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Selection of Superior Quality Annona Species by Means of Bioactive Compounds and Antioxidant Capacity

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Abstract Present study evaluated different parts of (leaves, seeds, bark, roots, ripen fruits, unripen fruits) 6 species of Annona (*Annona cherimola, Annona muricata* L., *Annona reticulata* L., *Annona squamosa* L (green and red varieties) and *Annona glabra* L. by means of total antioxidant capacity (TAC), total phenolic content (TPC) and total flavonoid content (TFC) in order to select superior quality species of Annona for commercial cultivation. TAC, TPC and TFC were determined using Ferric Reducing Antioxidant Power (FRAP) assay, Folin-Ciocalteu method and colorimetric method, respectively. It was observed that all tested parts of all tested species contained appreciable amount of TAC, TPC and TFC. Significantly higher TPC were recorded in roots of custard apple (82.08 ± 0.74^{a} mg GAE/g DW) followed by roots of soursop (73.10 ± 0.72^{b} mg GAE/g DW), leaves of soursop (55.18 ± 0.18^{a} mg GAE/g DW) respectively. It was interesting to see that the highest TAC was observed in root extracts of soursop (194.98 mg TE/g DW followed by bark extracts of pond apple (134.37 mg TE/g DW) and leaf extracts of soursop (122.67 mg TE/g DW respectively. Total flavonoid content of leaf extracts of six different species were varied as soursops>sugar apple R>pond apple>sugar apple G> custard apple >cherimoya respectively. **S**trong positive correlations were observed between TAC values and TPCs of leaves, seeds, barks and roots (R^2 = 0.78; p<0.001). Based on the results of bioactive molecules present in different species and their parts, it could be concluded that soursop and custard apple could be recommended as superior quality Annona species for commercial cultivation.

Keywords: *Annona muricata, Annonaceae, bioactive molecules, total phenolic content*

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1. Introduction

Genus Annona (family Annonaceae) consist of 119 species exist in tropical America (109 species), 10 species to tropical Africa and seven species and one hybrid are grown for domestic and commercial use [1,2]. The plants belonging to genus Annona are tap rooted, evergreen or semi deciduous tropical trees or shrubs. These plants are erect or somewhat spreading in habit, with grey brown bark, often rough. Among existing commercially grown Annona species, *Annona cherimoya* Mill. (Cherimoya) *Annona muricata* L.(Soursop), *Annona reticulata* L. (Custard apple), *Annona squamosa* L. (Red and green Sugar apples) and *Annona glabra* L.(Pond apple) are popular among cultivators due to their distinguished organoleptic, therapeutic properties and commercial value [3,4,5,6]. Even though Annona species are considered as underutilized fruit crops in many countries, they are rich with an array of phytochemical groups such as alkaloids, flavonoids, phenolic compounds, essential oils, essential amino acids, pigments, vitamins and hence they demonstrate board spectrum of therapeutic activities including antifungal, antimicrobial, anti-diabetic, hepato-protective, antimalarial, cytotoxic, antitumor, anti-parasitic and antioxidant activities [2,7,8]. In Sri Lanka, there are 6 Annona species recommended for commercial cultivation by Department of Agriculture**.** Out of these six species, only very few studies were conducted on morphological and antioxidant capacity of only one species *A. muricata* [14]. Therefore, the current study was aimed to select superior quality Annona species by means of bioactive compounds and antioxidant capacity for commercial cultivation.

2. Materials and Methods

2.1. Sample Collection

Well ripen fruits, fruits at immature stage (two to three month after flowering), leaves, roots and barks of six Annona species were collected from authenticated mother plants maintained at Fruit Crop Research & Development

Station Gannoruwa, Peradeniya, Sri Lanka. Samples were labelled and immediately transported to the laboratory in cool conditions and stored under refrigerator condition (-4°C) until further studies (Table 1 & Figure 1).

Table 1. Details of the Annona species used in the current study

Vernacular name	Common name	Botanical name
Cherimoya	Cherimoya	*Annona cherimola* Mill.
Katuanoda	Soursop	*Annona muricata* L.
Welianoda	Custard apple	*Annona reticulata* L.
Sinianoda (G)	Sugar apple	*Annona squamosa* L.
Sinianoda (R)	Sugar apple	*Annona squamosa* L.
Welaththa	Pond apple	*Annona glabra* L.

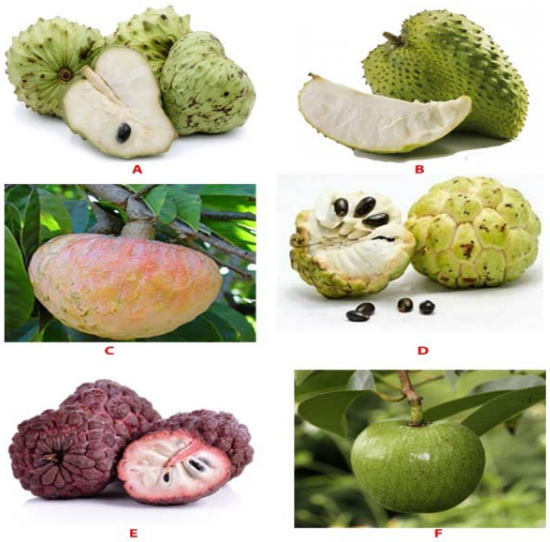

Figure 1. Fruit samples of collected Annona species [A- Cherimoya; B- Soursop; C- Custard apple; D-Sugar apple (Green); E- Sugar apple (Red); F-Pond apple]

2.2. Chemicals and Reagents

Folin-Ciocalteu Reagent, Gallic acid, Rutin, 2, 4, 6-trypyridyl-2-try-azine (TPTZ), 6-hydroxy-2, 5, 7, 8-tetramethy-chroman-2-carboxilic acid (Trolox) and ferric chloride (FeCL3.6H2O) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

2.3. Preparation of Samples

The fresh leaves, barks, roots and fruits were thoroughly washed under running water. Then leaves, barks, roots and seeds were cut into small pieces and air dried for three days.

2.4. Extraction of Phytochemicals from Leaves, Roots, Bark and Seeds

Air dried samples were powdered using coffee blender (Khalaf *et al*., 2008) and sieved using 0.25 mm mesh. Powdered sample (0.1 g) was mixed with 5 mL of 80% methanol, vortexed for 15 min. Then it was placed in a water bath at 60°C for 40 min. and vortex procedure repeated in 10 min. intervals. After centrifugation at 4,000 rpm for 5 min, the supernatant was decanted into a 15 mL centrifuge tube and the remaining was re-extracted with 5 mL of 80% methanol. Supernatants were pooled and stored at -20 °C until analysis.

2.5. Extraction of Phytochemicals from Mature and Immature Fruits

Extraction of phytochemicals from mature and immature fruits was carried out as follows. Briefly, 10 g of fruit pulp was weighed into beaker. Then, the sample was homogenized with chilled 80% methanol using a fruit blender (Sun *et al*., 2002) for 5 min under chilled conditions. Then the sample was further homogenized using homogenizer (Witeg, 0400189139T002, German) for additional 2 min. The homogenate was filtered through No.1 Whatman filter paper on a Buchner funnel under vacuum. Then the filtrate was recovered with chilled 80% methanol to final volume of 50 mL. The extracted samples were stored at -20°C until analysis.

2.6. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using FRAP assay described by Benzie and Strain [9] with slight modifications. Briefly, 100 µL of methanolic extracts of samples was mixed with 900 µL of freshly prepared FRAP (Mixing 25mL of 300 mM Sodium acetate buffer, 2.5 ml of 10 mM TPTZ solution and 2.5 ml of 20 mM ferric chloride solution) reagent of pH 3.6 and incubated for 4 min. Absorbance were measured at 593 nm using spectrophotometer (Shimadzu, UV Mini 1240, Japan). Total antioxidant capacity was calculated using the standard trolox curve and express as milligrams of trolox equivalents (TE) per g of DW and FW.

2.7. Determination of Total Phenolic Content (TPC)

Total phenolic contents of all samples were determined using a modified Folin -Ciocalteu method, briefly, 4 mL of distilled water and 0.5 mL of extract was added into a test tube. Then the same amounts of (0.5 mL) of 0.2N Folin-Ciocalteu reagent was added and allowed react for 3 min. Then 1 mL of saturated sodium carbonate solution was mixed and incubated in a water bath for 2 hr at 30°C. The absorbance was measured at 760 nm using a UV visible spectrophotometer. Total phenolic content was calculated using the standard gallic acid curve and expressed as milligrams of gallic acid equivalents (GAE) per g of DW and FW.

2.8. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by a colorimetric method with slight modifications [10]. Briefly, a volume of 0.5 mL of a plant extract was added to centrifuge tube containing 3.5 mL of distilled water. Then solution was mixed with 0.3 mL of 5% $NaNO_2$. After 6 min 0.3 mL of 10% Al $(NO_3)_3.6H_2O$ solution was

added and allowed to stand another 6 min, and then, 2 mL of 2 M NaOH added. The reaction mixture was diluted with 1.4 mL of distilled water and absorbance measured immediately using a spectrophotometer at 510 nm. Total flavonoid content was calculated using the standard rutin curve and express as milligrams of rutin equivalents (RE) per g of DW and FW.

2.9. Statistical Analysis

To verify the statistical significance of all parameters, means values and ± SD were calculated. Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Tukey Multiple Range Test using SAS (SAS Institute, 1999). The P values less than 0.05 were adopted as statistically significant.

3. Results and Discussion

In the current study, total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC) of different parts of six Annona species grown in Sri Lanka were scientifically analysed in order to select the superior species for commercial production. Since all samples were collected from the plants grown under similar cultural practices in the same soil and climatic conditions, the observed results demonstrate unbiased results from soil and environmental conditions.

As shown in Table 2, all tested parts of six species of Annona demonstrated the marked content of TPC. Significantly higher TPC was recorded in roots of Custard apple (82.08±0.74^{a} mg GAE/g DW) followed by roots of Soursop (73.10±0.72^{b} mg GAE/g DW), leaves of Soursop (55.18±0.18^{a} mg GAE/g DW) respectively. TPC of leaves of Annona were ranged from 8.83-55.18 mg GAE/g DW, while roots exhibited 10.70- 82.08 mg GAE/g DW). Comparatively higher TPCs were reported in roots, leaves and bark while seeds and fruit sample of Annona showed the lower contents of TPC (Table 2). Our results on TPC of *A, muricata* are in agreement with previous researchers [11,12,13], who investigated the TPC content of *A. muricata*.

3.1. Total Antioxidant Capacity (TAC)

Total antioxidant capacity of different parts and fruits of six Annona species are given in Table 3.

Total antioxidant capacity of leaves of six Annona species showed that it was varied from 18.87 to 122.67 mg TE/g DW and the highest TAC was observed in soursop leaf extracts (122.67 mg TE/g DW), while the lowest value was observed from Cherimoya 18.87 mg TE/g DW. It was interesting to see that the highest TAC was observed in root extracts of Soursop (194.98 mg TE/g DW followed by bark extracts of Pond apple (134.37 mg TE/g DW) and leaf extracts of Soursop (122.67 mg TE/g DW respectively. However, seed, ripen fruits and unripen fruits exhibited comparatively lesser TAC content in leaf extracts of soursop compared to the current study. Further, our results of lower TAC in fruits and seeds are in agreement with Widyastuti et al., [14], who reported the comparatively lower content of TAC in seeds and fruits of Soursop. Further, Wang et al., [15] reported higher antioxidant capacity in immature fruits compared to the mature fruits.

3.2. Total Flavonoid Contents (TFC)

Total flavonoid content presence in different parts (leaves, seeds, barks, roots, ripen fruits and unripen fruits) of six species of Annona are presented in Figure 2. As demonstrated in Figure 2. significantly higher TFCs were observed in roots of Soursops (317.22±3.47 mg RE/ g DW), followed by bark of Pond apple (270.83±1.67 mg RE/ g DW), bark of Soursops (201.17 RE/ g DW) and leaves of Soursops (181.94 mg RE/ g DW) respectively. Out of the tested parts, the lowest total flavonoid content was reported from seed extracts. Total flavonoid content of leaf extracts of six different species were varied as Soursops>Sugar apple R>Pond apple>Sugar apple G> Custard apple >Cherimoya respectively. Results of the current study on leaf flavonoid content is in agreement with Nguyen et al., [16], who compared TFC of soursop leaves and found that it was 209.52±1.88 mg/g.

Table 2. Total phenolic content (TPC) of the leaves, seeds, bark, roots, ripen fruits and unripen fruits of six Annona species

Species	TPC (mg GAE /g DW)				TPC (mg GAE /g FW)	
	Leaf	Seed	Bark	Root	Ripen Fruit	Unripen Fruit
Cherimoya	8.83±0.09^{f}	3.79±0.08^{c}	4.51±0.06^{e}	10.70±0.05^{e}	0.22±0.00^{f}	0.17±0.01^{f}
Soursop	55.18±0.18^{a}	3.96±0.13^{c}	22.82±0.11^{a}	73.10±0.72^{b}	1.19±0.02^{c}	0.68±0.00^{d}
Custard apple	20.76±0.09^{c}	3.15±0.09^{d}	13.77±0.13^{b}	82.08±0.74^{a}	2.72±0.02^{a}	1.81±0.01^{c}
Sugar apple (G)	12.20±0.11^{e}	4.78±0.08^{b}	12.52±0.09^{d}	13.03±0.04^{d}	0.57±0.00^{d}	2.76±0.02^{b}
Sugar apple (R)	13.28±0.10^{d}	3.65±0.09^{c}	12.92±0.06^{c}	38.51±0.03^{c}	1.29±0.03^{b}	2.94±0.01^{a}
Pond apple	25.44±0.25^{b}	7.83±0.19^{a}	12.52±0.02^{d}	12.17±0.10^{d}	0.50±0.00^{e}	0.55±0.00^{e}

Means denoted by the same letters in a column represent non-significant differences ($p<0.05$); TPC – Total phenolic content; GAE- Gallic Acid Equivalents; DW-Dry Weight; FW-Fresh Weight.

Table 3. Total antioxidant capacity (TAC) of the leaves, seeds, bark, roots, ripen fruits and unripen fruits of Annona species

Species	TAC (mg TE/g DW)				TAC (mg TE/g FW)	
	Leaves	Seeds	Bark	Root	Ripen Fruits	Unripen Fruits
Cherimoya	18.87±0.33^{f}	4.51±0.06^{e}	11.28±0.09^{f}	35.15±0.59^{e}	0.25±0.00^{f}	0.37±0.0^{f}
Soursop	122.67±1.4^{a}	4.89±0.04^{d}	114.64±1.46^{b}	194.98±1.29^{b}	1.95±0.03^{b}	2.01±0.0^{d}
Custard apple	30.09±0.25^{e}	5.08±0.05^{c}	69.39±0.41^{c}	337.70±0.85^{a}	5.00±0.01^{a}	5.89±0.1^{c}
Sugar apple (G)	53.39±0.48^{d}	6.43±0.00^{a}	33.09±0.54^{e}	63.75±1.36^{d}	0.94±0.01^{d}	6.71±0.0^{b}
Sugar apple (R)	79.04±0.38^{b}	5.10±0.05^{c}	60.07±1.14^{d}	105.54±0.90^{c}	1.68±0.02^{c}	6.88±0.0^{a}
Pond apple	58.03±0.29^{c}	5.56±0.02^{b}	134.37±1.33^{a}	32.66±0.42^{e}	0.85±0.01^{e}	1.08±0.0^{e}

Means denoted by the same letters in a column represent non-significant differences ($p<0.05$); TAC – Total antioxidant capacity; TE- Trolox Equivalents; DW-Dry Weight; FW-Fresh Weight.

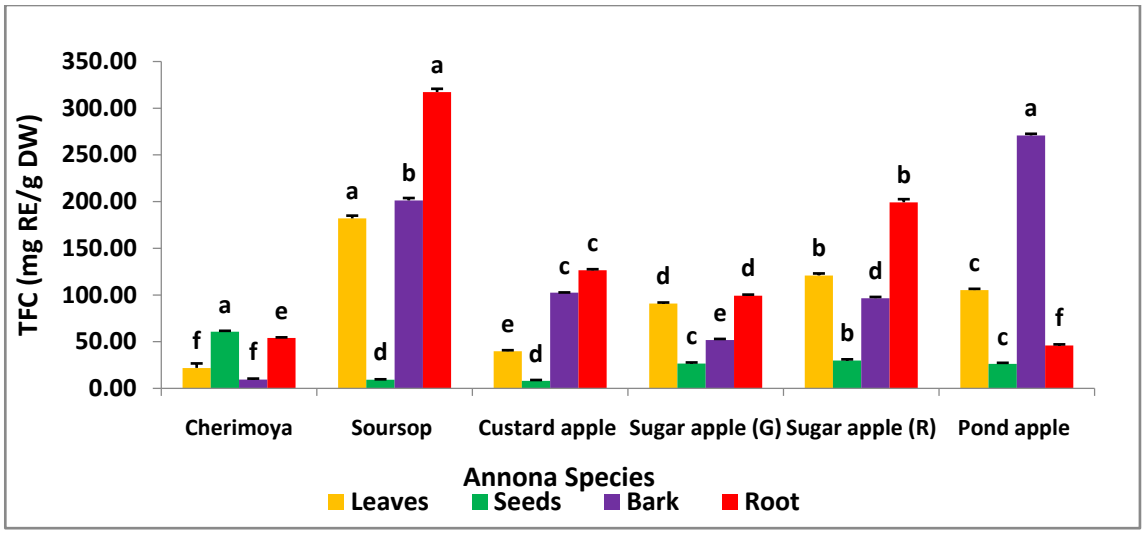

Figure 2. Total flavonoid content (TFC) of the leaves, seeds, bark, roots, of Annona species

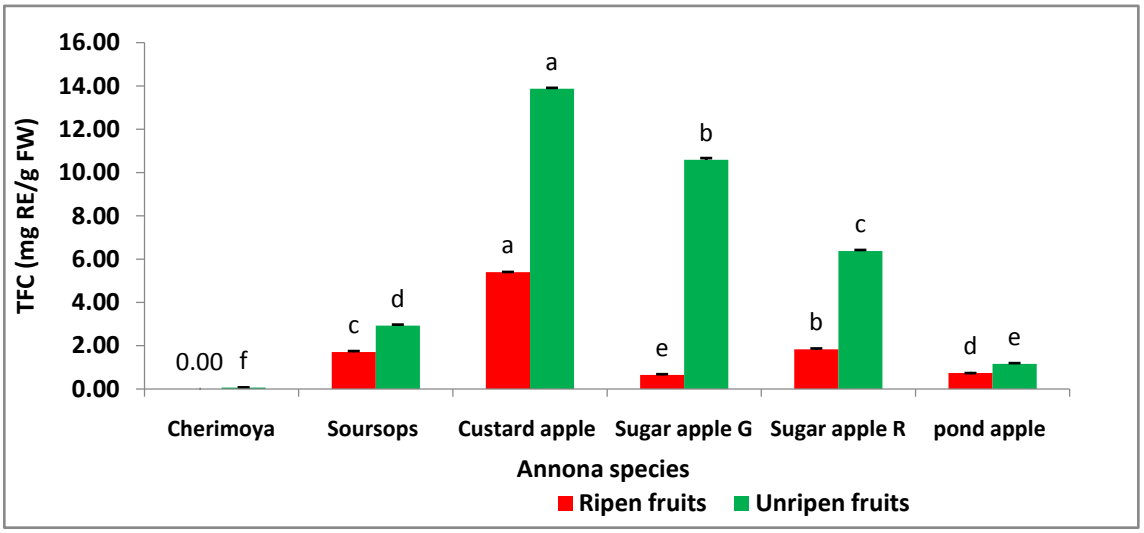

Figure 3. Total flavonoid content of the ripen fruits and unripen fruits of six Annona species

As demonstrated in Figure 3, significantly the higher content of TPC was observed in extracts of unripen fruits compared to the ripen fruits. The order of TPC content of unripen fruits was Custard apple> Sugar apple G> Sugar apple R> Pond apple> Cherimoya. respectively. According to the results it is clear that Antioxidant may be probably due to high amount of secondary metabolites presence in unripen fruits than ripen fruits.

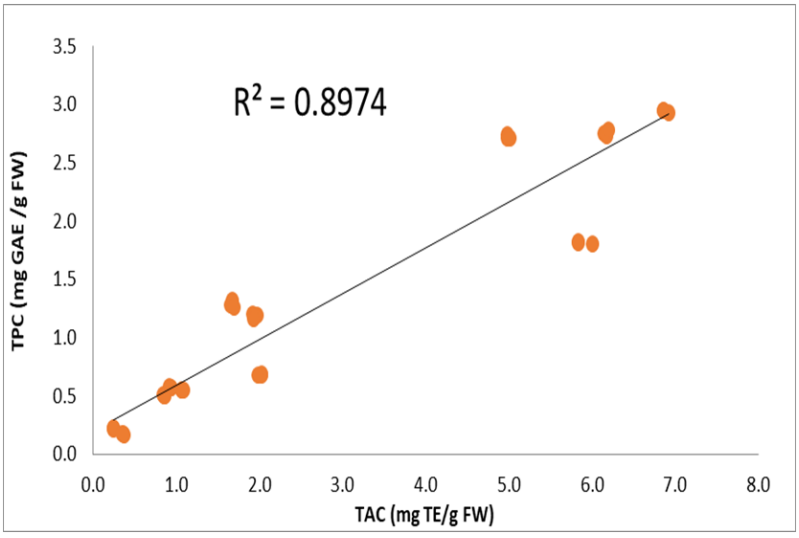

Figure 4.

3.3. Correlation of TAC with TPC and TFC

In this study strong positive correlations were observed between TAC values and TPC of leaves, seeds, bark and roots (R^2 = 0.78; p<0.001), TAC values and TPCs of ripen and unripen fruits (R^2 = 0.90; p<0.001) and TAC values and TFCs of ripen and unripen fruits (R^2 = 0.78; p<0.01).

Our results are in agreement with Recuenco et al [17], who observed strong positive correlation between TAC and TFC contents. However, TAC values and TFCs of leaves, seeds, barks and roots showed fairly a low correlation (R^2 = 0.49; p<0.01). Previous workers reported that there is a strong positive correlation of TPC of *Annona squamosa* fruit peel (R2 = 0.995; p< 0.01 with total antioxidant capacity [17,18]. Further, Kothari and Sephardi [19] observed that the flavonoid and phenolic contents of the seed extracts of Annona demonstrated a linear correlation with the total antioxidant capacity. These positive correlations suggest that the phenolic components contribute significantly to the antioxidant capacity of different plant parts.

Results of the bioactive compounds analysis of different parts of Annona species revealed that the significantly higher total phenolic content, total flavonoid content and total antioxidant capacity were observed in leaves of Soursops. Further, roots of Soursop exhibited the highest TFC, second highest TAC and TPC while bark demonstrated the highest TPC, second highest TAC and TFC compared to all other Annona species tested. Moreover, different parts of Custard apple and Sugar apples also exhibited substantial amount of TAC, TPC and TFC contents. Also, bark of pond apple and seeds of cherimoya exhibited marked amount of TPC, TAC and TFC contents.

4. Conclusions

In this study, all the tested Annona species showed marked amounts of TAC and TPC. Significantly higher TAC, TPC and TFC were observed in bark and roots of all species. Very low levels of TAC, TPC and TFC were observed in ripen and unripen fruits. Among all six species soursops, Custard apple, Sugar apple (Red) and Pond apple showed higher amounts of TAC, TPC and TFC. Based on the results of bioactive molecules of TAC, TPC and TFC of different plant parts of six Annona species, it could be concluded that Soursops (*Annona muricata*) could be recommended for commercial cultivation for Annona based value added industries. In addition to the fruits, elevated TAC, TPC and TFC were available in leaf, root and bark of Soursops. Therefore, it is encouraged to commence leaf, root and bark based value added products.

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