Journal of Soil Science and Plant Nutrition, 2012, 12 (3), 463-469

Determination of mineral contents of *Digitalis purpurea* L. and *Digitalis lanata* Ehrh

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Abstract

Digitalis purpurea and *Digitalis lanata* are commonly used in treatment of heart diseases, the species has recently introduced for cultivation in the state of Uttarakhand, India. The mineral contents of cultivated plants of these species have to be within permissible limit for its effectiveness. Using inductively coupled plasma mass spectrometry (ICP-MS), this paper describes analysis of selected minerals [viz. Boron (B), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Copper (Cu), Arsenic (As) and Lead (Pb)] in various plant parts of *D. purpurea* and *D. lanata* at pre- and post flowering stages. Our results revealed that the concentration of B, Cr, Mn, Co, Ni, Cu, As and Pb as 8.16±0.04 to 27.18±1.11, 7.30±0.03 to 21.16±0.20, 62.69±1.45 to 247.27±5.29, 0.65±0.08 to 6.13±0.05, 9.19±0.01 to 16.15±0.05, 0.02±0.0 to 25.27±0.20, 0.83±0.04 to 4.98±0.06 and 4.70±0.02 to 8.19±0.04 ug g^{-1}, respectively. The study reports that the mineral contents were well within the permissible range for medicinal uses. Therefore, the species are recommended for large scale cultivation.

Keywords: Mineral elements, ICP-MS, Microwave Digestion, *Digitalis purpurea*, *Digitalis lanata,* Scrophulariaceae.

1. Introduction

Minerals present in medicinal plants are of great importance to understand their pharmacological actions (Serfor-Armah *et al*., 2002). Mineral elements play an important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living organism which will lead to the formation of active organic constituents (Serfor-Armah *et al*., 2001). Their deficiency causes diseases, whereas the excess presence may cause toxicity (Hashmi *et al*., 2007). Mineral contents of several medicinal plants have been determined by applying several techniques such as atomic absorption spectroscopy (Negi *et al*., 2010), atomic emission spectrometry (Razic *et al*., 2003), electrothermal atomic absorption spectrometry (Gomez *et al*., 2004), X-ray emission (Mohanta *et al*., 2003), X-ray fluorescence (Salvador *et al*., 2003), inductively coupled plasma mass spectrometry (Falco *et al*., 2003) and neutron activation analysis (Yamashita *et al*., 2005).

Digitalis purpurea and *D. lanata* (family Scrophulariaceae) are reported to contain various cardiac and steroidal glycosides localized in roots and leaves (Lungeanu *et al*., 1963). Digitoxin is the main active constituent of these species (Hiermann *et al*., 1977). These species are used in the treatment of heart failure (Reddy, 2010), and extracts are reported to perform cardiovascular (Navarro *et al*., 2000), antitumour (Lopez-Lazaro *et al*., 2003), antimicrobial (Benli *et al*., 2009) and antiinflammatory activities (Nikolov *et al*., 1962). There are many reports on organic constituents of the genus *Digitalis*, however little attention has been given to analyze their mineral contents (Roca-Perez *et al*., 2006). Because of high market demand these species have been introduced for cultivation in the state of Uttarakhand, India, in recent years and successfully grown at farms with excellent biomass and seeds production capacity. It is important to analyze the mineral contents of these species before recommending them for large scale cultivation and medicinal uses. Considering that, present study was designed to analyze the mineral elements viz, Boron (B), Chromium (Cr), Manganese (Mn), Cobalt (Co), Nickel (Ni), Copper (Cu), Arsenic (As) and Lead (Pb) in different parts of *D. purpurea* and *D. lanata* that are raised in farms of Herbal Research and Development Institute.

2. Materials and methods

2.1 Plant Sample Collection and Processing

For analysis of mineral contents of two *Digitalis* species (viz. *D. purpurea* and *D. lanata*), the plant samples were collected at pre- and post flowering stages from herbal garden of Herbal Research and Development Institute, Gopeshwar, Uttarakhand, India. For each stage samples were collected from three different places of same population with nearly same maturity. The samples were separately air dried and grinded to powder of homogenized particles. Samples (0.5 g each) were taken and transferred to acid washed teflon PFA digestion tubes separately. Thereafter 10 ml of concentrated HNO_3 and H_2SO_4 (4:1) was added and the tubes were heated in a microwave oven using digestion program (Table 1) in microwave digestion system (Aurora MW 800). After completion of digestion all the samples were cooled for about 1hour. The digested samples were transferred to 100 ml acid washed volumetric flask and make up the volume using demineralized water (Millipore). Thereafter the samples were filtered and stored in polypropylene flasks prior to analysis.

Table 1. The digestion program for *Digitalis* samples.

Step	Time (S)	Start Temp. (°C)	Target Temp. (°C)	Pressure (psi)
1	100	0	100	400
2	600	100	160	620
3	600	160	170	640

2.2 Preparation of Standard Solutions

Multi-element working standard solutions (50, 100, 150, 200 µg/l) were prepared by appropriate dilution of the stock ICP multi-element standard (Merck) 10000 µg/l. All analyses were performed on ICP-MS (Perkin Elmer NexION 300X) using both Standard and Kinetic Energy Discrimination (KED) modes. The operating conditions for the ICP-MS are summarized in Table 2. All standard solutions and digested samples of both *Digitalis* species were run one by one. The calibration curves were obtained using NexION software. The data were arranged in tabular form using standard deviation.

Table 2. Instrumental Conditions for ICP-MS.

Component/Parameter	Type/Value/Mode
Sample Uptake Rate	0.5 mL min^{-1}
Flush Delay	35 sec
Read Delay	90 sec
Wash	40 sec
Neublizer	Glass concentric
Spray Chamber	Glass cyclonic
RF Power	1600 W
Sample Run Time	3 min/sample
Plasma gas flow	17.0 L/min
Auxiliary gas flow	4.1 L/min
Nebulizer gas flow	0.98 L/min
Modes	Standard and KED (He)
Replicates	3

3. Results and discussion

The concentration of B, Cr, Mn, Co, Ni, Cu, As and Pb analyzed in different plant samples of two *Digitalis* species are presented in Table 3. The concentration of essential minerals (B, Cr, Mn, Co, Ni and Cu) in the studied samples were found to be higher than the toxic ones (As and Pb). It was estimated that the concentration of most of the minerals was higher at post flowering stage than that of pre flowering stage. The concentration of boron ranged between 8.16±0.04 to 27.18±1.11 µg/g, which was higher in the leaves of *D. lanata*. The B content was higher in post flowering stage than the pre flowering stage except for roots of *D. perpurea* where it exhibited reverse stand (Table 3). Among the studied species the leaves of *D. lanata* had higher concentration than the leaves of *D. perpurea*. Contrarily roots of *D. perpurea* had higher B concentration than the roots of *D. lanata.* In medicinal plants, WHO (2007) limits had not yet been established for B, however, Krejcova and Cernohorsky (2003) found the range of B between 2.71±0.13 to 27.7±0.9 mg/kg in tea and coffee samples. Comparison of our results with these findings clearly shows that our results are well within the range. In case of chromium, leaves and roots of *D. perpurea* at post flowering stage comprised higher concentration, whereas for *D. lanata* the Cr concentration more or less at pre- and post-flowering stages (Table 3). There is no set limit for Cr in medicinal plants by WHO, however in Canada 2 ppm permissible limit was set for raw medicinal plant material (WHO, 2007). Ashraf *et al*. (2010) estimated the range of Cr between 1.325 to 28.28 ppm in *Artemisia maritime* and *A. turanicum*. Comparison of our results with these findings clearly shows that our results are also within the range. Interestingly for manganese the leaves comprised higher concentration at pre flowering stage, whereas for roots the Mn concentration was higher at post flowering stage for both species. From

this study, it is concluded that roots of both species are rich source of Mn at post flowering stage and leaves at pre flowering stage. For medicinal plants, WHO (2007) limits have not yet been established for Mn.

The concentration of cobalt was higher in *D. perpurea* than in *D. lanata* (Table 3). Among plant parts it was estimated higher at post flowering stage for *D. perpurea.* Contrarily, for *D. lanata* the concentration was higher in pre flowering stage for both plant parts (Table 3). Cobalt is an important component of vitamin B_{12} which participates as a coenzyme in various enzymatic reactions (Berdanier, 1994). For Co there is no set limit for their concentration in medicinal plant. The nickel content was more or less similar among plant parts as well as among species. The permissible limit for Ni is not established by WHO, although, daily dietary intake for Ni is estimated to be 100 µg/day. Ni deficiency is rare in humans as Ni requirements are low and availability high from dietary sources. Nickel deficiency causes depressed growth, reproductive changes and altered lipid and glucose levels in the blood (Onianwa *et al*., 2000). The copper content was minimal in roots particularly at post flowering stage in both the species. Among species Cu content was higher in *D. lanata* than *D. perpurea* (Table 3). Copper has been recognized as an essential element for living organism due to its presence in important proteins and enzymes (Onionwa *et al*., 2001). Cu deficiency in humans caused bone demineralization, depressed growth, depigmentation, gastro-intestinal disturbances, dermatitis and neurological disorders, while, excessive intake has been reported to cause liver cirrhosis and toxicity (Reynold *et al*., 2008). Thus *Digitalis* roots and leaves can be used to compensate Cu deficiency. The permissible limit set by WHO in edible plants is 3 ppm. After comparison of the metal limit in the studied plants with those proposed by WHO, it was found that most of the samples accumulates Cu above this limit. However, for medicinal plants, the WHO (2007) limits had not yet been established for Cu. Although in medicinal plants, permissible limits for Cu set by Singapore was 150 ppm (WHO, 2007). Reddy and Reddy (1997) pointed out that the range of Cu contents in the 50 medicinally important leafy material growing in India were 17.6 to 57.3 ppm.

Table 3. Concentration of mineral elements (µg/g) observed in *Digitalis* species.

Sample	Plant Part	Plant stage	Concentration of Elements (Mean ± SD µg/g of dry weight of samples)							
			B	Cr	Mn	Co	Ni	Cu	As	Pb
D. purpurea	leaves	Pre-fl	8.16±0.04	7.30±0.03	118.04±1.79	0.65±0.08	9.19±0.01	8.58±0.03	0.83±0.04	6.72±0.03
		Post-fl	13.76±0.22	12.82±0.13	62.69±1.45	1.04±0.01	9.24±0.10	7.84±0.08	1.20±0.01	5.99±0.01
	roots	Pre-fl	24.78±0.57	13.98±0.10	152.44±2.03	1.63±0.01	12.33±0.05	17.90±0.13	1.78±0.01	6.41±0.04
		Post-fl	17.89±0.97	21.16±0.20	215.07±4.03	6.13±0.05	16.15±0.05	ND	4.98±0.06	8.19±0.04
D. lanata	*leaves*	Pre-fl	13.06±0.08	10.16±0.09	174.03±3.13	0.81±0.03	9.48±0.05	9.03±0.02	0.96±0.05	7.43±0.01
		Post-fl	27.18±1.11	11.91±0.12	64.55±1.06	0.75±0.01	10.10±0.13	10.54±0.13	3.12±0.45	4.70±0.02
	roots	Pre-fl	10.31±0.38	12.88±0.12	86.83±2.14	1.46±0.02	10.94±0.09	25.27±0.20	1.38±0.02	7.43±0.54
		Post-fl	23.27±0.27	12.60±0.10	247.27±5.29	0.92±0.01	10.56±0.05	0.02±0.00	2.52±0.20	6.93±0.04

fl= flowering stage, SD= Standard Deviation, ND=Not detected.

The two toxic minerals also exhibited different level of concentration among different plant parts as well as different plant stage. The arsenic content was higher in *D. perpurea* than in *D. lanata* and was higher in roots than leaves (Table 3). The lead content was estimated higher in root parts than leaves for both species. The concentration of Pb decreased in leaves and roots at post flowering stage except for roots of *D. perpurea* where it exhibited reverse stand. Arsenic is considered as toxic mineral, however it exhibits beneficial actions in low quantities as it affects taurine and polyamine formation in plasma and tissues. The permissible limit for As and Pb in herbal medicines and products are 5 and 10 mg/kg, respectively (WHO, 2007). Our values are lower than these showing good qualities of material. The data through ICP-MS provides more accurate estimation than other techniques. Therefore, implications of our values are high for suggesting plant material for medicinal uses.

4. Conclusion

The microwave digestion method has considerable advantages, which includes good precision and accuracy, reduced contamination, speed and safety. The uses of concentrated nitric acid and sulfuric acid (4:1) mixtures allowed the complete digestion of samples. Mean intake of B, Cr, Mn, Co, Ni, Cu, As and pb are falls within the recommended range. The results obtained showed significant differences among the elemental concentrations in both species of *Digitalis*. The mineral contents were well within the permissible range for human consumption, therefore, recommended for medicinal uses. It is also suggested that large scale cultivation of these species may be promoted in the state.

Acknowledgments

The authors are thankful to Agriculture and Processed Food Products Export Development Authority, Ministry of Commerce and Industry, Govt. of India, provided financial support for equipments (Grant no. FLR/059/2006-07/13692). Thanks are also to Director, HRDI for providing facilities.

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