INTERNATIONAL JOURNAL OF PHYTOTHERAPY RESEARCH
ISSN 2278 – 5701

Original Research Article

DETERMINATION OF HEAVY METAL IN MEDICINAL PLANTS BY ATOMIC ABSORPTION SPECTROSCOPY (AAS)

Siddique NA[1], Mujeeb M[1]*

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, JamiaHamdard(Hamdard University), New Delhi, India.

ABSTRACT:
Accumulation of heavy metals (HMs) like cadmium, lead, arsenic and mercury were determined in medicinal plants namely *Mucuna pruriens* L (Velvet beans; Fabaceae), *Delphinium denudatum* Wall (Jadwar; Ranunculaceae) and *Portulaca oleracea* L. (Purslane; Portulaceae) by atomic absorption spectroscopy. The objective of the study was to determine the concentration of heavy metals in plants 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. Of the three plants samples, all were found negative for cadmium, lead, arsenic and mercury. This study confirm that the risk of HMs contamination to medicinal herb like *M. pruriens, D. danudatum and P. oleraceae* appears low but the presence of HMs in these plants 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, *Delphinium denudatum*, Heavy metal, *Mucuna pruriens*, *Portulaca oleraceae*

INTRODUCTION:

The plants *Mucuna pruriens* L (Velvet beans; Fabaceae), *Delphinium denudatum* Wall (Jadwar; Ranunculaceae) and *Portulaca oleracea* L. (Purslane; Portulaceae) have the potential for wide therapeutic applications. *M. pruriens*, a herbaceous forage and food legume used as remedy for various diseases such as diabetes, arthritis, dysentery, and cardiovascular diseases [1]. The seeds of *M. pruriens* are high in protein, carbohydrates, lipids, fibre, and minerals along with minor amounts of methylated and nonmethylated tetrahydroisoquinolines (0.25 %) [2]. *D. denudatum* is one of the important medicinal drugs used as indigenous medicine in India [3]. The root is used in various medical formulations in Unani and Ayurveda to reduce the nervine disorders, respiratory problems, increasing the sperm counts and keeps check on urine related problems [4]. Chemically *D. denudatum* contains two alkaloids namely delphinine and stahisagrine besides this it contains some others alkaloids including delphocurarine, staphisagrine, delphine, condelphine, denudatin, talatizidine, acetylhetero-phylistine and a diterpenoid alkaloid identical to condelphine [5]. *P. oleracea* is a widely distributed weed grows in different areas of the world including India. The seeds have been used

ISSN 2278 – 5701

as a folk medicine in many countries in the treatment of urinary problems, digestive problems and cardiovascular diseases [6]. The seeds have been reported to be rich in a variety of bio constituents, including catecholamines, l-noradrenaline, dopamine, α-amyrin, β-amyrin and portuloside A [7]. Heavy metals (HMs) pollution is a result of increasing industrialization throughout the world, which has penetrated into all sectors of the food industry [8]. 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]. The HMs cause metabolic disturbance and the excess produce serious consequences on human health. It's widely acceptable that the metals may react directly with DNA and produces cross links between the DNA strands as was observed after exposure [13]. Men, animals and plants through air, water and food take up these metals from the environment [14]. The plants are widely used as raw materials for pharmaceutical preparations and as a supplement for dietetic products and especially for 'self-medication' in the general population [15]. There is little information available about the safety of herbal plants and their products in respect to heavy metals contamination. Due to the use of enormous amount of herbal medicine, it is important to know the toxic metal contents in these products. The objective of this work is to investigate the magnitude of heavy metals (cadmium, lead, arsenic and mercury) contamination in selected medicinal plants. Despite the numerous uses and the various biologically active chemical constituents reported in *M. pruriens*, *D. denudatum* and *P. oleracea*, no data on heavy metal detection of these plants are available in the literature. In order to ensure safety and quality we have collectively developed a cost efficient, flexible and rapid method for the quantification of heavy metals as these plants are commonly consumed by people without any awareness of their safety [16]. There exist no well-accepted scientific methods for proper determination and quality control of heavy metals in selected plant materials. The aim of this study was to screen the content of heavy metals as well as providing a sensitive, accurate and reproducible analytical method for the detection of heavy metals. Further, the purpose of this study is to provide useful information on heavy metals levels in *M. pruriens*, *D. denudatum* and *P. oleraceae* and to achieve a confirmatory method for quick determination of HMs by atomic absorption spectrophotometer.

MATERIAL 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.

Sample collection

All sample species were collected from a local market of New Delhi, India and were authenticated by Dr. H.B. Singh (Chief Scientist & Head, Raw materials Herbarium & Museum, NISCAIR, New Delhi). A

ISSN 2278 – 5701

voucher specimen (RHMD/1704/04) has been deposited in the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi. All samples were ground to powders and kept in moisture proof paper bags to keep their water content constant before analysis. The herbs and their parts used are summarized in Table 1.

Samplespreparation

Five grams air dried and fine powder (□2mm) from each plant sample (Table 1) was placed in different crucibles and oven dried at 105 Ċ 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 Ċ 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 Ċ and the heating was continued for 2 h. The flask was cooled to room temperature and then decomposes in a 10 ml solution of6 M nitric acid (HNO_3) and 1 mL 60% hydrogen peroxide (H_2O_2) with 2 mL of concentrated sulphuric acid (H_2SO_4). 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 werecadmium, lead, arsenic, and mercurysummarized in (Table 1). For each of the selected metals a standardlinear calibration curve of various concentrations ranging from 0.5000 ppm, 1.0000 ppm and 1.5000 ppm (three points) were analysed 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 in (Table2).

RESULTS AND DISCUSSION:

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 was found robust for the separation of arsenic and mercury (Table 2).

Optimisation of the atomic absorption spectra

Atomic absorption spectrometry detection was carried out on positive ionisation mode because this mode gave sharp and sensitive signals. It was optimised by using a standard linear calibration curve for various concentrations ranging from 0.5000 ppm,

ISSN 2278 – 5701

Table 1. Summary of heavy metals and herbs used in this study

Standard heavy metals	Tested herb		
	Species	**Family**	**Part used**
Cadmium (Cd)			
Lead (Pb)	*M. pruriens*	Fabaceae	Seed
Arsenic (As)	*D. denudatum*	Ranunculaceae	Root
Mercury (Hg)	*P. oleraceae*	Portulacaceae	Seed

Table 2. 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)	2.5 L/min (Acetylene)	5.5L/min (Argon)	5.5 L/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 3. Contamination levels of heavy metals in different herbs (mg/kg) obtain from atomic absorption spectrophotometer (AAS)

Name of herb	Cd	Pb	As	Hg	MDL[a]
Mucuna pruriens	ND	ND	ND	ND	0.01mg/kg
Delphinium denudatum	ND	ND	ND	ND	0.01mg/kg
Portulaca oleraceae	ND	ND	ND	ND	0.01mg/kg

ND: Not detected; MDL: Minimum detection limit; [a]n=3

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 compound. The heavy metals (cadmium, lead, arsenic, and mercury) were analysed 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 *M. pruriens*, *P. oleraceae* and *D. denudatum* were observed (Table 3). The method developed was subsequently applied to determine the occurrence of heavy metals in selected widely used commercial medicinal plants samples (Table 1). We found that neither *M. pruriens* nor *P. oleraceae* and *D. denudatum* show 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 contamination [17]. In particular, contamination levels of some samples i.e. root of *D. denudatum*, seeds of *M. pruriens* and *P. oleraceae* caught our attention, suggesting that herbal products rich in root, rhizomes and seeds should be carefully monitored. Heavy metal toxicity is frequently the result of long term low level exposure to pollutants common in our environment [18]. Cadmium is absorbed by the roots of many plants, can not be removed by washing and is concentrated particularly in the kidneys, liver, blood forming organs and the lungs [19]. Due to their high affinity for proteins, the lead ions consumed bond with the hemoglobin and the plasma protein of the blood. This leads to inhibition of the synthesis of red blood cells and thus of the vital transport of oxygen [15]. 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 Pb [8]. 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 cancer [20].

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Bioactive Natural Product Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, INDIA and Council of scientific and industrial research (CSIR) Government of India for financial assistance (09/591(0106)/2011-EMR-I).

REFERENCES

1. Bhaskar A, Vidhya V, Ramya M. Hypoglycemic effect of *Mucuna pruriens* seed extract on normal and streptozotocin-diabetic rats. Fitoterapia. 2008; 79(7-8): 539-543.
2. Spencer JPE, Jenner P, Halliwell B. Superoxide dependent GSH depletion by L-DOPA and dopamine. Relevance to Parkinson's disease. Neuro Repor.t 1995; 6(11): 1480-1484.
3. Qudsia N, Jafri MA. Unani drug, Jadwar (*Delphinium denudatum*Wall.) - A review. IJTK. 2006; 5(4): 463-467.
4. Zafar S, Aftab MA, Siddiqui TA. Jadwar (*Delphinium denudatum* Wall.) Roots – A Boon in Unani Medicine. Hamdard Med. 2003; 6(2): 9-14.
5. Oh KB, Chang IM, Hwang KJ, Mar W. Detection of antifungal activity in *Portulaca oleracea* by a single cell bioassay system. Phytother Res.2000; 14(5): 329-332.
6. 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.
7. Chan K, Islam MW, Kamil M, Radhakrishnan R, Zakaria MN, Habibullah M, Attas A. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L.subsp. Sativa (Haw.) Celak. J Ethnopharmacol. 2000; 73(3): 445-51.
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. Husain I, Khan F, Khan L,Wali-Ullah. Determination of heavy metal in medicinal plants. J Chem Soc Pak. 2006; 28(4): 347-351.
14. Hifsa M, Naeem I, Taskeen A, Zeb S. Investigation of heavy metal in commercial spices brands. N Y Sci J. 2009. 1554-2200.
15. Jarup L. Hazards of heavy metal contamination. Impact of environmental pollution on health: Balancing risk. Brit Med Bull. 2003; 68(1):167-182.
16. Siddique NA, Mujeeb M, Ahmad S, Panda BP. Determination of aflatoxins in medicinal plants by High-Performance Liquid Chromatography–Tandem

ISSN 2278 – 5701

Mass Spectrometry. J Pharm Pharm Sci.2013; 16(2): 321–330.
17. 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.
18. 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.
19. Poletti S, Gruissem W, Sautter C. The nutritional fortification of Cereals. Curent Openion Biotecnol. 2004; 15(2): 83-166.
20. 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.