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HEAVY METAL AND MINERAL CONTENT ASSESSMENT IN *SKIMMIA ANQUETILIA* AND *DICTAMNUS ALBUS* IN KASHMIR HIMALAYA: A COMPARATIVE STUDY

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Abstract

The heavy metal contamination in plants is one of the major issues faced throughout the world and requires keen attention because heavy metals above their normal ranges are extremely threatened to both plant and animal life. It was therefore of keen interest to conduct a study to quantitatively analyze the levels of heavy metals in two medicinally important plants grown in Kashmir Himalaya-*Skimmia anquetilia* and *Dictamnus albus*. The study investigates the elemental profiling in different parts (Leaf, stem, root and fruit) of both the plant species. The dried powdered plant material was subjected to nitric acid digestion for the preparation of test samples, which were then analyzed by atomic absorption spectrophotometer. The present study was devised for the first time to analyze the heavy metal content in *Skimmia anquetilia and Dictamnus albus*, with the aim that the useful information obtained from the work will help educate the masses and to provide an insight to the scientific community about the levels of contaminants in the plants. The results of the present investigation revealed that the levels of heavy metals were far below the internationally accepted permissible limits in all the parts of the plants analyzed. Besides, both the plants are rich in essential minerals, especially magnesium and iron.

Key words: Flame atomic absorption spectrometer, nitric acid digestion, *Skimmia anquetilia*, *Dictamnus albus*, toxicity, WHO limit.

Introduction

Plants are the largest biochemical and pharmaceutical stores ever known on our planet. The use of plants as medicine is known to humanity since antiquity and has been used in all cultures throughout history (Barnes *et al*., 2007). In the past two decades, nearly two thirds of approved new drugs were obtained from natural plant products (Newman and Cragg, 2007). Majority of the world's human population relies on medicinal plants for its primary form of healthcare (McChesney *et al*., 2007; Owolabi *et al*., 2007). Today there is a wide spread interest in drugs derived from plants because of the potential health hazards associated with synthetic drugs. However, the use of herbal medicines has come under scrutiny due to their perceived long term toxicity among other considerations. The causes of the toxicities could be attributed to the chemical and mineral content of the plants. The human body requires a number of minerals for their growth and other activities which are obtained from plants, since plants absorb and accumulate minerals from the environment which is necessary for their growth. Among the different type of minerals, traces of heavy metals have been detected and reported in plants and foodstuffs. The plants accumulate metals in various parts and transfers it from soil to the food web (Singh *et al*., 2014). This accumulation is one of the most serious environmental concerns because of the potential harmful effects that toxic metals could have on animals and human health. Some metals like zinc, iron, copper, chromium and cobalt are toxic only at higher concentrations, while others like lead, mercury and cadmium are exclusively toxic (Radojevic and Bashkin, 1999; Gajalakashmi *et al*., 2012).

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Therefore it is necessary to measure and establish the levels of metallic elements in the herbal plants. The World Health Organization also emphasized the need to ensure the quality of plant and its products by using modern techniques and applying suitable standards (Ajas *et al*., 2004). Also by monitoring the level of metals in medicinal plants one can be able to indicate the level of environmental pollution in that area (Kirmani *et al*., 2011).

The potential intake of the toxic metal can be estimated on the basis of the level of its presence in the product and the recommended or estimated dosage of the product. A simple, straightforward determination of heavy metals can be found in many pharmacopoeias and is based on colour reactions with special reagents such as thioacetamide or diethyldithiocarbamate and the amount present is estimated by comparison with a standard (WHO, 1988a; Kunle *et al*., 2012). Instrumental analyses have to be employed when the metals are present in trace quantities, in admixture, or when the analyses have to be quantitative. Generally, the main methods commonly used are atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP) and neutron activation analysis (NAA) (Watson, 1999).

Kashmir Himalaya, often referred to as "terrestrial paradise" on earth supports a rich and spectacular biodiversity of great scientific curiosity and during the present study two important medicinal plant species of family Rutaceae viz., *Skimmia anquetilia* N.P. Taylor and Airy Shaw and *Dictamnus albus* L. have been selected on account of their medicinal importance and wide distributional range in Kashmir Himalaya. In Kashmir Himalaya, Rutaceae is represented by two genera (*Skimmia* and *Dictamnus*) wherein each genera is represented by single species only viz., *S. anquetila* and *D. albus*.

The purpose of the present work was to investigate the presence and concentration of essential elements and toxic heavy metals in different parts of *Skimmia anquetilia* and *Dictamnus albus*, with the hope that a useful information obtained will help educate the masses and to provide an insight to the scientific community about the levels of contaminations in them.

Material and Methods

Sample collection

Two medicinally important plants- *Skimmia anquetilia* and *Dictamnus albus* were collected from Gulmarg and Sonamarg areas of Kashmir Himalaya respectively. The plant materials were identified by Dr. Anzar A. Khuroo, Assistant Professor, Department of Botany, University of Kashmir. A voucher specimen has been deposited in Kashmir University Herbarium (KASH) under voucher number 2037-KASH and 2688-KASH respectively.

Reagents and standard

All solutions used in the study were prepared from analytical reagent grade chemicals. The standard solutions of analytes for calibration procedure were produced by diluting a stock solution of 1000 parts per million (ppm) of all the investigated elements.

Preparation of the plant samples and standard solution

The samples were thoroughly washed with deionized water, shade dried at room temperature, ground into powder with a mechanical grinder and homogenized. Ten milliliters of concentrated HNO_3 (ultrapure 65%) was added to 1g sample and allowed to stand overnight at room temperature. The samples were then heated for 4 h at 120°C, after which the temperature was increased to 140°C. The digestion was continued at this temperature until about 1ml of acid remained. After cooling, the suspension was filtered in a 50ml volumetric flask and diluted to the mark (Abou-Arab and Abou-Donia, 2000). The standard stock solutions (1000ppm) were diluted to obtain working standard solutions of 25, 50 and 100ppm.

Analysis of the sample

Mg, Mn, Fe, Zn, Cu, Co, Pb, Cr, Cd, Ni contents were measured using flame atomic absorption spectrometry. The calibration curves were plotted between measured absorbance and concentration. The mineral and heavy metal concentrations were expressed in parts per million (ppm) with respect to the dry weight of the plant materials.

Results and Discussion

Different parts of *Skimmia anquetilia* and *Dictamnus albus* were investigated for their mineral and heavy metal content. In this respect a total of ten elements Mg, Mn, Fe, Zn, Cu, Co, Pb, Cr, Cd, Ni in the units of ppm (parts per million) have been determined. The results (Tables 1, 2, 3, 4) are discussed as:

- **Magnesium (Mg):**

Intracellular free magnesium is involved in the energy reactions of phosphorylation and is necessary for the activation of hundreds of enzymatic reactions concerning adenosine-5'-triphosphate (Dube and Granry 2003). From the present study it was clearly evident that the concentration of magnesium, an important element, was higher than all the other analyzed elements. The highest content is present in the leaves of *Dictamnus albus* (19.715ppm) and the lowest in case of stem sample of

Table 1: Mineral content (ppm) of different parts of *Skimmia anquetilia*.

Mineral	Plant part				Permissible limit of mineral
	Leaf	Stem	Root	Fruit	
Magnesium (Mg)	4.461	0.817	2.252	3.163	No regulatory limits by WHO
Manganese (Mn)	0.098	0.021	0.043	0.026	No regulatory limits by WHO
Iron (Fe)	0.454	0.121	0.223	0.057	NMT 20.0 ppm
Zinc (Zn)	0.042	0.029	0.046	0.058	NMT 27.4 ppm
Copper (Cu)	0.046	0.004	0.010	0.027	NMT 3.0 ppm
Cobalt (Co)	0.037	0.030	0.036	0.042	No regulatory limits by WHO

Table 2: Mineral content (ppm) of different parts of *Dictamnus albus*.

Mineral	Plant part				Permissible limit of mineral
	Leaf	Stem	Root	Fruit	
Magnesium (Mg)	19.715	3.771	1.900	5.913	No regulatory limits by WHO
Manganese (Mn)	0.088	0.015	0.040	0.025	No regulatory limits by WHO
Iron (Fe)	1.130	0.447	1.723	0.359	NMT 20.0 ppm
Zinc (Zn)	0.055	0.044	0.034	0.052	NMT 27.4 ppm
Copper (Cu)	0.023	0.018	0.010	0.021	NMT 3.0 ppm
Cobalt (Co)	0.016	0.035	0.038	0.036	No regulatory limits by WHO

Skimmia anquetilia (0.817 ppm).

• Manganese (Mn):

Manganese is an essential micronutrient playing a vital role in the photosynthesis, nitrogen and lipid metabolism and in the activation of many enzymes (Mousavi *et al.*, 2011). It is less toxic than any other metal, however, can cause neurological disorders if its concentration exceeds 5 mg/m^3 due to continuous exposure to manganese dust and fumes. In the present study, a wide variation was found to occur in Mn concentration in different parts of both the plant species, with highest content in leaves of both the plants. Further Mn concentration was more in *Skimmia anquetilia* as compared to *Dictamnus albus*.

• Iron (Fe):

Iron is a most crucial element for growth and survival of almost all living organisms (Valko *et al.*, 2005), however, its overdose is associated with symptoms of dizziness, nausea and vomiting, diarrhea, joints pain, shock and liver damage (Martin and Griswold, 2009). The present study has shown a wide variation in Fe concentrations in the plant species. Among these two plant species, highest content of Fe was found in the stem and leaves of *Dictamnus albus*.

• Zinc (Zn):

Zn is a crucial micronutrient involved in many physiological functions in plants. It is required for the optimum crop growth and it is taken in divalent form by the plants (Tripathi *et al.*, 2015). Zinc is an essential component of thousands of proteins in plants, although it is toxic in excess quantities (Bakar and Bhattacherjy, 2012). Maximum amount of zinc was found in fruit samples of *Skimmia anquetilia* followed by leaf and fruit samples of *Dictamnus albus*. From the present investigation it was evident that the concentration of Zn in the aforementioned samples do not show a huge variation.

• Copper (Cu):

Cu is one of the essential metals which is required for the normal plant growth and development. It is a co-factor for metalloproteins and play a major role in several metabolic pathways. Though it is essential, the excess levels of copper in the plant would inhibit its growth and alters certain cellular processes (Yruela, 2005). The present work revealed that the concentration of Cu was higher in case of leaves of both the plants in comparison to other parts of plants. Further this accumulation was more in case of *Skimmia anquetilia* (0.046ppm) as compared to *Dictamnus albus* (0.023ppm).

• Cobalt (Co):

Cobalt is a trace element beneficial for humans as it is a part of vitamin B12 (Zhang *et al.*, 2009). However, too high concentrations of cobalt may damage human health. From the present study it was revealed that the concentration of Co was almost comparable in majority of the samples of both the plant species (0.035 ppm - 0.042ppm).

• Lead (Pb):

It is the most frequently occurring and stable heavy metal in nature. It is highly hazardous for plants, animals

Table 3: Heavy metal (ppm) analysis of different parts of *Skimmia anquetilia*.

Heavy metal	Plant part				Permissible limit of mineral
	Leaf	Stem	Root	Fruit	
Lead (Pb)	0.338	0.416	0.146	0.182	NMT 10.0 ppm
Cadmium (Cd)	0.006	0.005	0.006	0.006	NMT 0.3 ppm
Chromium (Cr)	0	0	0	0	NMT 2.0 ppm
Nickle (Ni)	0.075	0.061	0.067	0.086	NMT 1.63 ppm

Table 4: Heavy metal (ppm) analysis of different parts of *Dictamnus albus*.

Heavy metal	Plant part				Permissible limit of mineral
	Leaf	Stem	Root	Fruit	
Lead (Pb)	0.204	0.229	0.254	0.276	NMT 10.0 ppm
Cadmium (Cd)	0.006	0.006	0.005	0.007	NMT 0.3 ppm
Chromium (Cr)	0	0	0	0	NMT 2.0 ppm
Nickle (Ni)	0.105	0.098	0.111	0.131	NMT 1.63 ppm

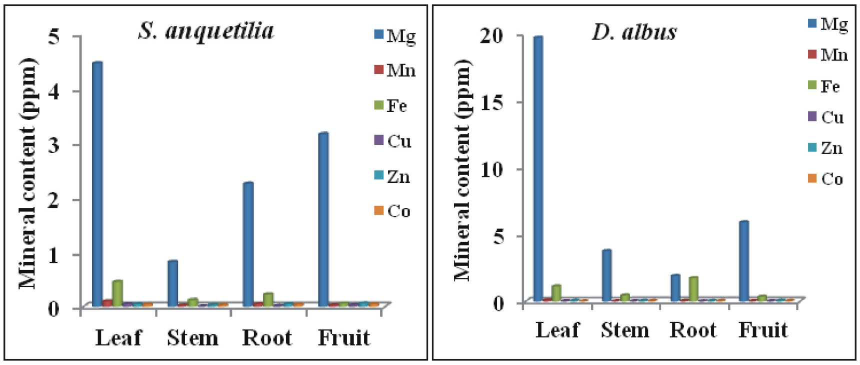

Fig. 1: Mineral content analysis in *Skimmia anquetilia* and *Dictamnus albus.*

and micro-organisms. Exposure to lead is of concern mainly because of its acute toxicity even at trace levels and numerous studies have revealed that it can adversely affect the central and peripheral nervous system, growth and development, renal system, blood circulation, mental retardation, reproductive health and even cause death (Gorbanova-Vorotto and Gorbanov, 2004; Shanthi *et al*., 2011). The permissible limit set for lead by the Ayurvedic Pharmacopoeia of India (Anonymous, 2009; Singh *et al*., 2014), WHO, (2007), China, Malaysia and Thailand is 10 ppm (Jabeen *et al.*, 2010). In the present study, lead was detected in all plant samples but the values were below 0.5ppm which was far below the prescribed limit.

- **Cadmium (Cd):**

Cadmium is one of the hazardous heavy metal which can cause significant reduction in plant yield (Kulhari *et al*., 2013). The major sources leading to accumulation of cadmium in soil and plants are phosphate fertilizers, non-ferrous smelters, lead and zinc mines, sewage sludge application and combustion of fossil fuels (Kulhari *et al*., 2013). At high concentrations, cadmium produces serious effects on the liver and vascular and immune system (Maobe *et al*., 2012). All the samples analyzed in the study had Cd concentration within the acceptable range of 0.3 ppm recommended by WHO, (2007) for raw herbal material. The present investigation depicted that the cadmium concentration was almost similar in both the plants- *Skimmia anquetilia* and *Dictamnus albus*.

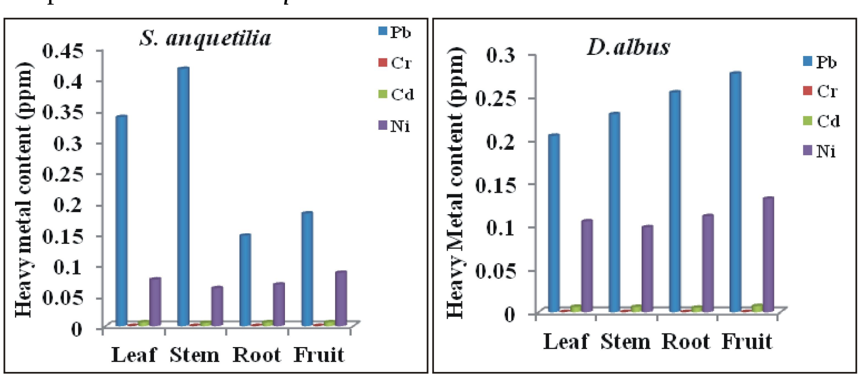

Fig. 2: Heavy metal analysis in *Skimmia anquetilia* and *Dictamnus albus.*

- **Chromium (Cr):**

Chromium is one of the known environmental toxic pollutants in the world. In plants, the toxicity of chromium is based on its valence state *i.e*. Cr (III) is less toxic when compared to Cr (VI) (Shankar *et al*., 2005). Chromium is not absorbed directly by plants rather it gets accumulated by carrier ions like sulfate or iron. Chromium toxicity alters the plant germination, its complete growth by affecting photosynthesis, other metabolic processes and the total dry matter production (Shankar *et al*., 2005; Gajalakshmi *et al*., 2012). Exposure to chromium is of concern mainly because of its acute toxicity even at trace levels. The permissible limit for Cr in raw herbal materials is 2.0 ppm (WHO, 2007). Chromium was not detected in any of the samples analyzed for the present study.

- **Nickle (Ni):**

Ni is directly coordinated by proteins. It regulates the mineral metabolism, enzyme activity and several other metabolic processes in plants. High concentrations of nickel cause severe chlorosis and necrosis in plants and a host of other growth abnormalities and anatomical changes (Bakar and Bhattacherjy, 2012). Generally Nickel and its salts do not affect the human body but in some cases it has been recorded to cause allergic problems and was recognized as an allergen of the year in 2008 by the American Contact Dermatitis (Bhat *et al*., 2010). In the present investigation, Ni concentration was found to be highest in *Dictamnus albus* as compared to *Skimmia anquetilia*. Furthermore, this accumulation is more in the fruit samples followed by the root samples.

Conclusion

Therapeutic plants are sources of a countless dynamic active principles of herbal and present day medication. However, persistent increase in environmental pollution leads to the built up of various pollutants including heavy metals in different plant parts which eventually enter the human food chain. Therefore, standard and regular screening of raw material is must to check the levels of these contaminations in the plant parts before using them for human consumption. The results obtained, revealed that both the plants are rich source of important elements, therefore, can act as supplements of macro and micro nutrient elements in the body. The results showed that minerals and heavy metals were found to be below the recommended maximum acceptable levels as given by World Health Organization. Studies have also shown that the plants do not accumulate chromium at all. The wide variations in metal concentrations in the analyzed herbs could be attributed to differences in the plant metal uptake and translocation capabilities. The uptake of metals by plants depend on a few variables including the plant species and their stage of growth and development, the soil type and the type of metals absorbed (Orisakwe *et al.*, 2012; Verma *et al.*, 2007). Thus, it is essentially required that each therapeutic plant ought to be checked for contaminant load before processing it for further pharmaceutical purposes and for local human consumption.

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Conflict of Interest

Authors have no conflict of interest.

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