FERROUS CHELATION ACTIVITY OF *Annona muricata* PLANT EXTRACTS

N.I. Shanilka Dilshan, Sanjeewa K. Rodrigo*
Department of Chemistry, The Open University of Sri Lanka, Sri Lanka

INTRODUCTION

Annona muricata is an evergreen terrestrial tree in the family Annonaceae and native to the warmest tropical areas in South and North America. It is also widely distributed throughout tropical and subtropical parts of the world. All portions of the plant are used in traditional medicine in treating various diseases and ailments including rheumatism, diabetes, inflammation, hypertension and parasitic infestation. In addition to medical uses, the fruits are used for the preparation of beverages, candy, ice cream, shakes and syrup. *A. muricata* fruit can provide essential nutrients and elements to the human body. This plant has widely been used as a source of chemically active metabolites owing to their various properties. Extensive chemical investigations of *A. muricata* plant parts have resulted in isolation of a great number of Phyto constituents and compounds including alkaloids, megastigmanes, flavanol triglycosides, phenolics, cyclopeptides and essential oils. In addition, various recent studies have investigated properties of this plant parts including anti-arthritic activity, anticancer, anticonvulsant, antidiabetic, antioxidant, antihypertensive and wound healing activities (Moghadamtousi et al, 2012). Hence, *A. muricata* can be considered as a good candidate to be used in complementary medicine.

Heavy metals are essential for maintaining functions of living organisms. As an example, Iron (Fe) can be found in hemoglobin, myoglobin and in many enzymes. However, high levels of Fe could be a major health issue, which is known as heavy metal poisoning quite common in young children. Chronic Fe overload can be encountered in β - Thalassemia patients (Vacca et al, 2004). The heart and liver can be highly affected by Fe overload. In case of metal poisoning, metal chelating therapy is the most important and primary clinical treatment. Desferrioxamine B is one of the current drugs being used to reduce the iron level (harris et al, 1981).

In this research study, the Fe chelation potential of methanol extracts of *A. muricata* leaves, seeds and bark is tested using percentage inhibition of 1,10 – Phenanthroline – Fe^{2+} complex. In addition, Fe chelation ability is compared with respect to plant part and extraction method.

METHODOLOGY

A. Collection and Preparation of the extracts

Bark and leaf samples of *Annana muricata* were collected from a tree located in the Imbulgoda area, Gampaha. Seeds were collected from the Pettah, Colombo fruit market. Collection of plant material with contamination with microorganisms like lichens, and fungus was avoided as much as possible. Plant materials were identified against specimens at the national herbarium, Royal Botanical Garden, Peradeniya. Each plant material was dried under mild sunlight inside the laboratory, and was extracted with methanol using Soxhlet, sonicator and bottle shaker methods.

B. Preparation of test solutions

A concentration series range (1 to 0.01 ppm) of each of the dried extracts was prepared in methanol.

C. Fe chelation assay

The Fe ion chelating activity of the plant extracts was tested as described by Minotti and Aust (Minotti and Aust, 1987) with slight modifications. Plant extracts with the appropriate concentration (2 mL) were added to 3.36 mL of Tris HCl (0.1M, pH 7.4) followed by the addition of 4 mL of NaCl and 1 mL of $FeSO_4$. The mixture was incubated for 5 minutes, and 2.5 mL of 1,10-Phenanthroline was added into it. The absorbance was recorded at 510 nm after 24 h. Same reaction mixture except the plant extracts was used as negative control. Ethylenediaminetetraacetic acid (EDTA) was used as the positive control instead of plant extracts. Ferrous chelation activity was calculated as given below.

% Ferrous chelation activity = $[(A_0 - A_t)/A_0] \times 100$

A_0 – absorbance of negative control, A_t – absorbance of extracted sample

All measurements are expressed as mean ± standard error mean of independent experiments. All tests were carried out in triplicate.

RESULTS AND DISCUSSION

Ferrous chelating activity of *A. muricata* plant extracts are given in Table 1-3 as inhibition percentages of 1,10-Phenanthroline-Fe^{2+} complex

Table 1: Ferrous chelating activity of *A.muricata* plant extracts prepared using Soxhlet method

Concentration (mg/mL)	inhibition percentages of 1,10-Phenanthroline-Fe^{2+} complex			
	Bark (BE)	**Leaves (LE)**	**Seed (SE)**	**EDTA**
1.00	86.35±0.59	46.63±0.92	38.77±0.90	85.31±0.74
0.50	84.19±0.26	42.22±0.65	29.70±0.39	83.07±0.52
0.25	84.27±0.65	37.30±0.24	25.47±1.76	78.06±0.53
0.10	68.04±0.79	31.17±1.32	25.21±0.88	69.60±1.23
0.05	60.61±0.89	22.02±2.23	21.32±0.65	59.49±1.52
0.01	54.74±0.39	20.12±2.61	23.57±0.52	47.84±0.38

Table 2: Ferrous chelating activity of *A.muricata* plant extracts prepared using Sonicator method

Concentration (mg/mL)	inhibition percentages of 1,10-Phenanthroline-Fe^{2+} complex			
	Bark (BE)	**Leaves (LE)**	**Seed (SE)**	**EDTA**
1.00	87.04±1.29	52.67±0.61	31.86±0.78	85.31±0.74
0.50	88.15±1.29	50.17±0.89	27.20±0.45	83.07±0.52
0.25	65.45±1.05	43.26±2.81	24.00±0.75	78.06±0.53
0.10	59.15±1.16	39.81±0.12	23.57±0.00	69.60±1.23
0.05	47.92±0.26	41.88±1.67	24.52±0.39	59.49±1.52
0.01	44.98±0.54	33.33±1.53	23.91±1.05	47.84±0.38

Table 3: Ferrous chelating activity of *A.muricata* plant extracts prepared using Shaker method

Concentration (mg/mL)	**inhibition percentages of 1,10-Phenanthroline-Fe^{2+} complex**			
	Bark (BE)	**Leaves (LE)**	**Seed (SE)**	**EDTA**
1.00	91.35±1.27	49.74±1.12	28.32±0.90	85.31±0.74
0.50	74.77±1.32	47.49±0.68	25.73±0.74	83.07±0.52
0.25	62.94±1.86	39.11±1.21	24.95±0.39	78.06±0.53
0.10	48.18±3.42	37.39±0.27	23.22±0.83	69.60±1.23
0.05	46.80±1.22	41.70±1.12	20.63±1.42	59.49±1.52
0.01	45.16±0.74	32.90±1.52	17.43±0.83	47.84±0.38

Ferrous chelation activities of all samples including plant extracts and EDTA are shown in Figure 01.

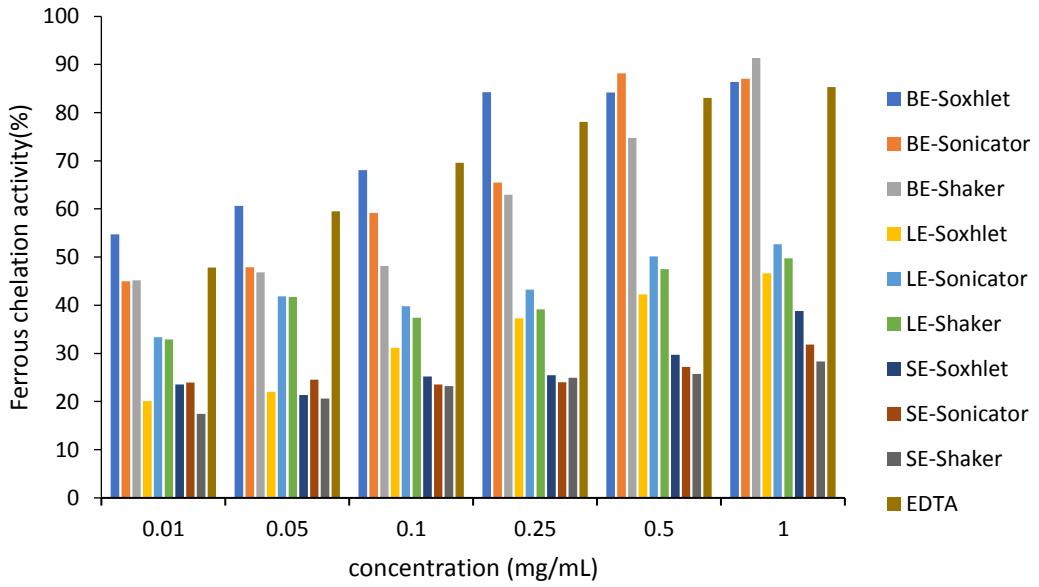

Figure 01: Ferrous chelation activity of *A. muricata* plant extracts and EDTA

The ferrous chelating activity is measured based on the absorbance measurement of 1,10-phenanthroline-Fe^{2+} complex, which gives a red color with a maximum absorbance at 510 nm. The chelating agents in the test samples are able to capture ferrous ions before 1,10-phenanthroline. Chelating agents of plant extract disrupt the formation of the complex due to which the red colour of the complex decreases. This red colour reduction is the estimation of the presence of another chelating agent. *A. muricata* Bark extracts showed promising chelating capacity (91% at 1 mg/mL, shaker method) while EDTA only showed 85% activity at 1 mg/mL. The sonicator-leaves extract showed 53% at 1 mg/mL and 39% at 1 mg/mL was observed for Soxhlet-seed extract. Leaves and seed extracts showed a lower capacity to chelate Fe ion compared to EDTA. In addition, the chelating potential of all extracts is increased with extract concentration. The extracts were observed to chelate the Fe in a dose, plant material and extraction method dependent manner. It was evident from the study that, *A.*

muricata bark possesses a higher metal chelating capacity than other parts and this might play a protective role against Fe toxicity.

CONCLUSION

The results show that the *Annona muricata* extracts contain ferrous chelation activity that varies with the plant part and extraction method. Bark extracts have a significant ferrous chelation activity among the different extracts and the activity decreases in this order: bark > leaves > seeds. Shaker extraction method is effective for bark extraction whereas sonicator extraction method for leaves. Soxhlet method is suitable for seed extraction. There seems to be a promising potential in *Annona muricata* plant extracts in combating oxidative stress as well as reduction of heavy metal levels. However, further studies including the isolation of active compounds and their identification, in *vivo* studies on the chelating agents should be done in order to get further insight to this promising activity.

ACKNOWLEDGMENT

The authors are thankful to the Department of chemistry, Open University of Sri Lanka for providing all the necessary laboratory facilities.

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