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Analysis of Metal Ion Levels in Ashwagandha and Thipala Ayurveda Powdered Drugs in Sri Lankan Herbal Market

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ABSTRACT

Over the years, herbal products have been used in various parts of the world for the cure of human disorders. Although herbal products are often demanded to be beneficial and free of side effects there have been reports of acute and chronic toxicity resulting from their use. One of the main causes of toxicity of herbal medicines is the existence of heavy metals. Therefore the main objective of this study is to analyze Ayurvedic powdered drugs, Ashwagandha churna (AC) and Thipala churna (TC) of three different brands available in Sri Lankan herbal market for the quantitative analysis of essential trace and heavy metals. The samples were prepared by wet digestion method using hydrogen peroxide and nitric acid treatment. The presences of metals were analyzed by Flame Atomic Absorption Spectroscopy using a HITACHI ZA3000 Polarized Zeeman Atomic Absorption Spectrometer. The results were compared with the extraneous maximum residue limit recommended by the Food and Agriculture Organization (FAO) /World Health Organization (WHO) for herbal medicines. Lead and cadmium in brand A of AC were not detected in the samples. However cadmium concentrations in other samples were below the extraneous maximum residue limit. The extraneous maximum residue limits of the FAO/WHO have not been established yet for copper, nickel, chromium and zinc in herbal medicine. The results reveal that among the trace elements zinc (33.25 mg/kg) found in highest amount, but this was below the acceptable daily intake limit (60 mg/day). Chromium was not detected in brand C of AC and TC samples. In conclusion, the quality of herbal churna products sold in Srilankan market is safe. However there is need for continuous monitoring of branded herbal churna products sold for human consumption.

Key words: Ayurvedic churna, Heavy metals, Trace elements, AAS

INTRODUCTION

World trend is moving towards nature based solutions rather than artificial solutions. As per the studies conducted by organic trade association in USA organic food industry is in the peak of its growth and further the studies conducted by global industrial analysis Inc. proven that Asia-pacific region as a high potential market growth in herbal medicines. Almost one third of the pharmaceuticals segment is captured by herbal medicinal products.

This mainly due to growing concern about the side effect of western drugs, aging population also drives the consumption and emphasis on healthy living benefits. The world health organization (WHO) estimates that 80% of the world population relaying herbal medicines as their primary health care intervention.[1]

Ayurveda is an ancient medicine system originated more than 5,000 years ago,[2] before the father of medicine, Hippocrates was born even. Ayurveda is literally a life science, derived from the Sanskrit term 'Ayus' and 'Veda' meaning life and science together translating to science of life. It can be defined as a system which uses the inherent principles of nature to help maintain health of a person by keeping the body mind and spirit in perfect equilibrium with nature.[3]

Churna is prepared by mixing powdered form of single or mixture of several herbs and spices.[4] Churna is a Sanskrit word meaning powder. Usually churnas are taken with milk, water, honey, curd or butter milk.[1]

One of the major reasons to monitor levels of toxic metals in medicinal plants is that the contamination of the general environment has increased.[4]Herbal medicines made out from contaminated herbal plants can be toxic and produce undesirable side effect to human being. Therefore it is important to maintain good quality medicinal herbs to prevent consumer from being exposed to metal ion contamination by using these herbal products.[5] Contamination of plants by heavy metals (cadmium, copper, nickel, zinc, lead and copper) has increased due to mining industry, smelting, agricultural fertilizers and pesticides, traffic emission, urban runoff, sewage discharge and industrial effluents.[6]

Ashwagandha churna possesses anti- inflammatory, anti-oxidizing, anti -stress, sleep inducing and drug withdrawal qualities[7]as well as it is used to treat various diseases such as anemia, chronic inflammatory diseases, arthritis and diabetes.[8] Thripala churna is made out of three fruits. They are *Emblica officinalis* (Nelli), *Terminalia chebula* (Aralu) and *Terminalia bellirica* (Bulu).[9]The ideal combination of the three herbs is a natural remedy for constipation and also it helps to cure excessive gas and other intestinal problems such as digestive disorders.[10]

Although heavy metals are naturally occurring elements that are found throughout the earth's crust, they cannot be degraded or destroyed.[11]The effect of any metal on a living system is always dependent on the concentration of it available to cells. Therefore there are no any metals

that are always toxic. Some metal ions are essential for metabolism of cells at low concentrations. These are called essential micronutrients.[12]

In general most common method for the determination of concentration of an interest metal element is Atomic Absorption Spectrometer (AAS). It makes use of the absorption of light by these elements in order to measure their concentration.[13]

The aim of the present study is to analyze and quantify the metal ion content and its traceability of two main churna ayurvadic products available in local market. It will be an initiation steps to begun to measure the quality and toxicity of the herbal medicines which will be the major threat for ayurvadic medicines in future.

MATERIALS AND METHODS

Sample Collection

Three different brands of Ashwagandha churna (AC) and Thripala churna (TC) were taken for the analysis, purchased from an ayurvedic pharmacy shops in Colombo area.

Reagents and Chemicals

Nitric acid (69% AR grade) was purchased from Sisco research laboratories and Hydrochloric Acid (35%) was purchased from [Merk] Mubmai, India. Hydrogen peroxide (30%) was purchased from research lab India. Metal standard solutions 1000 ppm was purchased from [Inorganic ventures] Christiansburg,United States. The water used was deionized water.

Digestion of sample

Samples were digested by wet digestion method. Briefly, 10 ml of nitric acid was added to 2g of accurately weighed dried sample in a 100 ml beaker and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for additional 30 min at 95°C.

The last step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 2 ml of deionized water and 3 ml of 30% hydrogen peroxide was added. The beaker was covered with watch glass. The sample was heated gently to start the peroxide reaction. If effervescence becomes excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1 ml increments, followed by gentle heating until the effervescence was subsides. Concentrated hydrochloric acid 5 ml and 10 ml of deionized water were added and the sample was heated for additional 15 min without boiling. The sample was cooled and filtered through a Whatmann No 41 filter paper[4] and diluted to 50ml with deionized water.

Preparation of standard metal solutions

Standard dilutions for each metal were prepared in different concentrations by appropriate dilutions of their respective stock solutions (1000 ppm) to obtain calibration curves. All the measurements were run in triplicate.

Standard solutions of Pb^{2+} were prepared in four different concentrations, 4 ppm, 8 ppm, 12 ppm and 16ppm. Standard solutions of Cd^{2+} were prepared in five different concentrations, 10 ppb, 20 ppb, 30 ppb, 40 ppb, and 50 ppb. Standard solutions of Cu^{2+} were prepared in four different concentrations, 10 ppm, 15 ppm, 20 ppm and 30 ppm. Standard solutions of Ni^{2+} were prepared in four different concentrations, 5 ppm, 10 ppm, 15 ppm and 20 ppm. Standard solutions of Zn^{2+} were prepared in four different concentrations, 1 ppm, 2 ppm, 3ppm and 4 ppm. Standard solutions of Cr^{2+} were prepared in four different concentrations, 10 ppm, 20 ppm, 30 ppm and 40 ppm.

Analysis of metal ions by Atomic Absorption Spectrometer

Digested samples were analyzed for Pb, Cd, Cr, Zn, Cu and Ni using Flame Atomic absorption spectrometer (HITACHI ZA3000 Polarized Zeeman Atomic Absorption Spectrometer).

Table 1: Instrumental conditions for analysis

Parameter	**Pb**	**Cu**	**Ni**	**Cr**	**Zn**	**Cd**
Wavelength	283.3	359.3	232.0	359.3	213.9	228.8
Lamp Current	7.5	7.5	10	7.5	5	5.0
Slit Width	1.3	1.3	0.2	1.3	1.3	1.3
Burner Head	Standard	Standard	Standard	Standard	Standard	Standard
Burner Height	7.5	7.5	7.5	7.5	5.0	7.5
Oxidant Gas Pressure	160	160	160	160	160	160
Fuel Gas Flow Rate (L/min)	2.0	1.8	1.8	2.9	1.8	1.8
Light source	HCL	HCL	HCL	HCL	HCL	HCL
Flame type	A	A	A	A	A	A
R^2	0.9998	0.9965	0.9923	0.9974	0.9968	0.9955

HCL- Hollow Cathode Lamp A- Air/C_2H_2

Results

Table 2: Metal Concentration of Ashwagandha Churna

Brand	Cd (µg/kg)	Pb (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Brand A						
1	BDL	BDL	8.75±1.767	11.75±0.707	9.38±0.530	14.63±1.590
2	BDL	BDL	12.25±1.767	11.63±0.176	9.63±0.530	14.75±0.353
3	BDL	BDL	11.88±0.176	9.88±0.883	9.88±0.176	12.5±2.474
Brand B						
1	0.00±0.000	BDL	10.50±0.354	10.75±1.414	10.75±0.000	30.25±0.707
2	NA	BDL	5.87±3.005	11.13±0.176	9.25±0.176	25.50±3.535
3	NA	BDL	9.00±2.475	11.38±0.530	9.50±0.353	25.37±2.652
Brand C						
1	37.00±21.313	BDL	BDL	10.50±0.000	1.50±0.353	12.25±1.060
2	0.00±0.000	BDL	BDL	10.13±0.176	1.63±0.176	11.37±1.237
3	64.00±21.213	BDL	BDL	10.13±0.176	1.50±0.353	11.87±0.177

Values are expressed as mean± SD, n=2, SD= Standard deviation n= no of readings taken per sample

Table 3: Metal Concentration of Thripala Churna

Brand	Cd (µg/kg)	Pb (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Brand A						
1	99.00±21.213	BDL	BDL	10.63±0.177	1.88±0.177	4.13±0.884
2	127.00±1.414	BDL	BDL	10.75±0.000	2.13±0.177	3.38±1.590
3	157.50±24.748	BDL	BDL	10.75±0.000	2.38±0.177	NA
Brand B						
1	106.00±11.314	BDL	BDL	10.63±0.177	10.75±0.000	28.25±8.485
2	60.50±12.020	BDL	BDL	10.88±0.177	9.25±0.354	20.25±4.949
3	86.00±5.657	BDL	BDL	11.50±0.354	9.50±0.354	17.65±6.894
Brand C						
1	146.00±18.385	BDL	BDL	12.00±0.354	2.50±0.354	15.88±3.005
2	163.00±4.242	BDL	BDL	11.75±0.000	2.63±0.177	10.00±0.707
3	203.00±46.667	BDL	BDL	12.00±0.354	2.63±0.884	NA

Values are expressed as mean± SD, n=2, SD= Standard deviation n= no of readings taken per sample

BDL = Below Detectable Limit NA = Not Applicable

NA- Standard deviation is higher than average

Discussion

According to FAO/WHO the extraneous maximum residue limits of heavy metals in herbal plants have been fixed for cadmium (0.3 mg/kg) and lead (10mg/kg). The regulatory limits of the FAO/WHO have not been established yet for copper, nickel, chromium and zinc in herbal medicine.[14]

Lead was not detected in both AC and TC (**Table 2 and 3**). Lead has no biochemical and physiological importance. It was considered as toxic pollutant.[15] The level of Lead obtained in this study does not indicate a potential health hazard to users. Cadmium was not detected in brand A of AC, but brand B and C of AC showed maximum cadmium levels of 3.4 µg/kg and 86.9 µg/kg respectively (**Table 2**). The maximum cadmium levels reported for TC were 157.4 µg/kg, 105.9 µg/kg and 202.9 µg/kg for brands A, B and C respectively (Ta**ble 3**). All reported values were below the extraneous maximum residue limit permitted for herbal medicines. The maximum Copper levels reported for AC were 11.75 mg/kg, 11.375 mg/kg and 10.5 mg/kg for brand A, B and C respectively (**Table 2**). For TC maximum Copper levels were 10.75 mg/kg, 11.5 mg/kg and 12 mg/kg for brand A, B and C respectively (**Table 3)**. Copper is an essential element for the human metabolic system. It regulates various biological processes inside the body like oxidation-reduction (redox) reactions, energy production, connective tissues formation, iron metabolism, synthesis of neurotransmitter etc.[16] Nickel is considered as highly mobile element within the plant. Accumulation of Nickel takes place only in the leaves. Nickel toxicity in human is not very common occurrence as it absorption by the body is very low.[17]The amount of maximum concentration of Nickel in brand A, B and C of AC were 9.875 mg/kg, 10.75 mg/kg and 1.621mg/kg respectively (**Table 2**). The maximum Nickel levels reported for TC were 2.375 mg/kg, 10.75 mg/kg and 2.625 mg/kg for brand A, B and C respectively (**Table 3**).

The maximum Zinc levels reported for AC were 14.75 mg/kg, 30.25 mg/kg and 12.25 mg/kg for brands A, B and C respectively (**Table 2**). For TC maximum Zinc levels reported for brand A, B and C were 4.125 mg/kg, 28.25mg/kg and 15.875 mg/kg respectively (**Table 3**). Zinc is essential to all organisms and has an important role in metabolism, growth, development and general well-being. It is an essential co- factor for a large number of enzymes in the body.[18]

Chromium was not detected in brand C of AC, but brand A and B of AC showed maximum cadmium levels of 12.25 mg/kg and 10.5 mg/kg respectively (**Table 2**). Chromium was not detected in TC (**Table3**).

Conclusion

The present study show that metal ion contamination of product AC is higher than the metal ion contamination of product TC. These results were concluded that the metal ions content of both products in different brands were within the acceptable value defines by FAO/WHO. The contamination of heavy metals and variations may be due to improper selection, identification,

area of collection, variation in the weight of the drug added to the formulation and processing conditions.

REFERENCES

(1) Krishna, V.; Meyayanathan, S.; Suresh, B. Estimation Of Copper, Zinc and Lead In Choornas By Flame Atomic Absorption Spectroscopy. *Ayurvedic Herb. Med. J.* **2012**, *2* (1), 176–179.

(2) Lad, V. *Textbook of Ayurveda*, 1st ed.; Crowther, G., Peet, S., Eds.; The Ayurvedic Press: Malayasiya, 2002.

(3) Lokhande, R.; Singare, P.; Andhele, M.; Acharya, R. Study of Some Indian Medicinal Plants by Application of INAA and AAS Techniques. *Nat. Sci.* **2010**, *2* (1), 26–32.

(4) Garg, M.; Singh, J. Quantitative AAS Stimation of Heavy Metals and Trace Elements In Marketed Ayurvedic Churna Preperation In India. *Pharm. Sci. J.* **2012**, *3* (05), 1331–1336.

(5) Maobe, M.; Gatebe, E.; Gitu, L.; Rotich, H. Profile of Heavy Metals in Selected Medicinal Plants Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. *Glob. J. Pharmacol.* **2012**, *6* (3), 245–251.

(6) Mahmoud, E. K.; Ghoneim, A. M. Effect of Polluted Water on Soil and Plant Contamination by Heavy Metals in El-Mahla El-Kobra, Egypt. *Solid Earth* **2016**, *7* (2), 703–711.

(7) Archana, R.; Namasivayam, A. Antistressor Effect of Withania Somnifera. *Ethnopharmacol. J.* **1998**, *64* (1), 91–93.

(8) Umadevi, M.; Rajeswari, R.; Rahale, C. S.; Selvavenkadesh, S.; Pushpa, R.; Kumar, K. P. S. Traditional And Medicinal Uses of Withania Somnifera. *Pharma Innov. J.* **2012**, *1* (9), 102–110.

(9) Bais, S. K.; Chandewar, A. V. Comparative Evaluation of Endosulfan Content in Triphala Churna Marketed in Yavatmal District of India by HPLC Method. *Pharm. Pharm. Sci. J.* **2011**, *3* (SUPPL. 3), 35–40.

(10) Maheshwari, R. K.; Chaudhary, K.; Verma, N. Ingenious Triphala : A Curative Preparation for Healthcare. *Chem. Pharm.* **2014**, *2* (2), 175–185.

(11) Duruibe, J. O.; Ogwuegbu, M. O. C.; Egwurugwu, J. N. Heavy Metal Pollution and Human Biotoxic Effects. *Int. J. Phys. Sci.* **2007**, *2* (5), 112–118.

(12) Appenroth, K.-J. Heavy Metals and Their Role in Biological Systems. *Soil Biol.* **2010**, *19* (Im), 1–18.

(13) Hadeishi, T.; McLaughlin, R. D. Hyperfine Zeeman Effect Atomic Absorption Spectrometer for Mercury. *Science (80-.).* **1971**, *174* (4007), 404–407.

(14) WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. In *World Health*; WHO: Switzerland, 2007; p 118.

(15) Lanphear, B. Childhood Lead Poisoning Prevention. *Am. Med. .Journal* **2005**, *89* (7), 1129–1130.

(16) Hamsphire, N. Copper Health Information Summary. *Environ. Serv. J.* **2013**, *603*, 271–1370.

(17) Duda-chodak, A.; Blaszczyk, U. The Impact of Nickel on Human Health. *Elem. J.* **2008**, *13* (4), 685–696.

(18) Temple, V. J.; Masta, A. Zinc in Human Health. *P. N. G. Med. J.* **2014**, *47* (3-4), 146–158.