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Evaluation of Heavy Metals in Two Major Ingredients of Ampucare

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ABSTRACT

The World Health Organization (WHO) estimates that 4 billion people or 80 percent of the world population, presently use herbal medicine for some aspect of primary health care and 25% of modern medicines are made from plants first used traditionally. 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, chromium, nickel, arsenic and mercury and this problem has become a matter of concern. The present study was done to check the presence of lead, cadmium, chromium, nickel, arsenic and mercury in two major ingredients (*Azadirachta indica* and *Curcuma longa*) of a polyherbal product- Ampucare. These ingredients were procured from different cities of India and subjected to analysis by Atomic Absorption Spectrophotometer (AAS). Results were compared with the permissible limits (PL), acceptable daily intake (ADI) and provisional maximum tolerable daily intake (PMTDI) as set by World Health Organization (WHO), Food and Drug Administration (FDA) and Joint FAO/WHO expert committee on food additives (JECFA). All the metals were within these limits. Mean levels were evaluated with respect to their place of procurement. Delhi samples had highest mean metal contamination followed by Lucknow, Chandigarh and Chennai samples. Lowest metal contamination was found in Srinagar samples. These results were also in correlation with the safe limits as well as pollution prevailing in these cities. Overall study confirmed the safety of Ampucare.

Keywords: Ampucare, *Azadirachta indica, Curcuma longa,* cadmium, lead.

INTRODUCTION

Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal teas, 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.

Lead, cadmium, chromium, nickel, arsenic and mercury 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. [1-6]

Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration. [7-8] Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, haemorrhagic injuries, anemia and cardiovascular disorders. [9-11]

Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity [12-14] whereas mercury causes neurological disorders, paralysis, digestive-tract inflammation, uremia, acrodynia and immunotoxicity. [15] Nickel has been reported to cause contact dermatitis, nasal, sinus and lung cancers, kidney disorders, chronic bronchitis, acute respiratory distress syndrome and pulmonary fibrosis. [16]

Chromium is known to cause nephrotoxicity, nasal and lung ulcers, skin ulcers, hypersensitivity reactions and "chrome holes" of the skin. [17-18]

Presence of these heavy metals in various herbal ingredients and products have been reported by several researchers such as in some therapeutically important medicinal plants [2]; herbal teas [3], herbal ingredients [4]; ginseng products [1]; rhizome at *Radix Notopterygii* [19]; Nigerian herbal remedies [5]; African potato- *Hypoxis hemerocallidea* [20]; natural drugs. [21] These reports throw light on the fact that medicinal plants which are considered harmless and are used as starting material for any herbal product may also contain toxic metals.

World health organization (WHO) has laid emphasis on the need of quality assurance of these herbal preparations for heavy metals such as lead, cadmium, chromium, nickel, arsenic and mercury.

In the present study, attempts have been made to study the presence of lead, cadmium, chromium, nickel, arsenic and

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mercury in two major ingredients (*Azadirachta indica* and *Curcuma longa*) of a polyherbal product- Ampucare.

MATERIAL AND METHODS

Sampling site description

Two major ingredients of Ampucare, *Azadirachta indica* and *curcuma longa*, were procured from different sources and were subjected to analysis by atomic absorption spectrophotometer (AAS). Table 1 gives detailed information on medicinal properties, therapeutic uses and place of procurement of these ingredients.

The samples were air-dried and powdered prior to digestion and analysis. All the glasswares were of Borosil "A" grade. Deionised water was used throughout the study. All the chemicals used were of analytical grade (AR). Mixed working standard (1 and 10 µg/ml) solutions were freshly prepared by diluting the stock solutions of 1000 µg/ml (Merck India).

Estimation of Cd, Pb, Cr and Ni

One gram of powdered sample was weighed accurately on electronic balance (Shimadzu LIBROR AEX 200G) The samples were put in a 100 ml digestion flask and 5 ml of mixture was added to it and heated on a hot plate in the fuming chamber. A digestion mixture comprising of concentrated HNO_3 and perchloric acid in the ratio of 6:1 was used for wet digestion of the samples. Blanks and spiked samples were also processed and analyzed simultaneously. The flasks were firstly heated slowly and then vigorously till a white residue is obtained. The residue was dissolved and made up to 10 ml with 0.1 N HNO_3 in a volumetric flask. The samples were then analyzed on Atomic Absorption Spectrophotometry (AAS). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. [24]

Estimation of As and Hg

Cold digestion for volatile heavy metals was followed and the method was developed and standardized in the laboratory. Weighed powdered sample (0.1 g) was digested in Erlenmeyer flask (100 ml) and the flask was left overnight after adding 10 ml of conc. Sulphuric acid. It was then incubated at 70°C in a water bath for one hour. The flask was then placed in an ice bath with constant shaking saturated aqueous potassium permanganate solution was added slowly. The process was continued till the colour of the permanganate persisted. After the flask reached room temperature, one ml of hydroxylamine hydrochloride 20 % w/v in distilled water) was added to reduce excess potassium permanganate. This solution was made to desired volume by deionized water and used for estimation of As and Hg.

RESULTS AND DISCUSSION

Azadirachta indica A. juss. Syn. *Melia azadirachta* Linn. is a scared tree of India and is one of the major ingredients of Ampucare. It is bitter tonic, antimicrobial, antifungal, astringent, antiperiodic; useful in wounds and skin infections and was procured from five different cities of India (Table 1). Cd was found to be highest in Delhi sample (0.30±0.04 mg/kg) and lowest in Srinagar sample (0.17±0.03 mg/kg). It was below the permissible limit of 0.3 mg/kg as prescribed by WHO [25] in all the samples. Pb ranged from 0.57±0.04 mg/kg (Srinagar sample) to 1.20±0.28 mg/kg (Delhi sample) and was far below the permissible limit of 10 mg/kg as prescribed by WHO [25] in all the samples. Ni ranged from 0.15±0.02 mg/kg (Srinagar sample) to 0.96±0.11 mg/kg (Delhi sample). Cr level was found to be highest in Delhi samples (0.84±0.10 mg/kg). It was found to be 10.5 folds higher than that of Chennai samples (0.08±0.01 mg/kg) whereas it was not detected in Srinagar samples. As was detected only in three samples and ranged from 0.20±0.03 mg/kg (Chandigarh samples) to 0.47±0.04 mg/kg (Delhi samples). It was below the permissible limit of 0.5 ppm as set by FDA. [26] Hg was also detected only in three samples and ranged from 0.31±0.04 mg/kg (Chandigarh samples) to 0.75±0.05 mg/kg (Lucknow samples). Hg in all the samples was found to be below the permissible limit of 1.0 ppm as prescribed by FDA. [27] The mean value of Cd, Pb, Ni, Cr, As and Hg in *Azadirachta indica* samples were found to be 0.24, 0.81, 0.64, 0.34, 0.19 and 0.33 mg/kg respectively. Acceptable Daily Intake (ADI) for these metakls is 0.21 mg/day for Pb, 0.015-0.10 mg/dayfor Pb, 0.40 mg/day for Ni and 0.20 mg/day for Cr. [25, 28-30] Similarly Provisional Maximum Tolerable Daily Intake (PMTDI) for Cd, Pb, Cr and Ni is 0.05, 0.18, 0.05 and 0.005 mg/kg body weight. [25, 28-30] Metal content in all these samples was found to be within these limits.

Curcuma longa Linn. Is also a reputed medicinal herb of India which has been in use since time immemorial by the traditional healers of India as an aromatic, tonic, astringent, stimulant, carminative, antiseptic, antifungal and antibacterial medicine. It is useful in skin infections, burns, inflammations, eczema, ringworm and bleeding ulcers (Table 1).

Cd ranged from 0.15±0.04 mg/kg (Srinagar samples) to 0.28±0.03 mg/kg (Delhi samples). It was found to be below the permissible limit of 0.3 mg/kg as prescribed by WHO [25] in all the samples. Pb was found to be highest in Lucknow samples (0.91±0.13 mg/kg) which was found to be 5.4 times higher than that of Srinagar samples in which lowest level of Pb was recorded (0.17±0.04 mg/kg). Pb in all the samples was far below the permissible limit of 10 mg/kg as prescribed by WHO. [25] Ni was found to be highest in Delhi samples (0.98±0.16 mg/kg) which were 9.8 folds higher than that of Srinagar samples (0.10±0.02 mg/kg) in which lowest level of Ni was recorded. Level of Cr was found to be highest in Delhi samples (0.67±0.08 mg/kg) and lowest in Chandigarh samples (0.23±0.03 mg/kg) almost 2.9 folds higher than that of Chandigarh samples. As was detected in four whereas Hg was detected in three samples. Both metals were found to be within permissible limits of 0.5 ppm [26] and 1.0 ppm [27] respectively.

The mean value of Cd, Pb, Ni, Cr, As and Hg in *Curcuma longa* samples were found to be 0.19, 0.70, 0.63, 0.36, 0.21 and 0.36 mg/kg respectively which was found to be below the Permissible Limits (PL), Acceptable Daily Intake (ADI)and Provisional Maximum Tolerable Daily Intake (PMTDI) [25, 28-30] all these samples.

Mean levels of these metals were also evaluated with respect to their place of procurement. Highest level of metal contamination was found in Delhi samples followed by Lucknow, Chandigarh and Chennai samples whereas lowest level of heavy metal contamination was recorded in Srinagar samples (Fig. 3). This contamination may be due to air, water and soil pollution prevailing in these cities. According to CPCB [31], vehicular pollution load of Delhi is 1046.30 tonnes/day and it is among the top cities of India. According to an another report [32], New Delhi topped the list of cities

Table 1 : Major herbal ingredients of Ampucare

Botanical name	Common name and family	Part used	Place of procurement	Chemical constituents	Medicinal properties and uses [22-23]
Azadirachta indica A. juss. Syn. *Melia azadirachta* Linn.	Margosa tree (Meliaceae)	Bark	Lucknow, Chandigarh, Delhi, Srinagar, Chennai	Nimbin, nimbidin, nimbinin and gallotannins	Bitter; tonic, antimicrobial, antifungal, astringent, ant tiperiodic; useful in wounds and skin infections
Curcuma longa Linn.	Turmeric (Zingiberaceae)	Rhizome	Lucknow, Chandigarh, Delhi, Srinagar, Chennai	Essential oil (turmerone and zingiberone), curcumin etc.	Aromatic, tonic, astringent, stimulant, carminative, antiseptic, antifungal, antibacterial; useful in skin infections, burns, inflammations, eczema, ringworm and bleeding ulcers

Table 2 : Level of heavy metals in *Azadirachta indica* and *Curcuma longa* procured from different cities of India

Ingredients	Place of procurement	Cd	Pb	Ni	Cr	As	Hg
Azadirachta indica A. juss. Syn. *Melia azadirachta* Linn.	Lucknow	0.28±0.03	0.85±0.25	0.80±0.10	0.40±0.05	0.31±0.04	0.75±0.05
	Chandigarh	0.23±0.03	0.65±0.08	0.83±0.15	0.36±0.05	0.20±0.03	0.31±0.04
	Delhi	0.30±0.04	1.20±0.28	0.96±0.11	0.84±0.10	0.47±0.04	0.60±0.05
	Srinagar	0.17±0.03	0.57±0.04	0.15±0.02	BDL	BDL	BDL
	Chennai	0.22±0.04	0.80±0.09	0.48±0.06	0.08±0.01	BDL	BDL
Curcuma longa Linn.	Lucknow	0.20±0.03	0.91±0.13	0.87±0.14	0.33±0.04	0.27±0.03	0.67±0.07
	Chandigarh	0.19±0.05	0.30±0.05	0.75±0.15	0.23±0.03	0.20±0.03	BDL
	Delhi	0.28±0.03	1.34±0.18	0.98±0.16	0.67±0.08	0.41±0.05	0.88±0.12
	Srinagar	0.15±0.04	0.17±0.04	0.10±0.02	0.30±0.04	BDL	BDL
	Chennai	0.16±0.02	0.78±0.10	0.45±0.05	0.25±0.03	0.18±0.02	0.24±0.03

Values are Mean±S.D. of 3 determinations in each case.

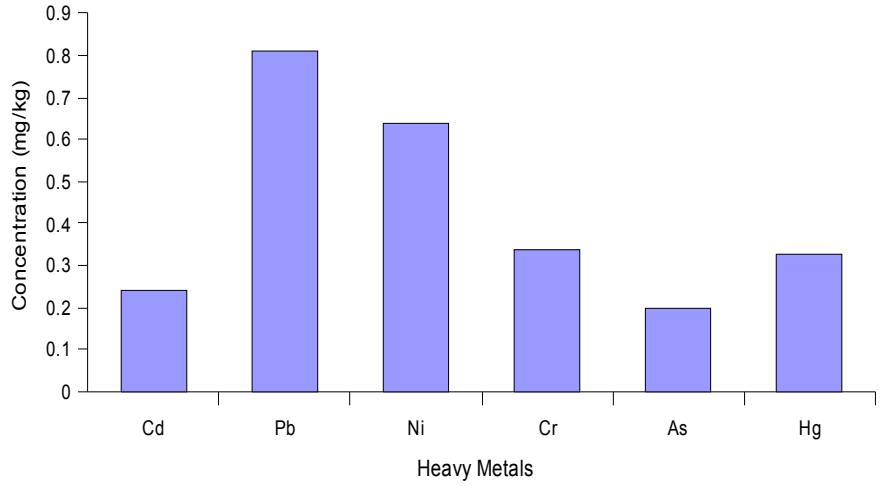

Fig. 1: Comparative mean levels of cadmium, lead, nickel, chromium, arsenic and mercury in *Azadirachta indica* procured from different sources

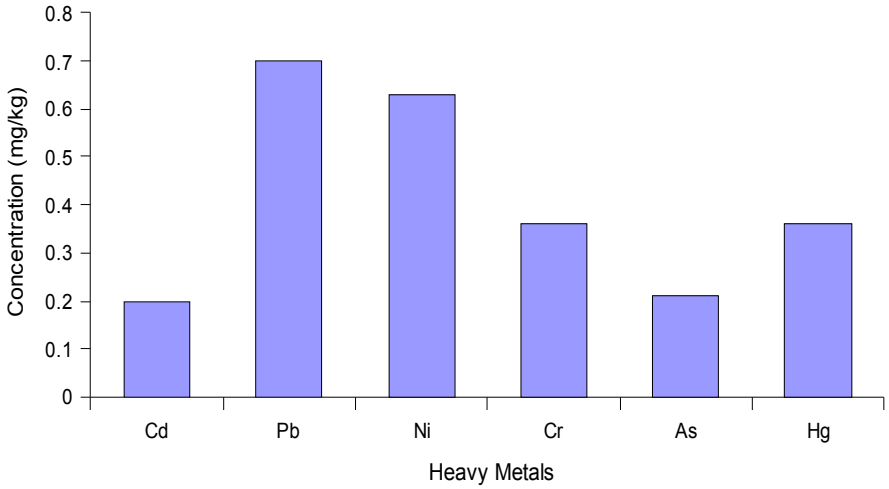

Fig. 2: Comparative mean levels of cadmium, lead, nickel, chromium, arsenic and mercury in *Curcuma longa* procured from different sources

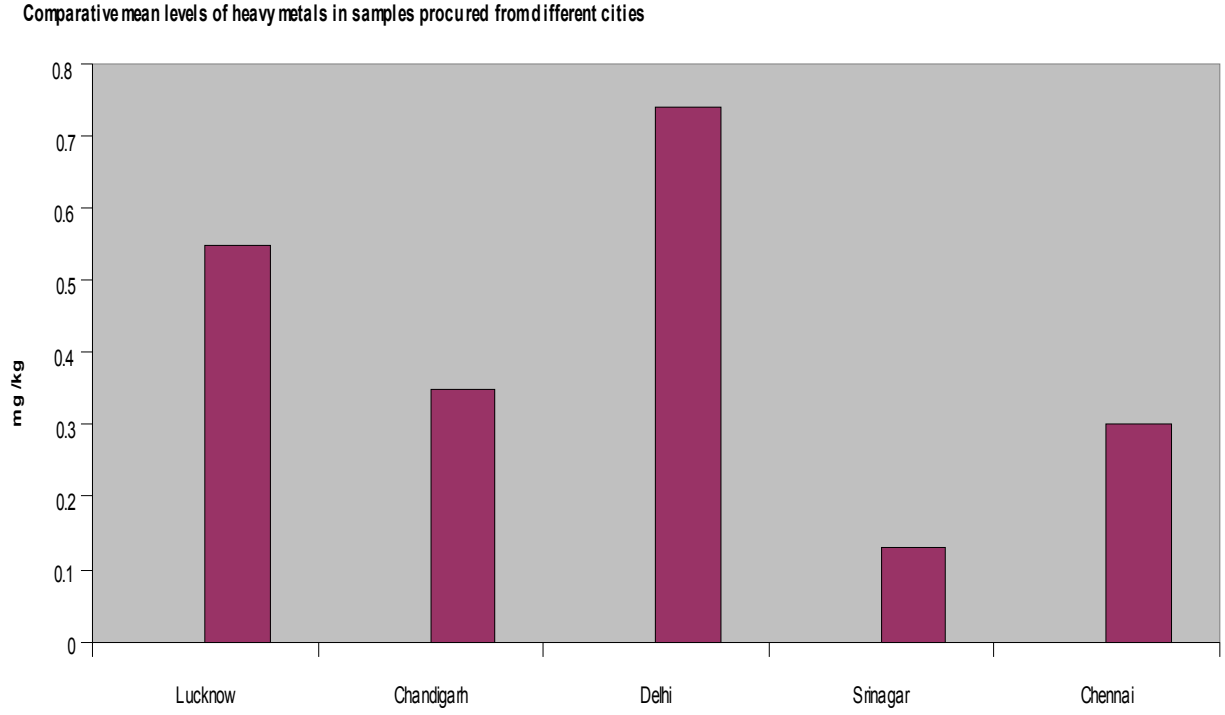

Fig. 3: Comparative heavy metal contamination in samples from different cities

with unacceptably high levels of suspended particulate matter (SPM) between 350 and 800 µg of suspended particulates per m^3 which is more than that of limit prescribed by the World Health Organization i.e., less than 50 µg. In our study, highest contamination of metal was seen in Delhi samples which are in correlation with above mentioned reports. According to a survey conducted by Industrial Toxicology Research Centre, Lucknow, concentrations of PAHs, especially the levels of suspended particulate matter (SPM) present in air and respiratory suspended particulate matter (RSPM) were both very high. PAH in the city was 11 times the permissible limit for residential areas and four times as high for industrial areas. [33] Heavy metal contamination in Lucknow samples was also found to be next to Delhi samples as compared to other cities.

Water and air, pollution in Chennai is also increasing. [34] A case study was carried out to assess Respirable dust particles (RDP) concentration present in the ambient air in Chennai city during the year 2000-2002. A high value of 607.1 microgram/m^3 was recorded during October 2001 and 90 % of the observed values exceeded the recommended values of National Ambient Air Quality Standards (NAAQS). [35] Some of the Chennai samples showed higher metal contamination as compared to the samples from other cities. Lowest metal contamination was seen in samples from Srinagar which is a hilly area and is comparatively less polluted.

Overall study confirms the safety of two major ingredients of Ampucare - *Azadirachta indica* and *Curcuma longa* as metal content of all the samples were within the limits prescribed by WHO, JECFA and FDA.

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