ELEMENTAL ANALYSIS OF INDIGOFERA GERARDIANA WALL BY ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

Shafiq Ahmad Tariq[1], Tayyaba Bashharat[2], Naeem Khan[1], Yaqoob ur Rehman[3]

[1] Institute of Basic Medical Science, Khyber Medical University Peshawar

[2] Department of Pharmacology, Khyber Medical College Peshawar

[3] Department of Pharmacology, PCSIR

Address for correspondence:
Shafiq Ahmad Tariq
PhD
Associate Professor
Department of Pharmacology,
Institute of Basic Medical Sciences,
Khyber Medical University,
Peshawar, Pakistan. E-mail:
shafiq_tariq2000@yahoo.com

ABSTRACT

Background: Increase in frequency of environmental pollution particularly soil contaminated with heavy metals direct their uptake in human food through plant parts. Tissue precipitation of macro-nutrients and micro-nutrients during utilization of produces adversely affects one's health. The current study is directed to assess and quantify the presence of metallic elements in whole plant of Indigofera gerardiana.

Methodology: The fresh specimen of Indigofera gerardiana Wall was collected from upper Dir. The sample of the plant was dried under shadow, cut into small pieces and grinded to coarse powder. Powder was then used to analyse for mac-ro nutrients by flame photometry and for micronutrients by atomic absorption spectrophotometer.

Results: The window of acceptability for metallic ion is being exceeded by Fe, Ni, Cr & Co in certain parts of plants but normal in others. Mostly, the micro-nutrients detected in leaves were beyond normal limits.

Conclusions: Our study showed the presence of substantial concentrations of micro- and macro-nutrients in samples of root, leaves and stem.

Keywords: Indigofera gerardiana, Medicinal plants, Micronutrients, Macro nutrients, AAS (Atomic Absorption spectrophotometer)

This article may be cited as: **Tariq SA, Bashharat T, Khan N, Rehman YU. Elemental analysis of indigofera gerardiana wall by atomic absorption spectrophotometer (aas). Adv Basic Med Sci. 2017; 1(1): 21-25.**

INTRODUCTION

Flora of Pakistan is rich in medicinal plants, which are therapeutically used. More than 1,000 species pos-sess medicinal properties which are used by local communities to cure different diseases[1]. These plants are commonly used due to their affordability and less side effects. World Health Organization (WHO 2002) esti-mates that in developing countries about 80 % of the total population uses phytotherapy for their primary health care, and hence it is mandatory to check these plants for their safety, efficacy and potency[2]. The In-digofera gerardiana is generally known as Ghorega, which is a species of Family Fabeaceae (Leguminosae) and sub-family Faboideae. It is widely distributed in northern areas.

The whole plant extract is mostly used in hepatitis and whooping cough[3]. It is also used as an antispas-modic[4], tonic and hypoglycaemic agent[5]; the flowers, leaves and small shoots have a cooling and demulcent effect. They are used in treatment of leprosy and cancerous infection. Its leaves are used to cure ab-scesses[6]. The alcoholic extract of the dried shoots is reported to have an anti-inflammatory activity[7]; the root bark if chewed, helps to relieve the abdominal pain[8]; it is also reported that its bark, leaves and roots have an anti-bacterial activity[6,9].The current study is directed to check the presence of metallic elements and also quantify it, in different parts of indigofera

gerardiana.

MATERIAL AND METHODS

Collection of Plants

The fresh specimen of Indigofera gerardiana wall was collected from upper Dir, (Khyber Pakhtunkhwa) Pakistan during the month of August as a whole plant. The sample plant was identified by a plant taxonomist.

Chemical

Reagent used in elemental analysis were of analytic grade including nitric acid, perchloric acid, potassium iodide, sulphuric acid, oxalic acid, ascorbic acid, and standard test metals Ca, Na, K, Zn, Cu, Cr, Co, Cd, Fe, Pb, Ni and Mn (Sigma Aldrich).

Method

Standard protocol was followed to arrange the samples (10). 1.00 ± 0.05 gram test sample powder was put in a flask to which 10 ml of conc. HNO3 was added and was kept for 12 hours at room temperature, then 4 ml of HClO4 was added. The solution was concentrat-ed at 60oC on a hot plate until 1 ml of clear solution was left. After cooling, the deionized double distilled water was added followed by filtration through What-man (# 42) filter paper. Finally, 100 ml solution was made with deionized water as the stock solution[2]. The test sample was analyzed for the analysis of micro-nutrients using Flame Atomic Absorption Spectropho-tometer (polarized Zeeman Hitachi 2000) and Flame Photometer (Jenway PFP7, UK) was used for the de-termination of macronutrients. Then the stock solu-tion of standard test metals, containing 1000 ppm of each metal, was used. Afterwards, the diluted stock solution was prepared for the construction of standard calibration curve. All chemicals and reagents used in the study were of analytical grade.

RESULTS

Elemental analysis results

High level of Ca concentration was found in all parts of the plant. However for Na and K the values were within the permissible limits. Na concentration was 348.84, 376.26 and 589.99 ppm in stem, root and leaf respectively whereas K concentration was 3822.08, 4903.81 and 8199.91 in root, stem and leaf respective-ly. The results for the micronutrient contents of the materials are listed in Table 1.

High concentration of Fe was found in all parts of the plant IG. However in Ni the concentration is high in leaf, i.e. 2.199 and within limit in stem and root, i.e. 1.59 and 1.28 respectively. Significant amount of Co was also observed. Cu values are within the permis-

Table 1: Micronutrient of the plant

	Macronutrients			
	Leaves	Stem	Root	Permissible Limit (ppm)
Na	589.99	348.84	376.26	2610-51340 (11)
K	8199.91	4903.81	3822.08	6380-36,600 (11)
Ca	32999.67	17342.76	22774.08	44-614 (11)

Table 2: Macronutrients

Micronutrients				
Metal	Leaves	Stem	Root	Permissible Limit (ppm)
Fe	1435.98	332.90	604.00	36-241 (11)
Ni	2.199	1.59	1.28	1.5 (12)
Cu	6.499	7.57	4.45	10 (12)
Cr	3.299	1.096	0.495	1.5 (12)
Pb	1.299	6.578	Nil	10 (2)
Mn	45.49	8.47	14.258	200 (12)
Co	1.599	1.196	0.9901	0.2 (12)
Cd	Nil	Nil	Nil	0.3 (2)

sible limits i.e. 6.499, 7.57 and 4.45 in leaf, stem and root respectively. Similarly, Pb and Mn values are also within the permissible limits. However, Cr levels were found high in leaf only whereas in stem and root they are within the range. The results for the micronutrient contents of the materials are listed in Table 2.

DISCUSSION

Micronutrients

Iron is the most abundant essential element in body tissues. Animals, plants and microorganism require optimal Fe levels for their body maintenance[13]. Accord-ing to WHO, approximately 48 % of world's pregnant women and 46% of the children suffer from anaemia[14]. Iron deficiency affects immune response in different ways and causes alteration of brain functions i.e. irre-versible[15,16] . All plant parts possess significant concen-trations of iron. The plant under study is not supposed to be a good natural source of iron because it exceeds the permissible limits as described in table 2.

Earth's crusts are natural source of Nickel. Its deficiency is not that much common as it is abundantly found in almost all types of food. Abnormalities associated with Nickel intoxication are skin allergies, variable degrees of cardiovascular and kidney system poisoning, lung fibrosis and activation of neoplastic transformation[2]. Liver disorders may also be associat-ed with its deficiency[13]. Pancreas is its main source and has an important role in the synthesis of insulin. Permissible limit for Ni in plants is 1.5 ppm. Our data suggests that concentration of Nickel was exceeding permissible limit except in plant roots as shown in ta-ble 2.

Copper is a crucial element for both plants and animals. It is important for different human metabolic systems. It is required for regulation of various bio-logical systems i.e. energy production, oxidation-re-duction reaction, connective tissue formation, neu-rotransmitter synthesis and iron metabolism[2]. Industry and sewage sludge, pesticides and fertilizers are the most abundant sources of copper in soils. Melting and cutting of copper may generate fumes, dust and inha-lation or exposures to which is hazardous to health. In-toxication of Cu may cause liver and kidney damage[13]. Samples of all parts of the plant do not exceed the permissible limit (10 ppm).

Chromium is crucially required for the metabolism of proteins, carbohydrates and lipids, biosynthesis of cholesterols and fatty acids, and also facilitates the action of insulin[17]. For the very reason, supplements having Cr are used to improve weight loss[17]. Chromium exposure is related with Cr toxicity and symptoms are skin rashes, respiratory problems, liver and kidney damage, weakened immune systems, stomach upset, ulcers, mutation of genetic material, lung cancer and ultimately death. Permissible limit of Cr in plants is 1.5 ppm. Leaves of plants exceed the normal permissible limits i.e. 3.29 ppm, while stem and root have Cr in normal range i.e. 1.096 and 0.495 ppm, respectively.

Lead (Pb) is highly poisonous metal and affects al-most every organ of the body[18]. Long term exposure to lead may alter function of nervous system. In pregnant women increase level of lead may cause miscarriage and in male reduces fertility[19]. Excessive level of lead may be associated with delaying the puberty in girls[20]. A permissible limit in plants is 10ppm[2]. The leaves and stem of Indigofera gerardiana have lead in normal permissible limits i.e. 1.29 and 6.5 ppm respectively, while absent in roots.

One of other essential metallic element for growing plant and animal is Manganese. Parkinsonism is associated with intoxication of Mn, which is usually progres-sive and irreversible, showing some extent of perma-nent destruction of neurologic structures[21] . All parts of plant have normal levels of Mn i.e. not exceeding permissible limits as shown in table 2. Permissible lim-it of Mn in plants is 200 ppm.

Cobalt is essential element for all animals and is key constituent of cobolamine (vit B12)[22]. Its deficien-cy in sheep may lead to "Bush sickness"[23]. Excessive level of cobalt in body may lead to cardiomyopathy[24] and contact dermatitis[25]. Normal intake of cobalt is 0.2mg/kg[22]. Permissible limits for cobalt is 0.2 ppm[2]. All parts of plant possesses excessive level of cobalt which exceed the permissible limits i.e. 1.59, 1.16 and 0.99 in leaves, stem and roots respectively.

Macronutrients

Calcium is an essential macronutrient and in com-bination with phosphorus, is a structural component of teeth, bones and soft tissues[26]. It also provides thermodynamic stability to proteins by binding to the human surface of growth hormones[13]. Ca is necessary for the regulation and monitoring of nerve functions and muscle, blood vessel dilation and contraction and glandular secretions.

Bone fractures are associated with low level of Ca. Its deficiency may also cause abnormality in heart beat that may lead to heart failure and spasm of skel-etal muscle.

All parts of the plant possess Ca levels that exceed

normal limits as mentioned in table 1. According to "Food and Nutrition Board"[27] , 1000 mg/day Ca is re-quired to our body. Thus, for dietary Ca it may not be a good source.

One of the most critically important macronutrient for human body is sodium, and table salt (NaCl) is its main dietary source. It plays a vital role in the main-tenance of normal physiology. Optimal ingestion of Na is mandatory for growth, and is the most abundantly found cation in the extracellular fluid; its fluctuation alters overall balance of fluid and distribution[13].

Na deficiency may reflect changes in mood, fa-tigue, muscle cramps, hair loss, dehydration and hy-potension[13]. Significant sodium concentration was ob-served in all plant parts and not exceeding permissible limit i.e. 589.9, 348.8, 376.2 ppm in leaves, stem and root respectively. Daily intake of 1–3.8 mg/day of Na is recommended[27]. Hypertension is associated with high levels of Na.

Potassium is an important macronutrient in humans. It is crucial because it regulate the action potentials and also take part in intercellular signaling in electri-cally active cells. Irrespective of cell excitability, K channels regulate different functions including trans-duction of signals, insulin secretion, membrane poten-tial regulation, cell volume and immune response, reg-ulation of vascular tone, and hormone release[13]. For adult men the average range of daily potassium intake is 3100 mg/day and 2300 mg/day for adult women[27]. Our study explores that significant level of K is there in all plant parts, which did not exceed the permissible limit.

CONCLUSION

Current study showed that all parts of the plant contain significant concentrations of macro- and mi-cro-nutrients. However, the levels of some metallic elements e.g. Fe, Ni, Cr, Mn, Cu and Pb, do exceeded permissible limits. Long term health complications are associated with excessive level of these ions, and even could be lethal. It is, therefore, compulsory to per-form proper safety evaluation of therapeutically used medicinal plant i.e. determination of heavy metal content prior to formulation into finished medicines.

REFERENCES

1. Hussain J, Khan FU, Ullah R, Muhammad Z, Rehman N, Shinwari ZK, et al. Nutrient evaluation and elemental analysis of four selected medicinal plants of Khyber Pa-khtoonkhwa, Pakistan. Pak J Bot. 2011;43(1):427-34.
2. Saeed M, Muhammad N, Khan H. Assessment of Heavy Metal Content of Branded Pakistani Herbal Prod-ucts. Tropical Journal of Pharmaceutical Research. 2011;10(4):499-506.
3. Shinwari ZK, M. Rehman, T. Watanabe and Y. Yoshikawa. Medicinal and Aromatic Plants of Pakistan (A Pictorial Guide). Kohat, Pakistan: Kohat University of Science and Technology; 2006.
4. Hamayun M, Khan A, Khan MA. Common medicinal folk recipes of District Buner, NWFP, Pakistan. Ethnobotani-cal Leaflets. 2003;2003(1):14.
5. Sittie AA, Nyarko AK. Indigofera arrecta: safety evalua-tion of an antidiabetic plant extract in non-diabetic hu-man volunteers. Phytotherapy Research. 1998;12(1):52-4.
6. Nisar M, Tariq SA, Marwat IK, Shah MR, Khan IA. Anti-bacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on Indigofera gerardiana. Jour-nal of enzyme inhibition and medicinal chemistry. 2009;24(1):224-9.
7. Amala BE, Ganga N, Arivudainambi R, Santhanam G. Anti-inflammatory activity of Indigofera aspala-thoides Vahl. The Indian journal of medical research. 1982;76:115.
8. Esimone C, Adikwu M, Muko K. Antimicrobial properties of< i> Indigofera dendroides</i> leaves. Fitoterapia. 1999;70(5):517-20.
9. Dahot MU. Antibacterial and antifungal activity of small protein of< i> Indigofera oblongifolia</i> leaves. Jour-nal of ethnopharmacology. 1999;64(3):277-82.
10. Khan MA, Ahmad I, Rahman IU. Effect of environmen-tal pollution on heavy metals content of Withania somnifera. Journal of the Chinese Chemical Society. 2007;54(2):339-43.
11. Ajasa AMO, Bello MO, Ibrahim AO, Ogunwande IA, Olawore NO. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry. 2004;85(1):67-71.
12. Srivastava SK, Rai V, Srivastava M, Rawat A, Mehrotra S. Estimation of heavy metals in different Berberis species and its market samples. Environmental monitoring and assessment. 2006;116(1-3):315-20.
13. Saeed M, Khan H, Khan MA, Khan F, Khan SA, Muhammad N. Quantification of various metals and cytotoxic profile of aerial parts of Polygonatum verticillatum. Pak J Bot.

2010;42(6):3995-4002.

14. Benoist Bd, McLean E, Egll I, Cogswell M. Worldwide prevalence of anaemia 1993-2005: WHO global database on anaemia: World Health Organization; 2008.

15. Beard JL. Iron biology in immune function, muscle me-tabolism and neuronal functioning. The Journal of nutri-tion. 2001;131(2):568S-80S.

16. Cook JD. Diagnosis and management of iron-deficiency anaemia. Best Practice & Research Clinical Haematolo-gy. 2005;18(2):319-32.

17. Lukaski HC, Siders WA, Penland JG. Chromium picolinate supplementation in women: effects on body weight, composition, and iron status. Nutrition. 2007;23(3):187-95.

18. Tepper LB, Levin LS. A survey of air and population lead levels in selected American communities. Environmen-tal quality and safety Supplement. 1974;2:152-96.

19. Golub MS. Metals, fertility, and reproductive toxicity: CRC Press; 2005.

20. Schoeters G, Den Hond E, Dhooge W, Van Larebeke N, Leijs M. Endocrine disruptors and abnormalities of pu-bertal development. Basic & clinical pharmacology & toxicology. 2008;102(2):168-75.

21. Wang D, Du X, Zheng W. Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders. Toxicology letters. 2008;176(1):40-7.

22. Schwarz F, Kirchgessner M, Stangl G. Cobalt requirement of beef cattle—feed intake and growth at different lev-els of cobalt supply. Journal of Animal Physiology and Animal Nutrition. 2000;83(3):121-31.

23. Underwood E. Trace metals in human and animal health. International Journal of Food Sciences and Nutrition. 1981;35(1):37-48.

24. Morin Y, Tětu A, Mercier G. QUÉBEC BEER-DRINK-ERS'CARDIOMYOPATHY: CLINICAL AND HEMODYNAMIC ASPECTS*. Annals of the New York Academy of Sciences. 1969;156(1):566-76.

25. Basketter DA, Angelini G, Ingber A, Kern PS, Menné T. Nickel, chromium and cobalt in consumer products: re-visiting safe levels in the new millennium. Contact Der-matitis. 2003;49(1):1-7.

26. Shapiro R, Heaney R. Co-dependence of calcium and phosphorus for growth and bone development under conditions of varying deficiency. Bone. 2003;32(5):532-40.

27. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary refer-ence intakes: vitamin A, vitamin K, arsenic, boron, chro-mium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Journal of the Amer-ican Dietetic Association. 2001;101(3):294-301.