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## COMPARATIVE TRACE AND HEAVY METAL ANALYSIS OF *RAUWOLFIA SERPENTINA* USING ATOMIC ABSORPTION SPECTROSCOPY

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### Abstract

The stem of Indian medicinal plant *Rauwolfia serpentina* were digested with HNO<sub>3</sub> and HClO<sub>4</sub> (4:1) and the contents of trace elements such as As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn were determined by atomic absorption spectroscopy. The presence of beneficial element i.e., Ca, Fe, K, Mg, Mn and Zn are within limit but the concentration of Co and Ni are high in all the samples. The concentration of heavy trace elements Cd and Cu are within limit but the concentration of As, Cr and Pb are high in sample of Agra but concentration of As, Cr and Pb are within limit in northern Himalayan samples.

**Keywords:** *Rauwolfia Serpentina*, atomic absorption spectroscopy, essential trace elements, heavy metals.

### 1. Introduction

*Rauwolfia serpentina* L. Benth is a tropical plant of family Apocynaceae. IUCN (International Union for the Conservation of Nature and Natural Resources) assigned it as an endangered species. It is commonly known as sarpagandha in India. It is also known as chandrabagha, snake root plant, chotachand, Harkaya, Chandrika etc<sup>1</sup>. It is an herb of medicinal importance and finds its application in Ayurveda, Siddha, Unani and Western system of medicines<sup>2</sup>. In India it is found in Northern Himalayas especially in Gharwal region, Punjab, Gangetic plains, Andaman and Nicobar Islands. It is also found in Indonesia, Sri Lanka, Pakistan, Thailand etc<sup>3</sup>. It is an evergreen, woody, globrous shrub with height up to 60 cm. Its roots, leaves, seeds and fruits are used in the treatment of pneumonia, body aches, burns, skin disease, excitement, epilepsy, anxiety, schizophrenia, epilepsy, asthma, high blood pressure and snakebite etc in Ayurvedic medicinal system<sup>4-6</sup>. Its leaves are used in removal of opacities of cornea. It is used as antifungal, anti-inflammatory, antiproliferative, anticancerous, antidiuretic, antidysentery, antihypotensive, antioxidant, tranquilizing agent, antiarrhythmic etc<sup>7-12</sup>. More than 70 compounds are known in *Rauwolfia serpentina* among which the

reserpine, are used for control of high blood pressure, whereas ajamaline are used for cardiac disease under modern system of medicine.

Deficiency and excess of heavy and trace elements result serious effect on human health. Now a day's people are very conscious towards their health. All over, worldwide people depend on plant products for curing their diseases. Due to imbalance in environment our plant products are contaminated with harmful elements. So before using these products, harmful metals must be removed from them. Trace metals play an important role in chemical physiological, biochemical, metabolic, catabolic and enzymatic reactions. They have great significance due to their tendency to accumulate in vital human organs over prolonged period of time. In many countries all over the world several attempts have been made to determine the essential and trace elements in the herbal plants<sup>13-17</sup>. In the present work, atomic absorption spectroscopy is used to determine the presence of trace and heavy metals in stem of *Rauwolfia serpentina*.

## 2. Materials and Methods

Stem of *Rauwolfia Serpentina* are collected from Institute of Basic Science Khandari Agra, Chalesar Agra, Kankhal Haidwar, and Nainital. For fourteen days the leaves were dried under shade. Stem were powdered with the help of kitchen mixer grinder. In a 250 ml conical flask, 2.5 gm ground dried plant stem was placed and in it 2.5ml concentrated nitric acid was added slowly with constant shaking. On a hot plate, the mixture was heated until the evolution of brown fumes ceases. Then it is cooled and 10 ml. of 70%

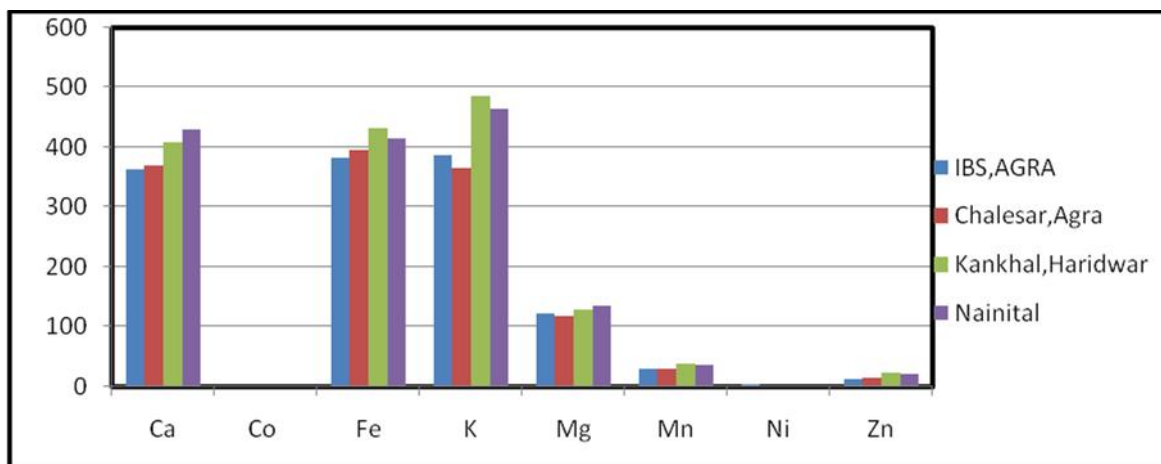
HClO<sub>4</sub> was added. It was heated again very gently until the solution turns colorless and was allowed to evaporate in to a small volume. Now, it is cooled and in it double distilled water was added and filtered into a 100 ml flask, using Whatman filter paper. Now, the volume was diluted with double distilled water. The total concentration of essential trace elements i.e., Ca, Co, Fe, K, Mg, Mn, Ni, Zn and harmful heavy metals i.e., As, Cr, Cd, Cu and Pb were analyzed by atomic absorption spectroscopy (Model: Perkin Elmer A Analyst 100).

## 3. Results

**Table-1 Essential Trace Elements in the stem of *Rauwolfia Serpentina***

Elements	IBS Khandari, Agra	Chalesar, Agra	Kankhal, Haridwar	Nainital
Ca (ppm)	362.384±3.12	370.586±4.21	408.214±4.75	430.562±4.36
Co (ppm)	2.160±0.25	2.142±0.11	1.166±0.10	0.982 ± 0.22
Fe (ppm)	382.454±3.54	396.232±3.62	432.385±5.36	414.462±5.58
K (ppm)	386.180±4.25	365.646±2.90	485.892±5.28	464.240±5.42
Mg (ppm)	122.462±2.52	118.225±1.66	128.724±3.11	134.021±2.54
Mn (ppm)	29.482±4.26	30.908±3.65	38.136±4.58	35.244±3.85
Ni (ppm)	3.426±0.48	2.968±0.28	2.042±0.25	1.826±0.10
Zn (ppm)	13.234±1.22	14.864±0.26	21.702±2.10	20.564±3.18

Values are expressed as mean ± SD (n=3), SD= Standard Deviation



**Fig. 1 Concentration of essential trace elements in stem of *Rauwolfia serpentina***

**Table-2 Harmful Heavy Metals in the stem of *Rauwolfia Serpentina***

Elements	IBS, Khandari Agra	Chalesaar, Agra	Kankhal, Haridwar	Nainital
As (ppm)	3.320±0.45	2.848±0.03	0.043±0.21	0.035±0.01
Cr (ppm)	2.480 ±0.35	2.524±0.42	ND	ND
Cd (ppm)	0.168 ±0.02	0.130±0.01	0.114±0.02	0.098±0.01
Cu (ppm)	9.544± 0.08	10.058±1.12	6.565±0.53	5.986±0.42
Pb (ppm)	1.514± 0.04	1.762±0.02	ND	ND

Values are expressed as mean ± SD (n=3), SD= Standard Deviation

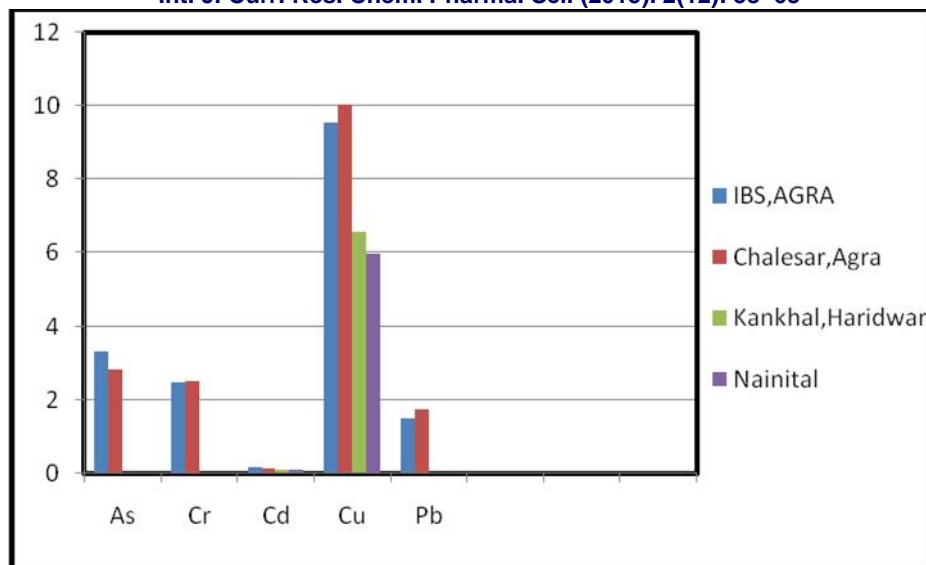


Fig. 2 Concentration of heavy metals in the stem of *Rauwolfia serpentina*

#### 4. Discussion

The concentration of arsenic in the stem of *Rauwolfia serpentina* ranges from 0.035 to 3.320 ppm. Arsenic is required in ultra trace amount. Arsenic poisoning may cause death through enzyme inhibition. The recommended concentration of arsenic in medicinal plants is less than 1.0 ppm. Toxicity of arsenic causes inactivation of 200 enzymes, especially those which are involved in cellular energy pathways, DNA synthesis and repair. Excess of arsenic results in dermatitis, metabolic disorder, lung cancer, cardiovascular effects, neurological effects etc<sup>18</sup>. Acute arsenic poisoning causes vomiting, abdominal pain, severe diarrhea, encephalopathy and peripheral neuropathy. Arsenic is more than permissible limit in the sample of Agra but within limit in the sample of Haridwar and Nainital.

The concentration of chromium in the stem of *Rauwolfia serpentina* is 2.480 to 2.524 ppm but it is absent in samples of northern Himalayan regions. The permissible limit for chromium set by FAO/WHO (1984) in edible plant was 0.02 ppm. Chromium plays an important role in synthesis of fatty acids and cholesterol. Deficiency of chromium causes disturbance in glucose lipids and protein metabolism. Chromium is found in pancreas, which produces insulin<sup>19</sup>. Excess of chromium causes asthma, shortness of breath, liver and kidney damage, allergic reactions etc<sup>20</sup>. In the stem sample of *Rauwolfia serpentina* of Agra chromium is more than permissible limit.

The concentration of cadmium in the stem of *Rauwolfia serpentina* ranges from 0.098 to 0.168 ppm. The permissible limit set by World Health Organization for cadmium is 0.21 ppm. Excess of cadmium damages kidneys and liver and causes high blood

pressure, gastrointestinal irritation, vomiting, diarrhoea. Cadmium poisoning causes a disease called itai-itai which results in softening of bones, anaemia, renal failure and ultimately death<sup>21</sup>. Cadmium is within permissible limit in the stem of *Rauwolfia serpentina*.

The concentration of copper ranges from 5.986 to 10.058 ppm in the stem of *Rauwolfia serpentina*. Copper is the third most abundant trace element in the human body. Copper is a component of many enzymes i.e., ceruloplasmin, cytochrome oxidase, lysyl oxidase, superoxide dismutase. Alzheimer's disease, Wilson's disease, Prion disease is due to the excess of copper. For normal synthesis of hemoglobin, traces of copper are required. Copper is needed for neurotransmitter synthesis, formation of myelin<sup>22</sup>. The permissible limit set by FAO/WHO (1984) for copper in edible plants was 3.00 ppm. WHO (2005) set no limit for copper but limit set by China is 20 ppm and Singapore is 150 ppm WHO (2005). But according to Reddy and Reddy<sup>23</sup> copper concentration in medicinal plants in India were 17.6 ppm to 57.3 ppm. Thus, copper is within limit in all the samples.

The concentration of calcium is 362.384 to 430.562 ppm. Calcium is necessary for normal functioning of cardiac muscles, regulation of cell permeability, blood coagulation. Excess of calcium in blood results in calcification of several internal organs. Deficiency of calcium causes diseases like rickets, osteoporosis<sup>24</sup>. It is also required for strong bones and teeth. It is within limit in the stem of *Rauwolfia serpentina*.

The concentration of cobalt ranges from 0.982 to 2.160 ppm. The permissible limit of cobalt in plants is 0.2 ppm. There is no limit set by WHO for cobalt. The limit of cobalt consumption is 0.04 mg per day for human. Excess of cobalt causes cardiomyopathy,

hyperglycemia, memory loss, allergic dermatitis etc. Deficiency of cobalt causes pernicious anaemia, severe fatigue, and hyperthyroidism. It is a part of vitamin B<sub>12</sub> which is essential for human health. It is more than permissible limit in all the samples.

The concentration of iron ranges from 382.454 to 432.385 ppm. FAO/WHO (1984) set 20 ppm permissible limit for iron. But WHO (2005) limits not yet been set for iron. In Egypt 261 ppm to 1239 ppm limit set for iron in medicinal plants. Iron is required for synthesis of hemoglobin and myoglobin. Deficiency of iron causes anaemia, poor resistance to infection, weakness. Iron is essential components of many proteins and enzymes in the human body. Iron is within permissible limit in all samples.

The concentration of potassium in the leaves of *Rauwolfia serpentina* ranges from 365.646 to 485.892 ppm. Potassium helps in the proper function of brain and nerves, so it helps in prevention of stroke. It regulates acid-base and water balance in the blood and tissues. It is required for bone and in prevention of osteoporosis<sup>25</sup>. High potassium diet lowered blood pressure in individuals with raised blood pressure. Potassium is essential in protein bio-synthesis by ribosomes.

The concentration of magnesium ranges from 118.746 to 134.021 ppm in stem of *Rauwolfia serpentina*. Magnesium is required for synthesis of protein and function of enzymes, energy transport, contraction and relaxation of muscles. Lack of magnesium is associated with abnormal irritability of muscles and convulsions and excess of magnesium cause depression in the central nervous system.

The concentration of manganese ranges from 29.482 to 38.136 ppm. Deficiency of manganese causes tissue damage and impairs CNS functions. For medicinal plants the WHO (2005) limits not yet been set for manganese. In Egypt 446 ppm to 338 ppm limit set for manganese in medicinal plants. The permissible limit of manganese in plants is 200 ppm. The average daily dietary intake of manganese is 2.8 mg per day. Excess of manganese causes pneumonia, affects reproductive system, which may lead to infertility, adverse effects primarily on the lungs and on the brain. Manganese is within permissible limit.

The concentration of nickel ranges from 1.826 to 3.426 ppm. The permissible limit set by FAO/WHO (1984) in edible plants was 1.63 ppm. For medicinal plants the WHO (2005) limits not yet been established for nickel. Nickel can cause cancer of different organs such as nose, prostate, lungs, thus it is carcinogen. Nickel is required for production of insulin. It is component of several enzymes i.e., carbon monoxide dehydrogenase, urease, hepatic microsomal enzymes

etc. Excess of nickel causes allergic dermatitis known as nickel itch. It is more than permissible limit in all the samples.

The concentration of lead is 1.514 to 1.762 ppm in the sample of Agra but it is absent in the sample of Haridwar and Nainital. Lead has no beneficial effects in humans. The permissible limit set by FAO/WHO (1984) for lead in edible plants was 0.43 ppm. Excess of lead causes anaemia, headache, central nervous system disorder, brain damage etc. The concentration of lead is absent in the sample of Haridwar and Nainital but more than permissible limit in the sample of Agra.

The concentration of zinc is 13.234 to 21.702 ppm. The permissible limit set by FAO/WHO (1984) is 27.4 ppm. About 100-300 enzymes contain zinc. Zinc is known to govern the contractibility of muscles and helps to avoid prostrate problems. Zinc acts as a co-factor for enzymes in the body and also takes part in synthesis of DNA, proteins and insulin. Zinc deficient diabetic person loss their sense of touch and smell. Zn is an important trace element and one of the several important micronutrients that is essential for proper functioning of the body. Zinc neutralizes the toxic effects of cadmium. High concentration of zinc is neurotoxin. The maximum tolerable daily intake of zinc is 0.3-1 mg/Kg. It is within limit in all the samples.

Thus on the basis of above results, it is found that stem of *Rauwolfia serpentina* contains useful trace elements Ca, Fe, K, Mg, Mn, Zn are within limit but the concentration of Co and Ni are high. Harmful heavy metals Cd and Cu are within limit in all the samples but the concentration of As, Cr and Pb are high in samples of Agra but concentration of all these heavy elements are within limit in northern Himalayan region samples. Stem of *Rauwolfia serpentina* are rich in some essential minerals like iron, magnesium, potassium which are essential for the health of human. The variation in concentration of element is due to the composition of the soil in which the plant cultivated, use of fertilizers, irrigation of water and climatic conditions<sup>26</sup>. FAO/WHO strongly recommends heavy metal analysis in the herbal medicines along with other necessary biological, chemical and environmental analysis in their guidelines and also documented the dietary allowances, absorption, elimination and toxic profiles of heavy metals<sup>27-29</sup>. Northern Himalayan region samples contain less amount of harmful heavy metals like arsenic, chromium and lead but concentration of these elements in the samples of Agra are higher.

## 5. Conclusion

Stem of *Rauwolfia serpentina* are used in different ayurvedic and modern system of medicines. So by this study it is recommended that harmful heavy metals



and trace metals must be removed from this medicinal plant before using them in preparation of medicines. Further Northern Himalayan region samples contains less concentration of heavy metals so plants of these regions are much safer in comparison to plants of city of Taj Mahal i.e., Agra.

### Conflict of interest statement

We decline that we have no conflict of interest.

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