IJPSR (2021), Volume 12, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 15 September 2020; received in revised form, 29 May 2021; accepted, 22 June 2021; published 01 September 2021

DETERMINATION OF INORGANIC TOXICANTS IN NUTRACEUTICALS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY

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Keywords:

Moringa, Heavy metals, Microwave digestion and Inductively coupled plasma-mass spectroscopy

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ABSTRACT: *Moringa oleifera* and chywanprash are two main nutraceuticals used worldwide because of their large medicinal application. The quality of these nutraceutical products is in question, especially due to the inorganic contaminants like heavy metals. In the present study, six Moringa herbal powder and five chywanprash samples were collected in India and further analyzed for As, Cd, Hg, and Pb. All samples were microwave digested and analyzed by ICP-MS. In Moringa As, Cd, Hg and Pb were found in the range of 0.027-5.264 µg/mL, 0.014-0.039 µg/mL, 0.049-45.225 µg/mL and 0.323-12.487 µg/mL respectively. In chywanprash As, Cd, Hg and Pb were found in the range of 0.012-0.021 µg/mL, 0.004-0.029 µg/mL, 0.01-0.103 µg/mL and 0.06-0.171 µg/mL respectively. The concentration of these heavy metals were found to be more than the maximum permissible limit given by the WHO for herbal medicines. Hence strong regulatory guideline needs to be prepared to improve quality of nutraceuticals.

INTRODUCTION: Food is a vital element of life and is required to nourish and improve physical growth and combat against diseases in the human life cycle. Micro and macro elements are requiring for the nourishment of health. The rate of development of the Indian economy has increased in the last few years because the Indian community experiences improvement in their daily lifestyle. Health issues like malnutrition and obesity are caused by the lack of awareness of the harmful effect of fast-food eating habits. Up to 50% of the burden of disease is attributable to food and nutrition [1, 2]. Hence, that leads the Indian community to face daily lifestyle disorders like diabetes, obesity, heart attack, stork, *etc.*

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(9).4832-42

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(9).4832-42

According to the world health organization, in 2016, 39% of women and 39% of male above 18 years aged were overweighed [3]. Recent study demonstrated that India secured the third position after the United States of America and China for the highest number of obese people in the country [4] pharmaceuticals and nutraceuticals treat these disorders.

Pharmaceuticals are "chemical substances used in the treatment" which involve the use of drugs that are chemically prepared, whereas nutraceuticals make use of "food and part of food that provides medical and health benefits hence help in the disease treatment" [5]. Long-time consumption of pharmaceuticals that make use of a chemically synthesized drug shows some side effects; hence, people generally prefer nutraceuticals over pharmaceuticals. These disorders required lifetime intervention of nutraceuticals in their daily routine; therefore, there is an increase in the nutraceutical market. In India, the Food Safety Standard Authority of India regulates the use and manu-

facturing of health supplements and nutraceuticals through the food and safety standard act 2016. The Drug marketing and manufacturing association estimates that the Indian nutraceuticals market is likely to grow by 16% compounded annual growth rate over the next five years. India's nutraceuticals market is expected to double in size to US$ 4 billion by 2020 on account of the strong demand for dietary supplements from the upper and middle class [6]. According to a 2018 Assocham Knowledge report, the Indian nutraceuticals market is expected to grow from $4 billion in 2017 to $18 billion in 2025 [7].

The concept of nutraceutical is gaining more popularity more recently, and their roots can be traced to the ancient Indian system of medicine, 'Ayurveda'. Such preparations have been referred to as 'Aajasrik Rasayana' [8, 9]. Ayurveda is the traditional medicine of India that has been practiced since ancient times *i.e.* having a track record of 20 years according to the estimates made by European scholars [10, 11]. The World Health Organization (WHO) estimates that about 5.6 billion people, representing 70-80% of the world's population, depend on medicinal plants as part of the repertoire of their primary health care needs [12, 13]. The concept of nutraceutical was started from the survey in U.K., Germany, and France [14].

Stephen DeFelice coined the term nutraceutical in 1989, the founder and chairman of the foundation for innovation in medicine, Cranford, which is an American organization that encourages medical health that defines Nutraceutical as any substance that may be considered a food or part of food providing medical or health benefits including the prevention and treatment of disease [9, 15, 16]. Such medicinal products are made up of non-toxic food and their parts. A nutraceutical product is defined as a substance with physiological benefits or protection against chronic diseases. Nutraceuticals are used to improve health, delay the aging process, prevent chronic diseases, increase life expectancy, or support the structure or function of the body above and beyond their basic nutritional function [8, 17, 18]. They include herbal medicines (Ayurvedic, Chinese, Tibetan, African, Amazonian, Herbalism), naturopathy, vitamin and mineral therapy, and homeopathy [15]. Nutraceuticals include products such as dietary supplements (products enhanced with vitamins, protein and mineral); supplements made from herbal, algal and plant extracts, Chyawanprash, energy and sports drinks, fortified foods and juices, *etc.* [19] chyavanprash, Arjuna ksheerpaka, phalaghrita, shatavari Gharita and Rasona Ksheerpaka are few commonly used Ayurvedic nutraceutical used for the treatment of different illness such as in the treatment of respiratory disorders, cardioprotection, improvement in reproduction health, *etc.* [8]

Because of medicinal applicability of plant, it has been entrenched that the plants which naturally synthesize some secondary metabolites, like tannins, volatile oils, alkaloids, and containing minerals and vitamins, used as a traditional medicine for the treatment of various diseases and illness such as skin diseases which includes eczema, acne vitiligo, hives, respiratory distress, ear and dental infections, hypertension, diabetes, anaemia, and cancer *etc.* [20] Medicinal plants are mostly used for therapeutic or medicinal benefits [21]. Every consumer expects that whatever product they are consuming, it should be of good quality without and health hazard contaminants.

According to the FDA, "it is the manufacturers responsibility to mention the possible contaminant present in the product like dietary supplements and manufacturers are prohibited to sale the products which contain unsafe ingredient or contaminants". This will be helpful for the regulatory authority to form new guidelines [22, 23]. consumers are very careful while selecting specific nutraceuticals in their diet especially plant products. Contaminated products may show some adverse effects, and these effects are classified into two groups, *i.e.*, intrinsic and extrinsic effects. The intrinsic effect is due to the overdose and pharmacological interaction of drug with the body, whereas extrinsic effects are due to the presence of contaminants, adulteration, lack of standardization, *etc.* [15] contaminants like heavy metals introduce in the product while manufacturing process through weighing, dispensing and processing operation [19].

Heavy metal is a naturally occurring substance which is distributed throughout the land in different concentration [22]. Essential metal and toxic metal are two main categories of metals. Essential metals such as iron, copper, and zinc are required for

different body functions. The presences of these essential elements in plants possess some therapeutic properties and is used in Phytotherapy. Toxic metals consist of lead, mercury, cadmium, and arsenic even if taken in acute quantity [17]. heavy metals introduce in the soil because of urbanization and industrialization processes. If any medicinal plant grows in this area, then there is a chance of entrapment of these heavy metals in the plants, and the same will reflect in the final product.

Hence it is important for every country to monitor such products and establish the standard regulatory guideline for the identification of heavy metals, and it is also important to establish the maximum permissible limit for each possible heavy metal. Heavy metal limit for Canada's natural health product is 0.14 µg/Kg in arsenic and it salts, cadmium- 0.09 µg/kg, lead- 0.29 µg/kg and mercury- 0.29 µg/kg [22]. According to the world health organization, the maximum permissible limit for heavy metals in the herbal formulation is given as, for arsenic- 3 µg/mL, lead- 10 µg/mL, cadmium- 0.3 µg/mL, and mercury- 3 µg/mL [24]. According to Association of Southeast Asian Nations (ASEAN) the limits of contaminants in traditional medicines and herbal supplement is lead- 10mg/kg, arsenic 5 mg/kg, mercury 0.5 mg/kg, and cadmium 0.3 mg/kg [25].

Heavy metals are considered carcinogenic in nature, and these causes various diseases, such as cardiovascular diseases, *e.g.*, Coronary artery diseases such as angina and myocardial infection, bone diseases such as osteoporosis, rickets, and Paget disease, kidney diseases such as acute kidney injury, kidney cysts, and kidney stones, gastrointestinal diseases such as constipation, haemorrhoid, and perianal infection, reduced general intellectual capacity, and cancer. Serious health problems can occur because of excessive accumulation of heavy metals when consumer eats contaminated herbal plants. Due to the toxicity of heavy metals in herbal plants, permissible limits of various heavy metals have been set for its regulation [6].

Heavy metal is currently one of the important pollution problems and increasingly extensive in our daily life, which should never be neglected. The possible cations present in the environment is Copper (Cu), Manganese (Mn), Zinc (Zn), Chromium (Cr), Cadmium (Cd) and Lead (Pb), whereas anionic metals are arsenic (As), boron (B), molybdenum (Mo), selenium (Se) (USDA, 2000). Arsenic is used as a semiconductor, animal feed additive, herbicides and wood. Cadmium is used as colour pigment and a rechargeable battery. Lead is used as a building material, paint and antiknock in petrol, and Mercury is used as a whitening cosmetic, syphilis treatment and filling teeth [26]. World Health Organisation regulated maximum permissible limits of heavy metals like arsenic, cadmium, and lead to 0.1, 0.3, 5.0 mg/kg, respectively [24].

In present study chywanprash and Moring oleifera powder selected to determine the presence of heavy metals in it. Both are common nutraceutical used because of their different therapeutic properties. *Moringa oleifera* belonging to family Moringaceae, it is one of the important medicinal plants. It is also commonly known as Sahjana, Sainjna in Hindi, Sajina in Bengali, ben oil tree, miracle tree, and mothers' best friend, 'Drumstick' and horseradish tree in English. It is mostly found in sub-Himalayan tracts of Northern India diffuse in tropical and subtropical areas at altitudes up to 20000 m. it is a fast-growing tree also found in Bangladesh, Sri Lanka, East and West Africa, and Pakistan [27-29].

Tyrosine, valine, methionine, isoleucine, histidine, leucine, cysteine, tryptophan, alanine, threonine and phenylalanine are some amino acids commonly found in Moringa. Fatty acids such as linolenic acid, heneicosanoic acid, linolenic acid, palmitic acid, and capric acid are some amino acids present in moringa, and several minerals such as zinc sulphur, sodium, iron, selenium, copper, potassium, calcium, phosphorus and magnesium. The leaves also reported consisting of carotene, condensed tannins, vitamin A, vitamin B vitamin A, and polyphenols [30].

Different parts of the plant such as leaves, roots, fruits, flowers, bark can be used for the treatment of different medical conditions such as it has antimicrobial activities against bacteria, yeast, dermatophytes, and helminthes [31] and it has been used in the treatment of asthma, and it also possesses antidiabetic, anti-anaphylactic, antibiotic,

antithyroid antiulcer, cholesterol-lowering, anti-HSV, antihypertensive, hepatoprotective antitumor activity [32-34]. It is reported that leaves of the moringa plant consist of antiatherosclerosis, antioxidant and hypoglycaemic activities [12]. The seed extract of the moringa consists of anti-inflammatory, diuretic, and antispasmodic activities [30]. It is used by ancient Egyptians in the preparation of cosmetic and skincare products. The undetectable level of heavy metals in the Moringa seed oil might be due to the growing conditions of Moringa [35].

At present, India's nascent market is incorporating traditional herbal ingredients into the nutraceutical segment, such as Chywanprash ($74.5 million in 2010) [1]. Chywanprash household remedy has been traditionally used in Ayurveda under the category of rasayanaand it is popularly used because of its nutritional value.The word chywanprash composed of two words, "chywana" and "prasha" former stands for the name of a sage means "degenerative change. Later word denotes a drug or diet, which is fit for ingestion. Sweet and tangy taste with fine aroma [16, 36]. It helps to strengthen the liver, improve the digestive system, relieves nausea, vomiting, help to correct hyperacidity, dyspepsia and flatulence, peptic ulcers, and gastritis, boost memory power, promote cardio fitness by supporting your heart, and helps in slow down the rate of normal aging and promote longevity.

It also cleanse your blood and aid in the elimination of toxins, rejuvenate and promote a healthy-looking complexion, support healthy bones and teeth through calcium absorption, help tone your muscles through protein synthesis, provide relief from occasional menstrual discomfort and boost your immune system to improve vigor, vitality, wisdom, and glow.

It has the hepatoprotective effect, and it reduces postprandial glycemia and blood cholesterol levels. It is made up of around 50 herbs, including Amla *i.e.,* Indian gooseberry as a key ingredient which is a rich source of vitamin C with sugar syrup, sesame oil, and clarified butter [8, 37, 38]. The end product has a fruit-like consistency, which is sweet, sour, and slightly spicy in nature [39, 9]. *Crepidium acuminatum* (D. Don) and *Crepidium acuminatum* (D. Don) are some common plants used in the preparation of chywanprash [40, 41].

Quality assessment study related to nutraceuticals associated with qualitative and quantitative assessment of an active ingredient and toxic components such as heavy metal detection hence quality control of nutraceuticals.

It is important to trace and eliminate these heavy metals from human consumption. Asia, Europe and the US are a few countries wherein toxicity due to heavy metal is reported.

Renal damage, gastrointestinal disorder, anaemia, irreversible brain damage, kidney damage, heart problem, chronic health problem, and teratogenic effects on the foetus are few examples of toxicity effects reported due to long-term exposure to lead and cadmium [42, 30, 43]. For that purpose, different Instrumental techniques are used to detect heavy metals and commonly used ones are Flame (GAAS), Inductively coupled plasma- Atomic emission spectroscopy and Inductively coupled plasma- mass spectrometry.

In ICP-MS, ICP is used to ionized the sample, whereas MS separate ion formed according to their mass/charge ratio [30]. The present study showcases the presence of heavy metals in nutraceuticals like chywanprash and *Moring oleifera* in India.

MATERIALS AND METHODS: Four Moringa herbal medicines were collected from online shopping websites, and two Moringa leaves samples were collected from Gujarat and Maharashtra each within March-April 2019. These are labelled as S-1 to S-6, and five chywanprash were collected from different glossary shops of Gujarat and labelled as C-1 to C-5 within the period of March-April 2019.

Supra-grade hydrochloric acid and nitric acid were used for digestion. Anton Paar- Thermo scientific microwave digester, Thermo scientific iCAP-RQ-ICP-MS was used for analysis.

Herbal medicines dried at 80 ºC and homogenized with mortar pestle and stored in zip lock bags. 0.1 gm of powder sample was accurately weighed and microwave digested with 3 mL of nitric acid and 1 mL of hydrochloric acid with conditions given in table no. 1 and further analyzed with ICP-MS with conditions given in **Table 2**.

TABLE 1: STEPS FOR MICROWAVE DIGESTION

S. No	Step	Temperature °C	Time (min)	Fan Level
1	Temperature ramp	100	10	1
2	Temperature hold		5	1
3	Temperature ramp	185	10	1
4	Temperature hold		30	1
5	cooling	65	20	3

TABLE 2: ICP-MS ANALYSIS PARAMETERS

Sr. No	Parameter	Value
1	No. of Replicates	3
2	Delay Time	60 Seconds
3	Sample Flush Time	60 Seconds
4	Nebulizer	PFA Micro Flow Nebulizer
5	Spray Chamber	Cyclonic Quartz
6	RF Power	1550 W
7	Plasma Gas Flow	14 L/m
8	Auxiliary Gas Flow	0.8 L/min
9	Flush Pump Rate	100 rpm
10	Analysis Pump Rate	40 rpm
11	Nebulizer Flow	1.183 L/min
12	Helium Flow (KED)	4.585 mL/min

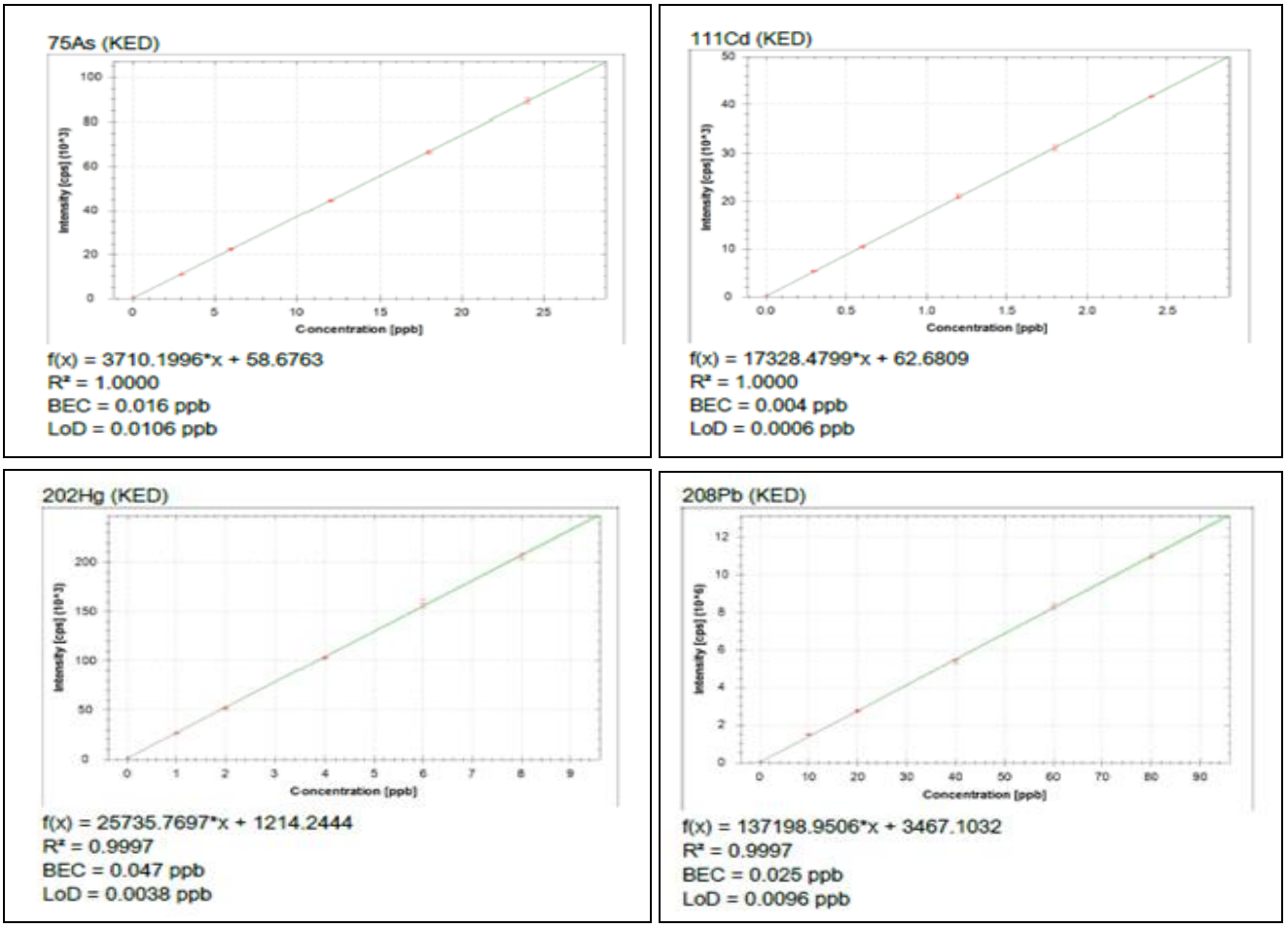

FIG. 1- CALIBRATION GRAPHS FOR ARSENIC, CADMIUM, MERCURY AND LEAD

RESULTS AND DISCUSSION:

Method Validation: The developed ICP-MS method was validated with their performance characteristics like specificity, accuracy, precision, linearity, selectivity, and sensitivity. Specificity is assessed by the ability of ICP-MS to detect the standard elements of Arsenic, Cadmium, lead and mercury and identify same in the sample matrix.

Accuracy of developed method assessed by the % recovery for arsenic, cadmium, mercury, and lead standard and it was found in the range of 90-100%, 100-101%, 99-100%, and 99-101% respectively. After analysis of every three samples, standard solution (3) was analyzed to check the accuracy of the results by the recovery of % recovery of concentrations of standard solution (3) and % recovery for the same found in the range of 95-100%. A recovery study was conducted by spiking standard solution (3) in each sample of moringa and chywanprash and further analyzed by ICP-MS, and recovery percentage was obtained. In ICP-MS, percentage recovery should be greater than 70% to check the accuracy of obtained results. The percentage recovery of As, Cd, Hg, and Pd obtained in sample S-1 was 80.890%, 83.329%, -187.179%, and 90.678%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample S-2 was 81.779%, 89.644%, 87.965%, and 101.494%, respectively.

The percentage recovery of As, Cd, Hg and Pd obtained in sample S-3 was 84.098%, 102.345%, 93.016% and 105.767%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample S-4 was 80.304%, 89.614%, 89.634%, and 101.702%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample S-5 was 82.309%, 90.922%, 89.535% and 100.286%, respectively. The percentage recovery of As, Cd, Hg, and Pd obtained in sample S-6 was 86.004%, 92.195%, 90.843% and 102.838%, respectively. Similarly, the Percentage recovery of As, Cd, Hg and Pd obtained in sample C-1 was 81.522%, 107.042%, 83.414%, and 101.478%, respectively.

The percentage recovery of As, Cd, Hg, and Pd obtained in sample C-2 was 79.132%, 102.525%, 84.116% and 103.777%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample C-3 was 75.897%, 101.313%, 80.236%, and 98.472%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample C-4 was 77.574%, 103.768%, 83.220% and 102.734%, respectively. The percentage recovery of As, Cd, Hg and Pd obtained in sample C-5 was 79.365%, 106.440%, 84.054% and 103.407%, respectively. Precision was assessed by the concertation relative standard deviation (RSD). Concentration RSD (Relative Standard deviation) obtained for standard solution (1) mixture of As, Cd, Hg and Pd were 0.7%, 0.5%, 1% and 1%, respectively. Concentration RSD obtained for standard solution (2) mixture of As, Cd, Hg, and Pd were 2%, 1.5%, 1.3% and 2%, respectively. Concentration RSD obtained for standard solution (3) mixture of As, Cd, Hg and Pd were 9 %, 1.7%, 1.3% and 2.1%, respectively. Concentration RSD obtained for standard solution (4) mixture of As, Cd, Hg, and Pd were 8%, 1.8%, 3% and 1.9%, respectively. Concentration RSD obtained for standard solution (5) mixture of As, Cd, Hg and Pd were 1.5%, 1 %, 1.7% and 1.1%, respectively.

Linearity assessed by external calibration of heavy metals with regression coefficient obtained for As, Cd, Hg, and Pd were 1, 1, 0.997, and 0.9997, respectively. Sensitivity is determined by the limit of detection (LOD) and background equivalent concentration (BEC). Limit of detection (LOD) obtained for As, Cd, Hg, and Pd was 0.0106 µg/L, 0.006 µg/L, 0.0038 µg/L, and 0.0096 µg/L respectively and background equivalent concentration (BEC) obtained for As, Cd, Hg, and Pd were 0.016 µg/L, 0.004 µg/L, 0.047 µg/L, and 0.025 µg/L respectively.

Sample Analysis: As per the World Health Organization, 80% of the total population relies on herbal medicine for the treatment of any disease and illness. *Moringa oleifera* is taken as a nutritional supplement. Heavy metal analysis in Mor*inga oleifera* plant leaves was studied by Limmatvapirat (2013, 2014, 2015), Gupta *et al.*, (2014), Gidado *et al.*, (2016), Khan *et al.* (2017), and Valdez-solana *et al.* (2015). Different Authors selected different parts of plants and different plant forms for the analysis. Limmatvapiratt (2013) had analyzed heavy metals in dried seeds, tea leaves, Moringa tea, leaf powders, and functional drinks. Gupta *et al.* (2014) had analyzed heavy metals in the Stem bark of *Moringa oleifera*. Gidado *et al.* (2016) had analyzed heavy metals in Bark, leaves, and roots of *Moringa oleifera*. Most of the studies found to be conducted on natural plants, whereas few studies are conducted on herbal medicines such as Limmatvapirat (2015) conducted a study on analysis of heavy metals in Moringa leaf capsules collected in the market. For heavy metal analysis it is important to first digest the sample with acids,

and this can be done by three different methods such as wet digestion, dry digestion, and microwave digestion. Nitric acid, hydrochloric acid, sulphuric acid, and perchloric acid are some commonly used acids for digestion [21]. Wet digestion is a process in which samples are mixed with single acid and a mixture of acid and further heated on the water bath for 3-4 h depending upon the sample matrix. In the dry digestion method sample to be analyzed first heated in Muffle furnace at high temperature of 500-1000 ºC and further digested with acids on hot water bath. Limmatvapirat *et al.*, (2015) and Gupta *et.al.* (2014) had used the wet digestion method in which they had digested samples in Nitric acid and perchloric acid. Gidado *et al.*, (2016) had used dry digestion in which Author had first heated the sample in Muffle furnace at 400-450 ºC and further digested it with Nitric acid. In contrast, Valdez & Ahmed Ali (2015) used a Microwave digestion system to extract heavy metals from the sample matrix. Microwave digestion systems have some advantages over routine wet and dry digestion systems. Microwave digestion systems require a small amount of extracting solvent, a time-consuming process, reduces exposure to hazardous acids, reduces environmental contamination due to acid fumes, and reduces the rate of sample contamination.

After digestion next step is to analyse the digested sample with suitable instrumental method and for that instruments like Atomic Absorption Spectroscopy (AAS) Annan, Dickson, Amponsah, & Nooni (2013) [44], Flame Atomic Absorption spectroscopy by Kaba & Goroya., (2019) [45], Inductively coupled Plasma-Mass Spectroscopy (ICP-MS) Agboola, Orji, Olatunji &Olowoyo (2016) [15], Inductively coupled plasma- Atomic emission spectroscopy (ICP-AES) and Neutron Activation Analysis (NAA) can be used. Gupta *et. al.* (2014), Gidado *et al.* (2016) and Khan *et al.* (2017) had used Atomic absorption spectroscopy for heavy metal analysis, whereas Limmatvapirat (2015) and Valdez- Solana *et al.* (2015) had used ICP-MS for the analysis of heavy metals. NAA is a quite expensive technique as compared to ICP-MS, ICP-AES, and AAS, whereas AAS required a separate excitation source for each element that increases the analysis time. The advantage of ICP-MS over ICP-AES is the extremely low detection limit.

TABLE 3: CONCENTRATION HEAVY METALS IN MORINGA HERBAL MEDICINE IN µG/ML

Sample	S-1	S-2	S-3	S-4	S-5	S-6
As	5.264	0.555	0.027	0.073	0.085	0.124
Cd	0.024	0.025	0.014	0.016	0.053	0.039
Hg	45.225	0.141	0.062	0.049	0.073	0.078
Pb	12.487	0.941	0.662	0.323	1.197	1.76

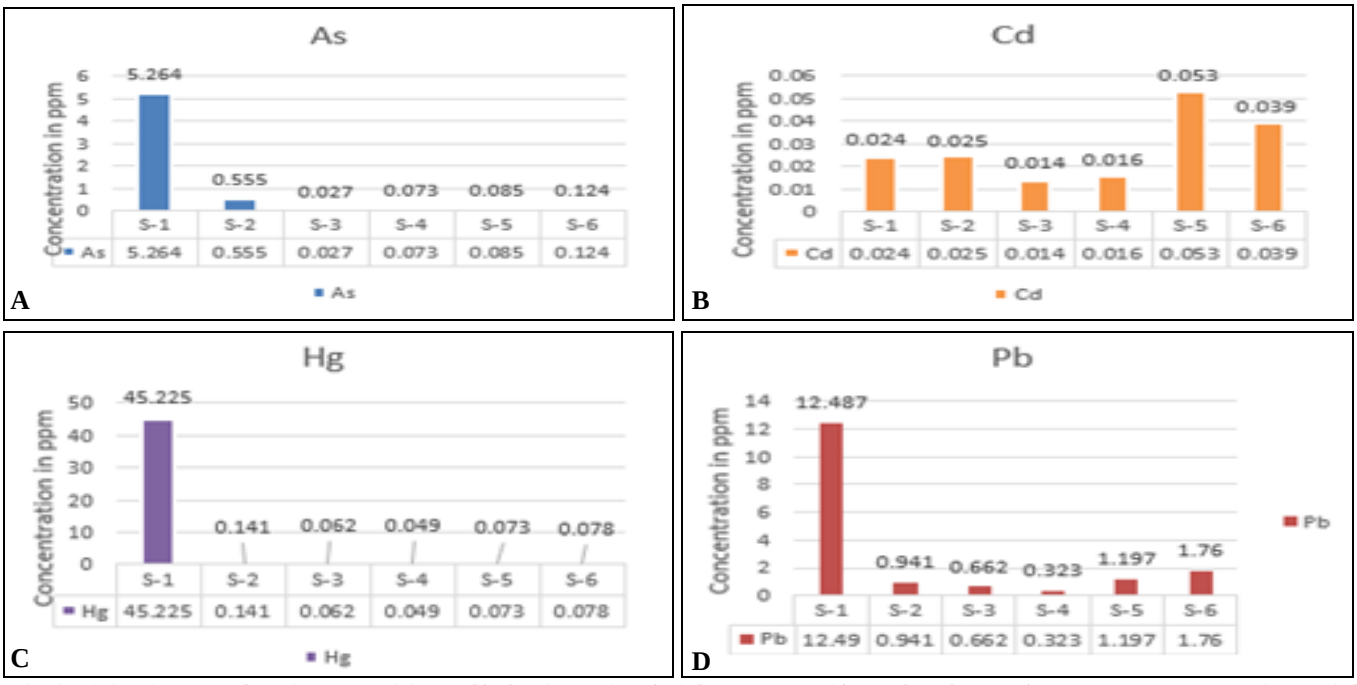

FIG. 2: CONCENTRATION IN µG/ML A) ARSENIC, B) CADMIUM, C) LEAD AND D) MERCURY IN MORINGA MEDICINE POWDER

In the present study, six different Moringa samples were collected. Four out of six were Moring herbal Medicines which were collected from the online shopping market which were taken as nutritional supplements, and two out of six samples were the Moringa leaves collected from Maharashtra and Gujarat to see the comparison between manufactured herbal product and naturally available Moringa Plants. Samples collected were first digested with 3 mL of Nitric acid and 1 mL of Hydrochloric acid according to the conditions given in the procedure. For concentration determination, it is required to produce a calibration curve with known concentrations of standard heavy metals. This analysis was conducted according to the guideline given by the World Health Organization for the presence of Heavy metals in Herbal products. According to that guideline the maximum permissible limit of As, Cd, Hg and Pb should be in the range of 3 µg/mL, 0.3µg/mL, 1 µg/mL, and 10 µg/mL respectively.

Based on that permissible limit, five different concentrations were prepared; each standard solution contained a different concentration of As, Cd, Hg and Pd. Standard (1)-0.25 Joint which contains (As- 3 µg/L , Cd- 0.3 µg/L , Hg- 1 µg/L , and Pd- 10µg/L), Standard (2)-0.5Joint which contains (As- 6 µg/L , Cd- 0.6 µg/L , Hg- 2 µg/L , and Pd- 20 µg/L), Standard (3)-1Join which contains (As- 12 µg/L , Cd- 1.2 µg/L, Hg- 4 µg/L, and Pd- 40 µg/L), Standard (4)- 1.5 Joint which contains (As-18 µg/L , Cd- 1.8 µg/L , Hg- 6µg/L, and Pd- 60 µg/L) and Standard (5)-2 Joint which contains (As- 24 µg/L, Cd- 2.4 µg/L, Hg- 8 µg/L , and Pd- 80 µg/L) Internal standards like Germanium (Ge), Tellurium (Te), Beryllium (Be), Scandium (Sc), Yttrium (Y), and Bismuth (Bi) were added in each sample and each standard to trace the internal loss of any heavy metal during analysis.

Gold (Au) was added in each sample and each standard to stabilize the Mercury. Each sample analysed trice, and the average value was obtained for each standard and sample to check the reproducibility of the results, and further concentration RSD was obtained for each standard solution and sample. Arsenic was found in the range of 0.027 µg/mL to 5.264 µg/mL. The highest concentration of Arsenic was found in S-1 (5.264 µg/mL), and the lowest concentration of Arsenic was found in S-3 (0.027), S-1 exceeded the maximum permissible limit given by WHO *i.e.*, 3 µg/mL. A similar study was conducted by Valdez-Solana *et al.* (2015), they had microwave digested the sample and analyzed by ICP-MS; the author had found the concentration of Arsenic in two Moringa leaves cultivated at two different sites as 0.0055 mg/100 g and 0.28 mg/100 g which is in highest concentration.

Cadmium was found in the range of 0.014 µg/mL to 0.039 µg/mL. The highest concentration of Cadmium was present in S-6 (0.039 µg/mL), and the lowest concentration of Cadmium was present in S-3 (0.014 µg/mL); these values are found to be within the permissible limit given by WHO. Mercury was found in the range of 0.049µg/mL to 45.225µg/mL.

The highest concentration of mercury was found in S-1 (45.225 µg/mL), and the lowest concentration of Mercury was found in S-4 (0.049 µg/mL). The concentration of Mercury in S-1 exceeded the maximum permissible value given by WHO *i.e.*, 1 µg/mL, and Lead was found in the range of 0.323 µg/mL to 12.487 µg/mL. The highest concentration of Lead was found in S-1 (12.487), and the lowest concentration of Lead was found in S-4(0.323 µg/mL).

A similar study also conducted by Limmatvapirat et.al (2015), they had digested the sample with acid digestion method analyzed by ICP-MS, they had found the concentration of As in the range of 0.012-1.574 mg/kg , Cd in the range of 0.12-0.598 mg/kg, Hg in the range of 0.010-0.366 mg/kg, and Pb in the range of 0.001-24.032 mg/kg.

A similar study also conducted by Limmatvapirat *et al.* (2013), they had compared the concentrations of 11 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) in almost all *M. oleifera* leaf samples collected from urban gardens and rural garden.

They had found that metal concentrations in urban garden were higher than those from rural gardens. They had found the concentration of As in the range of 0.003- 1.014 mg/kg, Cd in the range of 0.002-0.007 mg/kg, Hg in the range of 0.001-0.472 mg/kg and Pb in the range of 0.012- 3.459 mg/kg.

TABLE 4: CONCENTRATION HEAVY METALS IN CHYWANPRASHIN µg/mL

Sample	C-1	C-2	C-3	C-4	C-5
As	0.012	0.021	0.012	0.012	0.015
Cd	0.029	0.015	0.004	0.006	0.006
Hg	0.016	0.035	0.103	0.018	0.01
Pb	0.026	0.171	0.06	0.087	0.092

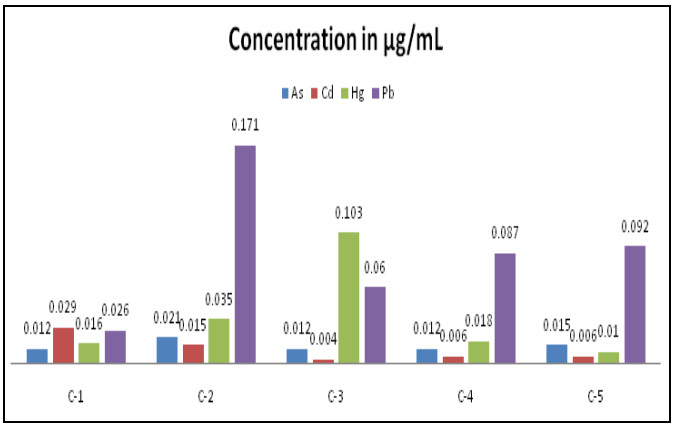

FIG. 3: CONCENTRATION ARSENIC, CADMIUM, LEAD AND MERCURY IN µG/ML IN CHYWANPRASH

In sample C-1, Arsenic was found in the range of 0.012 to 0.021 µg/mL, the maximum quantity was found in sample C-2, and minimum quantity was found in samples C-1, C-3, and C-5. Cadmium was found in the range of 0.004 to 0.029 µg/mL, maximum quantity was found in sample C-2 and minimum quantity was found in sample C-1.

Lead was found in the range of 0.06 to 0.171 µg/mL, maximum was found in sample C-2, and minimum quantity was found in sample number C-3. Mercury was found in the range of 0.01 to 0.103µg/mL, Maximum quantity was found in sample C-3, and minimum quantity was found in sample C-5. All the concentration was found within maximum permissible limit given by WHO.

Adams *et al.* (2017) Conducted a study on *Crepidium acuminatum* (D. Don) which is used as a part of chyawanprash formulation; microwave digested and analyzed by ICP-MS. Arsenic, Cadmium, Mercury and Lead was found in the range of 0.211 µg/g, 0.017 µg/g, 0.044, and 0.807µg/g, respectively [40].

K. Srilatha Srinivas and A.A. Saraf (2011), conducted a study on *Oroxylum indicum* L.(Vent.), which is one of the key ingredients used in the chyawanprash formulation, microwave digested and analyzed by ICP-MS, in that lead and cadmium were not detected [41].

CONCLUSION: From the above study, it is concluded that microwave digester is a highly efficient method to extract heavy metals from any type of sample, such as solid, liquid, viscous and semi-solid substances. It is also proved that ICP-MS is highly reliable, sensitive, and accurate technique used to determine the concentration of metals, mainly heavy metals like Arsenic, Lead, Cadmium, and Mercury, up to µg/L level. In collected samples, few samples found to be contained a high concentration of Arsenic, Mercury, and Lead. These medicines should be prepared under the specified guideline given by the herbal medicines regulatory authority. The present study proved a need for strong regulation of heavy metals present in herbal medicines to reduce their harmful effect on the consumers. Further research could be carried out on the presence of heavy metals with reference to geographical origin, environmental pollution, and parts of the plant. The present study could be highly significant to the research laboratory involved in Ayurveda and herbal drug laboratory.

ACKNOWLEDGEMENT: The author has acknowledged Mr. Ayan Bhardwaj, a scientist at ThermoFisher Scientific company, for the useful analytical assistance.

CONFLICTS OF INTEREST: The author has no conflicts of interest in this work.

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How to cite this article:
Satpute A and Pandey A: Determination of inorganic toxicants in nutraceuticals by inductively coupled plasma mass spectroscopy. Int J Pharm Sci & Res 2021; 12(9): 4832-42. doi: 10.13040/IJPSR.0975-8232.12(9).4832-42.