



Mineral composition, nutritional properties, vitamins, and bioactive compounds in *Annona squamosa* L. grown at different sites of Egypt

Nutritional properties in *Annona squamosa* L.

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Abstract: *Annona squamosa* L. is an important fruit crop worldwide, which has become an important crop as a result of its tasty flavor, nutritional value and antioxidant activities. This study aimed at determination of the phytochemicals and antioxidant compounds present in fruits, seeds and leaves of *A. squamosa* grown in four sites in Egypt (Mnofia, Giza, Alexandria and Mansoura). The highest levels of carbohydrates, folic acid, potassium and calcium of fruits were recorded at Mansoura. The highest contents of protein and sodium were recorded at Alexandria. Flavonoids, total phenols and vitamin C as well as the activity of POX and PPO enzymes were the highest at Mnofia. The fruits at Giza had a high content of iron, beside the high enzymatic activity of CAT at Giza and also at Alexandria. In seeds, carbohydrates, vitamin C and the activity of CAT, POX and PPO enzymes recorded the highest values at Giza. Flavonoids, total phenol and folic acid were the highest at Mnofia, beside the high content of protein was found also as well as at the Alexandria. Vitamin C recorded a high content at Mansoura. In leaves, the highest values of bioactive compounds (flavonoids, total phenol, folic acid, vitamin C.), PPO activity and K⁺ content were the highest at Giza. The total pigments, protein and Na⁺ recorded the highest levels at Mansoura. Meanwhile, total carbohydrates as well as CAT and POX activities have a high value at Mnofia. Ca²⁺ and Fe²⁺ have the highest content at Alexandria. Potassium, sodium, calcium and iron of roots recorded the highest contents at Mansoura followed by Alexandria and Giza.

keywords: *Annona squamosa*, antioxidants, ions, vitamins

1. Introduction

Plants synthesize a wide variety of natural medicinal compounds and thus attracted considerable interest as a main source for many bioactive metabolites [1]. Plant organs like fruits have potential human health benefits and consequently they have become increasingly important in human nutrition. The nutritional and health values of fruits are attributed to their high content of bioavailable nutrients and phytochemicals [2].

Recently, the term “functional food” has been introduced to describe foods containing components that induce particular physiological responses along with their nutritional functions and sensory acceptance [3]. It has been

advocated that a diet rich in vegetables can lead to a longer and healthier life [4].

The inclusion of fruit and their nutritional derivatives in dietary recommendations requires that they provide appreciable quantities of vitamins, minerals and many other nutrients of health benefits. For instance, folic acid can significantly reduce the risk of coronary heart disease and maintain a healthy body weight [5].

Plant extracts have been used as a source of medicinal compounds for thousands of years.

Custard apple (*Annona squamosa* L.) is called sugar apple or sweetsop and produces edible fruits that are consumed in many countries. Custard apple is a short (4 to 6 m

high) deciduous tree with many branches. [6]. The leaves are 5 to 15 cm long and 2 to 6 cm wide, with bluish-green lower surface and a bright green upper surface. Custard apple is the most important tropical fruit and is the most widely distributed among the annonaceous fruits. The plant can withstand rough climatic conditions [7]. The fruits are eaten and are also used in the preparation of beverages and ice creams [8]. The importance of the fruit is attributed to its sweet pulp which has medicinal applications. It also serves as a good source of carbohydrates (23.5 %), proteins (1.6 %) and minerals (0.9 %) [9]. Along with these nutritional constituents, the fruit contains large amounts of vitamins such as folic acid and ascorbic acid as well as minerals such as calcium, phosphorus and iron. [10]. In addition, the custard apple is a good source of natural antioxidant compounds, and all the plant parts are used in folk medicine worldwide [11]. The antioxidant activities of fruit extracts as well as the role of custard apple in preventing oxidative damage have been reported [11, 12]. Custard apple fruits contain a considerable amount of polyphenolic compounds with antiviral, antimicrobial, and anti-inflammatory activities along with their antioxidant properties [13, 14].

2. Materials and methods

Collection of plant samples

The above-ground parts of (*A.squamosa*) including the fruits and some parts of the root were collected from different sites in Egypt that is Mnofia, Giza, Alexandria and Mansoura during the fruiting season of the year (September-October, 2017). In addition, soil samples were collected from each site.

Sample treatments

The mature fruits, leaves and roots 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 80 °C and grinded 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.

1. Chemical analysis of the soil

Air dried soil samples were used to determine the soil pH, organic matter, calcium carbonate and total soluble salts, as well as chlorides, carbonates, bicarbonates, sulfates, as Na^+ , K^+ , Ca^{2+} and Fe^{2+} . A 1:5 soil: distilled water suspension was used to determine pH and soluble salts of the soil. The soil suspension was shaken for one-two hours and filtered through Whatman No. 1 filter paper and the filtrate was used for soil chemical analysis.

1.1. Preparation of soil

Soil reaction (pH) was determined using P.W 9418 pH meter PYE Unicam [15]. Electrical conductivity was monitored using YSI MODEL 33.S.C.T. Meter [16]. Calcium carbonate content was determined in the dry soil using the method described by [17]. Oxidizable organic carbon (as indication of the total organic matter) was estimated using Walkely and Block rapid titration method as described by [18]. The total nitrogen was determined by the conventional semi-micro propagation of kjeldahl method of [19] and adopted by [20]. Total soluble salts were calculated gravimetrically [21]. Sulfates in the soil extract were estimated gravimetrically by using 1% BaCl_2 [18]. Carbonates and bicarbonates were determined in the soil extract according to the method described by [15]. Estimation of chloride was done by titration against silver nitrate using potassium chromate solution as indicator [18]. The extractable cations Na^+ , K^+ , Ca^{2+} and Fe^{2+} were assayed in air dried soil using ammonium acetate solution at pH 7 as described by [15]. Na^+ and K^+ were determined using a flame photometer with a propane burner (type Biologie PHF-808) but Ca^{2+} and Fe^{2+} were determined using atomic absorption spectrophotometry (PHF 80B biology Spectrophotometer) [22].

Soil analysis of different sites in the selected four governorates was recorded in table (1).

2 Determination of relative water content (R.W.C)

RWC was estimated by the method of [23]. Leaf discs were cut from the center of the blade and their fresh weight (FW) was determined. The discs were then floated on distilled water for 4 hours, blotted and their fully turgid weight (TW) was recorded. The discs were oven-dried

at 80 °C till constant weight and their dry weight (DW) was determined. RWC was calculated as:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Water content (WC) estimated according to [24] of other plant organs (fruit, seed and root) calculated according to the formula:

$$WC = (FW - DW) / FW$$

2. Estimation of photosynthetic pigment

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined by the spectrophotometric method as described by [25] for chlorophylls, [26] for carotenoids

Table1: Chemical properties of selected soil from different sites in Egypt (Mnofia, Giza, Alexandria and Mansoura).

Parameters	Sites			
	Mnofia	Giza	Alexandria	Mansoura
pH	7.7	8.0	7.4	8.1
EC(μS/cm)	0.22	0.60	0.67	0.15
Organic carbon (%)	41.2	12.02	13.48	15.33
Total nitrogen (%)	0.105	0.128	0.134	0.118
C/N	390.17	94.14	100.34	128.02
T.S.S (%)	0.664	0.165	0.116	1.87
SO ₄ ²⁻ (%)	0.98	1.07	6.50	0.74
CaCO ₃ (%)	35.5	34.5	35.5	28.0
HCO ₃ ⁻ (%)	0.305	0.122	0.244	0.183
CO ₃ ⁻ (%)		Not detected	detected	Detected
Cl ⁻ (%)	10.0	10.0	12.5	10.0
K ⁺ (μmol g ⁻¹ D.wt)	1.69	1.49	0.794	3.99
Na ⁺ (μmol g ⁻¹ D.wt)	9.02	11.15	16.26	29.23
D Ca ²⁺ (μmol g ⁻¹ D.wt)	3.66	3.40	6.55	10.96
Fe ²⁺ (μmol g ⁻¹ D.wt)	0.068	0.011	0.067	0.0004

Estimation of carbohydrates

Dried tissue samples (leaves, fruits and seeds) were incubated in 10 cm³ of 80% (v/v) ethanol at 25 °C overnight with periodic shaking. The mixture was filtered, and the filtrate was made up to a known volume and kept in the refrigerator for analyses of the different sugar fractions. Glucose was assayed using the O-toluidine procedures of [27]. Fructose was estimated using the resorcinol

method [28]. Sucrose was estimated using the procedures adopted by [29]. Total soluble

sugars (TSS) content was determined using modification of the procedures of [30]. The content of polysaccharides was determined using the method of [31]. Total carbohydrates were determined by summation of the total soluble sugars and polysaccharides.

3. Total protein determination

The method of extraction and spectrophotometric determination of protein

content in the plant extract was that described by [32].

4. Determination of ionic contents.

The powdered dried matter was digested in concentrated HNO₃ and made up to volume with deionized water as described by [33]. K⁺ and Na⁺ concentrations were measured by Flame-Emission Spectrophotometer. Ca²⁺ and Fe²⁺ concentrations were measured by Atomic Absorption Spectrophotometry (PHF 80B biology Spectrophotometer). Data were calculated as μmol g⁻¹ dry weight.

5. Estimation of phenolic compounds

5.1. Determination of total phenolics

The total phenolics content of fruit extract of was determined according to the method described by [34].

5.2. Determination of total flavonoids

The total flavonoids content was estimated using AlCl₃ colorimetric assay as described by [35].

6. Estimation of vitamins

6.1. Determination of ascorbic acid

Ascorbic acid was quantified according to the method described by [36].

6.2. Estimation of Folic acid

Folic acid was determined according to the spectrophotometric method described by [37].

7. Estimation of antioxidant enzymes activity

7.1. Estimation of Catalase Activity (CAT, EC 1.11.1.6)

CAT activity was determined by slightly modified procedure of [38]. The reaction mixture contained 1 cm³ of 0.01 M phosphate buffer (pH 7.0), 0.4 cm³ of 0.5 M H₂O₂ and 0.5 cm³ of the plant extract. The absorbance was measured at 610 nm. One enzyme unit is defined as mmol H₂O₂/min./g f.wt.

7.2. Estimation of peroxidase (POX, EC 1.11.1.7)

Peroxidase activity was estimated at pH 6 as the increase in absorbance at 420 nm due to the formation of purpurogallin according to the modified method of [39].

7.3. Polyphenol oxidase (PPO, EC 1.14.18.1)

Polyphenol oxidase activity was assayed at pH 7 as the increase in absorbance at 420 nm due to the formation of purpurogallin in according to the modified method of [39].

I. Statistical analysis

The data were statistically analyzed using the procedures reported by [40] and means were compared using the multiple range tests at the 5 % level of probability.

II. Results and discussion

Fruits contribute significantly to the security of food and nutrition. Their important role depends on their typical flavor, taste and nutritional values.

Today, there is an increasing health concerns among consumers about the synthetic foods and their related chemical ingredients. As a result, natural foods and their derivatives are receiving much attention.

1. Relative water content

Annona species are native to the tropical and subtropical regions like tropical America, eastern Africa and Asia also, but few species are found in the temperate regions [41]. It occurs as an invasive species in some areas. Nowadays it is cultivated in Egypt, Lebanon, Sudan, Saudi Arabia, Oman, Jordan and Palestine [42].

The present results (Table 1), suggest that, *Annona Squamosa* L. grows well in the four sities of Egypt (Mnofia, Giza, Alexandria and Mansoura) which exhibited diverse characterized with some chemical properties. In this concept [41] stated that, *Annona* species can grow in a wide range of soil types, from sandy soils to clay loam.

WC of fruits had the highest value at Mnofia followed by Giza, Mansoura and Alexandria. Meanwhile, the WC of seeds was highest at Mnofia followed by Alexandria, Mansoura and Giza. In this concept, Generally the moisture content of Nigerian sample of *Punica granatum seeds* is 48.40% indicating that the sample has low moisture content compared to that of Saudi Arabia sample 57.83% [43]. The result is different from those reported by other researchers [44] on juice and seed of the fruits to be 72.6-86.4%. Mohammed *et al.*, (2010) [45] reported 52.3% on seeds, pulp and peel of pears fruits. The low moisture content signifies the higher dry matter yield as reported by [46]. The low moisture content of fruits does not favor growth of microorganisms, but aids in the long shelf life period of the produce [47]. On the other word, WC of roots and RWC of leaves showed high elevation at Alexandria and high decreasing at Mansoura. These differences may be due to the differences in the soil properties.

The pH ranged between 7.4 at Alexandria to 8.1 at Mansoura and; EC range from 0.15 at Mansoura to 0.67 at Alexandria. In this context, [48] found that, *Annona* species are grown in calcareous soil. Calcareous soils have high pH (from 7.5 to 8.5), high concentration of bicarbonate and generally low organic matter content. This was confirmed with our results (Table 1), except the organic matter that was high in our result. Organic carbon ranged between 12.02 % and 41.21% at Giza and Mnofia. For total nitrogen, its contents ranged from 0.105 % to 0.134% at Mnofia and Alexandria. The highest C/N ratio was recorded at Mnofia region where C/N ratio considered as a limiting factor for best growth. A common problem in these soils is an inadequate supply of soluble Fe²⁺, which results in Fe²⁺ deficiency in many crops [49].

Table 2: Fresh weight (g), dry weight (g) and relative water content (%) of different *Annona squamosa* in leaves, fruits, seeds and roots growing at different sites of Egypt (Mnofia, Giza, Alexandria and Mansoura). Each value is the mean of 3 samples \pm s.e

The groups that share the same letter are not significant

Organ and measurement	Site			
	Monofia	Giza	Alexandria	Mansoura
Root				
FW(gm)	0.04 \pm 0.01	0.03 \pm 0.03	0.04 \pm 0.01	0.02 \pm 0.03
DW(gm)	0.02 \pm 0.02	0.02 \pm 0.02	0.014 \pm 0.01	0.019 \pm 0.2
WC (%)	50.0 \pm 0.279 ^b	45.9 \pm 0.02 ^c	65.0 \pm 0.01 ^a	24.0 \pm 0.02 ^d
Leaf				
FW(gm)	0.04 \pm 0.02	0.18 \pm 0.04	0.17 \pm 0.01	0.037 \pm 0.02
DW(gm)	0.02 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.02	0.02 \pm 0.01
RWC (%)	50.0 \pm 0.03 ^c	67.5 \pm 0.04 ^a	64.7 \pm 0.05 ^b	45.9 \pm 0.03 ^d
Fruit				
FW(gm)	5.3 \pm 0.02	17.4 \pm 0.02	10.0 \pm 0.1	2.96 \pm 0.1
DW(gm)	1.2 \pm 0.02	5.6 \pm 0.03	3.3 \pm 0.1	0.95 \pm 0.1
WC (%)	76.1 \pm 0.01 ^a	67.8 \pm 0.01 ^b	67.0 \pm 0.2 ^b	67.7 \pm 0.04 ^b
Seed				
FW(gm)	1.0 \pm 0.01	1.14 \pm 0.01	2.0 \pm 0.01	0.51 \pm 0.1
DW(gm)	0.48 \pm 0.02	0.965 \pm 0.03	0.117 \pm 0.02	0.33 \pm 0.1
WC (%)	52.0 \pm 0.2 ^a	31.7 \pm 0.02 ^d	41.5 \pm 0.3 ^b	35.5 \pm 0.3 ^c

2 Photosynthetic pigments

Total chlorophyll and total pigments of *A.squamosa* leaves were highest at Mansoura followed by Giza, Alexandria and Mnofia. The Ch. (a/b) ratio showed non-significant difference between all sites (<0.05). The carotenoids content of *A.squamosa* leaves recorded the highest content at Giza followed by Mansoura, Alexandria and Mnofia. Chlorophyll plays an important role in plant performance particularly photosynthesis. Remote sensing can provide farmers with location-specific information of chlorophyll content as a representative of crop response to nitrogen application during different growing phases of the plant [50]. Modifications in the main parameters of the photosynthetic process occur in response to environmental changes. These modifications regulate photosynthesis during the day [51].

2. Carbohydrate contents

Although *A. squamosa* is reported to possess important applicable properties due to its insecticidal, antiovolatory and antitumor activities, there is no previous report about the nutritive effect of its fruit pulp [52]. Our present study offers the first scientific report regarding the nutritive value of the fruit pulp of *A. squamosa*.

A.squamosa recorded the highest content of glucose in leaves, seeds and fruits at Mnofia and Alexandria, respectively; while the content of glucose was the highest in fruits, seeds and leaves, respectively at Giza. The content of glucose of Mansoura region; in the fruits and leaves was; nearly; the same and had a higher content than that in seeds.

Fructose content in *A.squamosa* recorded the highest amount in seeds, fruits and leaves at Mnofia and Alexandria. Meanwhile, Giza and Mansoura regions recorded the highest fructose content in fruits followed by seeds and leaves.

Sucrose content of fruits was highest at Giza and Mansoura while that of seeds was highest at Mnofia and Alexandria. The lowest content of sucrose at all sites was that in leaves.

The highest content of total soluble sugars at Giza and Mansoura was found in fruits while the least content was that in leaves. At Monoufia, the highest content was found in seeds while the content of total soluble sugars was arranged ascending as seeds leaves and fruits at Alexandria.

Increased carbohydrates content in growing parts of the plant reflects metabolic regulation associated with enhanced enzyme activity which helps plants to withstand environmental conditions and to promote their growth [53].

The highest values of TSS were recorded in plant parts growing in the soil with high sand content which are exposed to water stress due to low soil water holding capacity. Accumulation of high soluble sugars levels have also been demonstrated in shoots of different plant species under stress conditions [54].

The highest content of polysaccharides was found in fruits while the least content was in leaves at Giza and Alexandria and in seeds at Mansoura and Mnofia.

Total carbohydrates concentration was in the order fruits > seeds > leaves at Giza and Mansoura. But in the order leaves > fruits > seeds was at Mnofia; and seed > fruits > leaves was at Alexandria.

In general the total soluble sugars in the fruits at Giza and Mnofia were higher than those at Alexandria and Mansoura. Meanwhile, the total carbohydrates in fruits at Mansoura and Giza were higher than at Mnofia and Alexandria. The soluble sugar content represented a mix of fructose and glucose (11.75%) in addition to sucrose (9.4%). The fiber combines cellulose, hemicellulose and pectic substances. The degree of fruit ripening does not interfere with these proportions, suggesting that fiber content is determined early ontogeny [41].

3. Protein contents

A.squamosa at Mnofia and Mansoura recorded the highest content of proteins in leaves followed by seeds and fruits. Meanwhile plants growing at Alexandria and Giza recorded the highest protein content in seeds followed by leaves and fruits. In general, the protein content in fruits was higher at Alexandria and Mansoura than Giza and Mnofia.

4. Vitamins contents

A.squamosa growing at Mnofia, Alexandria and Mansoura recorded the highest content of vitamin C in seeds followed by leaves and fruits. But plants growing at Giza recorded the highest content in leaves followed by fruits and seeds. The fruits at Mnofia have a higher content of vitamin C than the other sites. Babu (2014) [55] stated that, *A.squamosa* is an excellent source of vitamin C. The content of ascorbic acid in custard apple was reported to be in the range of 34 to 44 mg 100 g⁻¹ [56].

Folic acid content was found in leaves of *A.squamosa* followed by seeds and fruits at the four regions. Meanwhile the fruits at Mansoura have higher content of folic acid than the other sites.

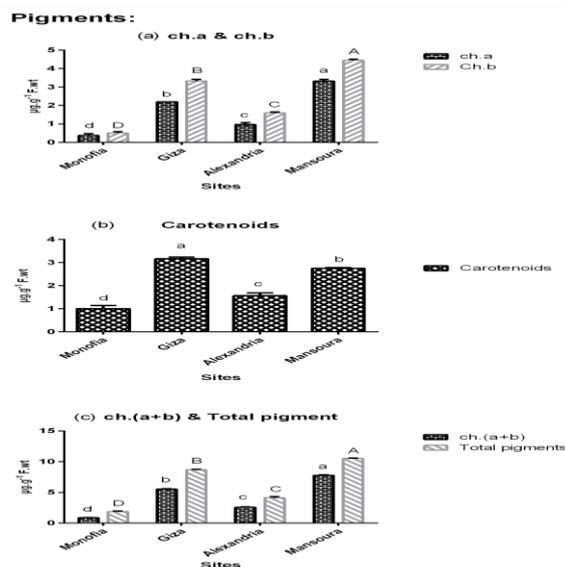


Figure 1: Photosynthetic pigment content in the leaves of *Annona squamosa* growing at different sites in Egypt (Mnofia, Giza, Alexandria and Mansoura). Each value is the mean of 3 replicates ± S.E.

The columns that share the same letter are not significantly different at P<0.05.

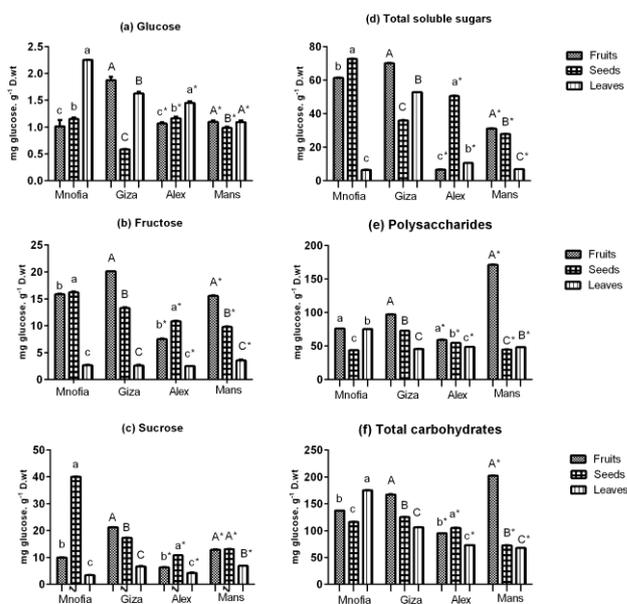


Figure 2: Carbohydrate contents in fruits, seeds and leaves of *Annona squamosa*, growing at different sites in Egypt (Mnofia, Giza, Alexandria and Mansoura). Each value is the mean of 3 replicates.

The columns that share the same letter are not significantly different at $P < 0.05$.

Table 3: Proteins, folic acid and vitamin C concentrations of leaves, fruits and seeds of *Annona squamosa* growing at different sites of Egypt (Mnofia, Giza, Alexandria and Mansoura). Each value is the mean of 3 replicates \pm S.E.

Organ and site	Protein (mg g ⁻¹ FW)		Vitamins
		Folic acid (μ g g ⁻¹ FW)	Ascorbic acid (mg g ⁻¹ FW)
Leaf			
Monofia	13.47 \pm 0.11 ^a	8.12 \pm 0.10 ^a	14.42 \pm 0.02 ^b
Giza	6.80 \pm 0.11 ^b	10.47 \pm 0.11 ^a	16.78 \pm 0.13 ^b
Alexandria	3.52 \pm 0.10 ^b	10.27 \pm 0.10 ^a	12.43 \pm 0.13 ^a
Mansoura	17.26 \pm 0.02 ^a	10.39 \pm 0.1 ^a	11.98 \pm 0.20 ^b
Fruit			
Monofia	0.737 \pm 0.025 ^c	0.319 \pm 0.002 ^c	1.07 \pm 0.002 ^c
Giza	0.754 \pm 0.06 ^c	0.189 \pm 0.003 ^c	0.382 \pm 0.001 ^c
Alexandria	1.41 \pm 0.05 ^c	0.175 \pm 0.003 ^c	0.881 \pm 0.01 ^b
Mansoura	1.21 \pm 0.02 ^c	0.388 \pm 0.001 ^c	0.602 \pm 0.01 ^c
Seed			
Monofia	10.50 \pm 0.06 ^b	1.91 \pm 0.002 ^b	36.53 \pm 0.03 ^a
Giza	7.87 \pm 0.13 ^a	1.33 \pm 0.004 ^b	48.08 \pm 0.11 ^a
Alexandria	10.59 \pm 0.13 ^a	1.33 \pm 0.001 ^b	0.789 \pm 0.03 ^c
Mansoura	4.57 \pm 0.14 ^b	0.372 \pm 0.001 ^b	47.87 \pm 0.01 ^a

The means that share the same letter are not significantly different at <0.05 in the fluid balance and nerve impulse transmission within human cells [58].

5. Phenolic contents

A.squamosa growing at Mnofia, Alexandria and Mansoura recorded the highest content of flavonoids and total phenols in fruits followed by leaves and seeds. But plants growing at Giza recorded the highest content in seeds followed by leaves and fruits. The highest total flavonoid and phenols in fruits contents were found at Mnofia.

6. Enzymes activates

Activities of some antioxidant enzymes revealed that the highest activity of CAT and POX was in leaves of *A.squamosa* followed by the seeds and fruits at all sites (Table 4), meanwhile, PPO recorded highest activity in the seeds followed by the leaves and fruits.

7. Ionic contents

Fruits are nutrient suppliers, acting on the metabolism of several functions in humans, and their main nutrients (minerals and vitamins) influence the performance of these functions. Hence, several researchers [57] have conducted studies on the mineral composition of different fruits.

Potassium concentration of plant tissue at different sites was highest in roots followed by fruits and leaves except at Alexandria where the highest content was in fruits, followed by leaves and roots. Potassium concentration of fruits was highest at Mansoura. Potassium aids

Sodium concentration of plant tissue at Mansoura was highest in the roots, followed by leaves and fruits. But at the other three sites it was highest in roots followed by fruits and leaves. Fruits had the highest Na⁺ concentration at Alexandria relative to the other sites.

The (K⁺/Na⁺) ratio of plant tissue was highest in fruits followed by leaves and, roots at all regions except at Alexandria where the highest content was in leaves followed by fruits and roots. K⁺/Na⁺ ratio of plant tissue was highest in fruits followed by leaves and roots at all regions except at Alexandria which has the highest content in leaves followed by fruits and roots. K⁺/Na⁺ ratio of fruits was higher at Mnofia and Mansoura than the other two sites

Calcium concentration of plant tissue was highest in leaves followed by roots and fruits at all regions except at Mansoura which has the highest concentration in leaves followed by fruits and roots. Iron concentration of plant tissue was highest in roots followed fruits and seeds while at Mansoura, it was the highest in leaves followed by roots and fruits. The calcium concentration of fruits was highest at Mansoura.

Calcium is essential for blood clotting and muscle contraction [58]. The fruits at Giza had a rich content of Fe²⁺. The importance of assure

adequate bio-available dietary Fe²⁺ arises from the severe consequences associated with iron deficiency and anemia, including reduced immunity and resistance to infection, retardation of body development [59]. Therefore, the *Annona* fruit becomes important in view of the fact that its regular consumption might ensure adequate supply of iron level into the body. Also, mineral content (Na⁺, Ca²⁺, K⁺ and Fe²⁺) of the deferent organs of *A.squamosa* differed in the different sites. The predominant mineral in *A.squamosa* fruits was K⁺ which

occurred in higher levels than the other fruits such as banana and apple [60].

Roots, leaves, fruits, seeds and bark custard apple has several applications [62]. The fruits are with high caloric value, minerals and vitamins. Athletes can use it for their high energy content. The seeds have major insecticidal properties and could be used for removing head lice [62] and as a pesticide in agriculture and horticulture. In folk medicine, fruits are effectively used as anti-depressant and antioxidant [63].

Table 4: Flavonoids and total phenols content and activities of Catalase, peroxidase and polyphenol oxidase in the leaves, fruits and seeds of *Annona squamosa* growing at different sites of Egypt (Mnofia, Giza, Alexandria and Mansoura).

Organ and site	Phenolics		Antioxidant Enzyme activities		
	Flavonoids (µg g ⁻¹ Dw)	Total phenols (mg gallic acid g ⁻¹ Dw)	CAT (mmol H ₂ O ₂ . min ⁻¹ . g ⁻¹ Fw)	POX (µmol pyrogallol .min ⁻¹ .g ⁻¹ F.wt)	PPO(µmol pyrogallol .min ⁻¹ .g ⁻¹ F.wt)
Leaf					
Monofia	2.820.02 ^{c±}	2.820.02 ^{c±}	386.620.12 ^{a±}	215.500.11 ^{a±}	40.360.20 ^{b±}
Giza	4.930.07 ^{c±}	4.930.07 ^{c±}	372.33±0.12 ^{a±}	182.90.10 ^{a±}	49.300.10 ^{b±}
Alexandria	1.08±0.13 ^b	1.08±0.13 ^b	318.42±3.7 ^a	168.50±0.12 ^a	45.50±0.03 ^b
Mansoura	1.92±0.20 ^b	1.92±0.20 ^b	368.99±0.21 ^a	145.49±0.11 ^a	48.72±0.14 ^b
Fruit					
Monofia	9.67±0.10 ^a	9.67±0.10 ^a	11.35±0.10 ^c	34.66±0.21 ^c	19.10±0.12 ^c
Giza	1.60±0.04 ^b	1.60±0.04 ^b	18.49±0.21 ^c	10.77±0.11 ^c	11.80±0.16 ^c
Alexandria	5.47±0.12 ^a	5.47±0.12 ^a	18.65±0.12 ^c	6.50±0.11 ^c	11.50±0.14 ^c
Mansoura	4.60±0.14 ^a	4.60±0.14 ^a	13.18±0.04 ^c	7.53±0.10 ^c	16.00±0.03 ^c
Seed					
Monofia	5.25±0.11 ^b	5.25±0.11 ^b	113.80±0.10 ^b	75.33±0.0 ^b	70.20±0.0 ^a
Giza	0.700±0.03 ^c	0.700±0.03 ^c	145.89±0.14 ^b	92.63±0.01 ^b	75.00±0.02 ^a
Alexandria	0.975±0.02 ^c	0.975±0.02 ^c	82.45±0.11 ^b	79.96±0.01 ^b	51.13±0.12 ^a
Mansoura	1.10±0.04 ^c	1.10±0.04 ^c	132.76±0.03 ^b	65.30±0.0 ^b	66.20±0.22 ^a

Each value is the mean of 3 replicates ± S.E. The means that share the same letter are not significantly different at P<0.05.

Table 5: Ionic content of leaves, fruits and roots of *Annona squamosa* growing at different sites in Egypt (Mnofia, Giza, Alexandria and Mansoura). Each value is the mean of 3 replicates ± S.E.

The means that share the same letter are not significantly different at P<0.05.

Organ and site	µmol g ⁻¹ DW				K ⁺ /Na ⁺ ratio
	K ⁺	Na ⁺	Ca ²⁺	Fe ²⁺	
Fruit					
Monofia	639.09±0.279 ^a	73.36±0.26 ^b	13.52±0.13 ^c	56.69±0.26 ^b	8.71±0.03 ^a
Giza	619.07±0.28 ^a	97.14±0.41 ^b	14.92a±0.08 ^c	85.30±0.10 ^b	6.37±0.02 ^a
Alexandria	419.68±0.88 ^a	145.75±0.38 ^b	14.47±0.06 ^c	62.00±0.86 ^b	2.87±0.00 ^b
Mansoura	778.77±2.83 ^a	89.58±0.13 ^c	19.47±0.11 ^b	15.02±0.31 ^c	8.69±0.03 ^a
Leaf					
Monofia	252.47±0.86 ^c	61.2a±0.27 ^c	78.51±0.27 ^a	39.90±0.47 ^c	4.12±0.00 ^b
Giza	386.98±0.43 ^c	70.01±0.05 ^c	160.29±0.05 ^a	40.33±0.21 ^c	5.52±0.01 ^b
Alexandria	315.25±0.48 ^b	85.86±0.12 ^c	171.65±0.13 ^a	49.55±0.21 ^c	3.67±0.01 ^a
Mansoura	314.42±0.21 ^c	95.18±0.08 ^b	113.21±0.08 ^a	37.230±.16 ^a	3.30±0.03 ^b
Root					
Monofia	460.16±2.20 ^b	218.91±0.22 ^a	74.71±0.15 ^b	75.47±1.8 ^a	2.10±0.01 ^c
Giza	393.53±1.11 ^b	212.13±0.58 ^a	88.46±0.30 ^b	171.5±1.8 ^a	1.85±0.01 ^c
Alexandria	298.94±4.33 ^c	235.42±0.84 ^a	73.91±0.17 ^b	81.61±0.24 ^a	1.26±0.02 ^c
Mansoura	472.14±11.66 ^b	157.78±0.40 ^a	60.840±0.13	31.72±0.34 ^b	2.99±0.07 ^c

III. Conclusion and Recommendation

The results of this study indicate that the fruits of *A.squamosa* are well endowed with

essential nutrients for humans. The presence of high mineral concentrations in the fruits gives a new insight into their potential use as a medicinal plant and to compensate for mineral deficiencies in human and animals. The present study reveals that the different parts of *A.squamosa* is rich in carbohydrates, vitamins, minerals, phenolics and protein; therefore they can be harnessed in human and animal nutrition. The climatic influence on fruit and seed components would also be studied. It would then be possible to determine specific potential of the seed and to recommend large scale cultivation of the plant. Unlike orange, mango and banana which are widespread in Egypt, custard apple (*A.squamosa*) trees are rarely cultivated; therefore we recommend large scale cultivation of this fruit tree.

IV. References

1. Yan, X.; Yang, J.; Sohn, K. and Lee, H. (2015). Attribute Conditional Image Generation from Visual Attributes. CoRR, abs/1512.00570 .
2. Albuquerque, T.G.; Santos, F.; Sanches-Silva, A.; Oliveira, M.B.; Bento, A.C.; and Costa, H.S. (2016). Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: potential health benefits. *Food Chem.*, **193**:187–195.
3. Zeraik , M.I.; Pereira, C.A.M.; Zuin, V.G. and Yariwake, J.H. (2010). Maracujá: um alimento funcional? *Revista Brasileira de Farmacognosia, Curitiba*, **20**: 459-471.
4. Melo, E.A.; Maciel, M.I.S.; de Lima, V. L. A.; and Rodrigues de Araujo, C. (2008).Capacidade antioxidante de frutas. *Revista Brasileira de Ciências Farmacêuticas, São Paulo*, **44**: 193-201.
5. Nicklas,T.A.; O’neil, C.E.; Liska, D.J.; Almeida, N.G. and Fulgoni, V.L. (2011). Modeling dietary fiber intakes in US adults: implications for Public Policy. *Food and Nutrition Sciences, Irvine*, **2**:925-931.
6. Kiill, L.H.P. and Costa, J.G. (2003). *Biologia floral e sistema de reproduc, aõde Annona squamosa L. (Annonaceae) na regiaõ de Petrolina-PE. Cie`ncia Rural*, **533**: 851–856
7. Shete, M.; Bhosale, A. and Supe, A. (2009). *Sitafal lagwad. 7th All Maharashtra State Custard Apple Symposium, Souvenir*, pp. 1-4.
8. Chikhalikar, N.V.; Sahoo, A.K.; Singhal, R.S. and Kulkarni, P.R. (2000). Studies on frozen pourable custard apple (*Annona squamosa* L.) pulp using cryoprotectants, *J. of the Science of Food and Agriculture*, **80**: 1339-1342.
9. Bressy, F.C; Brito, G.B.; Barbosa, I.S.; Teixeira, L.S.G. and Korn, M.G.A. (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.
10. Dembitsky, V.M.; Poovarodom, S.; leontowicz, H.; leontowicz, M.; Vearasilp, S.; Trakhtenberg, S. and Gorinstein, S. (2011). The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food Research International, Ottawa*, **44**:1671-1701.
11. Nunes, C.R.; Bernardes, N.R.; Glória, L.L. and Oliveira, D.B. (2012). Flavonoides em Annonaceae: ocorrência e propriedades biológicas. *Revista Vertices, São Paulo*, **14**: 39-57.
12. Ronowicz J.; Kupcewicz, B. and Budzisz, E. (2014). Implementation of chemometric techniques for evaluation of antioxidant properties of *Camellia sinensis* extracts. *Cent Eur J Chem.*, **12**: 700-710.
13. Roesler, R.; Malta, L.G.; Carrasco, L.C.and Pastore, G. (2006). Evaluation of the antioxidant properties of the Brazilian Cerrado fruit *Annona crassiflora* (Araticum). *J. Food Science*, **71**: C102–C107.
14. Ignat, I.; Volf, I. and Popa, V.I.(2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem.*, **126**: 1821-1835.
15. Jackson, M. (1967). *In soil chemical analysis*. Prentica Hall. Inc. Inglewood cliffs London, PP. 498.
16. Jackson, M.; Gillette, D.; Danielsen, E., Blifford, I., Bryson, R.; and Syers, J (1973). Global dust fall during the

- quaternary as related to environments. *J. of Soil Science*, **116**: 135-145.
17. Allen, S.; Terman, G.; Clements, L. and Mikkelsen, R. (1976). Greenhouse techniques for soil-plant-fertilizer research. *J. National Fertilizer Development Center, Tennessee Vally Authority. Bull. Y-104. Muscle Shoals, AL.*
 18. Piper, C.S. (1966). Soil and plant analysis; A laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants. Hans Publications, Bombay.
 19. Pirie, N.W. (1955). Proteins. In: Modern methods of plant analysis Peack, K. and Tracey, M.V. (eds). Springer Verlage, Berlin.
 20. Delory, M. (1949). Colorimetric estimation of ammonia. In: Inorganic chemistry (Vogel, H.J. ed.). Longman, London, 126- 132.
 21. Pansu, M. and Gautheyrou, J. (2006). Handbook of soil analysis mineralogical, organic and inorganic methods. Springer-Verlag, Heidelberg.
 22. Chapman, H.D. and Pratt, P.F. (1978). Methods of analysis for soils, plants and waters. University of California, Division of Agricultural Sciences. *J. of Agronomy*, **56**:133-136.
 23. Weatherly, P.E. and Barrs, C. (1962). A re-examination of relative turgidity technique for estimating water deficits in leaves. *Australian J. of Biological Science*, **15**: 413- 428.
 24. Sridhar, M. B. B.; Han, F. X.; Diehl, S. V.; Monts, D. L., and Su, Y. (2007). Effects of Zn and Cd accumulation on structural and physiological characteristics of barley plants. *Brazilian J. of Plant Physiology*, **19**: 15–22.
 25. Horvath, G.; Kissimon, J. and Faludi-Daniel, A. (1972). Effect of light intensity on the formation of carotenoids in normal and mutant maize leaves. *Phytochemistry*, **11**: 183- 187.
 26. El-Shahaby, O.A.; Younis, M.E.; Hasaneen, M.N.A. and Gaber. A.M. (1993). Plant growth, metabolism and adaptation in relation to stress conditions: XVII Influence of different water treatments on stomatal apparatus, pigments and photosynthetic capacity in *Vicia faba*. *J. of Arid Environments*, **25**: 221-232.
 27. Feteris, A.W. (1965). A serum glucose method without protein precipitation. *American J. of Medical Technology*, **31**: 17- 21
 28. Roe, J. H. (1934). A colorimetric method for the determination of fructose in blood and urine. *J. Biol. Chem.*, **107**: 15- 22.
 29. Handel, E.V. (1968). Direct microdeterminations of sucrose. *Analytical Biochemistry*, **22**: 280- 283.
 30. Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates by anthrone. *Biochemical journal*, **57**:508-514.
 31. Sadasivam S, Manickam A (1996). *Biochemical Methods*, New Age International Publishers (P) Ltd.; New Delhi, India.
 32. Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein-dye binding. *Anal. Biochem.* **72**: 248–254.
 33. Motsara, M.R. and Roy, R.N. (2008). Guide to Laboratory establishment for plant nutrient analysis. FAO Fertilizer and Plant Nutrition Bulletin. Food and Agriculture Organization, Rome.
 34. Kosem, N.; Han, Y.H., Moongkarndi, P. (2007). Antioxidant and cytoprotective activities of methanolic extract from *Garcinia mangostana* hulls. *Science Asia*, **33**: 283–292.
 35. Atanassova, M.; Georgieva, S.; and Ivancheva, K. (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *J. U. Chem. Technol. Metall*, **46**: 81–88.
 36. Elgailani, I.E.H.; Elkareem, M. A. M. G.; Noh, E. A. A.; Adam, O. E. A. and Alghamdi, A. M. A. (2017). Comparison of Two Methods for the determination of Vitamin C (Ascorbic Acid) in Some Fruits. *American Journal of Chemistry*, **2**:1-7
 37. Ruengsitagoon, W. and Hattanat, N. (2012). Simple Spectrophotometric Method for Determination of Folic Acid. 4th Annual Northeast Pharmacy Research

- Conference of Pharmacy Profession in Harmony, 11-12:281.
38. Sinha, K.A. (1972). Colorimetric assay of catalase. *Anal Biochem*, **47**: 389-394.
 39. Devi, P. (2002). Principles and methods in plant molecular biology, biochemistry and genetics, 2nd edition. Agrobios, India 71-72.
 40. Snedecor, G.W. and Cochran, W.G. (1982). Statistical Methods. The Iowa State University Press 7 th. Edit., 2nd printing. PP 507.
 41. Pinto, A.C.Q.; Cordeiro, M.C.R.; Andrade, S.R.M.; Ferreira, F.R.; Filgueiras, H.A.C.; Alves, R.E. and Kinpara, D.I.; (2005). Annona Species. International Centre for Underutilised Crops, University of Southampton, Southampton, UK. pp. 6-7.
 42. Fischer, M.R.; Wong, L.J. and Pagad, S.(2017). Global Register of Introduced and Invasive Species- Egypt. Published by Invasive Species Specialist Group ISSG REGISTERED SEPTEMBER 28: 2017.
 43. Dangoggo, S. M.; Bunu, M.I.; Uba, A. and Saidu, y. (2012). Study of Proximate, mineral and anti-nutrient composition of Punica granatum seeds from North-Western Nigeria, and Saudi Arabia. *Researcher*, **4**:4-9.
 44. Morton, I. J. (1987). Soursop. In I. J. Morton (Ed.), Fruits of warm climates (pp. 69-72). Miami, Florida: Media Incorporated.
 45. Mahammad, M.U.; Kamba, A.S ;Abubakar, L.and Bagna, E.A.(2010). Nutritional composition of pear fruits (*Pyrus ommunis*). *African J. of Food Science and Technology* (ISSN: 2141-5455). **1**: 76-81.
 46. Bamigboye, A.Y.; Okafor, A.C. and Adepoju, O.T. (2010). Proximate and mineral composition of whole and dehulled Nigerian sesame seed. *Afr. J. Food Sci. Technol.*, **1**:76-81.
 47. Ruberto, G. and Baratta, M.T.; (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, **69**: 167 - 174.
 48. Lucena, J.J. (2000). Effect of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. *J. Plant Nutr.* **23**: 1591-1606
 49. Tagliavini, M., Rombolá, A.D. and Marangoni, B. (1995). Response to iron-deficiency stress of pear and quince genotypes. *J. Plant Nutr.* **18**: 2465-2482.
 50. van Evert, F.K.; Booij, R.; Jukema, J. N.; ten Berge, H.F.M.; Uenk, D.; Meurs, E.J.J.; van Geel, W.C.A.; Wijnholds, K.H. and Slabbekoorn, J.J. (2012) .Using crop reflectance to determine sidedress N rate in potato saves N and maintains yield. *Europ. J.Agronomy*, **43**: 58-67.
 51. Geiger, D.R. and Servaites, J.C (1994). Diurnal regulation of photosynthetic carbon metabolism in C₃ plants. *Annu Rev Plant Physiol Plant Mol Biol* **45**:235-256.
 52. Rajasekaran, S.; Sundaramoorthy , P.and Sankar Ganesh , K. (2015). Effect of FYM, N, P fertilizers and biofertilizers on germination and growth of paddy (*Oryza sativa*. L), **8**:59-65.
 53. Patil, R.N.; Patil, R.Y. and Ahirwar, D. (2010) Study of Some Medicinal Plants for Antidiabetic Activity in Alloxan Induced Diabetes. *Pharmacology online* **1**: 53-60.
 54. Bakry, A.B.; Elewa, T.A. and Ali, O.A.M.; (2012). Effect of Fe Foliar Application on Yield and Quality Traits of Some Flax Varieties Grown Under Newly Reclaimed Sandy Soil. *Australian J. of Basic and Applied Sciences*, **6**: 532-536.
 55. Babu, C. Devi, P.R. Kombiah, P.(2014): Screening of biological actives extracts from *Annona squamosa*. *International J.of Biological Research*, **2**:18-20.
 56. Alwazeer, D.; Delbeau, C. Divies, C.and Cachon, R.(2003). Use of redox potential modification by gas improves microbial quality, color retention and ascorbic acid stability of pasteurized orange juice. *International J. of Food Microbiology*. **89**:21-29.
 57. Tufuor, J.K.; Bentum ,J.K.; Essumang, D.K. and Koranteng-Addo ,J.E. (2011) Analysis of heavy metals in citrus juice from the Abura-Asebu- Kwamankese District, Ghana *J. Chem. Pharm. Res.* **3**: 397-402.

58. Whitney, E. and Rolfes, S.R.(2005). Understanding Nutrition. Thompson Learning Inc. New York, U.S.A.
59. Cooper, M.J.; Cockell, K.A. and Labbe, M.R.(2006). The iron status of Candian adolescent and adults: Current knowledge and practical implications. *Can J Diet Pract Res*, **67**: 130-138.
60. Damodaran, S.; Parkin, K.L and Fennema, O.R. (2008). Fennema's Food Chemistry, 4thed. Taylor & Francis Group, LLC, 1160 p.
61. Pandey, N. and Barve, D.(2011). Phytochemical and Pharmacological Review on *Annona squamosa* Linn, *International J. of Research in Pharmaceutical and Biomedical Sciences*, **2**:1404-1412.