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Screening of Indian Withania Plant and marketed products for trace elements, heavy metals for quality and efficacy

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ABSTRACT
An attempt was made to analyze some heavy metals and trace elements like As, Hg, Cd, Pb, Zn, Ca and Cu present in *Withania somnifera.* A total of 30 samples representing *Withania somnifera* fresh roots, dried roots and marketed formulations (ten each) collected from different places were subjected to heavy metal analysis using Atomic absorption spectrometry (AAS). The study showed differences in metal concentrations according to the categories of samples analysed (dried roots, fresh roots and marketed formulations). The obtained results which showed the predominance of Pb in almost all the analysed samples followed by As, Hg and Cd. However among the trace elements the Ca was found to be dominant in concentration followed by Zn and Cu.

Keywords: Atomic absorption spectrometry, Digestion, Heavy metals, Trace elements.

1. Introduction
Withania somnifera (Dunal), also known as Ashwagandha, is an important member of family Solanaceae. Withania products have long been used for medicinal purposes [1]. Roots of this plant are considered most active for therapeutic purposes by virtue of significant accumulation of active constituents, withanolides [2]. Many herbs including *Withania somnifera* have been shown to have anti-inflammatory, antitumor, antioxidant, anti-arthritic and immunomodulatory activities etc [3-9].
Heavy metals are classified in two main categories i.e. essential and toxic heavy metals. Essential heavy metals (Cu, Zn Cr, Fe and Co) are required in very trace quantities for the proper functioning of enzyme systems, haemoglobin formation, and vitamin synthesis in men and for the growth and development and photosynthesis in plants. Metabolic disturbances are encountered in case of both deficiency and excess of these essential metals. On the other hand toxic metals for example Pb, Cd, As, and Hg are not required by the body and they produce deleterious effects upon exposure even at very low concentrations [10-13].
Heavy metal contamination in herbal and synthetic drugs can cause serious health hazards such as injury of kidney, renal failure, and liver damage [14]. Physiological concentration of trace elements must always be maintained for proper maintenance of cellular functions of animals. However, the normal concentration of trace elements in different cells mainly depends on the dietary concentration, absorption, and homeostatic control mechanism of the body [15].
Unfortunately less attention is paid towards the legislation and quality control parameters of herbal drug industry as compare to allopathic system of medicines. FAO/WHO has highlighted this critical issue and strongly recommends heavy metal analysis in the herbal medicines along with other necessary biological, chemical, and environmental analysis in their guidelines (WHO 1989, 1993, 1998, 2005) and also documented the dietary allowances, absorption, elimination, and toxic profiles of heavy metals [16-19].
Very little information is available on the trace element and heavy element profile of genus Withania. Therefore, the present study was designed to estimate the essential trace elements and heavy metals in *Withania somnifera* by Atomic absorption spectrometry (AAS).

2. Materials and Methods

2.1 Plant Material

Withania somnifera fresh roots, dried roots and different marketed herbal formulations (ten each) containing Ashwagandha as main component were collected from different places.

2.2 Preparation of samples

Dry plant materials (2 g) were digested with 10 ml of nitric acid at 95 °C for 15 min or until the volume reduced to about 5 ml in digester. The digested residues were dissolved in ultra-pure Milli-Q (Millipore) water, filtered, and the volume was made up to 50 ml and used for determination of elements. These digestion was achieved according to standard procedure recommended by the Royal Committee of Experts [20].

2.3 Instruments

Atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) attached with Hydride Generator and hollow-cathode lamps for Cd, Hg, Ca, Cu, Zn (ECIL) and As, Pb (Photron) were employed as radiation source. The fuel used was air/acetylene. Nitrogen was used as carrier gas. Cadmium, Lead and trace elements were analyzed by flame atomic absorption spectrometry using air/acetylene flame. Mercury was analyzed by cold vapour atomic absorption spectrometry. All the measurements were run in triplicate for the samples and standard solutions.

3. Results and Discussion

The estimation of micro elements viz. Zn, Ca and Cu was carried out by atomic absorption spectrometry. AAS has been successfully used for the quantification of above mentioned elements in different samples of *Withania somnifera* commonly used in the treatment of various ailments. The concentrations of As, Hg, Cd, Pb, Zn, Ca and Cu observed in fresh roots, dried roots and marketed formulation samples of *Withania somnifera* are summarized in Table 1, 2 and 3.

Table 1: Distribution of heavy metal and trace elements in samples of marketed formulations (ppm)

Sample I D	As	Hg	Cd	Pb	Zn	Ca	Cu
1	1.48±0.106	2.12±0.01	1.04±0.304	BDL	4.94±0.76	5255±251.048	10.35±2.66
2	0.42±0.075	1.13±0.01	0.725±0.12	13.5	4.18±0.6	5071.17±147.3	6.18±0.43
3	11.35±2.80	BDL	0.61±0.26	30.53±2.94	17.62±0.35	7381.67±52.04	25.07±0.73
4	0.52±0.044	0.69	0.15±0.041	BDL	16.34±1.5	7235±297.153	1.15±0.53
5	6.23±0.649	4.9±0.06	0.911±0.24	47.65±11.1	27.67±1.72	815.83±24.023	7.4±1.27
6	1.63±0.226	1.09±0.02	0.396±0.04	38.81±11.9	11.76±1.96	1339.75±137.5	2.75±0.51
7	1.78±0.151	BDL	0.43±0.11	9.35	41.51±4.22	2560.17±65.24	32.93±2.98
8	2.33±0.043	3.41±0.02	0.28±0.09	97.5±11.11	15.35±1.54	2840.67±9.24	9.37±0.85
9	2.47±0.422	BDL	0.39±0.126	71.09±6.43	10.44±0.53	2289±19.56	8.63±1.13
10	2.75±0.075	5.25±0.038	0.73±0.07	68.28±5.96	44.99±4.98	2722±117.19	15±1.13

Values are expressed as mean ±SD (n=3), BDL = Below Detectable Limit, SD = Standard Deviation.

Table 2: Distribution of heavy metals and trace element in dried roots of Ashwagandha

Sample I D	As	Hg	Cd	Pb	Ca	Zn	Cu
1.	9.47±0.62	10.95±0.65	2.01±0.29	11.41±1.01	7470±125.8	35.91±3.22	12.96±0.33
2.	14.66±0.52	10.73±0.38	2.03±0.31	14.01±1.63	6506.7±68.3	52.97±2.79	34.82±2.23
3.	14.15±0.98	11.39±0.38	2.84±0.06	19.67±0.81	5975±79.4	36.79±3.88	12.17±1.37
4.	10.54±0.38	13.99±2.29	2.77±0.23	23.44±1.41	6993.3±163.6	26.93±0.81	14.54±0.90
5.	9.47±0.64	11.82±1.64	3.45±0.19	30.43±1.42	6233.3±113.7	18.67±2.30	17.02±1.49
6.	12.14±0.47	13.59±0.6	3.57±0.1	38.07±2.94	7360±17.3	35.73±2.13	10.98±0.68
7.	14.73±0.42	14.43±0.1	3.68±0.31	30.43±1.41	6181.7±17.5	35.38±1.32	11.22±3.44
8.	11.41±0.64	10.95±0.65	4.16±0.39	37.6±1.4	5103.3±59.7	31.51±1.33	12.37±1.23
9.	13.42±0.47	10.66±0.17	4.01±0.31	33.35±4.25	5501.7±67.9	28.88±1.69	14.93±0.59
10.	10.96±0.74	12.67±0.19	4.53±0.39	35.24±4.08	5885±58.9	25.88±1.09	17.68±1.5

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

Table 3: Distribution of heavy metals and trace elements in fresh roots of Ashwagandha

Sample I D	As	Hg	Cd	Pb	Ca	Zn	Cu
1	9.13±0.31	7.59±0.27	3.89±0.27	32.45±2.01	7555±58.9	18.84±0.93	9.3±0.75
2	3.5±0.37	14.2±1.15	3.88±0.09	32.89±1.34	7111.6±127.9	14.46±0.56	5.05±0.22
3	7.34±0.37	8.12±0.38	4.07±0.33	21.05±0.75	7705±102.6	17.2±1.25	6.68±0.38
4	7.97±0.98	13.37±0.73	4.02±0.19	26.64±1.33	8058.3±423.1	30.21±1.31	6.93±0.57
5	4.08±0.27	8.34±0.65	4.07±0.27	35.65±1.29	6586.7±328.6	16.85±0.43	6.092±0.21
6	8.64±0.16	9.36±0.27	4.23±0.14	42.09±0.86	5861.7±768.3	40.28±0.45	9.8±0.57
7	9.25±0.37	7.91±0.75	4.06±0.19	34.45±1.28	5308.3±153.7	23.05±0.99	4.26±0.29
8	10.6±0.28	9.43±1.64	3.63±0.16	44.3±1.01	5413.3±68.3	11.86±0.27	4.9±0.21
9	15.48±0.34	6.82±0.38	3.72±0.14	36.69±3.65	6653.3±171.3	15.02±1.6	8.8±0.43
10	7.52±0.59	9.86±0.38	3.5±0.21	34.66±2.03	7046.7±151.2	15.94±1.4	12.68±0.65

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

Data reveals that there is variation among heavy metal content and trace elements in roots collected from different places. The obtained results which showed the predominance of Pb in almost all the analysed samples followed by As, Hg and Cd. However among the trace elements the Ca was found to be dominant in concentration followed by Zn and Cu.

Comparatively among the heavy metals, the dried root samples contain highest (14.73 ppm) and marketed samples contains lowest

(0.42 ppm) concentration of arsenic whereas lead conc. was higher (97.5 ppm) in marketed formulation and lower (11.41 ppm) in dried roots. Further in case of trace elements, fresh root samples were found to have higher conc. of calcium (8058.3 ppm) whereas zinc (52.75 ppm) and copper (32.9.3 ppm) conc. was highest in dried roots and marketed formulation respectively.

The above described variation in conc. in different samples of *Withania somnifera* may be due to improper storage, storage under unhygienic conditions or improper manufacturing practices.

4. Conclusion

Most of the herbal drugs that are being sold in the local markets were found toxic and unsafe for human consumption due to elevated levels of heavy metals especially Pb. Comparative studies on the heavy metal contents of dried roots, fresh roots and marketed formulations of *Withania somnifera* revealed wide variation in the results. The presence of essential trace elements in *Withania somnifera* may readily account for the most of the therapeutic efficiencies. The data obtained for individual elements concentration in *Withania somnifera* will be useful in deciding the dosage of herbal drugs prepared from Withania species in the treatment of variety of diseases.

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