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Evaluation of Heavy Metals and Microbial Contamination of Selected Herbal *Churna* Marketed Formulations

Research Article

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Abstract

Herbal formulations are popular in India and across the world due to their medicinal properties. *Churna* is powdered formulations that are consumed widely due to their low cost and ease of administration. *Brahmi Churna*, *Satavari Churna*, and *Jethimadh churna* of 3 different brand manufacturers were procured from the local market. The main objective of the study is the determination of heavy metals and microbiological evaluation as per Ayurvedic Pharmacopoeia. Inductively Coupled Plasma Mass Spectroscopy was performed for the detection of heavy metals. Microbial evaluation was done as per Ayurvedic Pharmacopoeia. The heavy metal content of 7 out of 9 samples complies with the test for heavy metals. Two samples of *Shatavari Churna* were above the permissible limit of Ayurvedic Pharmacopoeia. The microbial study showed all samples within the allowable limits of Ayurvedic Pharmacopoeia. *Brahmi Churna* and *Jethimadh Churna* were found to be safe for human consumption. Quality checks of *Shatavari churna* should be performed to ensure the heavy metals are within the permissible limit.

Key Words: *Churna,* Heavy metals, Microbial contamination.

Introduction

The traditional form of medicine is extensively used for the prevention and treatment of various ailments and chronic diseases (1). In many parts of the world including India, people depend on traditional medicine to fulfil their health care requirements (2). There are a variety of herbal formulations available in the market. *Churnas* are popularly consumed for various health benefits. *Churnas* are powdered formulations having a fine particle size which provides better bioavailability and dissolution (3). Safety and efficacy of Herbal formulations are crucial to confirm the quality of herbal drugs used in the formulation (4). Trace elements like Iron, Copper, Manganese, and Zinc are highly required by the human body for various body functions (5). Heavy metals like Cadmium, Arsenic, Lead, and Mercury are highly toxic for human beings and may cause lethal effects like cancer, hepatoxicity, alopecia, cardiac arrest, myocardial infarction etc. (6, 7). Heavy metals are found to lower renal excretion rates and even in low concentrations, they can cause toxic effects (8). Heavy metals are not easily metabolized by the body and cause accumulation in soft tissues and cause various health issues such as seizures, headache and coma (9, 10). The main source of the presence of metals in the formulation is due to environmental pollution like industrial emission, dung containing cadmium, pesticides, fungicides containing mercury, lead arsenate insecticides (8). The permissible limits of World Health Organization (WHO) depend on the nature of the sample and contaminants. The national limits vary for the individual countries based on raw material or finished formulations. Ayurvedic Pharmacopoeia has provided permissible limits of heavy metals in formulations to ensure the safety and efficacy of herbal formulations (11).*Churna* formulations are easy to be adulterated. Attempts are made to determine the presence of heavy metals in *Churna*. *Churnas* having immunomodulator activity has been popular choice amongst consumers after COVID 19 pandemic. *Satavari Churna, Brahmi Churna, and Jethimadh Churna* reported for good immunomodulator activity. Therefore, we had selected these three *churnas* for determination of heavy metals and microbial evaluation in the present study. *Shatavari* (*Shatamull*) is also known as *Asparagus racemosus* Willd. and is a member of the Liliaceae Family (12). Roots of the plants are dried and powdered to form *Churna*. *Satavari Churna* is beneficial as a reproductive tonic, rejuvenating effects, diuretic, antioxidant, anti-inflammatory, immunity booster, and various medicinal advantages (12, 13). *Brahmi* (Indian pennywort) is also known as *Bacopa monnieri* (Linn.) Pennell and a nootropic plant, member of the family Scrophulariaceae (14). The whole plant is dried and powdered for *Churna*. The benefits of *Brahmi Churna* are Immunity

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booster, Cognitive promoter, Anti-inflammatory, and Antiaging effects (15, 16). It has a noteworthy therapeutic role to cure various neurodegenerative diseases like Alzheimer's disease (17), Parkinson's disease (18), Schizophrenia (19), Epilepsy (20), and dementia (21). *Jethimadh* (*Mulethi*, Liquorice) also known as *Glycyrrhiza glabra* L., member of the Leguminosae family (22, 23). Roots of the plants are dried and powdered to form *Churna*. Benefits of *Jethimadh Churna* are numerous Respiratory and skin diseases, Immunity boosters, Hemorrhagic diseases, rheumatism, jaundice, etc. (24, 25). In the present study, *Brahmi Churna*, *Satavari Churna*, and *Jethimadh Churna* of 3 different brand manufacturers were selected for Heavy metals determination by Inductively Coupled Plasma Mass Spectroscopy and microbial contamination as per Ayurvedic Pharmacopoeia. The objectives of this study is to determine microbial contamination and heavy metal impurities in three marketed samples of *Brahmi Churna*, *Satavari Churna*, and *Jethimadh Churna.*

Materials and Methods

Sample collection

Marketed formulations of 3 different brands of *Churna* were selected for the present study. A total of 9 samples were considered for heavy metal and microbial analysis.

Samples of *Brahmi Churna* (Sample ID- BC1, BC2, BC3), *Shatavari Churna* (Sample ID- SC1, SC2, SC3), and *Jethimadh Churna* (Sample ID- JC1, JC2, JC3) were procured from the popular herbal stores in Ahmedabad city.

Chemicals and reagents

Reagents that were used for the heavy metal analysis were from reliable sources and details are as follows:

Nitric acid Ultrapure grade (Merck), Hydrogen peroxide 30 % (Merck) used for microwave-assisted digestion, Milli – Q Ultrapure Water Type -1 (Merck Millipore), Heavy metal Certified Reference Material's (CRM's) National Institute of Standards & Technology (NIST) Traceable (Sigma Aldrich).

Media

Sterile buffered Sodium chloride peptone solution pH 7, Nutrient agar medium, Soyabean casein digest agar medium, Cetrimide Agar medium, Baird Parker Agar medium, MacConkey agar medium, Xylose-lysine-deoxy-cholate agar medium, Brilliant Green Agar (BGA) and Bismuth Sulphate Agar medium, Tetrathionate broth medium were procured from Hi media.

Instrumentation

A microwave digestor (Anton Paar) was used for microwave-assisted sample digestion of all herbal churna samples. Inductively Coupled Plasma Mass Spectrometry (Agilent 7700x) was used for determining metal concentration in samples. Neuation iswix VT used for mixing of samples.

Autoclave (Sharma Scientific Pvt. Ltd.) was used for sterilization of media; Incubators (Sharma Scientific Pvt. Ltd.) were used to cultivate and preserve microbial cell cultures of herbal *churna* samples. Laminar airflow to provide a clean work area and aseptic condition.

Heavy metal analysis

Microwave-assisted sample digestion:

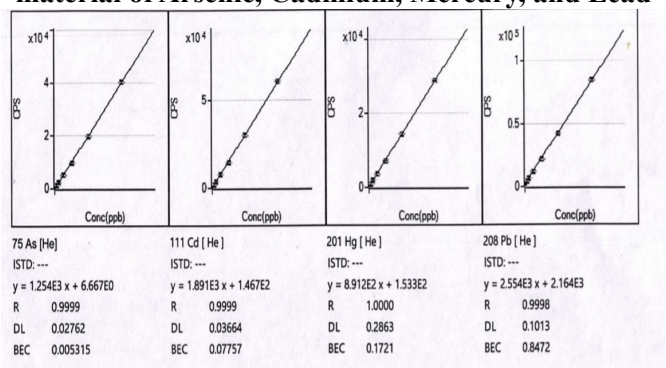
Microwave-assisted sample digestion was performed for all herbal *churnas*. The procedure is described as below:

Powdered sample of 0.50 g is taken in digestion container. Two reagent blanks of nitric acid and hydrogen peroxide without a sample is taken in each digestion series. 4 ml of HNO_3: 1 ml H_2O_2 is added for microwave assisted digestion to each container. The samples were diluted and used in the quantitative determination of Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As) by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Elemental Quantitative Analysis

Prepare 0.5 mg/l of the mixture of working standards from the individual stock solution in 4% nitric acid. Working solution was diluted to prepare calibration standards of concentration 0.001, 0.002, 0.004, 0.008, 0.016, 0.032 mg/ml respectively. Standard curves of certified reference material were shown in Figure 1. The concentration of samples was calculated by calibration curve regression equation in the same manner as parts per million and is shown in Table 1.

Figure 1: Standard curves for Certified reference material of Arsenic, Cadmium, Mercury, and Lead



Microbiological testing

Microbial analysis was carried out as per the standard procedure mentioned in Ayurvedic Pharmacopoeia of India. Microbial tests included for determination are Total plate count, Yeast and Mould count, and presence of pathogens like Escherichia coli, Staphylococcus aureus, Salmonella, and Pseudomonas aeruginosa for all samples.

Media preparations

Media specified were prepared and sterilized in accordance with Ayurvedic pharmacopoeia. These were then inoculated with samples and transferred to the aseptic area.

Media Inoculation

The individual samples of powdered *Brahmi Churna*, *Jethimadh Churna*, and *Satavari Churna* were individually transferred to 50 ml sterile media under a laminar flow cabinet. Serial dilutions of samples were prepared for Total plate count and yeast and mould count. After uniform mixing, the inoculated media were incubated for 1-5 days as per Ayurvedic Pharmacopoeia

Results and Discussion

The results showed 7 out of 9 samples complies with the Ayurvedic Pharmacopoeia limits for heavy metals. The samples of *Brahmi Churna* and *Jethimadh Churna* were found to be safe for human consumption.

Cadmium was found to be below the permissible level in all herbal churna samples which were considered for the study.

Two samples of *Shatavari Churna* were above the permissible limit of Ayurvedic Pharmacopoeia. Lead was above the Ayurvedic Pharmacopoeia permissible limit of 10 ppm in two samples of *Shatavari Churna* which were 12.9 ppm and 84.5 ppm respectively. Arsenic and Mercury were above allowable limits of Ayurvedic Pharmacopoeia in one sample of *Shatavari churna* 3.54 ppm and 2.86 ppm respectively. Quantitation reports of samples SC1 and SC2 are shown in figure 2 and figure 3 respectively.

Table 1: Results of Heavy metals found in Herbal churna of different brands

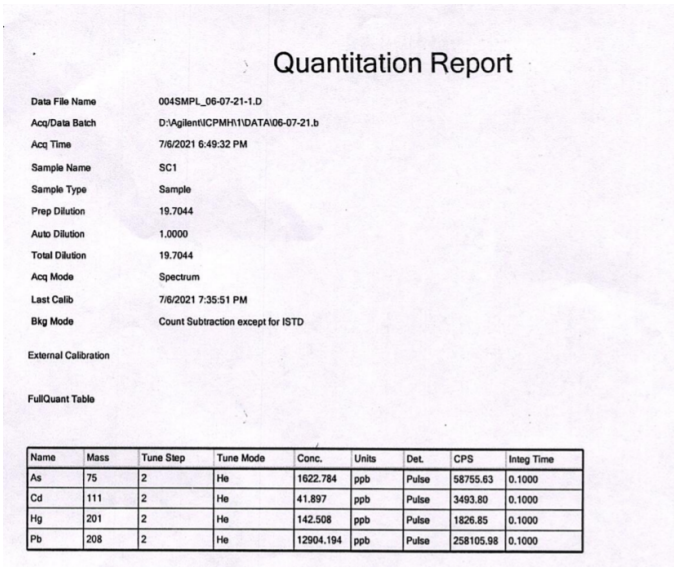
Herbal *Churna*	Sample ID	Heavy metals			
		Lead	Cadmium	Arsenic	Mercury
Brahmi Churna	BC1	2.95	0.21	0.99	0.06
	BC2	0.56	BLQ	0.40	BLQ
	BC3	2.49	BLQ	2.36	BLQ
Shatavari Churna	SC1	12.9	BLQ	1.62	0.14
	SC2	84.5	BLQ	3.54	2.86
	SC3	4.13	BLQ	1.34	0.12
Jethimadh Churna	JC1	4.34	BLQ	0.67	0.52
	JC2	0.70	BLQ	0.68	0.12
	JC3	0.71	BLQ	0.44	0.07

BLQ- Below Quantitation level

Limits as per Ayurvedic Pharmacopoeia for Heavy metals:

Lead: 10 ppm; **Cadmium:** 0.3 ppm; **Arsenic:** 3 ppm; **Mercury:** 1 ppm.

Figure 2: Quantitation report of *Shatavari Churna*- SC1 sample



Quantitation Report

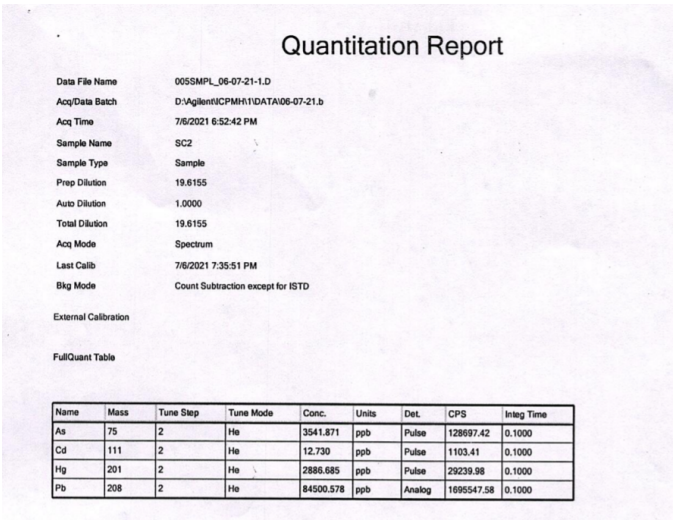
Data File Name	004SMPL_06-07-21-1.D
Acq/Data Batch	D:\Agilent\ICPMH\1\DATA\06-07-21.b
Acq Time	7/6/2021 6:49:32 PM
Sample Name	SC1
Sample Type	Sample
Prep Dilution	19.7044
Auto Dilution	1.0000
Total Dilution	19.7044
Acq Mode	Spectrum
Last Calib	7/6/2021 7:35:51 PM
Bkg Mode	Count Subtraction except for ISTD

External Calibration

FullQuant Table

Name	Mass	Tune Step	Tune Mode	Conc.	Units	Det.	CPS	Integ Time
As	75	2	He	1622.784	ppb	Pulse	58755.63	0.1000
Cd	111	2	He	41.897	ppb	Pulse	3493.80	0.1000
Hg	201	2	He	142.508	ppb	Pulse	1826.85	0.1000
Pb	208	2	He	12904.194	ppb	Pulse	258105.98	0.1000

Figure 3: Quantitation report of *Shatavari Churna*- SC2 sample



Quantitation Report

Data File Name	005SMPL_06-07-21-1.D
Acq/Data Batch	D:\Agilent\ICPMH\1\DATA\06-07-21.b
Acq Time	7/6/2021 6:52:42 PM
Sample Name	SC2
Sample Type	Sample
Prep Dilution	19.6155
Auto Dilution	1.0000
Total Dilution	19.6155
Acq Mode	Spectrum
Last Calib	7/6/2021 7:35:51 PM
Bkg Mode	Count Subtraction except for ISTD

External Calibration

FullQuant Table

Name	Mass	Tune Step	Tune Mode	Conc.	Units	Det.	CPS	Integ Time
As	75	2	He	3541.871	ppb	Pulse	128697.42	0.1000
Cd	111	2	He	12.730	ppb	Pulse	1103.41	0.1000
Hg	201	2	He	2886.685	ppb	Pulse	29239.98	0.1000
Pb	208	2	He	84500.578	ppb	Analog	1695547.58	0.1000

Heavy metals contamination in Herbal *churnas* could be caused by various reasons such as environmental pollution, fertilizers, and soil composition (26, 27). Heavy metals such as lead, arsenic, and cadmium could be linked to water used in irrigation, polluted soils, fertilizers and pesticides, industrial emissions, transportation, and harvesting and storage processes(28). The heavy metals in *Churnas* can be due to adulteration or inappropriate manufacturing practices (29). It may also be due to improper storage conditions across all herbal shops, retail pharmacies, and provisional stores. In polluted water, Arsenic found as one of the major pollutant (30). Suitable measures for assessment of sources of contamination and drug safety standards are to be followed (31). The concerned authorities have to create awareness amongst the manufacturers and distributors for the proper storage practices. Manufacturers should also ensure to perform a quality check for the finished herbal formulations.

Microbiological testing

Total plate count was within the permissible limit given by Ayurvedic Pharmacopoeia in all herbal *churna* samples. Total Yeast and Mould count also has very few colonies, complying with the Ayurvedic Pharmacopoeia standards for all herbal *churna* samples. Pathogens like Staphylococcus aureus, Salmonella, and Pseudomonas aeruginosa, and E.coli were absent in all the Herbal *Churna* samples. Quality checks need to be regularly performed and proper storage conditions should be maintained at all outlets.

Table 2: Results of Microbial Contamination found in Herbal churna of different brands

Herbal *Churna*	Sample ID	Microbial Contamination					
		Total Plate Count cfu/g	Total Yeast and Mould Count cfu/g	Staphylococcus aureus	Salmonella	Pseudomonas aeruginosa	E. Coli
Brahmi Churna	BC1	160	<10	Absent	Absent	Absent	Absent
	BC2	770	<10	Absent	Absent	Absent	Absent
	BC3	750	<10	Absent	Absent	Absent	Absent
Shatavari Churna	SC1	800	<10	Absent	Absent	Absent	Absent
	SC2	1400	<10	Absent	Absent	Absent	Absent
	SC3	20	<10	Absent	Absent	Absent	Absent
Jethimadh Churna	JC1	5800	<10	Absent	Absent	Absent	Absent
	JC2	930	<10	Absent	Absent	Absent	Absent
	JC3	800	<10	Absent	Absent	Absent	Absent
Limits as per Ayurvedic Pharmacopoeia for Microbial Contamination: Total Plate Count cfu/g: 10^5/g**; Total Yeast and Mould Count cfu/g:** 10^3/g**; Staphylococcus aureus:** Absent**; Salmonella:** Absent**; Pseudomonas aeruginosa:** Absent**; E.Coli:** Absent							

Conclusions

The majority of herbal *churnas* (77.78%) were within the acceptable risk. Herbal *churnas* complies as per Ayurvedic Pharmacopoeia standards except for two samples of *Satavari Churna*. *Brahmi Churna* and *Jethimadh Churna* were found to be safe for consumption. Heavy metals variation in *Churna* could be due to contamination due to improper handling and storage practices. The herbal raw material sellers should ensure routine quality checks. The Herbal manufacturers should maintain quality checks and proper seal-proof containers are important for the containment of finished products. Herbal manufacturers should also ensure proper storage conditions. Retail Pharmacies play a vital role in selling and advising consumers about quality, storage, and health risks associated with herbal formulations.

Acknowledgments

Not applicable

Conflicts of Interest

There is no conflict of interest

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