

Assessment of Heavy Metals in Crude Root Ethanolic Extract of *Crataeva magna* Lour (DC) by Atomic Absorption Spectrometer

R Meera*, S Venkataraman

Department of Pharmaceutical Chemistry, K.M. College of Pharmacy, Uthangudi, Madurai – 625 107. Tamilnadu, India.

Available Online: 25th August, 2017

ABSTRACT

Accumulation of heavy metals (HMs) like cadmium, lead, arsenic and mercury were determined in root bark of *Crataeva magna* Lour (DC) by atomic absorption spectroscopy. The objective of the study was to determine the concentration of heavy metals in root bark that are used in medicine by the local community. Analysis of the heavy metal in selected plant samples was performed by atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) for cadmium, lead, arsenic and mercury at wavelengths of 228.80 nm, 283.31 nm, 193.70 nm and 253.7 nm respectively. The analysis revealed the negative result for cadmium, lead, arsenic and mercury in root bark. The root bark was found negative for cadmium, lead, arsenic and mercury. This study confirm that the risk of HMs contamination of root bark of *Crataeva magna* Lour (DC) to appears low but the presence of HMs in this root bark needs to be analyzed every time before processing to confirm the absence of HMs. The consumer must be cautious while consuming these plants for medicinal purpose.

Keywords: Atomic absorption spectroscopy, Heavy metal, *Crataeva magna* Lour (DC).

INTRODUCTION

Medicinal plants play an important role in traditional medicine and are widely consumed as home remedies. The past decade has seen a significant increase in the use of herbal medicine due to their minimal side effects, availability and acceptability to the majority of the population of under developing countries and wild fruits to overcome the malnutrition¹. Since times immemorial, plant based drugs have been in use in the amelioration of various ailments ranging from common cold to cancer. Relatively high levels of essential elements, such as Fe, Mn, Zn, and Ca, have been demonstrated to influence the retention of toxic elements in animals and human beings^{2,3}. In recognition of the important role that major and trace elements play in health and disease of human body, in the building up and restoration phenomenon, it was observed that during the last few years remarkable progress has occurred in this area of health sciences⁴. Elements research has definitely been part of this explosion of scientific knowledge⁵. The quantitative estimation of various trace element concentrations is important for determining the effectiveness of the medicinal plants in treating various diseases and also to understand their pharmacological action⁶. The continuous intake of diets that are excessively high in a particular trace element can influence changes in the functioning, forms, activities of some organs or concentrations of such element in the body tissue and fluids can rise above the permissible limit⁷. Heavy metals (HMs) pollution is a result of increasing

industrialization throughout the world, which has penetrated into all sectors of the food industry⁸. In 1991 Codex Alimentarius Commission give safe and maximum allowable limits of elements in fruits and vegetables for Cd (0.2mg/kg dry weight), As (0.2mg/kg dry weight), Hg (10mg/kg dry weight), Cu (40mg/kg dry weight), and Zn (60mg/kg dry weight)(Codex Alimentarius Commission, 1991)⁹. WHO-2003 set the provisional tolerable weekly intake (PTWI) for As (0.015mg/kg body weight), WHO-1995 set intake for Pb (0.025mg/kg body weight) whilst WHO- 1991 set the total intake of mercury (0.005mg/kg body weight)¹⁰⁻¹². In the present study, an elemental assay was done by using FS Atomic absorption Spectrophotometer for the toxic elements like cadmium, lead, arsenic and mercury in the root bark of *Crataeva magna* Lour (DC).

Crataeva magna Lour DC (family Cappariaceae) is known as three leaved caper in English, Varuna in Sanskrit and Baruna in Hindi, a small tree with a much branched head, found to be distributed mainly in the warmer (tropical) parts of the world. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation¹³⁻¹⁵. Leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined¹⁶. The traditional plant used to treat

various ailments in particular to Urolithiasis¹⁷, Hepatoprotective¹⁸, Cardio protective¹⁹, anti arthritic and rubifacient²⁰⁻²². Bark juice of this plant is given orally to prevent childhood diseases among the inhabitants of the Kanyakumari district²³. The literature revealed that wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, dodecanoic anhydride, saponins, flavonoid, sterols, glucosilicates, cadabicine diacetate, lupeol, betulinic acid, glucocapparin, triacontane, triacontanol, cetyl and ceryl alcohol, octanamide, 12-tricosanone, rutin, quercetin, varunol, methyl pentacosanoate, kaemferol-3-O- α -D-glucoside and quercetin-3-O- α -D-glucoside have been reported from *C. magna*^{24,25}.

MATERIALS AND METHODS

Equipment and chemicals

The analysis was performed on Varian model AA 240 FS atomic absorption spectrophotometer (AAS), data were acquired and processed. Nitric acid, sulphuric acid, hydrogen peroxide and HPLC grade water were supplied by Merck (Darmstadt, Germany). Cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) were purchased from Sigma (St. Louis, MO). All other inorganic chemicals and organic solvents were of reagent grade.

Plant material

Root bark of *Crataeva magna* Lour DC were collected in and around local forest area of Kanyakumari, Tamilnadu and authenticated by the Botanist Prof. Chelladurai, Department of Botany, Govt. Siddha Medical College, Tirunelveli. A voucher herbarium specimen number KMCP/CM/01/2015 was also preserved in the K.M.College of Pharmacy, Madurai.

Sample preparation^{26,27}

Five grams air dried and fine powder of root bark of *Crataeva magna* Lour (DC) was placed in different crucibles and oven dried at 105 °C for 24 h. It was kept overnight. Dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550 °C and then left to ash at this temperature for 4 h to vaporize all other constituents and leave the heavy metals as a clean ash. The ash was transfer in to conical flask and added 20 ml of Aqua Regia to wet the sample in a flask. It was kept overnight. The

next day the temperature was raised to 120 °C and the heating was continued for 2 h. The flask was cooled to room temperature and then decomposes in a 10 ml solution of 6 M nitric acid (HNO₃) and 1 ml 60% hydrogen peroxide (H₂O₂) with 2 ml of concentrated sulphuric acid (H₂SO₄). The solution was subsequently heated and evaporated to half its volume using a hot plate. The resulting solution was then poured into a volumetric flask, and makeup the volume up to 25 ml with distilled water. The ash suspension was filtered into a 25 ml volumetric flask using Whatman filter paper No. 41.

Standard preparation

The selected heavy metals were cadmium, lead, arsenic, and mercury. For each of the selected metals a standard linear calibration curve of various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points) were analyzed by AAS and storing as stock solutions in a quartz flask.

Atomic Absorption Spectrophotometric Analysis

Analysis of the heavy metal in selected plant samples was performed by Varian model AA 240 FS atomic absorption spectrophotometer (AAS). Measurements were made using a hollow electron discharge lamp (EDL) for cadmium, lead, arsenic, and mercury at wavelengths of 228.80 nm, 283.31 nm, 193.70 nm and 253.7 nm respectively. Analysis was performed by testing samples at three different concentrations 0.5000 ppm, 1.0000 ppm and 1.5000 ppm to ensure that the method has wide adaptability and good accuracy. The slit width was adjusted for all metals at 0.5 nm and the parameters were discussed.

RESULTS

Selection of parameters

The AAS parameters were optimized by considering the wavelength, fuel gas as well supporting gas by using different EDL lamps. The wavelength for cadmium (228.80 nm), lead (283.31 nm), arsenic (193.70 nm) and mercury (253.65 nm) was found to be suitable for the detection of HMs. The fuel gas (acetylene) with supporting gas (air) in combination of 2.5: 15.0 L/min was found the best for the separation of cadmium and lead whilst the fuel gas (argon) with supporting gas (air) in combination of 5.5:15.0 L/min

Table 1: Parameters used for the analysis of heavy metal.

Parameters	Cadmium	Lead	Arsenic	Mercury
Instrument	Atomic Absorption Spectrophotometer	Atomic Absorption Spectrophotometer	Atomic Absorption Spectrophotometer	Atomic Absorption Spectrophotometer
Model No	AA 240 FS	AA 240 FS	AA 240 FS	AA 240 FS
Lamp	Cadmium EDL	Lead EDL	Arsenic EDL	Mercury EDL
Wavelength	228.80nm	283.31nm	193.70nm	253.65nm
Fuel gas	2.5L/min (Acetylene)	2.5L/min (Acetylene)	5.5L/min (Argon)	5.5L/min (Argon)
Support gas	15.0 L/min (Air)	15.0 L/min (Air)	15.0 L/min (Air)	15.0 L/min (Air)

EDL: Electron Discharge Lamp

Table 2: Contamination levels of heavy metals in root bark of *Crataeva magna* Lour (DC) Obtained from atomic absorption spectrophotometer (AAS).

Name of herb	Cd	Pb	As	Hg	MDL ^a
Crude extract	ND	ND	ND	ND	0.01mg/kg

ND: Not detected; MDL: Minimum detection limit; ^an=3.

was found robust for the separation of arsenic and mercury. (Table 1).

Optimization of the atomic absorption spectra

Atomic absorption spectrometry detection was carried out on positive ionization mode because this mode gave sharp and sensitive signals. It was optimized by using a standard linear calibration curve for various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points). The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each element. The heavy metals (cadmium, lead, arsenic, and mercury) were analyzed at their particular wavelength and the ion with the uppermost intensity was selected as the basic ion. The study revealed that no resultant spectral peaks of Cd, Pb, As and Hg in root bark of *Crataeva magna* Lour (DC) were observed (Table 2).

DISCUSSION

We found that neither or nor root bark of *Crataeva magna* Lour (DC) shows the presence of Cd, Pb, As and Hg. This is not unexpected because the soil and the environment has been the predominant source of the HMs contamination mainly Cd, Pb, As and Hg. This may account for the high incidence and concentrations of Cd, Pb, As and Hg compared with other HMs in the plants. HMs in roots, rhizomes, seeds and fruits were apparently higher than in flowers, indicating that the sample of these parts might be more favourable for HMs contamination²⁸.

Cadmium is absorbed by the roots of many plants, cannot be removed by washing and is concentrated particularly in the kidneys, liver, blood forming organs and the lungs²⁹. The high Pb value in plants were due to the uptake from the available Pb in the soil and in the above ground parts (leaves, stem and seeds) is due to air born Pb³⁰. Mercury has a particular affinity to become deposited in vital organs such as brain, nervous system, heart, liver, kidneys, bone marrow and also known to cause dementia, peripheral neuropathy, Parkinson's disease and cancer³¹.

CONCLUSION

In this study 4 elements were assessed in root bark of *Crataeva magna* Lour (DC) a medicinal plant. Among the various elements are not detected in the medicinal plant used in the treatment of various diseases. The data obtained in present study will be helpful in the synthesis of new modern drugs with various combinations of plants which can be used in the cure of many diseases ethno medicinally. However,

more detailed analysis of chemical composition of the following medicinal plants is required. The elemental analysis results of root bark of *Crataeva magna* Lour (DC) shows that the root bark contains elements of vital importance in man's metabolism and that are needed for growth, developments, prevention and treatment of many diseases. It is evident that they are important sources of essential mineral elements in reasonable concentrations which have required in treatment of many diseases.

REFERENCES

1. Kiran Yasmin Khan, Mir Ajab Khan, Rabia Niamat, Mamoona Munir, Hina Fazal, Paras Mazari, Nighat Seema, Tasmia Bashir, Ammarah Kanwal, Sidra Nisar Ahmed, Element content analysis of plants of genus *Ficus* using atomic absorption spectrometer, African Journal of Pharmacy and Pharmacology .2011;5(3):317-321.
2. Calabrese E .Nutrition and Environmental Health: The Influence of Nutritional Status on Pollutant Toxicity and Carcinogenicity. John Wiley and Sons, New York, 1981,p. 486.
3. Wang CF, Duo MJ, Chang EE, Yang JY. Essential and toxic trace elements in the Chinese medicine. J. Radioanal. Nucl. Chem. 1996; 211: 333-347.
4. Steiner RP . India's Traditional Medicine: Ayurveda in Folk Medicine: The Art and Science, American Chemical Society, Washington DC, 1986, p. 41.
5. Said S, Saeed HMA, D'Silva LA, Zubairy HN, Bano Z . Medicinal Herbal, A Textbook for Medical Students and Doctors. Published by Hamdard Foundation Pakistan Nazimabad, Karachi 74600, Pakistan, 1996; 1: 272-291.
6. Rajua GJN, Saritaa P, Murtya GAVR, Kumara MR, Reddya BS, Charlesa MJ, Lakshminarayanaa S, Reddya TS, Reddya SB, Vijayanb V. Estimation of trace elements in some anti-diabetic medicinal plants using PIXE technique. Appl. Radiation Isotopes. 2006; 64: 893-900.
7. Obiajunwa EI, Adebajo AC, Omobuwajo OR. Essential and trace element contents of some Nigerian medicinal plants. J. Radio analytical Nuclear Chem.2002; 252(3): 473-476.
8. Rose M, Knaggs M, Owen L, Baxter M. A review of analytical methods for lead, cadmium, mercury, arsenic and tin determination used in proficiency resting. J Anal At Spectrom. 2001; 16:1101-1106.
9. Codex Alimentarius Commission. Joint FAO/WHO Food Standards Program. Nineteenth Session. Rome.1991; 1-10.
10. WHO. Arsenic and Arsenic Compounds. Environmental Health Criteria, Geneva: World Health Organization. 2001; 224.
11. WHO. Lead. Environmental Health Criteria. World Health Organization, Geneva. 1995;165.
12. WHO. Inorganic Mercury. Environmental Health Criteria, Geneva: World Health Organization. 1991;118.

13. Sovan pattanaik, Sudam chandra si, Shiva shankar naik Evaluation of free radical scavenging activity, wound healing activity and estimation of phenolic, flavonoid and proanthocyanidine contents of the plant "*Crataeva magna*" Asian Journal of Pharmaceutical and Clinical Research, 2012;5: 168-171.
14. Gagandeep M. and Kalidhar SB. Chemical constituents of *Crataeva nurvala* (Buch-ham) leaves. In J.Ph.Sc., 2006; 68: 804- 806.
15. Kritikar K., Basu BD, Indian medicinal plant, 2nd Edition, Dehradun, International Book Publisher, 2005; 1: 190-192.
16. Inayathulla, Shariff WR., Karigar asif A., Sikarwar mukesh S.. Evaluation of anti diarrhoeal activity of *crataeva nurvala* root bark in experimental animals. International Journal of Pharmacy and Pharmaceutical Sciences. 2010;2:158-161.
17. Baskar R, Meenalakshmi M, Varalakshmi P. Effect of lupeol isolated from *Crataeva nurvala* stem bark against free radical-induced toxicity in experimental urolithiasis. Fitoterapia.1996; 67:121-125.
18. Sunitha S, Nagaraj M, Varalakshmi P. Hepatoprotective effect of lupeol and lupeol linoleate On tissue antioxidant defense system in cadmium-induced hepatotoxicity in rats. Fitoterapia.2001; 72:516-523.
19. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Lupeol and its ester ameliorate the Cyclophosphamide provoked cardiac lysosomal damage studied in rat. Mol Cell Biochem. 2006; 282: 23-29.
20. Geetha T, Varalakshmi P .Antiinflammatory activity of lupeol and lupeol linoleate in rats. J Ethnopharmacol.2001; 76:77-80.
21. Latha RM, Lenin M, Rasool M, Varalakshmi P. A novel derivative pentacyclic triterpene and ω 3 fatty acid [Lupeol-EPA] in relation to lysosomal enzymes glycoproteins and collagen in adjuvant induced arthritis in rats. Prostaglandins Leukot Essent Fatty Acids.2001;64(2):81-85.
22. Mhaskar KS, Blatter F, Caius JF (Eds.).In: Kirtikar and Basu's Illustrated Indian Medicinal Plants: Their usage in Ayurveda and Unani medicines. Delhi: Sri Satguru Publications.; 2000. p. 254-59.
23. Solomon Kiruba, Mony Mahesh, Zachariah Miller Paul, Solomon Jeeva, Preliminary Phytochemical screening of the pericarp of *Crataeva magna* Lour DC-a medicinal tree. Asian Pacific Journal of Tropical Medicine,2011,S129-S130.
24. Mantena RKR, Wijburg OIC, Vinduram polle C, Robins-Browne RM, Strugnell RA Reactive oxygen species are the major antibacterial against *Salmonella typhimurium* purine autotrophs in the phagosomes of RAW 264.7 cells. Cell Microbiology, 2008; 10(5): 1058-73.
25. Md Moniruzzaman, Mohammad Zafar Imam, Evaluation of anti nociceptive effect of methanolic extract of leaves of *Crataeva nurvala* Buch.-Ham, BMC Complementary and Alternative Medicine 2014, 14:354.
26. Kumar BSA, Prabhakarn V, Lakshman K, Nandeesh R, Subramanyam P, Khan S, Ranganayakalu D, Krishna NV. Pharmacognostical studies of *Portulaca oleracea* Linn. Braz J Pharmacogn. 2008; 18(4): 527-531.
27. Rose M, Knaggs M, Owen L, Baxter M. A review of analytical methods for lead, cadmium, mercury,arsenic and tin determination used in proficiency resting. J Anal At Spectrom. 2001; 16:1101-1106.
28. Miller JR, Hudson-Edwards KA, Lechler PJ, Preston D, Macklin MG. Heavy metal contamination of water, soil and produce within riverine communities of the Rio Pilcomayo basin, Bolivia. Sci Total Environ. 2004; 320(2-3):189-209.
29. Poletti S, Gruissem W, Sautter C. The nutritional fortification of Cereals. Curent Openion Biotechnol. 2004; 15(2): 83-166.
30. Tam YL and Singh B. 'Heavy metals availability at industrially contaminated soils in NSW, Australia', in A.L. Juhasz, G. Magesan and R. Naidu (eds.), Waste Management, Science Publishers, Plymouth. 2004; 97-120.
31. Weiss B, Clarkson TW, Simon W. Silent latency periods in methylmercury poisoning and in neurodegenerative disease. Environ Health Perspect. 2002; 110 (Suppl 5): 851-4.