ELEMENTAL ANALYSIS OF SIDDHA FORMULATION SAMBIRANI POO KULIGAI USING ICP-OES ANALYTICAL TECHNIQUE: RETROSPECTIVE ANALYTICAL APPROACH

M. Vijibala*[1], R. Karolin Daisy Rani[2], V. Velpandian[3] and M. Pitchiah Kumar[4]

[1]Siddha Consultant, Pranav Siddha Clinic, Kancheepuram, Tamil Nadu 603204, India.
[2]Lecturer, Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
[3]HOD, Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
[4]State Drug Licensing Authority (Indian Medicine) Post Graduate Department of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

*Corresponding Author: Dr. M. Vijibala
Siddha Consultant, Pranav Siddha Clinic, Kancheepuram, Tamil Nadu 603204, India.
Email ID: vijibala_m@yahoo.com,

ABSTRACT

As per World Health Organisation's (WHO) data, the percentage of people using medicinal plants is considerably increased to 70–80%. Herbal raw materials (leaves, herbs, rhizomes, roots, oils) can be sources of undesirable toxic components, including heavy metals. Increases in globalization, cultural remedies from siddha, ayurveda, unani and other traditions have become more available to international consumers. According to recent research literatures it was evident that traditional medicines have traces of lead, mercury and arsenic in concentrations 100–10,000 times higher than the allowable limit values. Heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are highly toxic even at much lower levels. Most common toxicity caused due to exposure of such heavy metals includes neurological disorders, kidney dysfunction, liver failure, reproductive disability, fetal abnormalities, cancer, atherosclerosis, reduce cognitive development etc. Hence it's highly essential to establish quality control on raw drugs as well as finished products to ensure the safety and level of heavy metals before exposing the formulation for clinical usage. Present research work aimed at establishing the monograph on elemental composition of the siddha formulation Sambirani Poo Kuligai by using ICP-OES (inductively coupled plasma optic emission spectrometry) analytical technique. Results of the current analytical study have clearly shown that the test drug SPK has no traces of heavy metals such as arsenic, mercury, cadmium, lead and nickel. Whereas the results further confirms the presence of calcium, sodium, potassium and phosphorus at trace level. From the data's of the present analysis it was concluded that the siddha formulation SPK has no toxic heavy metals and long term exposure of the drug will not attribute to any of the cumulative toxicity and considerably safe chronic usage.

KEYWORDS: WHO, Heavy metals, Toxicity, Siddha, Sambirani Poo Kuligai, ICP-OES.

1. INTRODUCTION

Heavy metal toxicity has proven to be a major threat and there are several health risks associated with it. The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes. Few metals, such as aluminium, can be removed through elimination activities, while some metals get accumulated in the body and food chain, exhibiting a chronic nature. Various public health measures have been undertaken to control, prevent and treat metal toxicity occurring at various levels, such as occupational exposure, accidents and environmental factors. Metal toxicity depends upon the absorbed dose, the route of exposure and duration of exposure, i.e. acute or chronic. This can lead to various disorders and can also result in excessive damage due to oxidative stress induced by free radical formation.[1]

Metals are found naturally in the earth's crust and their compositions vary among different localities, resulting in spatial variations of surrounding concentrations. The metal distribution in the atmosphere is monitored by the properties of the given metal and by various environmental factors.[2]

Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment.[3]

Arsenic is one of the most important heavy metals causing disquiet from both ecological and individual health standpoints.[4] It has a semimetallic property, is prominently toxic and carcinogenic, and is extensively available in the form of oxides or sulfides or as a salt of iron, sodium, calcium, copper, *etc*. Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds are lethal to the environment and living creatures. Humans may encounter arsenic by natural means, industrial source, or from unintended sources. Arsenic is a protoplastic poison since it affects primarily the sulphydryl group of cells causing malfunctioning of cell respiration, cell enzymes and mitosis.[5]

In arsenic biotransformation, harmful inorganic arsenic compounds get methylated by bacteria, algae, fungi and humans to give monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). In this biotransformation process, these inorganic arsenic species (iAs) are converted enzymetically to methylated arsenicals which are the end metabolites and the biomarker of chronic arsenic exposure.

On the other hand, heavy metals such as Pb, Hg, Cd, and As are toxic at much lower levels. Lead is known to induce renal tumors, reduce cognitive development, increase blood pressure and cardiovascular diseases in adults. The human brain is most affected by lead intake. Children appear to be especially sensitive to lead, and lead exposure has been correlated to decreased IQ and poor learning in children.[6-8] Organic mercury is more toxic than inorganic form since it is more readily absorbed through ingestion; it is very harmful to fetal and children developments.[9] However, high exposure to organic and inorganic mercury may cause neurological disorders including seizures and even death.[10] Cadmium excessive intake affects mostly the kidney and to a lower extent the reproductive system, while that of arsenic is known to cause cancer,[11] impairment of the reproductive system,[12] and atherosclerosis.[13]

With the rapid development of modern industry, a large number of heavy metals are discharged into the environment along with waste water, waste residue and waste gas from industrial and agricultural activities, resulting in environmental heavy metal contamination.[14] Heavy metals in the natural environment could enter human body via a variety of ways and can accumulate in the body.[15-16] affecting normal organism physiological functions and causing inflammation and a variety of diseases, including cancer.[17] Present research work aimed at establishing the monograph on elemental composition of the siddha formulation Sambirani Poo Kuligai by using ICP-OES (inductively coupled plasma optic emission spectrometry) analytical technique.

2. MATERIALS AND METHODS
2.1. Source of raw drugs
The Required raw materials were procured from a well reputed indigenous drug shop from Parrys corner, Kanda Samy Temple, Chennai, Tamil Nadu, India. All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India. The test drug *Sambirani poo kuligai* was prepared as per Agasthiar Paripuranam 400.

2.2. Ingredients
The siddha formulation *Sambirani poo kuligai* Comprises of the following ingredients

Sambirani [Styrax benzoin] - 250 g
Korosanai [Felbovinum purifactum] - 6 g
Kirambu [Syzygium aromaticum] - 20 g
Vetrilai [Piper betel] Juice - 50 ml

2.3. Purification of Raw Drug
- *Styrax Benzoin*[18]**:** The gums were purified by removing the sand, dust and odd particles.
- *Fel bovinum purifactum*: The unwanted debris substances were removed.
- *Syzygium aromaticum*[19]**:** The flower buds were removed and fried slightly.
- *Piper betle*: The stalk and the middle vein were removed.

2.4. Method of preparing *Sambirani poo kuligai*[20]
The purified *Styrax benzoin* was powdered well and was placed in a small pot. Then a paper was pasted on the inner surface of the big pot. The big pot was placed over the small pot and their mouths oppose each other. The gap between their mouths were covered by a seven layered muddy wet cloth and they allowed to dry. Then it was subjected to sublimation process for 12 hours (*4 samam*).After finishing sublimation process let the pot undisturbed to give away heat. Followed by this the seal were opened and the sublimed product was scrapped and collected.

2.4.1. Kuligai Process
Syzygium aromaticum and *Felbovinum* are powdered well and sieved through a white cloth. Finely powdered *Syzygium aromaticum* powder and *Felbovinum* powder are added along with the sublimate. Then all these substances are grounded well with *Piper betle* leaf juice for 48 minutes [2 *Nazhigai*]. The paste was made into pills in the size of seeds of *Abrus precatorius [Kundri size]* which was equivalent to 130 mg, dried in the shade and packed in bottle.

2.5.ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry) Analysis[21]
Manufacturer: Perkin Elmer.

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer.

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000– 10,000°C). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES.

3. RESULTS
3.1. ICP-OES result analysis of the sample SPK
Analytical results obtained from the ICPOES revealed that the sample SPK has no traces of heavy metals such as arsenic, mercury, cadmium, lead and nickel. Whereas the results further confirms the presence of calcium (2.130 mg/L), sodium (04.120mg/L), potassium (01.821 mg/L) and phosphorus (32.541 mg/L) at trace levels. Presence of calcium, phosphorus and potassium may render therapeutically beneficial effects on metabolism and electrolyte hemostasis on the biological system. The results were tabulated in the table 1.

Table 1: Elemental Analysis of the sample SPK by ICP-OES.

S. No.	Elements	Detected levels
1	Arsenic	BDL
2	Calcium	2.130 mg/L
3	Cadmium	BDL
4	Mercury	BDL
5	Potassium	1.821 mg/L
6	Sodium	4.120mg/L
7	Nickel	BDL
8	Lead	BDL
9	Phosphorus	32.541 mg/L

4. DISCUSSION
Bioaccessibility is defined as the proportion of a pollutant that can be dissolved in the gastrointestinal environment, indicating the relative amount of the pollutant in the matrix that can be absorbed by the body, and higher bioaccessibilities indicate greater potentials for absorbing pollutants. Therefore, the health risk of

heavy metal could be overestimated if the total content of the heavy metal is used to carry out risk assessment instead of the total absorption content. Thus, bioaccessibility may be more accurate in assessing risks of heavy metals present in traditional system of medicine.

The toxicity of trace metals on human health and the environment has attracted considerable attention in recent years. Plants are the main link in the transfer of heavy metals from the contaminated soil to humans. Heavy metals have a tendency to accumulate in the food chain. Heavy metals have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. Metals such as zinc, copper, iron, manganese, and chromium are essential nutrients; they are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic.[22-23] In general, a number of health problems were linked to excessive uptake of dietary heavy metals including a decrease in immunological defenses, cardiac dysfunction, fetal malformation, impaired psychosocial and neurological behavior, gastrointestinal cancer, and many others.[24-25]

Lead is known to be one of the highly toxic environmental pollutants. It can complex with various biomolecules and adversely affect their functions. Lead exposure may have an adverse effect on the blood, nervous, immune, renal, skeletal, muscular, reproductive, and cardiovascular systems causing poor muscle coordination, gastrointestinal symptoms, brain and kidneys damage, hearing and vision impairments, and reproductive defects.[26] Exposures to lead at early childhood and prenatally are associated with slowed cognitive development, learning deficits, and many other effects.[27] It was found from the current study that the sample SPK has no trace of lead. It was observed from the result of the present study that the sample SPK has no traces of lead and arsenic.

The high levels of cadmium possess a serious toxicological effect on human health. Kidney is the critical target organ in the exposed population. Excretion of cadmium is very slow and it accumulates in human kidney for a relatively long time, resulting in an irreversible impairment of the renal tract.[28] It was found from the current study that the sample SPK has no trace of cadmium. Cd induced oxidative damage to transport proteins and mitochondria which may induce apoptosis of tubular cells Cadmium affects the cardiovascular system in several ways. Cadmium has considerable endocrine disruption capacity, apparently deregulating all pituitary hormones. Cadmium exposure is a known risk factor for developing insulin resistance.[29-30]

The manufacturing of medicinal products requires extensive quality control, including the control of all manufacturing phases until the final product. Some

countries have set strict quality control regulations and many others failed.[31] Several regulatory agencies highlighted that some dietary supplements may induce health problems with regard to their quality, effectiveness and safety for human consumption.[32] Poor quality control increases the risk of contamination of these products by bacteria, fungi, heavy metals and metalloids.[33]

Phosphate is a fundamental mineral component of hydroxyapatite, the principal structural element of bone, according to research that dietary phosphate, a marker of the metabolic production of acid, is detrimental to bone.[34] Phosphate present in the sample SP was found to be 1.821 mg/L.

Adequate calcium intake is essential for the maintenance of bone health during growing phases[35] and the preservation of bone mineral density in elderly individuals.[36] Therefore, calcium supplementation is generally recommended to individuals who might be at risk of inadequate dietary calcium intake or osteoporosis regardless of age in order to prevent the deterioration of bone strength. In the preset study the level of calcium in the sample SPK is 2.130 mg/L.

5. CONCLUSION
Traditional medicines have become an essential for public health at a same time the content of heavy metals in each formulation should be monitored for toxic metal levels due to their natural geochemical association to provide patient the safe allowable amounts. Siddha formulation SPK has no traces of heavy metals such as arsenic, mercury, cadmium, lead and nickel. Whereas the results further confirms the presence of calcium, sodium, potassium and phosphorus at trace level. Present of essential element may be required for regulation of metabolism including maintenance of normal hemostasis. Hence it was concluded the sample SPK was found to be safer and possess certain essential components which action may have to be study in detail to justify the mechanism of action of the drug in future.

ACKNOWLEDGEMENT

6. REFERENCES
1. Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett, 2010; 8(3): 199–216.
2. Khlifi R, Hamza-Chaffai A. Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review. Toxicol Appl Pharmacol, 2010; 248: 71–88.
3. Lambert M, Leven BA, Green RM. New methods of cleaning up heavy metal in soils and water; Environmental science and technology briefs for citizens; Manhattan, KS: Kansas State University, 2000.
4. Hughes JP, Polissar L, Van Belle G. Evaluation and synthesis of health effects studies of communities surrounding arsenic producing industries. Int J Epidemiol, 1988; 17: 407–413.
5. Gordon JJ, Quastel GH. Effect of organic arsenicals on enzyme system. Biochem J., 1948; 42: 337–350.
6. Raman P, Patino LC, Nair MG. Evaluation of metal and microbial contamination in botanical supplements. J Agriculture Food Chemist, 2004; 52: 7822–7827.
7. Kauffman JF, Westenberger BJ, Robertson JD, Guthrie J, Jacobs A, Cummins SK. Lead in pharmeucitical products and dietary supplements. Regul Toxicol Pharmacol, 2007; 48: 128–134.
8. Ikem A, Egiebor NO. Assessment of trace elements in canned fishes (Mackerel, Tuna, Salmon, Sardine, and Herrings) marketed in Georgia and Alabama (United States of America) J Food Composition Anal, 2005; 18: 771–787.
9. Obeid PJ, El-Khoury B, Burger J, Aouad S, Younis M, Aoun A, El-Nakat JH. Determination and assessment of total mercury levels in local, frozen, and canned fish in Lebanon. J Environ Sci, 2011; 23(9): 1564–1569.
10. Garcia-Rico L, Leyva-Perez J, Jara-Marini ME. Content and daily intake of copper, zinc, lead, cadmium and mercury from dietary supplements in Mexico. Food Chem Toxicol, 2007; 45: 1599–1605.
11. Kitchin KT, Conolly R. Arsenic-induced carcinogenesis–oxidative stress as a possible mode of action and future research needs for more biologically based risk assessment. Chem Res Toxicol, 2010; 23: 327–335.
12. Rahman A, Persson LA, Nermell B, El Arifeen S, Ekstrom EC, Smith AH, Vahter M. Arsenic exposure and risk of spontaneous abortion, stillbirth, and infant mortality. Epidemiology, 2010; 21: 797–804.
13. Hsieh YC, Lien LM, Chung WT, Hsieh FI, Hsieh PF, Wu MM, Tseng HP, Chiou HY, Chen CJ. Significantly increased risk of carotid atherosclerosis with arsenic exposure and polymorphisms in arsenic metabolism genes. Environ Res, 2011; 111: 804–810.
14. Luo C, Shen ZG. The mechanisms of heavy metal uptake and accumulation in plants. Chin Bull Bot, 2003; 20: 59–66.
15. Kampa M, Castanas E. Human health effects of air pollution. Environ Pollut, 2008; 151: 362–367.
16. Zeng X, Xu X, Boezen HM, Huo X. Children with health impairments by heavy metals in an e-waste recycling area. Chemosphere, 2016; 148: 408–415.
17. Koedrith P, Kim H, Weon JI, Seo YR. Toxicogenomic approaches for understanding molecular mechanisms of heavy metal mutagenicity and carcinogenicity. Int J Hyg Environ Health, 2013; 216: 587.

18. Thiyagarajan.Sarakku Suthi Muraigal, Published by Siddha Maruthuva Nool Veliyitu Pirivu, Indian Medicine and Homoeopathy dept. First edition, 2008; 6-13.

19. Lohar DR. Protocol for testing: Ayurvedic, Siddha and Unani Medicines. Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad.

20. Kandasamypillai, History of Siddha Medicine edition, Thamarai Noolagam, 1998; 1: 917.

21. Montaser A, Golightly DW. Inductively Coupled Plasmas in Analytical Atomic Spectrometry. New York: VCH Publ. A, 1992.

22. Korfali S. I., Hawi T., Mroueh M. Evaluation of heavy metals content in dietary supplements in Lebanon. Chemistry Central Journal, 2013; 7(1, article 10).

23. Korfali S. I., Mroueh M., Al-Zein M., Salem R. Metal concentration in commonly used medicinal herbs and infusion by Lebanese population: health impact. Journal of Food Research, 2013; 2(2): 70–80.

24. Mahan L., Escott-Stump S., Raymond L. Krause's Food and Nutrition Care Process. 13th. St. Louis, Mo, USA: Saunders, 2012. (edited by: Y. Alexopoulos).

25. Singh R., Gautam N., Mishra A., Gupta R. Heavy metals and living systems: an overview. Indian Journal of Pharmacology, 2011; 43(3): 246–253.

26. Johnson F. M. The genetic effects of environmental lead. Mutation Research—Reviews in Mutation Research, 1998; 410(2): 123–140.

27. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Lead (Update)Atlanta, Ga, USA: Public Health Service, U.S. Department of Health and Human Services, 2007.

28. Li S.-M., Fang Y., Ning H.-M., Wu Y. X. Heavy metals in Chinese therapeutic foods and herbs. *Journal of the Chemical Society of Pakistan*, 2012; 34(5): 1091–1095.

29. Satarug S, Moore MR. Emerging roles of cadmium and heme oxygenase in type-2 diabetes and cancer susceptibility. The Tohoku Journal of Experimental Medicine, 2012; 228(4): 267–288.

30. Chen YW, Yang CY, Huang CF, Hung DZ, Leung YM, Liu SH. Heavy metals, islet function and diabetes development. Islets, 2009; 1(3): 169–176.

31. Tumir H, Bošnir J, Dragun Z, Tomić S, Puntarić D, Jurak G. Monitoring metal and metalloid content in dietary supplements on the Croatian market. Food Control, 2010; 21: 885–889.

32. Haidar S, Naithani V, Barthwal J, Kakkar P. Heavy metal content in some therapeutically important medicinal plants. Bull Environ Contam Toxicol, 2004; 72: 119–127.

33. Mazzanti G, Battinelli L, Daniele C, Costantini S, Ciaralli L, Evandri MG. Purity control of some Chinese crude herbal drugs marketed in Italy. Food Chem Toxicol, 2008; 46: 3043–3047.

34. Remer T, Manz F. Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. Am J Clin Nutr, 1994; 59: 1356–1361.

35. Krall EA, Dawson-Hughes B. Heritable and life-style determinants of bone mineral density. J Bone Miner Res, 1993; 8: 1–9.

36. National Institutes of Health (U.S.) Optimal calcium intake. Bethesda: National Institutes of Health, 1994; 1–31.