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Short Communication

Antioxidant Capacity and Total Phenol Content of Sri Lankan *Annona muricata* L.

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ABSTRACT: *Annona muricata L. (Annonaceae) is a tropical fruit tree in Sri Lanka and commercial utilization of this species is poor. The objective of the present study was to determine the antioxidant activity and the total phenol content of the ethanolic extract of fruit pulp of the Sri Lankan variety of A. muricata. Antioxidant activity was determined by using 2,2-diphenylpicrylhydrazyl free radical scavenging assay, ferrous ion chelating assay, and ferric reducing ability of plasma assay. The total phenol content was determined by Folin-Ciocalteu method using Gallic acid standard. The fruit pulp extract exhibited scavenging activity having an IC50 value of 725 ppm and EC50 value for ion chelating activity as 306 ppm. The ferric reducing ability of 1000 ppm of the fruit pulp extract was equivalent to that of 25 ppm of vitamin C. The total phenol content of the extract of fruit pulp was 139 mg Gallic Acid Equivelant /100 g. The ethanolic extract of Sri Lankan variety of A. muricata fruit pulp exhibited moderate antioxidant capacity in terms of redox properties.*

Keywords: *Annona muricata, antioxidant, Ferric reducing ability of plasma assay, 2,2-diphenylpicrylhydrazyl free radical scavenging assay, , total phenol,*

INTRODUCTION

Annona muricata L. belongs to the family Annonaceae is a tropical fruit tree in Sri Lanka. The ripened fruits of *A. muricata* are consumed as fresh fruits but commercial utilization of fruits is uncommon in Sri Lanka, hence categorized as an under-utilized fruit tree species. Fruits of *A. muricata* provide nutrients, phyto-chemicals and biological properties which are vital to maintain human health. Bioactive substances such as vitamin C, flavonoid, anthocyanins and carotenoids are also found in fruits among others (Cardozo *et al*., 2012). Biological properties including antioxidant activities are important to control and prevent many diseases including cancer, rheumatoid arthritis, cardio vascular and diseases related to degenerative process (Almeida *et al*., 2011; Dembitsky *et al*., 2011). Several studies have shown high correlation between the consumption of fruits in prevention and treatment of various diseases (Loizzo *et al*., 2012).

Most abundant antioxidants in the fruits are phenols, flavonoids, vitamins and carotenoids (Lim *et al*., 2007). Acetogenins are long chain fatty acids common in plants belonging to the Annonaceae family and they show broad range of biological properties such as cytotoxic, immunosuppressive, pesticidal, antiparasitic, antimicrobial and antioxidant activity (Lima *et*

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al., 2010). *Annona muricata* is popular for its edible fruit and is used as a folkloric herbal medicine in Philippines. The juice of ripe fruit is said to be a diuretic, decoction of powdered immature fruits is a dysentery remedy (Ragasa *et al*., 2012). There were no previous studies on bioactivity of *A. muricata* fruits in Sri Lanka. The objective of the present study was to determinate the antioxidant capacity and total phenol content of Sri Lankan *A. muricata* fruits.

METHODOLOGY

Collection of fruits

Multistage sampling method was used to collect the morphological information of *A. muricata* plants in three different climatic zones (dry, wet and intermediate zones) in the country. Nine different clusters were identified by using morphological characters from selected samples (Padmini *et al*., 2012). Many plants were grouped into the cluster seven and it was considered as the common group. Accession number 204, 239 and 257 of *A. muricata* plants from the germplasm collection center at the Regional Agricultural Research and Development Center, Makandura are also grouped under the cluster seven and fruits of these plants were assumed as representative samples. A mature fruit was collected from accession 204 and allowed to ripe for 2-3 days at room temperature (30 ± 2 ^{0}C) prior to analysis.

Total ethanolic extraction of fruit pulp

Total ethanolic extraction was used to separate organic compounds from the sample. The ripe fruit pulp (387.80 g) was soaked with 300 ml of ethanolic for 24 hours and stirred for 1 hour using a mechanical stirrer at room temperature. The ethanolic was filtered through a celite bed packed in a sinter funnel under vacuum. The extraction procedure was repeated thrice. The filtrates obtained after each extraction were combined and concentrated using a rotary evaporator under vacuum (Buchi rotary evaporator, pressure 40 mbar and temperature 50 ^{0}C) to obtain a dark brown gummy extract (61.33 g).

Determination of antioxidant activity using DPPH free radical scavenging assay

The 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. DPPH is a stable nitrogen centered free radical, the color of which changes from violet to yellow upon reduction by electron donation. DPPH reagent (40 ppm) was prepared freshly by dissolving 0.002 g of DPPH in methanol (50 ml). Ethanolic extract of *A. muricata* fruit pulp of cluster seven (0.06 g) was dissolved in methanol (10 ml) to prepare 6,000 ppm stock solution. The ethanolic extract of fruit pulp stock solution (40, 35, 30, 25 and 20 µl), methanol (60, 65, 70, 75, and 80 µl) and DPPH (200 µl) were introduced into micro wells (total volume of each micro well was 300 µl) and kept in dark for 30 minute at room temperature (30 ^{0}C).

Vitamin C (50 ppm) was used as the positive standard for the DPPH assay. DPPH (200 µl), Vitamin C (36, 30, 24, 18 and 12 µl) and methanol (64, 70, 76, 82 and 88 µl) were introduced into micro wells and kept in dark for 30 minute at room temperature. Methanol (300 µl) was used as the blank and it is required to correct any colour absorbance by the solvent. Control assay was carried out without adding the sample or extracts. Percentage inhibition was calculated with compared to the absorbance value of control assay. The control assay gives highest absorbance value according to colour of DPPH. The degree of discoloration indicates the scavenging potential of the antioxidant compound; it is indicated

as percentage of inhibition. Absorbance was measured at λ = 517 nm using a micro plate reader (Spectra max 340). Three replicates were carried out for each concentration of the sample. Ability of free radical scavenging activity (antioxidant activity) is expressed as fifty percent inhibition capacities (IC_{50}). The percentage inhibition value was calculated according to the Equation 1.

$$\text{Percentage Inhibition} = \frac{\text{absorbance of control - absorbance of sample X 100}}{\text{absorbance of control}} \quad \text{Equation 1}$$

Determination of antioxidant activity using ferrous ion chelating assay

The antioxidant activity was determined by using ferrous ion chelating assay. Ferrous ion chelating ability was determined by the decrease in the absorbance at λ = 562 nm of the Fe^{2+} ferrozine complex and EDTA was used as the positive standard for the assay (Stookey,1970; Guo *et al*., 2001; Lim *et al*., 2007; Shajiselvin & Muthu, 2011; Loizzo *et al*., 2012). All glassware including micro plates were cleaned and dipped overnight in 0.1M EDTA (300 ml) and washed with distilled water several times and dried well to ensure they are free of Ferrous.

In the EDTA positive standard for the assay, distilled water (100, 90, 80, 70, 60 µl), 100 ppm EDTA (10, 20, 30, 40, 50 µl) and 1.25 mM $FeSO_4.7H_2O$ (20 µl) were introduced in to micro wells. Blank assay was carried out by adding only distilled water (230 µl) and control assay was carried out without adding EDTA. Triplicates were carried out for each concentration. Micro titer plate was incubated at room temperature for 10 minute and reaction was initiated by the addition of 0.3125 mM ferrozine (100 µl) to each well and incubated at room temperature for 10 minutes. After incubation, ferrous ion chelating ability was determined by measuring the absorbance values at λ = 562 nm wave length by using Spectra max micro plate reader. The ability of EDTA to chelating ferrous ion was calculated relative to the control using the Equation 2 as percentage chelating effect.

$$\text{Percentage Chelating effect} = [(\text{Abs. C} - \text{Abs. S}) \text{ X } 100] / \text{Abs.C} \quad \text{Equation 2.}$$

Where: Abs. C= absorbance of control, Abs. S= absorbance of sample

The data, percentage chelating effect *vs* concentration of EDTA were plotted. The fifty percent effect of chelating (EC_{50}) was calculated by using the equation of linear regression. Same ethanolic extract of fruit pulp stock solution was prepared (6,000 ppm) and the volume range of ethanolic extracts used was 90, 80, 70, 60, 50, 40 µl. The chelating ability of ethanolic extract of fruit pulp was calculated relative to the control using the equation 2.

Determination of antioxidant activity using Ferric reducing ability of plasma (FRAP) assay

The FRAP assay was used to measure the antioxidant power of ethanolic extract of *A. muricata* fruit pulp. Ferrous TPTZ complexes (2, 4, 6-tripyridyl- *s*-triazine) form intensive blue colour at low pH (Benzie and Strain, 1996; Benzie and Szeto, 1999; Ibrahim *et al*., 2010; Garcia *et al*., 2009). In the presence of antioxidants in the sample Fe^{+3}- TPTZ reduced to Fe^{2+} TPTZ. FRAP value was obtained by comparing the absorbance change at λ = 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. All glassware were washed with 0.1M EDTA and cleaned and dried well. Freshly prepared FRAP reagent (200 µl), Acetate buffer (48.75, 47.5, 45, 40, 30 µl) and vitamin C (1.25, 2.5,

5, 10 and 20 µl) were introduced into micro wells (total volume was 300 µl) and the micro plate was vortexed for 8 minute. Absorbance was measured at λ = 593 nm wave length using micro plate reader. The absorbance values *vs* concentration of vitamin C were plotted. For each concentration, three replicates were used. Ethanolic extract of fruit pulp (6,000 ppm, 50 µl, 25 µl) were added to micro wells followed by, Acetate buffer (50 µl) and FRAP solution (200 µl) and vortexed for 8 minute. Absorbance was measured at λ = 593 nm wave length using a micro plate reader. The FRAP value of 1,000 ppm *A. muricata* fruit extract was calculated using vitamin C standard curve and the result was expressed as vitamin C equivalent concentration.

Determination of total phenol content (TPC)

Total phenol content of the ethanolic extracts of fruit of *A. muricata* was determined by using Folin-Ciocalteu method using gallic acid as a standard phenolic compound (Anesini *et al*., 2008; Babbar *et al*., 2011; Velioglu *et al*., 1998; Lee *et al*., 2003; Basniwal *et al*., 2009, Yu *et al*., 2002). Gallic acid dilution series (500, 250,125 and 62.5 ppm) were prepared from the 1,000 ppm stock solution. Ethanolic extract of fruit pulp (0.2 g) was dissolved in methanol (10 ml) to prepare 20,000 ppm solution.

Gallic acid (20 µl) from each dilution (500, 250,125 and 62.5 ppm), 0.2 N Folin-Ciocalteu reagent (110 µl) and 10% sodium carbonate solution (70 µl) were introduced into micro plate wells (200 µl), mixed well and incubated for 30 minute at room temperature. The absorbance was measured at λ=765 nm using a Spectra max micro plate reader. Three replicates were carried out for each concentration. Standard gallic acid curve was drawn using absorbance data obtained for standard concentrations of gallic acid i.e. 100, 50, 25, 12.5 and 6.25 ppm. Fruit pulp extract (20,000 ppm, 20 µl) was introduced into wells followed by Folin-Ciocalteu reagent (110 µl) and 10% sodium carbonate solution (70 µl) and incubated for 30 min at room temperature. The absorbance of mixture was measured at λ = 765 nm. The experiment was carried out in triplicates. The total phenol content in the ethanolic extract of *A. muricata* fruit pulp (g) was determined as mg of gallic acid equivalent (GAE) mg /g by using an equitation which was obtained from the standard gallic acid curve.

RESULTS AND DISCUSSION

Antioxidant activity using DPPH free radical scavenging assay

The mean percentage inhibition for vitamin C and ethanolic extract of *A. muricata* are given in Table 1. The data indicated that with increasing concentration of the sample, the percentage inhibition increased. According to regression analysis of percentage Inhibition *vs* vitamin C concentration, R^2 was 0.99. Vitamin C has high antioxidant capacity and showed free radical scavenging activity having IC_{50} value of 3.11 ± 0.04 ppm. According to the regression analysis of percentage Inhibition *vs A. muricata* fruit pulp extract concentration, R^2 was 0.97. Fruit pulp extract of *A. muricata* exhibited free radical scavenging activity having IC_{50} value of 724.98 ± 3.00 ppm. With compared to the IC_{50} value of Vitamin C, *A. muricata* fruit pulp extract showed the low ability of radical scavenging activity.

Table 1. Percentage Inhibition of vitamin C and *A. muricata* fruit pulp extract

Conc. of Vitamin C ppm	Average Absorbance & (SD)	% Inhibition	Conc. of Extract ppm	Average Absorbance		% Inhibition
6	$0.061 \pm 1.16x10^{-2}$	91.92	900	0.280	$\pm 2.74x10^{-2}$	60.33
5	$0.142 \pm 0.35x10^{-2}$	81.22	800	0.310	$\pm 0.61x10^{-2}$	56.16
4	$0.244 \pm 1.80x10^{-2}$	67.66	700	0.360	$\pm 0.72x10^{-2}$	49.12
3	$0.388 \pm 1.54x10^{-2}$	48.67	600	0.405	$\pm 0.81x10^{-2}$	42.69
2	$0.521 \pm 0.58x10^{-2}$	30.99	500	0.462	$\pm 0.12x10^{-2}$	34.69
			400	0.525	$\pm 0.95x10^{-2}$	25.68

Each treatment was measured in triplicate and expressed as mean absorbance

Antioxidant activity using ferrous ion chelating assay

Percentages of chelating effect for different EDTA concentrations and different concentration of ethanolic extract of *A. muricata* are given in the Table 2. According to the above analysis, EC_{50} value of standard EDTA was 7.902 ± 0.03 ppm and *A. muricata* fruit pulp EC_{50} value was 305.69 ± 4.71 ppm. Results indicated that the fruit pulp extract exhibited poor ferrous ion chelating activity when compared to EDTA. It indicates the available bioactive compounds such as phenols, vitamins, alkaloids, acetogenins, flavonoid, anthocyanins and carotenoids react as antioxidants (ferrous ion chelating compounds).

Table 2. Percentage Chelating effect of standard EDTA to ferrous ion chelating assay

[EDTA] (ppm)	Average Absorbance		Chelating Effect%	Fruit extract (ppm)	Average Absorbance		Chelating Effect%
2.17	0.596	$\pm 1.46x10^{-2}$	14.24	391.30	0.191	$\pm 2.07x10^{-2}$	72.44
4.34	0.464	$\pm 1.15x10^{-2}$	32.52	347.83	0.321	$\pm 1.96x10^{-2}$	53.63
8.68	0.292	$\pm 1.10x10^{-2}$	57.99	304.35	0.361	$\pm 2.56x10^{-2}$	47.86
13.02	0.102	$\pm 1.30x10^{-2}$	85.32	260.87	0.428	$\pm 1.08x10^{-2}$	38.29
17.36	0.006	$\pm 0.87x10^{-2}$	99.14	217.39	0.439	$\pm 7.44x10^{-2}$	36.60
				173.91	0.527	$\pm 2.92x10^{-2}$	24.00
				130.43	0.559	$\pm 2.16x10^{-2}$	19.29
				86.96	0.592	$\pm 1.44x10^{-2}$	14.53

Each treatment was measured in triplicate and expressed as mean absorbance

Antioxidant activity using ferric reducing ability of plasma (FRAP) assay

Standardization of FRAP assay was measured by reacting TPTZ with $FeSO_4$. The absorbance values for different ferrous ions concentrations and different vitamin C concentrations are given in the Table 3. Average absorbance values obtained for 1,000 and 500 ppm concentration of ethanolic extract of fruit pulp are 0.747 nm and 0.164 nm respectively. Accordingly, FRAP activity of 1,000 ppm *A. muricata* fruit pulp extract is equivalent to that of 24.51± 0.20 ppm of vitamin C. The available vitamin C content is 50.1 mg / 100 g in fresh weight of Sri Lankan *A. muricata* fruit pulp. It indicates the available bioactive compounds such as alkaloids, acetogenins, flavonoid, anthocyanins and carotenoids react as antioxidants.

Table 3. Absorbance values for standardization of TPTZ of FRAP assay

Vitamin C concentration ppm	Average Absorbance	Fruit pulp extract ppm	Average Absorbance
5	$0.310\pm 2.01x10^{-3}$	500	$0.164\pm 2.02x10^{-3}$
10	$0.414 \pm 3.06x10^{-3}$	100	$0.747\pm 1.42x10^{-3}$
20	$0.817 \pm 4.58x10^{-3}$		
40	$1.069 \pm 2.57x10^{-3}$		
80	$2.057 \pm 3.81x10^{-3}$		

Each treatment was measured in triplicate and expressed as mean absorbance

Out of three types of antioxidant assays, ferrous ion chelating assay and FRAP assay showed good antioxidant properties in term of redox properties for the fruit pulp extract of *A. muricata* (Table 4).

Table 4. Antioxidant activities of extract of *Annona muricata* fruit pulp

	DPPH assay IC_{50} (ppm)	Fe2+ chelating activity EC_{50} (ppm)	FRAP assay (1,000 ppm equivalent to vitamin C (ppm)
Fruit pulp extract	724.98± 3.00	305.69± 4.71	24.51± 0.20
EDTA	-	7.9± 0.03	-
Vitamin C	3.13± 0.04	-	-

The reported IC_{50} values radical scavenging activity of other annona varieties are 1,715 and 1,702 for white and deep pink *A. diversifolia,* 3,900 and 4,600 ppm for red and green *A. squamosa* respectively (Loaeza.*et al*.,2011). Comparing the results with above annona varieties, Sri Lankan *A. murircata* has better radical scavenging activity. Loizzo *et al.* (2007) reported antioxidant activity of ethanolic extract of *A. cherimola* fruit pulp IC_{50} value as 72.2 µg/ml, Fe^{2+} chelating activity $EC_{50,}$ as115.8 µg/ml and FRAP assay as 68.3 µM Fe(II)/g in Italy. According the Italian *A. cherimola,* Sri Lankan *A. murircata* has moderate antioxidant activity.

Total phenol content (TPC)

Average absorbance values for different gallic acid concentrations for standard gallic acid curve given in Table 05. Average absorbance value obtained for the extract of *A. muricata* fruit pulp (2,000 ppm) was 0.668 ± 0.01 nm. According to the linear regression analysis equation of standard gallic acid curve, the total phenol content of the 2,000 ppm ethanolic extract of fruit pulp was equivalent to 55.59 ± 1.05 ppm GAE. Accordingly, total phenol content of the ethanolic extract of Sri Lankan *A. muricata* fruit pulp was 138.93 mg GAE/100g. Recorded values of total phenols content of fresh weight of Brazilian *A. muricata* fruit pulp is 120 mg/100 g (Hassimotto *et al.,* 2005) and 54.8 mg/100 g (Almeida *et al.,* 2011). The total phenol content of fresh weight of the Sri Lankan *A. muricata* fruit pulp was 21.96 mg / 100 g.

Table 5. Average absorbance values for different gallic acid concentrations

Gallic concentration ppm	Average Absorbance	Extract of fruit pulp ppm	Average Absorb
6.25	0.160 ± 0.01	2000	0.668 ± 0.01
12.5	0.224 ± 0.01		
25	0.347 ± 0.02		
50	0.682 ± 0.01		
100	1.080 ± 0.03		

Each treatment was measured in triplicate

Babbar *et al.* (2011) reported total phenol concentration of extract could be influenced by geographical origin, cultivar, harvest and storage time as well as drying and extraction methods. Amararatne *et al.* (2011) reported total phenol content in pomegranates is vary in different locations in Sri Lanka as 7.7 - 13.7 mg/l in Southern dry zone (DL 1b) and 21.7 mg/l in North western dry zone (DL 3). Ma *et al.* (2011) reported that the total phenol content of different mango genotypes showed great variation as 8.71 - 193.36 mg / 100 g of fresh weight of fruit. With comparing fruits of pomegranates and mango genotypes, Sri Lankan *A. muricata* fruit showed low content of total phenol.

CONCLUSIONS

Ethanolic extract of Sri Lankan *A. muricata* fruit pulp exhibited moderate antioxidant activity as free radical scavenging activity having IC_{50} value of 724.98 ppm, EC_{50} value for ferrous ion chelating activity as 305.69 ppm and Ferric reducing ability of 1000 ppm *A. muricata* fruit pulp extract is equivalent to that of 24.51 ppm of vitamin C. The total phenol content of fresh weight of the Sri Lankan *A. muricata* fruit pulp was 21.96 mg / 100 g.

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REFERENCES

Almeida, M.M.B., de Sousa, P.H.M., Arriage, A.M.C., do Prado, G. M., Magalhaes, C.E.C., Maia, G.A. and Lemos, G.A. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil, *Food Res. Int.l*, *44*, 2155-2159.

Amararatne, D.I.M., Weerakkody, W.A.P. and Jayakody, J.A.L.P. (2011). Bioactive properties of fruit juice of Promegrante (*Punica grntum*) grown in dry regions of Sri Lanka, *Trop. Agric.l Res. 23(4)*, 370-375.

Anesini, C., Ferraro, G.E. and Filip, R. (2008). Total polyphenol content and antioxidant capacity of commercially available Tea (*Camellia sinensis*) in Argentina, *Journal of Agricultural and Food Chemistry*, *56*, 9225-9229.

Babbar, N., Oberoi, H.S., Uppal, D.S. and Patil, R.T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues, *Food Res.Int.l*, *4*, 391-396.

Basniwal, P.K., Suthar, M., Rathore, G.S., Gupta, R., Kumr, V., Pareek, A. and Jain, D. (2009). *In-vitro* antioxidant activity of hot aqueous extract of *Helicteres isora* Linn. fruits, *Natural Product Radiance*, *8(5)*, 483-487.

Benzie, I.F.F. and Strain, J.J. (1996). The Ferric reducing ability of plasm (FRAP) as a measure of antioxidant power the FRAP Assay, *Analytical Biochemistry, 239*, 70-76.

Benzie, I.F.F. and Szeto, Y.T. (1999). Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay, *Journal of Agricultural and Food Chemistry*, *47*, 633-636.

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

Cardozo, C.J.M., Lozano, V.V., Betancur, D.P.Y., Velasquez, H.J.C. and Valenzuela, J.R.C. (2012). Physiological and physio-chemical characterization of Soursop fruit (*Annona muricata* L. cv. Elita), *Revista Facultad Nacional de Agronomi Medellin, 65(1)*, 6477-6486.

Guo, J.T., Lee, H.L., Chiang, S.H., Ilin, F. and Chang, C.Y. (2001). Antioxidant properties of the extracts from different parts of Broccoli in Taiwan, *Journal of Food and Drug Analysis*: *9(2)*, 96-101.

Garcia, Y.D., Valles, B.S. and Lobo, A.P. (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace, *Food Chemistry 117*, 731-738.

Hassimotto, N.M.A., Genovese, M.I. and Lajolo, F.M. (2005). Antioxidant Activity of Dietary Fruits, Vegetables and Commercial Frozen Fruit Pulps, *Journal of Agricultural and Food Chemistry*, *53*, 2928-2935.

Ibrahim, W.I.W., Sidik, K. and Kuppusamy, U.R. (2010). A high antioxidant level in edible plants in associated with genotoxic properties, *Food Chemistry*, *122*, 1139-1144.

Lee, K.W., Kim, Y.J., Lee, H.J. and Lee, C.Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine, *Journal of Agricultural and Food Chemistry*, *51*, 7292-7295.

Lim, Y.Y., Lim, T.T. and Tee, J.J. (2007). Antioxidant Properties of several tropical fruits: A comparative study, *Food Chemistry, 103*, 1003-1008.

Lima, L.A.R.S., Pimenta, L.P.S. and Boaventura, M.A.D. (2010). Acetogenins from *Annona corrnifolia* and their antioxidant capacity, *Food Chemistry, 122*, 1129-1138.

Loaeza, J.A.P., Santos-Sanchez, N.F., Valadez-Blnco, R., Sanchez-Guzman, B.S., Sals-Coronado, R., (2011). Chemical composition, color, and antioxidant activity of three varieties of *Annona diversifolia* Safford fruits, *Industrial Crops and Products, 34*, 1262-1268.

Loizzo, M.R., Tundis, R., Bonesi, M., Menichini, F., Mastellone, V., Avallone, L. and Menichini, F. (2012). Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (Cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents, *Journal of Food Composition and Analysis, 25*,179-184.

Ma, X., Wu, H., Liu, L., Yao, Q., Wang, S. and Zhan, R. (2011). Polyphenolic compounds and antioxidant properties in mango fruits, *Scientia Horticulturae*, *129*, 102-107.

Padmini, S.M.P.C., Pushpakumara, D.K.N.G. and Samarasekera, R. (2012). Morphological variation in selected *Annona muricata* L. (soursop) germplasm in Sri Lanka, *Tropical Agricultural Research, in press.*

Ragasa, C.Y., Geneveve, S., Torres, O.B., Don, M.J., Shen C.C. (2012). Acetogenins from *Annona muricata*, *Pharmacognosy Journal, 4(32)*, 32-37.

Stookey, L.L. (1970). Ferrozine-A new spetrophotometric reagent for iron, *Anlytical Chemistry, 42(7)*, 779-781.

Shajiselvin, C.D. and Muthu, A.K. (2011). Antioxidant activity of various extracts from whole plant of *Bauhinia purpurea* (Linn): An in-vitro evaluation, *Journal of Advance Pharmaceutical Research*, *2(1)*, 31-37.

Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain products, *Journal of Agricultural and Food Chemistry*, *46*, 4113-4117.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. and Qian, M. (2002). Free radical scavenging properties of wheat extracts, *Journal of Agricultural and Food Chemistry*, *50*, 1619 - 1624.