food Chem. 193: 187-195.

Amerine, M.A and C. S. Ough. 1980. Methods for analysis of musts and wines. J. Wiley. 663.

Anuragi, H., B. Jain, H. Dhaduk and S. Kumar. 2017. Genetic association studies for fruit yield and its components and qualitative phytochemical screening in promising annona genotypes. Res. J. of Agric. Sci. 8:222- 227.

AOAC. 2010. Official Methods of Analysis of Association of Official Analytical Chemists, Washington, DC.

AOAC. 2012. Official Methods of Analysis, Association of Official Analytical Chemists, Washington, DC, USA.

Appiah, F., I. Oduro and W. Ellis. 2011. Proximate and mineral composition of Artocarpus altilis pulp flour as affected by fermentation Park. J. Nutr. 10: 653-657.

Bala, S., V. Nigam, S.S. Singh, A. Kumar and S. Kumar. 2018. Evaluation of nutraceutical applications of *Annona squamosa* L. Based Food Products. J. Pharmacogn. Phytochem. 7: 827-831.

Boakye, A. A., F. D. Wireko-Manu, J. K. Agbenorhevi and I. Oduro. 2014. Dietary fibre, ascorbic acid and proximate composition of tropical underutilised fruits. African J. Food Sci. 8: 305-310.

Bressy, F. C., G. B. Brito, I. S. Barbosa, L. S. Teixeira and M. G. A. Korn. 2013. Determination of trace element concentrations in tomato samples at different stages of maturation by ICP OES and ICP-MS following microwave-assisted digestion. Microchem. J. 109: 145-149.

Chamundeeswari, D., J. Vasantha, S. Gopalakrishnan and E. Sukumar. 2003. Free radical scavenging activity of the alcoholic extract of Trewia polycarpa roots in arthritic rats. J. Ethnopharmacol. 88: 51-56.

De Brum, T. F., M. Zadra, M. Piana, A. A. Boligon, J. K. Fröhlich, R. B. De Freitas, S. T. Stefanello, A.L.F. Froeder, B.V. Belke and L.T. Nunes. 2013. HPLC analysis of phenolics compounds and antioxidant capacity of leaves of Vitex megapotamica (Sprengel) Moldenke. Molecules 18: 8342-8357.

Dembitsky, V.M., S. Poovarodom, H. Leontowicz, M. Leontowicz, S. Vearasilp, S. Trakhtenberg and S. Gorinstein. 2011. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. Food Res. Inter. 44: 1671-1701.

Devi, P. 2000. Principles and Methods in Plant Molecular Biology, Biochemistry and Genetics. Agrobios.

El-Aidie, M. S. A., H. Hatem, A. M. Ewis and A. M. El-Nimer. 2020. Production of Nutraceutical Probiotic Whey–Based Beverage Fortified with *Annona squamosal* L (Custard Apple) Fruit *Annona squamosal* L. J. Food & Dairy Sci. 11: 171-177.

Emelike, N and O. Akusu. 2019. Quality attributes of jams and marmalades produced from some selected tropical fruits. J. Food Process. Technol. 10: 1-7.

Hwang, E.S and N. Do-Thi. 2014. Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera). Prev. Nutr. Food Sci. 19: 40-48.

Ibrahim, M.A.M. 2016. Biochemical consequences of some active ingredients isolated from Annona species grown in Egypt through the growing season. Ph. D. Thesis, Fac. Agric. Cairo University.

Ignat, I., I. Volf and V. I. Popa. 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem. 126: 1821-1835.

Iheanacho, K.M and A.C. Udebuani. 2009.Nutritional composition of some leafy vegetables consumed in Imo state, Nigeria. J. Appl. Sci. Environ. Manag. 13: 35–38.

Iqbal, K., A. Khan and M. Khattak. 2004. Biological significance of ascorbic acid (vitamin C) in human health-a review. Pakistan J. Nutr. 3:5-13.

Ishida, H., H. Suzuno, N. Sugiyama, S. Innami, T. Tadokoro and A. Maekawa. 2000. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (I*pomoea batatas* poir). Food Chem. 68: 359-367.

Krinsky, N. I., G. Beecher, R. Burk, A. Chan, J. Erdman, R. Jacob, I. Jialal, L. Kolonel, J. Marshall and P. Taylor Mayne. 2000. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Institute of Medicine 19: 95-185.

Kuijpers, T. F., T. Van Herk, J. P. Vincken, R. H. Janssen, D. L. Narh, W. J. Van Berkel and H. Gruppen. 2014. Potato and mushroom polyphenol oxidase activities are differently modulated by natural plant extracts. J. Agric. Food Chem. 62: 214-221.

Kumpulainen, J., A. M. Raittila, J. Lehto and P. Koivistoinen. 1983. Electrothermal atomic absorption spectrometric determination of selenium in foods and diets. J. Assoc. Off. Anal. Chem. 66:1129-1135.

Lanza, E and R. R. Butrum. 1986. A critical review of food fiber analysis and data. J. J. Am. Diet. Assoc. 86:732-741.

Mariguele, K.H and P. S. L. Silva. 2010. Relationship between fruit traits of custard apple trees (Annona squamosa L.). Rev. Ceres 57:476-479.

Martínez-Girón, J., A. Arias-Rodas, J. Hernández-Montaña, J. Agudelo-Burbano and B. Reina-Hernandez. 2019. Physicochemical, microbiological and sensory characterization of liquor obtained from atemoya fruit (*Annona cherimola*× *Annona squamosa*). Sci. et Tech. 24: 636-642.

Mazumdar, B. C and K. Majumder. 2003. Methods on Physico-chemical Analysis of Fruits. Daya publishing house Delhi. 2003: 137-138.

Melo, E. A., M. I. S. Maciel, V.L.A. Lima and R. J. Nascimento. 2008. Capacidade antioxidante de frutas. Revista Brasileira de Ciências Farmacêuticas Braz. J. Pharm. Sci. 44:193-202.

Mohammed, M., S. El-Gengaihi, A. Enein, E. Hassan, O. Ahmed and M. Asker. 2016. Chemical constituents and antimicrobial activity of different Annona species cultivated in Egypt. J. Chem. Pharm. Res. 8:261-271.

Muthu, S and B. Durairaj. 2015. Evaluation of antioxidant and free radical scavenging activity of Annona muricata. Eur. J. Exp. Biol. 5:39-45.

Nair, R and V. Agrawal. 2017. A Review on the Nutritional Quality and Medicinal Value of Custard Apple-An Under Utilised Crop of Madhya Pradesh, India. Int. J. Curr. Microbiol. Appl. Sci. 6:1126-1132.

Nicklas, T., C. O'Neil, D. Liska, N. Almeida and A.V. Fulgoni. 2011. Modeling dietary fiber intakes in US adults: Implications for Public Policy. Food Sci. Nutr. 2:925-931.

Olagunju, A. I and O. E. Sandewa. 2018. Comparative physicochemical properties and antioxidant activity of dietary soursop milkshake. Beverages 4: 38.

Omale, J and P. Okafor. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. Afr. J. Biotechnol. 7:3129- 3133.

Peters, I.S., B. Combs, I. Hoskins, I. Iarman, M. Kover Watson and N. Wolf. 2003. Recommended Methods of Manure Analysis. Univ. of Wisconsin, Cooperative extension Publ. Madison.

Pourmorad, F., N. Hossienimehr and N. Shahabimajd. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotech. 5:1142-1145.

Prieto, P., M. Pineda and M. Aguilar. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269:337-341.

Princewill-Ogbonna, I.L., P.C. Ogbonna and I.B. Ogujiofor. 2019. Proximate composition, vitamin, mineral and biologically active compounds levels in leaves of *Mangifera indica* (Mango), *Persea americana* (Avocado pea), and *Annona muricata* (Sour sop). J. Appl. Sci. Environ. Manage. 23:65-74.

Priyanka, H., T. Sakthivel, K. S. Shivashankar, D. MR and V. GM. 2019. Biochemical profiling of Annona species and Annona atemoya varieties. Int. J. Chem. Stud.7: 27-261.

Rafat, H., B. Cillard and N. Cilliad. 2008. Hydroxyl radical scavenging activity of flavonoids. J. Phytochem. 26: 2489-2491.

Ronowicz, J., B. Kupcewicz and E. Budzisz. 2014. Implementation of chemometric techniques for evaluation of antioxidant properties of Camellia sinensis extracts. Cent. Eur. J. Chem. 12: 700-710.

Rozan, M.A. 2017. Carotenoids, phenolics, antioxidant activity and sensory attributes of carrot jam: Effect of Turmeric Addition. Egypt. J. Food Sci. 45: 113 - 123.

Salvo, F., M.M. Tripodo and G. Dugo. 1984. Determination of fructose and glucose on Cucurbitaceae by h.p.l.c. J. Sci. Food Agric. 35: 212-214.

Santos, W. N. D., M. C. S. Sauthier, D. D. Cavalcante, C. M. Benevides, F. S. Dias and D. C. Santos. 2016. Mineral composition, nutritional properties, total phenolics and flavonoids compounds of the atemoya fruit (*Annona squamosa* L. x *Annona cherimola* Mill.) and evaluation using multivariate analysis techniques. An. Acad. Bras. Cienc. 88:1243-1252.

Sinha, A. K. 1972. Colorimetric assay of catalase. Anal. Biochem. 47:389-394.

Spanou, C. I., A. S. Veskoukis, D. Stagos, K. Liadaki, N. Aligiannis, A. Angelis, A. L. Skaltsounis, M. Anastasiadi, S. A. Haroutounian and D. Kouretas. 2012. Effects of Greek legume plant extracts on xanthine oxidase, catalase and superoxide dismutase activities. J. Physio. Biochem. 68:37-45.

Ugwu, O., O. Nwodo, P. Joshua, A. Bawa, E. Ossai and C. Odo. 2013. Phytochemical and acute toxicity studies of Moringa oleifera ethanol leaf extract. Int. J. Life Sci. Biotechnol. Pharm. Res. 2:66-71.

Virgen-Ceceña, L. J., L. M. Anaya-Esparza, A. V. Coria-Téllez, M. de Lourdes García-Magaña, H.S. GarcíaGalindo, E. Yahia and E. Montalvo-González. 2019. Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen dessert. Food Sci. Biotechnol. 28:1337-1347.

Waley, A.S., O.E. Shaltout, A.M. Abu – EL Yazeed, A.A. AL-bakry and A.M. Zeitoun. 2020. Bioactive Compounds and Physicochemical Properties of Fruit – Orange Juice Mixes with Rosemary Extract. Alex. Sci . Exch. J. 41: 141-153.

Yan, X., J. Yang, K. shhon and H. Lee. 2016. Attribute 2 image: conditional image generation from visual attributed. Computer Vision – ECCV.776-791.

Zhishen, J., T. Mengcheng and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64: 555-559.

الملخص العربي

التركيب الكيميائي والمركبات النشطة بيولوجيا والمركبات الفينولية في ثلاثة أصناف من فاكهة القشطة المنزرعة في مصر

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القشطة واحدة من الفواكه اللذيذة التي تنتمي إلى العائلة القشطية، يتزايد استهلاك مستخلصات الفاكهة الطازجة أو المشروبات المحضرة منها فى جميع أنحاء العالم، بسبب الاعتقاد بأنها تُسهم في نظام غذائي متوازن وأنها مصدر جيد لمضادات الأكسدة والمعادن مثل الكالسيوم، والفوسفور، والحديد، والماغنسيوم. اعتنت الدراسة الحالية بقياس التراكيب المورفولوجية والتركيب الكيميائى والمركبات النشطة بيولوجيا لثمار القشطة لأشهر ثلاثة أصناف من القشطه المنزرعه فى مصر. فيما يتعلق بالمعايير المورفولوجية، سجل الصنف عبد الرازق أعلى وزن للفاكهة (٦٢١,٤٣ جم) وكان وزن القشرة (١٢٩,٩٣ جم)، بينما احتلت ثمار الصنف البلدي أعلى نسبة من قشرة بالمقارنه بوزن الثمار (٠,٢٨٩ جم)، وكان متوسط عدد البذور ٥٥ بذرة في الثمرة. أما بالنسبة للصنف الهندي فقد سجل أعلى قيم لوزن البذور (١١,٧٤ جم/١٠٠ بذره). أما بالنسبه للتركيب الكيميائي فقد سُجل أعلى محتوى للدهون، والألياف، والكربوهيدرات ومعظم السكريات والمواد الصلبة الذائبة في الصنف عبد الرازق، وأشارت النتائج أن أعلى محتوى للرطوبة والرماد والبروتين كان فى الصنف الهندي. بينما كان محتوى الحموضة فى الأنواع الثلاثه متساويًا. وكان صنف عبد الرازق الأعلى في محتواه من العناصر المعدنية: الفوسفور، والبوتاسيوم، والحديد، والكالسيوم، والزنك والماغنسيوم مقارنة بالصنفين الآخرين، وكان صنف عبد الرازق أعلى في محتواه من المركبات النشطة بيولوجيًا: حمض الأسكوربيك والفلافونويدات الكلية، ومضادات الأكسدة، بينما كان الصنف هندي الأعلى فى محتواه من الفينولات الكلية و DPPH .

الكلمات المفتاحية: فاكهة القشطه، التركيب الكيميائي، المركبات النشطة بيولوجيا، المركبات الفينولية.