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Biochemical profiling of *Annona* species and *Annona atemoya* varieties

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Abstract

The objective of this study was to characterize the chemical composition of fruit pulp of the six widely known *Annona* species and also three *Annona atemoya* varieties. The trees maintained at the field gene bank, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru served as the experimental material. The biochemical analysis of *Annona* species and *Annona atemoya* varieties revealed the range of TSS (12.74 to 28.69⁰ B and 29.31 to 27.65⁰ B), acidity of pulp (0.13 to 0.43% and 0.243 to 0.265), TSS: Acidity ratio in accession 3/2 (29.60 to 130.92 and 104.64 to 118.11), ascorbic acid content (16.22 to 25.31 and 21.31 to 21.53), total sugars (10.95 to 23.54 and 23.42 to 23.99 g/100g), reducing sugars (8.13 to 19.89 and 20.12 to 20.51 g/100g) and non-reducing sugars content (2.90 to 3.83 and 3.11 to 3.48 g/100g). 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 and *Annona atemoya* varieties respectively.

Keywords: *Annona* species, *Atemoya* varieties, biochemical parameters, fruit pulp

Introduction

The genus *Annona*, belonging to family *Annonaceae* is one of the world's most exquisite, but less studied fruits in India. The *Annonaceae* or custard apple family comprises about 130 genera and has 166 species (Anon, 2013) ^[1], six of which have pomological significance. Among the edible *Annonas*, the cherimoya (*Annona cherimola* P. Mill.), sweetsop/sugar apple/custard apple (*Annona squamosa* L.) and *Annona atemoya* Hort. (a hybrid of *Annona cherimola* and *Annona squamosa*) are commercially significant and economically important fruits in several tropical and sub-tropical countries. Other species of *Annonaceae* are Ramphal (*Annona reticulate* L.), Soursop (*Annona muricata* L.) and Pond apple (*Annona glabra* L.).

Annona squamosa is native to tropical America but its exact native range is unknown. The sugar apple species name 'squamosa' refers to the knobby appearance of the fruit. Sugar apple is a small tropical tree originating in the New World tropics, probably Central America. It is one of the major *Annonaceous* fruits grown commercially in India with an area of 44,000 hectares with a production of 3,67,000 MT (Anon, 2017) ^[4]. The plant parts also contain numerous amounts of bioactive chemical substances such as acetogenins, alkaloids, terpenes, flavonoids, cyclopeptide, annomuricatin and oils (Pinto *et al.*, 2005) ^[20]. These compounds are very useful medicines because some acetogenins have anti-tumoral, antifertility, abortifacient, insecticidal, antibacterial, immuno-suppressant, pesticidal or antihelminthic properties. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. Ethanolic extracts of leaves and stem are reported to have an anticancerous activity. *A. squamosa* has phytopharmacological properties (Saleem *et al.*, 2009) ^[22].

Nutritional and commercial potential knowledge of the native species could be an economic alternative to the livelihood of native population from different regions (Silva *et al.*, 2013) ^[24]. There is an old saying "Eat your food like your medicines or else you will eat your medicines like food". Although, there is a crescent increase in studies with native fruits as well as development of new food products based on them, the information about the nutritional potential of the *Annonaceous* fruits are limited or, often do not exist, occurring a lack on scientific investments in this area (Souza *et al.*, 2012) ^[27]. Thus the current investigation was taken up to assess the biochemical profiling of selected six species of *Annona* and three varieties of *Annona atemoya*.

Materials and Method

The experiments were carried out at ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake, Bengaluru during 2015-2017. The fruits were harvested at full maturity and kept in laboratory for 2 days for ripening. Fresh pulp from ripe fruits was used for the biochemical analysis. The experiment was done in a completely randomized design using three replications and data generated through experiments were statistically analyzed by using the OPSTAT (Gomez and Gomez, 1983) [10]. The list of six species of *Annona* and three varieties of *Annona atemoya* used in the study are given in

Table 1: List of Six species of *Annona* and three varieties of *Annona atemoya* selected for the biochemical profiling

Treatments	Six species of <i>Annona</i>	Three varieties of <i>Annona atemoya</i>
	<i>Annona squamosa</i> L.	Island Gem
	<i>Annona reticulata</i> L.	Pink's Mammoth
	<i>Annona glabra</i> L.	Bullock's Heart
	<i>Annona muricata</i> L.	
	<i>Annona atemoya</i> Hort.	
	<i>Annona cherimola</i> Mill.	

Procedure followed for biochemical analysis are described below:

Total soluble solids [° Brix] of fruit pulp of all the genotype was recorded by using a digital Refractometer (Digital refractometer, DBX-55).

Titration acidity (%): The acidity of the 10 g pulp samples was determined by diluting an aliquot of the sample with distilled water and titrating with 0.1N NaOH using phenolphthalein as an indicator. The end point appeared as light-pink colour. The calculated acidity was expressed as per cent anhydrous citric acid (Ranganna, 1986) [21]. The TSS: acid ratio was calculated by dividing the TSS by titration acidity.

Ascorbic acid content was determined by 2, 6-Dichlorophenol indophenol (DCPIP) method (Malick and Singh, 1980). (AOAC, 967.21) (Association of Official Analytical Chemists, 2006). Ten grams of fruit pulp was mixed thoroughly with 4% oxalic acid solution, squeezed through a muslin cloth and volume was made up to 50 ml. Vitamin C content present in the solution was estimated by titrating a known quantity of the extract against DCPIP. The end point is the appearance of pink colour. Vitamin C content was calculated as mg of ascorbic acid equivalents per 100 g fresh weight using a standard curve of L-Ascorbic acid.

Total Sugars, Reducing and non-reducing sugars by Nelson-Somogyi Method (Somogyi, 1952 and Krishnaveni *et al* 1984) [26, 15]. The extract was taken and titrated against 10ml of mixed Fehling solution A and B using methylene blue as indicator. The results were expressed as percent of

reducing sugar. The sugar extract was hydrolyzed with concentrated hydrochloric acid and titrated against 10 ml of mixed Fehling's solution (5 ml Fehling A + 5 ml Fehling solution B) using methylene blue as indicator. Results were expressed as per cent total sugar. The amount of non-reducing sugar was calculated by subtracting reducing sugars from total sugar and multiplying the difference by factor 0.95 as suggested by AOAC (1980).

Antioxidant activity (Diphenyl-1-picryl hydrazyl radical scavenging ability (DPPH)): Extract was prepared by taking sample (5g) with 50 ml of 80% methanol. 0.2 ml of extract was taken in test tube, 0.3 ml of acetate buffer was added followed by 2.5 ml of DPPH solution and mixed. The absorbance of the solution was read spectro photometrically at 517 nm after 30 minutes of incubation (A1). The absorbance of DPPH solution without sample (A2) was taken. The difference in the absorbance of DPPH solution with and without sample (A2 – A1) was calculated and the decrease in absorbance with sample addition was used for calculation of antioxidant activity. A standard curve was developed using different concentrations of ascorbic acid (20-100 µg/ml). The results were expressed as ascorbic acid equivalent antioxidant capacity (Kang *et al.* 2002). the difference in absorbance of DPPH solution with and without ascorbic acid (b1-a1) was calculated and Divided the concentration of the ascorbic acid by the difference in absorbance to arrive at the amount of ascorbic acid per unit absorbance (µg/OD) - (a). The antioxidant concentration in the sample extract (µg/ml) was calculated by multiplying the absorbance of sample with (a) - (b).

Total phenols: The analysis of total phenols was carried out by Folin-Ciocalteu spectrophotometric method suggested by Singleton and Rossi (1965) [25]. Gallic acid equivalence method was used for determining the phenol content in the fruit juice. Total phenolics content was expressed as Gallic acid equivalents (GAE) in mg per 100g fresh weight of pulp

Total flavonoids: 5g of sample was Homogenized with 20 ml of methanol (80%) in a pestle and mortar 2-3 times. the extract was Pooled and the volume was made up to 50 ml. 1.0 ml of extract was Taken in tubes and 0.3 ml of 5% NaNO₂ was added. after 2 min 0.3 ml of 10% AlCl₃ was added. After another 2 min, 3.4 ml of NaOH was add and allowed to stand at room temperature for 10 minutes. The absorbance was Read at 510 nm against blank. Catechin was used as standard (Chun *et al.*, 2003) [18].

Results and Discussion

A considerable variation was observed in some of the biochemical and antioxidant properties of selected *Annona* species and atemoya varieties and are presented in Table 2 and 3.

Table 2: Biochemical characters of different *Annona* species

<i>Annona</i> species	TSS (°B)	Titration acidity (%)	TSS: Acidity	Ascorbic acid (mg/100g)	Total sugars (g/100g)	Reducing sugars (g/100g)	Non reducing sugars (g/100g)	Total Phenols (mgGAE/100g)	Total Flavonoids (mg Catechin equivalent /100g)	DPPH (mgAEAC/100g)
<i>Annona squamosa</i>	24.98	0.24	133.84	24.92	20.58	17.38	3.20	73.06	20.45	86.05
<i>Annona reticulata</i>	24.48	0.27	93.44	20.50	19.93	16.85	3.13	87.60	23.95	100.29
<i>Annona glabra</i>	16.56	0.13	134.63	19.12	12.73	9.83	2.90	74.96	26.90	81.15
<i>Annona muricata</i>	12.74	0.43	29.60	25.31	10.95	8.13	2.83	125.74	25.66	125.26
<i>Annona atemoya</i>	28.69	0.24	119.99	21.31	23.54	19.89	3.65	83.00	20.84	91.84

<i>Annona cherimola</i>	28.42	0.22	130.92	16.22	23.26	19.44	3.83	75.60	20.44	90.42
CD@5%	0.92	0.03	14.12	1.29	1.69	1.75	0.46	3.62	1.80	20.41
SEm	0.31	0.01	4.71	0.43	0.56	0.58	0.15	1.21	0.60	6.81

Table 3: Biochemical characters of different *Annona atemoya* varieties

<i>Annona atemoya</i> varieties	TSS (⁰ B)	Titration acidity (%)	TSS: Acidity	Ascorbic acid (mg/100g)	Total sugars (g/100g)	Reducing sugars (g/100g)	Non reducing sugars (g/100g)	Total Phenols (mgGAE/100g)	Total Flavanoids (mg Catechin equivalent /100g)	DPPH (mgAEAC/100g)
Island Gem	28.69	0.24	118.11	21.31	23.53	20.12	3.43	82.97	20.84	91.83
Pink's Mammoth	29.31	0.26	114.69	21.53	23.99	20.51	3.48	85.17	21.94	95.72
Bullock's Heart	27.65	0.27	104.64	21.48	23.42	20.31	3.11	80.72	24.03	94.70
CD@5%	0.53	0.01	4.86	0.15	0.36	0.28	0.04	1.65	1.38	-
SEm	0.18	0.004	1.62	0.053	0.12	0.09	0.016	0.55	0.46	7.84

When evaluating a fruit for consumer acceptance a breeder is not concerned with 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 custard apple, the fruits having perfect sugar-acid blend are preferred by consumers. Different kinds of organic acids and the extent of their concentration play an important role in the flavor of a fruit. Usually high acidity gives better blend and flavor.

The average TSS content for different species of *Annona* was recorded and significantly higher TSS content was recorded for *A. atemoya* (28.69 ⁰B) and *A. cherimola* (28.42 ⁰B), whereas significantly lower TSS content was recorded for *A. muricata* (12.74 ⁰B). In case of different varieties of *Annona atemoya* higher TSS content was recorded for Pink's Mammoth (29.31⁰B). Significantly higher acidity of pulp was recorded for *A. muricata* (0.43%) whereas significantly lower acidity of pulp was recorded for *A. glabra* (0.13%) among different species of *Annona*. Among different varieties of *Annona atemoya* acidity was recorded highest in Bullock's Heart (0.27%).

The variation in the values of TSS may be due to various factors like climatic condition and genetic characters of the genotype. The similar results were reported by Jagtap and Kokate (1991), Shete *et al.* (1991), Jadhav *et al.* (1992) and Dhumal *et al.* (1997) ^[13, 23, 12, 9] in *Annona*. Jalikop and Kumar (2000) ^[14] reported similar results that in Arka Sahan, interspecific hybrid the TSS was more than 30 ⁰B. Study of Othman *et al.* (2014) ^[18] also revealed titratable acidity ranging from 0.10-1.25% in freshly matured fruit. Since acidity in fruits plays an important role in taste, color and microbial stability of the fruit juice, it can be concluded that *Annona* fruits can have better acceptability for the consumers. For perfect blend, sugar and acid ratio is one of the important parameters which determine the taste of fruit. The TSS and acidity of fruits both have contribution towards fruit taste and flavour. The highest TSS: Acidity ratio was observed in the species *A. glabra* (134.63) which was at par with the species *Annona squamosa* (133.84) and Island Gem had the highest ratio (118.11) among the different varieties of *Annona atemoya*. Anonymous (2006) ^[2] reported variation for TSS, acidity and ascorbic acid in seventeen accessions of custard apple germplasm in Tamilnadu.

Ascorbic acid content was found maximum in *A. muricata* (25.31mg/100g) among the species and in Pink's Mammoth (21.53mg/100g) among *A. atemoya* varieties whereas, lowest ascorbic acid content was recorded in Island Gem (31.31mg/100g). The variation in the values of ascorbic acid may be due to various factors like climatic condition and genetic characters of the genotype. Pareek *et al.* (2011) ^[19]

also claimed 11.5 ± 5.5, 30.0, 29.4 ± 3 and 37.38 ± 4.62mg of ascorbic acid per 100g of pulp of cherimola, custard apple, soursop and sugar apple, respectively. Boake *et al.* (2014) ^[7] obtained 20.33 and 63.67 mg ascorbic acid per 100 g 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. Therefore, these fruits could be considered as good sources of ascorbic acid for purposes of human nutrition.

Sugars are the primary products of photosynthesis and perform multiple roles in plants as energy and carbon transport molecules, hormone like signalling factors, from which plants make proteins, polysaccharides, oils and woody materials. It is also responsible for resistance mechanism against biotic stresses. Sugars play important role in osmotic adjustment and in providing protection against various types of stresses. Sugar content of fruit is the only factor which determines the sweetness of pulp.

The highest total sugars and reducing sugar content was recorded in the species of *Annona atemoya* (23.54 g/100g and 19.89 g/100g respectively), while the non-reducing sugar was higher for *A. cherimola* (3.83 g/100g). The average total sugars (23.99 g/100g), reducing (20.51g/100g) and non-reducing sugars (3.48 g/100g) for different varieties of *Annona atemoya* were significantly higher in Pink's Mammoth.

Jalikop and Kumar (2000) ^[14] observed a total sugar in Arka Sahan as 22.80 per cent. Onimawo (2002) ^[17] reported that the total sugars in *Annona squamosa* was about 16.70 percent. The total sugar content varied from 21.02 to 24.88 percent in cultivars of custard apple (Anon., 2007) ^[3]. Vinay *et al.* (2016) ^[28] reported that Arka Sahan had highest total sugars and reducing sugars. Benkeblia *et al.* (2014) ^[6] also reported similar result of 11.98 to 17.88 mg reducing sugar in *A. reticulata*. Jadhav (2008) ^[11] reported that non-reducing sugars varied from 1.50 to 2.95 percent in *Annona* hybrids. The non-reducing sugars percentage of cultivars of custard apple under Rahuri conditions ranged from 2.17 to 3.50 per cent (Anon., 2007) ^[3].

Significantly higher total phenol was recorded for *A. muricata* (125.74 mgGAE/100g) and with respect to different varieties of *A. atemoya*, the highest total phenol was recorded for Pink's Mammoth (85.17 mgGAE/100g) and lowest total phenol was recorded for Bullock's Heart (80.72 mgGAE/100g). Above result indicated that *A. muricata* which possessed significantly higher phenol content can be a potential source of disease resistance and also for therapeutic purpose in pharmaceuticals.

Flavonoids are a group of compounds contributing to the total antioxidant capacity of fruits and vegetables (Chun *et al.*, 2003) [8]. Flavonoid content was found in a very less quantity. Significantly higher total flavonoids was recorded for *Annona glabra* (26.90 mg Catechin equivalent/100g) which was on par with *Annona muricata* (25.66 mg Catechin equivalent/100g) and lowest was recorded in *Annona cherimola* (20.44 mg Catechin equivalent/100g). Among different varieties of *A. atemoya*, significantly higher total flavonoid was recorded for Bullock's Heart (24.03 mg Catechin equivalent/100g) and lowest in Island Gem (20.84 mg Catechin equivalent/100g).

The antioxidant activity is an essential biological property of great interest because it reduces the toxic effects of oxidants and thus prevents the cellular damage caused by free radicals. The total antioxidant capacity for different species of *Annona* was recorded and significantly higher antioxidant capacity was recorded for *A. muricata* (125.26 mg AEAC/100g) followed by *Annona reticulata* and the lowest in *A. glabra*. Among different *A. atemoya* varieties, higher antioxidant capacity was recorded for Pink's Mammoth (95.72 mg AEAC/100g) which was on par with Bullock's Heart and the lowest was in Island Gem.

In addition to the antioxidant capacity, phenolics can influence the flavour determining fruit astringency and bitterness (Silva *et al.* 2013 and Anuragi *et al.*, 2017) [24]. Study of Benkeblia *et al.* (2014) [6] also revealed 9.83 to 18.71 mg phenols per gram of fruit pulp of custard apple (*A. reticulata*). Vijayaraghavan *et al.* (2013) reported 7.81 to 125.0 µg/ml antioxidant activities using DPPH assay in *A. squamosa* extracts.

Conclusion

From the study, it was revealed that species of *Annona viz.*, *A. cherimola*, *A. reticulata*, *A. muricata*, *A. atemoya* and different cultivars of *A. atemoya* can act as important source of compounds with a high potential to protect health. *Annona* species especially *A. muricata* possessed relatively higher amount of antioxidants but unfortunately the fruits of this species are not sweet. Fruits of species like *A. cherimola*, *A. atemoya* and *A. squamosa* were sweet enough and also possessed higher quantity of antioxidants. Thus, it can be suggested that the fruits of these species can be good source of natural antioxidants.

This study showed considerable variation in the biochemical properties of different *Annona* species as well as for different varieties. The variation could originate from the genetic and agro-climatic as well as environmental conditions. This study provides important data for compositional information of the fruits (e.g. reducing sugars, vitamin C, titrable acidity, antioxidant activity and etc), emphasizing that annona fruit can be a good source of nutrients.

In conclusion, a comparison of the results obtained by us with those found in other studies reveals that annona fruit contains important amounts of antioxidant and high amount of nutrients that play a valuable role in people's daily diet. The variability in the biochemical characteristics is mostly useful for improving the quality of *Annona* and thus helpful for the exploitation of heterosis.

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