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DETERMINATION OF HEAVY METALS IN FIVE MAJOR INGREDIENTS OF HERBAL MEDICINES

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ABSTRACT: Ayurvedic Medicine originated in India more than 2000 years ago and relied heavily on herbal medicine products. Approximately 80% of India's 1 billion populations use Ayurveda through more than one-half million Ayurvedic practitioners working in 2860 Ayurvedic hospitals and 22100 clinics. Ayurveda's popularity in Western countries has increased. There are several reports of adverse effects of these herbal preparations due to the presence of high level of heavy metals such as Lead, cadmium, arsenic, and mercury and this problem has become a matter of concern. The present study was done to check the presence of lead, cadmium, arsenic, and mercury in five major ingredients Ajwain (*Trachyspermum copticum*), Ginger (*Zingiber officinale*), Neem tree (*Azadirachta Indica* L.), Peppermint (*Mentha piperita*), Turmeric (*Curcuma longa*) are the most essential and regular used materials for preparation of herbal medicines. These ingredients were procured from different location of Delhi, India, and subjected to analysis by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption spectrometer-Vapor Generation Assembly (AAS-VGA). Results were compared with the permissible limits (PL), as set by the World Health Organization (WHO). The percent recoveries in all the case were within the acceptable limits of 70% to 120% as per regulatory guidelines.

INTRODUCTION: Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal tea, herbal oil, *etc*. These preparations are being used worldwide due to their therapeutic potential and as they are considered to be safe as compared to allopathic medicines. Ajwain, essential oil is extracted by steam distillation of the crushed seeds of ajwain. This oil is valued considerably in medicine on account of the presence of thymol, and its germicide and antiseptic properties are utilized in many cough syrups and throat lozenges.

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Ajwain seeds are reputed to be beneficial to asthma sufferers. Ginger is most commonly known for its effectiveness as a digestive aid. By increasing the production of digestive fluids and saliva, ginger helps relieve indigestion, gas pains, diarrhea, and stomach cramping. The known primary constituents of ginger root include gingerols, zingibain, bisabolenel, oleoresins, starch, essential oil (zingiberene, zingiberole, camphene, cineol, borneol), mucilage, and protein. Ginger's therapeutic properties effectively stimulate the circulation of the blood, removing toxins from the body, cleansing the bowels and kidneys, and nourishing the skin.

Neem tree uses to cure skin diseases such as boils, ulcers, eczema, and ringworm. Pastes and extracts from Neem trees have also proven effective in treating various skin fungus conditions, including athlete's foot and lesions in the mouth and vagina.

More serious diseases such as chicken pox and smallpox have been treated with neem tree pastes, and even people suffering from herpes and hepatitis B viruses have obtained relief from neem tree preparations. It also purified the blood, and it useful for treating liver and heart diseases. Peppermint, a popular flavoring for gum, toothpaste, and tea, is also used to soothe an upset stomach or to aid in digestion. Because it has a calming and numbing effect, it has been used to treat headaches, skin irritations, anxiety associated with depression, nausea, diarrhea, menstrual cramps, and flatulence.

Turmeric is a blood purifier and antiseptic. It is loaded with antioxidant, and thus its regular intake protects against free radical damage. It is also beneficial in the treatment of different skin problems like acne, skin rashes, spots, *etc*. It is quite effective for treating of Gallbladder problems, hepatitis, indigestion, infections, lack of appetite, scabies, Alzheimer's disease, arthritis, asthma, athlete's foot, boils, bursitis, breast cancer, colon cancer, cataracts, colic, dermatitis, diarrhea, eczema, fibrosis, gallstones, gas, hardening of the arteries, heart disease, high cholesterol, high triglycerides, inflammation, intestinal pain, irritable bowel syndrome, jaundice, lack of menstruation, lymph gland problems, menstrual pain, morning sickness, pain, psoriasis, sprains, ulcers, wounds, yeast infections. It is also being used for the treatment of bruises, for childbirth, eye inflammation, epilepsy, fever, hemorrhage, hemorrhoids, itching, and ringworm.

Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients [1-4]. Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count, and hepatotoxicity in higher concentration [5]. Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, hemorrhagic injuries, anemia, and cardiovascular disorders [6]. Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases, and neurotoxicity, whereas mercury causes neurological disorders, paralysis, digestive tract inflammation, uremia, acrodynia, and immunotoxicity [7-8]. World health organization (WHO) has emphasized the need for quality assurance of these herbal preparations for heavy metals such as lead, cadmium, arsenic, and mercury [9-10].

The present paper describes the determination of residues of toxic heavy metals mainly lead, cadmium, arsenic and mercury by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption Spectrometer-Vapour Generation Assembly (AAS-VGA) Agilent Technologies, Inc. from five major ingredients like Ajwain (*Trachyspermum copticum*), Ginger (*Zingiber officinale*), Neem tree (*L. Azadirachta Indica*), Peppermint (*Mentha piperita*),Turmeric (*Curcuma longa*), which are used as raw material for preparation of herbal medicines.

MATERIALS AND METHODS:

Chemicals and Reagents: Standards solution 1000 µg/ml of lead, cadmium, arsenic, and mercury (traceable to NIST) were procured from Scharlau Chemie, Spain. Samples for testing Ajwain, Ginger, Neem tree, Peppermint, and Turmeric were purchased from the local market of Delhi (India). Nitric acid, Sulfuric acid, and Hydrochloric acid AR grade were procured from Merck Specialist Chemical limited. All glassware used was "A" grade and calibrated. Calibrated micropipette with range 100µl to 1000µl was used. Whatman filter paper no. 41 was used for filtration.

Instrumentation: Atomic Absorption spectrometer-Vapor Generation Assembly (AAS-VGA 220), Agilent Technologies, Inc. equipped with Graphite Thermal Analyzer and Agilent Technologies, Inc. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) equipped with argon saturation assembly, CCD detector and 21 CFR 11 version 4.1.0 software for data acquisition and processing was used

Preparation of Calibration Standard solution:

Preparation of Calibration Standard Solution of Lead and Cadmium: 10 ml of standard reference solution of lead (1000µg/ml) and 1 ml of standard reference solution of cadmium (1000µg/ml) was pipetted into a 100 ml volumetric flask and diluted to volume with HPLC grade water. This gave a solution with a concentration of 100 µg/ml for lead (solution A) and 10 µg/ml for cadmium (solution B). This both solutions were then used as a stock solution for preparation of calibration standard

solution. Calibration standards for lead were prepared by adding appropriate volumes of standard working solution A with HPLC grade water at 5 levels in the range between 100 µg/l, 200 µg/l, 400 µg/l, 800 µg/l and 1000 µg/l. Similarly, calibration standards for cadmium were prepared by adding appropriate volumes of standard working solution B with HPLC grade water at 5 levels in the range between 10 µg/l, 20 µg/l, 40 µg/l, 80 µg/l and 100 µg/l respectively.

Preparation of Calibration Standard Solution of Arsenic and Mercury: 1 ml each of standard reference solution of arsenic and mercury (1000µg/ml) was pipetted into a 100 ml volumetric flask and diluted to volume with HPLC grade water. This gave a solution with a concentration of 10 µg/ml for arsenic and mercury (solution C). This solution was then used as a stock solution for the preparation of calibration standard solution. Calibration standards for arsenic and mercury were prepared by adding appropriate volumes of standard working solution C with HPLC grade water at 3 levels in the range between 5 µg/l, 10 µg/l and 20 µg/l respectively.

Sample Preparation: About 5.0 ±0.01 g (for lead, cadmium and arsenic) and 1.0 ±0.005 g (for mercury) dried homogenized powder samples of each ajwain, ginger, neem tree, peppermint, turmeric were weighed accurately in an iodine flask separately, 10 ml of concentrated HNO_3 was added into each flask (for mercury 0.5 ml of H_2SO_4 was added with this). The iodine flasks were refluxed for 1 h at 95 °C ± 5 °C. The sample solutions were cooled, and 5 ml of concentrated HNO_3 was added into each flask. The flasks were again refluxed for about 1 h at 95 °C ± 5 °C. Repeated the process until the digestion was completed. Evaporated the solution to 5 ml. Solutions were cooled, and 10 ml of concentrated HCl was added into each flask.

Kept the solutions for refluxed for about 15 min to remove the nitrous fumes. Cooled the digested sample solutions, 20 ml of HPLC grade water was added into each flask and filtered the digested solution through Whatman filter paper no. 41 into 50 ml volumetric flask and made up to the volume using HPLC grade water. Recovery study was carried out by fortifying known concentration of standard into the pre-analyzed sample.

Instrument conditions:

ICP-OES Conditions: Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with radial torch equipped with argon saturation assembly was used for the determination of lead and cadmium. High purity (99.99%) argon was used as plasma, auxiliary, and nebulizer gas. The gas flows were kept at 15.0 l/min for plasma, 1.50 l/min for auxiliary, and 0.56 l/min for nebulizer. Radio frequency (R.F) power of the plasma generator was 1.35 kW. The vertical height of the plasma was fixed at 7 mm.

Sample uptake time of 30.0 sec, the delay time of 5 sec, rinse time of 10 sec, initial stabilization time of 10 sec and time between replicate analyses of 5 sec was maintained throughout the studies for ICP-OES. All the observation of emission were recorded at 220.353 nm and 226.502 nm, which corresponds to the most sensitive emission wavelength of lead and cadmium, respectively. The instrument was calibrated for various parameters before the studies.

AAS-VGA Conditions: Atomic Absorption spectrometer-Vapor Generation Assembly (AAS-VGA), equipped with Graphite Thermal Analyzer, was used for determination of arsenic and mercury. High purity acetylene and zero air were used. For vapor generation, 6% Sodium Borohydride solution (w/v) and 50% Hydrochloride solution (v/v) were used for arsenic, and 25% Stannous Chloride ($SnCl_2$) was used for mercury. The gas flows were kept at 13.50 l/min for zero air and 2.10 l/min for acetylene. The lamp current 10.0 mA and spectral bandwidth of 0.5 nm was used for both the metals. All the observation were recorded at 193.7 nm and 253.7 nm, which corresponds to the most sensitive wavelength of arsenic and cadmium. The EHT volts were recorded 68 and 59 for arsenic and mercury, respectively.

RESULT AND DISCUSSION: Results obtained for Pb, Cd, As and Hg by the methods ICP-OES and AAS-GTA are tabulated in **Table 1**. The lead was present in all samples of Ajwain, Ginger, Neem tree, Peppermint, and Turmeric from all the sources. The concentration of lead in the samples were obtained in the range of 0.2 mg/kg to 1.3 mg/kg; the levels were within the maximum residual limits prescribed by the WHO, *i.e.* 10.0

mg/kg. All samples showed the presence of cadmium in the range of 0.02 mg/kg to 0.046, which were also in the range of maximum residue limits as per WHO, *i.e.* 0.3 mg/kg. Though, the different samples showed the presence of As and Hg, the levels are within the maximum residual limits, *i.e.* 1.0 mg/kg and 10.0 mg/kg, respectively. The concentration of heavy metals shown is higher in Turmeric as compared to other samples like Ajwain, Ginger, Neem tree, and Peppermint.

TABLE 1: RESULTS FOR THE PRESENCE OF Pb, Cd, As and Hg (mg/kg) IN SAMPLES OF AJWAIN, GINGER, NEEM TREE, PEPPERMINT AND TURMERIC FROM ALL THE SOURCES

Samples	Pb (ICP-OES)	Cd (ICP-OES)	As (AAS-VGA)	Hg (AAS-VGA)
Ajwain				
Source 1	0.2042	0.0325	0.0125	0.0444
Source 2	0.2663	0.0251	0.0152	0.0314
Source 3	0.2215	0.0452	0.0186	0.0519
Ginger				
Source 1	0.3420	0.0458	0.0294	0.0764
Source 2	0.3818	0.0389	0.0349	0.0846
Source 3	0.3301	0.0372	0.0421	0.0751
Neem tree				
Source 1	0.2415	0.0302	0.0051	0.0186
Source 2	0.1156	0.0315	0.0127	0.0192
Source 3	0.1527	0.0389	0.0103	0.0210
Peppermint				
Source 1	0.2172	0.0275	0.0187	0.0509
Source 2	0.2731	0.0213	0.0251	0.0659
Source 3	0.2459	0.0325	0.0283	0.0662
Turmeric				
Source 1	0.6485	0.0357	0.0348	0.2736
Source 2	1.2854	0.0412	0.0510	0.1192
Source 3	0.8495	0.0316	0.0459	0.5846
As per WHO guidelines	10.0	0.3	10.0	1.0

The calibration curve prepared using the pure standards of Pb, Cd, As and Hg were found to be linear with correlation coefficient (r) of more than 0.990. The recovery of Pb, Cd, As and Hg in spiked samples was calculated to study the effect of matrix on the determination of Pb, Cd, As and Hg. The recovery studies were carried out at three different concentrations, and results are given in **Table 2**.

TABLE 2: PERCENTAGE RECOVERIES (µg/kg) (AMOUNT RECOVERED) FROM THE SPIKED SAMPLES

Sample	Spike level	Lead		Cadmium		Arsenic		Mercury	
		% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Ajwain	1	85.1	4.2	84.7	4.8	80.4	7.2	80.7	4.8
	2	97.2	3.2	91.7	2.5	91.5	5.4	88.7	3.2
	3	98.0	2.5	94.2	2.1	96.2	5.2	92.5	3.0
Ginger	1	85.5	4.9	86.7	5.1	88.5	4.6	76.5	4.9
	2	94.9	5.4	91.2	4.9	94.5	4.3	85.4	4.4
	3	96.8	2.4	95.8	3.3	97.2	3.3	92.8	3.2
Neem tree	1	90.7	4.5	90.5	7.3	86.4	4.3	88.5	3.8
	2	97.4	3.1	96.8	4.4	92.5	3.8	97.2	3.9
	3	97.1	3.3	96.9	4.2	94.8	3.6	98.0	2.3
Peppermint	1	89.2	4.5	88.4	7.5	90.2	3.5	92.5	3.8
	2	91.9	3.6	92.9	6.0	96.5	2.8	95.8	3.4
	3	95.8	2.8	94.7	5.8	97.9	2.5	97.6	2.2
Turmeric	1	88.7	4.4	80.6	5.8	80.2	4.5	87.6	3.5
	2	92.5	3.2	92.9	3.9	92.6	3.9	92.8	3.2
	3	95.3	3.5	95.3	3.1	95.5	3.5	96.7	2.8

*Spike levels: 1) 100.0, 10.0, 5.0, 5.0 for Pb, Cd, As and Hg; 2) 200.0, 20.0, 10.0, 10.0 for Pb, Cd, As and Hg; 3) 400.0, 40.0, 20.0, 20.0 for Pb, Cd, As and Hg

The recoveries of Pd and Cd in samples are ranged between 85% to 98% and 80% to 97% respectively for ICP-OES method and the recoveries of As and Hg in samples is ranged between 78% to 98% and 76% to 98% respectively. The percent recoveries in all the case were within the acceptable limits of

70% to 120% as per regulatory guidelines. The contamination of heavy metals in herbal medicinal plants could be either due to intake by the roots or due to the surface deposit from the environmental pollution.

CONCLUSION: The studies have indicated that the presence of various toxic metals in the Ajwain, Ginger, Neem tree, Peppermint and Turmeric from the different location of Delhi market, has been well within the allowed limit. The use of ICP-OES and AAS-VGA to determine such toxic metals up to trace levels (ppb or sub-ppb levels) and thus indicate compliance to the regulations of permissible maximum residual limits as per the various regulatory authorities.

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CONFLICT OF INTEREST: Nil

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