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ATOMIC ABSORPTION SPECTROSCOPY FOR QUANTITATIVE EVALUATION OF HEAVY METALS AND TRACE ELEMENTS IN DARUHARIDRA: A RAPID AND COMPREHENSIVE RESTORATION SCHEME FOR QUALITY ASSURANCE OF HERBAL PLANTS FROM MARKET PLACE

S. TAMILSELVI* AND K P KANNAN

Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam - 638452, TamilNadu, India

ABSTRACT

With the recent increasing demand of herbal drugs among populations worldwide it is important to establish the identity, purity and quality assurance of herbs. Due to various factors such as soil, climatic conditions, human activities, presence and distribution of heavy metals is very likely. The present study reveals the heavy metal profile by atomic absorption spectrophotometry in various samples marketed as Daruharidra. Although all the metals studied viz., copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb) and zinc (Zn) have been found under permissible limits, the concentrations suggest the variation in the geographical location of the herbs collected thus indicating an evidence of the importance of ideal screening of metals in herbs. This study also indicates the simplicity and convenience of the AAS method which can effectively be adopted at an industrial level for the quality control and standardization of all herbs.

KEYWORDS: **Daruharidra, heavy metals, AAS, toxic.**

S. TAMILSELVI
[1]**Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam - 638452, TamilNadu, India**

INTRODUCTION

Herbal drugs are statistically gaining importance in contrast to synthetic drugs. This has been evidenced by the growing global and national markets for herbals indicating massive consumption. People rely more on herbal drugs than the synthetic drugs due to the latter's high prices and harmful side effects. This trend is growing, not only in developing countries, but also in developed countries as herbal drugs have proved to have a positive impact over the allopathic system of medicine.In India, medicinal plants have been widely used in various systems of medicine such as Unani, Ayurveda and Homeopathy. Efficacy and safety of raw drugs with respect to the source and quality plays an important role in the quality control of herbal drug formulations [1]. The therapeutic quality of these plants is largely affected by temperature, light, water availability, time and period of collection, and the consequent steps to drying, packaging and storing. Trace metal content of these plants are also considered essential for human nutrition and health as herbal drugs are mainly administered to maintain physiochemical processes in the body [2]. The WHO has passed several resolutions to ensure the quality control of the plants using various analytical techniques [3]. Metal contaminations have been largely found in herbal drugs due to unhygienic storage and packaging conditions resulting in toxicity. These contaminations may also occur due to high concentrations of these metals in the environment viz., air, water and soil resulting in the inhabitants accumulating metals leading to deleterious effects [4]. The increasing faith in herbal drugs is now leading to persistent efforts in maintaining and exporting herbal plants with the required quality assurance by negating the heavy metal contaminations. WHO has highlighted this issue as being critical and strong recommendations have been made to analyze the heavy metals in herbal medicines [5].Presence of trace elements can prove to be beneficial but presence of toxic heavy metals above permissible amounts surely has adverse effects on the consumer health who always take the herbal products with an impression of being safe because of the natural origin. Among the various methods in literature, atomic absorption spectrophotometry (AAS) is the most common method of estimating the heavy metals in herbs [6]. The concentration of atoms of an element by passing light, emitted by a hollow cathode lamp of that element, through a cloud of atoms from a sample is measured. Only those atoms that are the same as those in the lamp will absorb the light from the lamp. A reduction in the amount of light reaching the detector is seen as a measure of the concentration of that element in the sample [7]. *Berberis aristata* DC (Berberidaceae) known as Indian barberry/ Daruharidra (Ayurveda) is a well-known drug documented for use as anti-inflammatory, wound healing, antidysenteric, indigestion, immunopotentiating, in ENT infections, hepatoprotective, nephroprotective, antiulcer, anticancer, antipyretic, antimicrobial and antihyperglycemic properties [8,9]. *B.aristata* DC has proven to have substitutes like *Coscinium fenestratum*, *Berberis vulgaris, Berberis asiatica* and *Morinda umbellate* and is sold as Daruharidra in raw drug markets. All the plants are targeted for berberine; an alkaloid which has wide medicinal properties [10]. Demand of Berberis species is quite high in the herbal drug market due to its medicinal importance. Inorder to meet the market demand, herbal drug providers supply inferior quality samples or a mixture of different *Berberis species*, as 'Daruharidra' in local drug markets. This study is undertaken to estimate the heavy metals by AAS in different *Berberis species viz., B. aristata, Coscinium fenestratum* and market samples procured from different drug markets of South India to assure the quality of plants sold as Daruharidra.

MATERIALS AND METHODS

Collection of Plant Material

Eight stem samples of Daruharidra were obtained from local raw drug markets in the southern states of Tamil Nadu, Kerala and Karnataka, India. Authenticated stem samples of *B. aristata* and *C. fenestratum* were obtained from FRLHT-ENVIS Centre for Local Plants, Bangalore.

Plant sample digestion and analysis

The dried powder of plant samples was digested in microwave digestion system (Milestone Model 1200) using 10 ml of Nitric acid (69%) for 10 min, 1ml of Perchloric acid (70%) for 5min and 5ml of Hydrogen peroxide (30%) for 10 min at 250W power settings. The digested solutions were made up to 25 ml and stored in a well cleaned polythene vial in refrigerator till the time of analysis and analysed for metals, namely copper, cadmium, chromium, nickel, lead and zinc in an Atomic absorption spectrophotometer (Perkin Elmer, Model 800). AAS standards for all the metals were obtained from SRL, India and used for calibration studies using Winlab32AA software. The efficiency of digestion of plant samples was determined by adding the standard reference material of metals to different samples. After addition of standards, samples were digested and metals were estimated as described above. The analysis of each metal was carried out in triplicate for precision of results. The metal concentrations were calculated from each replicate absorbance value, which was then used to calculate an average metal concentration. Metal concentrations were expressed in ppm on dry weight basis of sample.

RESULTS

In the present study, use of two authenticated samples has been used as control. *B. aristata* and *C. fenestratum* were used as a standard for the testing of the stem samples obtained from various markets of Trivandrum, Coimbatore, Coonoor, Chennai and Trishur in the estimation of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb) and zinc (Zn) (Table 1). An intial comparision of the metal concentration among the authenticated samples was carried out as *B. aristata* and *C. fenestratum* have been seen to be localized to the southern and northern parts of India respectively. The permissible limit of the metal concentration of Pb and Cd is 10ppm and 0.3ppm respectively by WHO in 1999. Although the permissible levels of the other metals have not been decided by the WHO, the normal concentration of these metals in plants by Markert was taken as reference i.e., 1.5, 0.2, 10, 200, 1.5 and 50 ppm respectively for Cr, Co, Cu, Mn, Ni and Zn [11]. In the present study, it was found that all the metals analysed of the authenticated samples were below permissible limits The metal content estimated is shown in the Table 2. Authenticated samples at an average showed a higher concentration of copper and chromium. No cadmium was detected in all the authenticated and market samples except 0.0022 ppm of cadmium was seen in the stem samples of Daruharidra obtained from Trivandrum. The amount of chromium and lead present varied greatly, even amongst the authenticated samples. This may be attributed to the varied growing conditions of the plant in northern and southern India as well as within the state of Tamilnadu.

Table 1

Sources of the marketed plant samples from various parts of South India

Sample No.	Type of the Sample obtained	Place of Collection
MS1	Market sample	Trivandrum, Kerala, India
MS2	Market sample	Coimbatore, Tamilnadu, India
MS3	Market sample	Trishur, Kerala, India
MS4	Market sample	Coonoor, Tamilnadu, India
MS5	Market sample	Chennai, Tamilnadu, India
MS6	Market sample	Trishur, Kerala, India
MS7	Market sample	Chennai, Tamilnadu, India
MS8	Market sample	Coimbatore, Tamilnadu, India
AS1	Authentic sample (*B. aristata*)	FRLHT, Bangalore, India
AS2	Authentic sample (*Coscinium fenestratum*)	FRLHT, Bangalore, India

Table 2
Atomic absorption spectroscopic study of heavy metal distribution in different market samples of Daruharidra along with control. (in ppm)

S.No	Cu	Cd	Cr	Ni	Pb	Zn
MS 1	0.0094±0.00006	0.0022±0.00009	0.2008±0.00014	0.0043±0.00031	0.0045±0.00009	0.0080±0.00016
MS 2	0.0097±0.00003	ND	0.2219±0.00004	0.0020±0.00015	0.0037±0.00011	0.0088±0.00009
MS 3	0.0082±0.00007	ND	0.2466±0.00012	0.0044±0.00033	0.0031±0.00003	0.0074±0.00026
MS 4	0.0055±0.00014	ND	0.2065±0.00006	0.0034±0.00008	0.0077±0.00007	0.0074±0.00013
MS 5	0.0114±0.00008	ND	0.2065±0.00011	0.0028±0.00011	0.0160±0.00007	0.0076±0.00006
MS 6	0.0114±0.00012	ND	0.2201±0.00008	0.0027±0.00022	0.0019±0.00008	0.0069±0.00014
MS 7	0.0103±0.00003	ND	0.2178±0.00021	0.0031±0.00024	0.0019±0.00020	0.0073±0.00033
MS 8	0.0115±0.00017	ND	0.2572±0.00022	0.0026±0.00015	0.0050±0.00030	0.0089±0.00025
AS 1	0.0222±0.00020	ND	0.2402±0.00042	0.0057±0.00017	0.0131±0.00018	0.0080±0.00017
AS 2	0.0245±0.00006	ND	0.2952±0.00020	0.0049±0.00014	0.0059±0.00003	0.0013±0.00023

Values are arithmetic mean ± standard deviations of n=3; ND – Not detectable

DISCUSSION

Medicinal value of herbs results from the presence of biologically active substances as well as macronutrients and microelements. The pharmacological importance of these plants largely depends on their diversity, occurrence of some elements and certain diagnostic features related to their origin. As a result, the number of environmental samples submitted for analyses in the frame of routine monitoring or risk and sustainability assessment studies is continuously growing. The first step of a monitoring action is to determine whether the total metal content is within the range of background levels or over the concentration limits according to the national legislation [12]. Contaminations seen in medicinal plants make them impossible to be used in pharmaceutical preparations and productions. These contaminations being environmentally based can act as indicators for identifying the authentic samples from the fake samples sold for the benefit of commercial profit. Nickel and zinc have shown minor differences in their content amongst the market samples, whereas significant difference is seen in comparison of *B. aristata* with *C. fenestratum* indicating the pollution present in areas from where they were collected. The concentration of Zn and the pH of the soil is also seen to be essential in the uptake of Cd from the soil to the herbs [13]; therefore indicating that the concentration of metals not only indicate the environmental conditions but are also responsible for a synergistic or mutual effect of the herb.

CONCLUSION

The present study investigating the metal concentration of herbs available in the market emphasizes on the need of critical evaluation of its authenticity. Although all the samples showed permissible levels of metals in this study, it is highly important that one must be utmost careful at the site of selection of the drug from market dealers for crude drug analysis.

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