

**Research Article****HEAVY METAL ANALYSIS OF RAW KASEESA BY INDUCTIVELY COUPLED PLASMA (ICP)****Dilip Jani¹, Jasmine Gujarathi^{2*}, Pravin Ganorkar³, K. Rukmini⁴, Marian Patel⁵, Harsha Jani⁵, Mitesh Patel⁶**

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ABSTRACT

Due to global recognition of Ayurveda formulations, the herbo mineral formulations of Ayurveda are questioned because of heavy metal contents found in many studies. *Kaseesa* (green vitriol) chemically known as ferrous sulphate which is a well known mineral used in various formulations as haematinic and emmenagogue was studied for standardization before making one of its formulation. Raw *Kaseesa* was analyzed for heavy metals using sophisticated method of Inductively Coupled plasma and was found that it contained metals lead and copper outside permissible limit of pharmacopeia norms.

For this study various samples were collected and evaluated by the *Rasashastra* experts and later by Ayurvedic Pharmacopoeial methods. After the confirm identity, the samples were selected for the Heavy Metal Analysis. Inductively Coupled Plasma (ICP) method used for this study and results found in raw *Kaseesa* are Copper 7.2153 ppm, Lead 1288.0 ppm and Mercury, Arsenic, Cadmium were not detected. The authors recommend further study of *Kaseesa* after its *Shodhana* and other processes to study whether such heavy metal (Cadmium, Lead, Arsenic, Mercury and copper) contents are found in the finished product.

KEYWORDS: Raw *Kaseesa*, Inductively Coupled Plasma, Heavy metal analysis, Cadmium, Lead, Arsenic, Mercury and copper.

INTRODUCTION

Kaseesa (Green vitriol), is the antique mineral originated¹ product widely used in various therapeutic administrations in Ayurveda². An Ayurvedic branch denoted as "*Rasashastra*" have the detailed description regarding its therapeutic administrations and processing. It is included under the *Uparasa*³ in Ayurveda texts. It is documented as *Kasaya* (Astringent), *Tikta* (Bitter), *Amla* (Sour) taste. It is also mentioned that it is having *Ushna* (Hot) *Virya* (potency) and have *Katu* (Pungent) *Vipaka* (transformed state after digestion)⁴. The Ayurvedic actions are documented to be *Vata-Kapha har*, *Keshya* (good for hairs), *Netrya* (Good for eyes), *Keshya Ranjana* (hair dye), *Rajah Pravartaka* (Emmenagogue), *Balya* (increases physical strength) and *Rakta Vardhak* (increases hemoglobin). It is indicated for *Kandu* (itching), *Visha Roga* (disease due to poison), *Mutrakricchra* (dysuria), *Ashmari*

(calculus), *Shvitra* (leucoderma/ vitiligo), *Pitta Apasmara* (epilepsy due to *Pitta Dosha*), *Pandu* (Anaemia), *Plihavridhhi* (splenomegaly), *Krimi* (helminthiasis/ worm infestation), *Gudabhransha* (prolapse of rectum), *Visarpa* (erysepales), *Netra Roga* (disease of eyes) and various *Shleshma Roga* (disease due to *Kapha Dosha*)⁵. In Ayurveda, the *Kaseesa* is administered after a proper procedure which is labeled as *Shodhana* (purification)⁶ and *Marana* (incineration)⁷. Both these procedures make the raw material into therapeutically administrable form which is called as *Bhasma*. There are many methods of its purifications and incinerations mentioned in various literatures of *Rasa Shastra*. Ideally, the methods documented in *Rasa Ratna Samuchaya* and *Rasamritam* were followed by most of the industries and researchers. Now a day's the issue of standardization and safety aspect of

Ayurvedic drugs is discussed a lot among the society and scientific background. Hence it was decided to analyze the raw sample for its heavy metal study.

AIMS AND OBJECTIVES

The study was conducted with an aim and objective to identify the heavy metal (Cadmium, Lead, Arsenic, Mercury and copper)⁸ in *raw Kaseesa*

METHODOLOGY

For the experimental study the various samples were collected from the open market. The samples were evaluated by the *Rasashastra* experts and later by Ayurvedic Pharmacopoeial methods⁹. After the confirm identity, the samples were selected for the Heavy Metal Analysis.

Sample Preparation for Heavy Metal Analysis

For Lead, Copper, Arsenic and Cadmium

This procedure is intended for the determination trace metals, the acid digestion was carried out of the sample as mentioned in the Pharmacopoea. The accurately weighed 1 g of the coarse powder of the *Kaseesa* being examined, which was transfer into a casparian flask and added 10-20 ml of the mixture of nitric acid and perchloric acid (4:1). A small hopper was added on the flask-top and macerated the solution overnight. Later it was heated to slake on the electric hot plate and kept for somewhat-boiling. The solution was heated continuously by adding the mixture till the solution becomes clean and transparent. Later the temperature was increased to have thick smoke, till white smoke disperses. The slaked solution becomes colorless and transparent made it cool, transferred it into a 100 ml. The reagent blank solution was also prepared according to the above procedure¹⁰.

For Mercury

Transferred 1 g of the coarse powder of the substance for examination into a casparian flask, added 5-10 ml of the mixture solution of *nitric acid* and perchloric acid (4:1), mixed well and fixed a small hopper on the flask-top, immersed overnight. The slake was heated on the electric hot plate at 120-140° for 4-8 hours until slaking completely, cooled and added a quantity of 4 per cent sulfuric acid solution and 0.5 ml of 5 per cent potassium permanganate solution. Later it was shaken well and added 5 per cent hydroxylamine hydrochloride solution until the violet red colour disappears. It was diluted with 4 per cent sulphuric acid solutions to 25 ml, shaken well and the supernatant is used as the test solution. The reagent blank solution was also prepared according to the above procedure¹⁰.

Operating Conditions of Inductively Coupled Plasma (ICP)

The instrument was optimized with the operating condition as given below. Calibrations were performed using external standards prepared from 1000 ppm single element stock, made-up appropriate with 2% Nitric acid.

- Spectral Profiling: No
- Resolutions: Normal
- Read Time: Auto
- Min Time 2 Sec, Max Time-10 Sec
- Plasma Parameters: Source equilibrium Delay time 15 Sec
- Plasma Gas Flows: Plasma 13 L/min, Auxillary 0.5 L/min, Nebuliser 0.8 L/min
- Power: 1300 W
- Viewing Height: 15 min
- Plasma View : Radial
- Pump Parameters : Pump tubing : Black/ Red
- Sample Flow Rate: 1.5 ml/min
- Sample Flush Time: 20 Sec
- Sample Flush Rate: 2.5ml/min
- Sample Units : mg/kg (ppm) Max Decimal Places : 7 Max Significant Figures: 5

Procedure

The first step in the procedure is conversion of the molecules in the sample to individual atoms and ions using a high temperature radio frequency induced argon plasma. The sample is introduced into the plasma as a solution. Sample is pumped using a peristaltic pump to a nebulizer, where it is converted to a fine spray and mixed with argon in a spray chamber. The purpose of the spray chamber is to make sure that only droplets in a narrow size range make it through into the plasma. Most of the sample drains away from the chamber; the rest is carried into the plasma and instantly excited by the high temperatures (5000-10,000 K). Atoms become ionized with 99% efficiency (arsenic and selenium are a couple of exceptions, ionizing only at 52% and 33%). ICP-OES utilizes UV and visible spectrometry to image the plasma at the exact wavelength of ionic excitation of the element of interest.

Finally the ions reach the detector. In its most sensitive mode (pulse counting) the detector measures individual ions ('Counts'). If the number of ions is too high for the pulse counting (above 2000 ppb, depending on the element) the detector automatically switches to analogue mode, dampening the sensitivity by a factor of 10,000 to protect the detector. Even so, the detector has a finite life (1027 counts).



Figure 1: Raw Kaseesa

Results and observation

The results are expressed in percentage.

Heavy metal analysis of Kaseesa

S. No.	Elements	Kaseesa
1.	Arsenic	Not detected
2.	Cadmium	Not detected
3.	Copper	7.2153
4.	Lead	1288.0
5.	Mercury	Not detected

DISCUSSION

Standardization of Ayurvedic raw materials is one of the major steps before preparing the finished product. The quality of finished product is solely depend on the source of material, its textual characters and the entire process of handling the various process of preparation of finished product. There may a debate on the analytical-methods used for analyzing the raw materials subject to the conceptual administration of a finished product in the human beings. There is a controversy among the scholars about the selection of various analytical tools to screen Ayurvedic materials for heavy metal study. But without entering into the controversy a study was carried out to analyze the raw sample of Kaseesa (green Vitriol) through ICP-OES tool. The results of the study states that the sample has higher level of lead which is crossing the normal limits of heavy metals mentioned in the Ayurvedic Pharmacopoea¹¹. Even though the sample is observed to be standard with traditional parameters, but its analysis through ICP-OES tool, is seems to be not recommendable to proceed for further preparation. The study raises many questions like are these heavy metal observed in the study are really in an elemental form (trace element) which are going to administered in the patient? It is because that, the digestion process before analyzing the sample is itself is a destructive method, i.e. it is not the form of medicine (after digestion) which is really administered in the human beings. So, in such case

the credibility of identifying the higher level of heavy metals through the analysis is questionable especially subject to the administration of the same in human beings. Moreover, the raw material is going through the process called as *Shodhana* (purification) and *Marana* (incineration) as per Ayurvedic literature. This process involves various developments of triturating of raw material with some herbs and later incineration of the same into a specific temperature, converting it into oxidized / sulphated form, etc. Ultimately this oxidized or sulphated material which is called as *Bhasma* is an actual material administered in human being under a specific diseased condition assessed through the level of humors involved, age, sex, etc (*Prakriti & Vikriti Pareekshana*). Ayurveda has also mentioned a specific dietary patterns and daily regimen while consuming the medicines. So collectively, the actual chemical form of finished medicine, time of administration (*Bheshaja Kala*) of medicine, the daily and dietary regimen (*Ahara-Vihara*) recommended for the consumption of medicine, diseased condition while administering the medicines, etc are responsible for good or bad effect of the medicine in humans. Hence the presence of Heavy Metal is a very big issue which is to be discussed among the learned scholars and specific guidelines to be issued.

CONCLUSION

The results of the study proved that even though the sample is observed to be standard with traditional methods but its analysis through an ICP-OES technology is found to be crossing the normal limits mentioned in the Pharmacopoeia. The claim of toxicity of heavy metals analyzed through various technologies like ICP-OES or AAS are under debate among scholars. The cost of analysis tool is also very huge hence it becomes financial unaffordable to conduct the analysis of every sample used in preparation. A thorough study and discussion is highly desired on this issue among the stalwarts of scientific society.

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