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Elemental Characterization of Leaf Extracts of Three Different Species of Mulberry: *Morus. alba L., Morus. nigra L. and Morus. indica L.,* Using Inductively Coupled Plasma -Atomic Emission Spectroscopy (ICP-AES)

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ABSTRACT

The present study aimed to compare the elemental composition of leaf extracts from three different plant species of Mulberry, *Morus alba L., Morus nigra L., and Morus indica L.* The results showed that ten essential elements of biological importance for human metabolism, such as Copper, Chromium, Iron, Manganese, Sodium, Zinc, Calcium, Nickel, Lead, and Cadmium, were present in varying concentrations, well below the World Health Organization, (WHO's) daily intake limits. Mulberry leaves are an economical and readily available source of essential mineral elements vital for human health, which can be used to fortify functional foods, treat various diseases, and act as nutraceuticals. The information obtained could be used to determine the efficacy and dosage of herbal drugs manufactured from the leaf extracts of the Mulberry plant, making it a new contributor to food supplements and nutraceutical products.

Keywords: *Morus alba L, Morus nigra L. and Morus indica L.,* Elemental composition, Functional foods, Inductively Coupled Plasma -Atomic Emission Spectroscopy (ICP-AES)

I. INTRODUCTION

Medicinal Plants are commonly used in most indigenous systems of medicine for therapeutic purposes and have always been an important source of herbal drugs and many of the currently available drugs have been derived directly or indirectly from them. People started believing in ethno-pharmacognosy due to the adverse effects and microbial resistance to the chemically synthesized drugs. [1]

Thousands of bioactive principles exhibiting anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity, have been isolated from medicinal plants and have been found to be safe and effective, cheap, readily available, with less adverse effects and are socially more acceptable because of the credence that they are safer than normal medicines. [2]

Medicinal herbs, and plants are widely used to relieve pain and treat many diseases and are often

advertised as natural, harmless, herbal remedies that are free from adverse effects. [3]. Although medicinal plants are considered harmless by common people, these products may be contaminated with pesticides, microbial contaminants, heavy metals, and other chemical toxins, [4] hence the elemental composition of the raw plant material should be a required condition for evaluating for both immediate and long-term side effects to demonstrate the effectiveness of a bioactive compound. [5]

Heavy metals and microbial contaminants may be related to the geographical source, harvesting, or collection process of these plant materials. Moreover, they can be contaminated during the chemical treatment or associated with storage. [6]. The concentrations of essential elements in plants is not only affected by the geochemical characteristics of soil and ecological parameters such as rainfall, atmospheric dusts, plant protection agents, and absorbed fertilizers but also by the ability of plants to selectively accumulate elements. [7]. Therefore, identification and quantification determination of major and trace elements in medicinal plants is very important. Many trace elements play a significant role in the formation of bioactive constituents responsible for the medicinal properties, but deficiencies or imbalances of these elements may cause physiological disorders. Also, essential elements in high concentrations may also be toxic. [8]

According to World Health Organization (WHO), medicinal plants should be checked for the presence of heavy metals. It is an established fact that the overdose or prolonged ingestion of the medicinal plant leads to the chronic accumulation of different elements which cause various health problems. [9]

Taking into consideration the importance of trace elements in various human metabolic processes, The main aim of the present study is to determine the concentration of ten trace elements, copper, chromium, iron, manganese, sodium, zinc, calcium, nickel, lead and cadmium found in the leaf extracts of three different species of Mulberry, namely *Morus alba L, Morus nigra L. and Morus indica L.,* by using Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) technique. The concentration of toxic metals is one of the criteria that determines the application of suitable plants for the production of herbal medicines. Thus, due to urbanization and enhanced environment pollution levels, a thorough detection of toxic elements in medicinal herbs is absolutely essential. [10]

Almost all varieties of mulberry plant are traditionally recognized in Unani, Ayurveda, and Chinese systems of medicine with several pharmacological properties. The leaves of mulberry plant are the sole food source of silkworm, *Bombyx mori L.* [11] Mulberry leaves are considered as an excellent food resource with high content of protein, carbohydrate, vitamins, microelements and dietary fiber. Their bioactive compounds possess anti-HIV, antioxidative, antibacterial, anti-obesity, hypotensive, cytotoxic, hypoglycemic, hepatoprotective, neuroprotective and anti-inflammatory properties. Though mulberry is very popular for its varied medicinal uses, reports are lacking on its morphological characteristics and elemental (mineral) composition. Very little information about elemental composition of leaves from different mulberry species is available. Thus, this study involves a comparison between three different species of mulberry based on their elemental concentration.

II. MATERIALS AND METHODS

A. Plant material

The plant material was prepared from fresh and healthy leaves of *Morus alba L, Morus nigra L. and Morus indica L.,* obtained from Central Sericultural Research & Training Institute, Central Silk Board, Govt. Of India, Ministry of Textiles, Gallandar, Pampore, Srinagar, Jammu and Kashmir, India. They were rinsed thoroughly with deionized water to remove sand and debris, and then air-dried in shade at ambient temperature and ground to a fine powder using a mechanical grinder and stored in air tight glass bottles for further analysis.

B. Sample Preparation by acid digestion

Two grams leaf powder of each *Morus* species was dissolved in nitric acid and heated until the reddish-brown colored fumes disappear. Perchloric acid was then added to the above solution and heated for 5 min. This was followed by addition of aqua regia and heated. The volume was then made up to 25ml in a standard flask by adding deionized water.

C. Elemental analysis

Estimation of essential and trace elements namely, copper, chromium, iron, manganese, sodium, zinc, calcium, nickel, lead and cadmium were carried out using Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES).

Make- SPECTRO Analytical Instruments GmbH, Germany.

Model- ARCOS, Simultaneous ICP Spectrometer

III. RESULTS AND DISCUSSION

Trace elements are very important building blocks of plants, essential for various functional activities and act as cofactors in the production of essential enzymes. [12]. Minerals play a major role in regulating cardiovascular function. Essential elements are important for human health. Deficiency or higher uptake of electrolyte minerals such as Sodium, Calcium, Iron, Copper etc. are persistent and inherently perilous occurrences that may lead to the development of cardiovascular diseases. [13]. The absorption of elements by plants depends on several soil properties and chemical composition, besides highly mobile elements (Cd, Zn, and Mo) and less mobile elements (Cr, Ni, Pb, As, and Cu) [14].

The methods used by the traditional healers to prepare the herbal medicines are responsible for the total concentration of the minerals ingested. So, to identify the hazardous effects related to the toxicity of the use of the herbal medicines, estimated daily intake (EDI) of elements in mg/day were calculated and compared with the tolerable limit set by international organizations such as WHO/FAO. (Table 1).

The leaf extracts of *Morus alba L, Morus nigra L. and Morus indica L.,* were found to be a good source of trace and major elements. Since these trace elements constitute only a minute fraction of the extracts, ICP-AES analysis which is a sensitive and reliable technique was used to obtain precise and accurate data. The concentrations of 10 major and trace elements, copper, chromium, iron, manganese, sodium, zinc, calcium, nickel, lead and cadmium found in the leaf extracts of three different species of Mulberry, namely *Morus alba L, Morus nigra L and Morus indica L,* by using Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) technique are listed in Table 2.

Table 1: Elements, Permissible limits and Concentration in ppm after ICP-AES analysis.

Element	Permissible limits (FAO/ WHO)	Reported concentration (ppm)	Remarks
Calcium	Age (yrs) / mg/day 4-8 / 800 9-18 / 1300 19-50 / 1000 >50 / 1200	*Morus.alba*: >3325 *Morus.nigra*: >2817 *Morus.indica*: >2796	Mulberry leaf extracts are abundant in calcium and within the prescribed limits.
Iron	Medicinal plant: limits not decided. Dietary intake: 27 mg/day for pregnant women 18 mg /day for child bearing women 8mg/ per day for men	*Morus.alba*: 34.53 *Morus.nigra*: 37.40 *Morus.indica*: 30.90	Mulberry leaf extracts are rich in Iron and within safe limits.
Sodium	2.4 g/day	*Morus.alba*:57.31 *Morus.nigra*:43.22 *Morus.indica*: 39.80	Sodium level within prescribed limits.
Zinc	Edible plant: 27.4 ppm. Medicinal plant: Not decided Dietary intake: 8mg/day for women 11mg/day for men.	*Morus.alba*: 5.92 *Morus.nigra*: 7.60 *Morus.indica*: 3.47	Adequate amount of Zinc found in the extracts, within safe limits
Manganese	Medicinal plant: limits have not been set. Dietary intake: 1.8 mg/day for women. 2.3 mg/day for men.	*Morus.alba*: 6.17 *Morus.nigra*: 6.48 *Morus.indica*: 6.67	Mulberry leaf extracts found to be a good source of manganese.
Copper	Edible plant: 3 ppm Medicinal plant: 20 ppm.	*Morus.alba*: 1.65 *Morus.nigra*: 1.03 *Morus.indica*: 0.85	Concentration of copper within permissible limits.
Chromium	Medicinal plant: 2 ppm in raw medicinal plant material. 0.02 mg/day in finished herbal products.	*Morus.alba*: 2.16 *Morus.nigra*: 2.17 *Morus.indica*: 1.55	Chromium concentration at safe levels.
Nickel	Edible plant: 1.63 ppm Medicinal plant: have not been set.	*Morus.alba*: 0.052 *Morus.nigra*: 0.14 *Morus.indica*: 0.073	Within the FAO and WHO permissible limits.
Lead	Edible plant: 0.43 ppm. Medicinal plant:10 ppm.	*Morus.alba*: 0.15 *Morus.nigra*: 0.13 *Morus.indica*: 0.10	Well below the limit set by FAO and WHO.
Cadmium	Edible plant: 0.21 ppm. Medicinal herb :0.3 ppm. Or 0.006 mg/day	*Morus.alba*: ND *Morus.nigra*: ND *Morus.indica*: ND	Toxic cadmium concentration was less than 0.01 ppm.

Table 2: Elemental profile of leaf extracts of three different plant species of Mulberry, namely *Morus alba L, Morus nigra L. and Morus indica L.,* using Inductively Coupled Plasma -Atomic Emission Spectroscopy (ICP-AES).

Plant specie	**Mineral concentration in ppm**									
	Ca	Fe	Na	Zn	Mn	Cu	Cr	Pb	Ni	Cd
Morus. alba L.	>3325	34.53	57.31	5.92	6.17	1.65	2.16	0.15	0.052	ND
Morus. nigra L.	>2817	37.40	43.22	7.60	6.48	1.03	2.17	0.13	0.14	ND
Morus. indica L.	>2796	30.90	39.80	3.47	6.67	0.85	1.55	0.10	0.073	ND

Note: ND means less than 0.01 ppm

Out of all the 10 elements quantified, leaf extracts of *Morus alba L, Morus nigra L and Morus indica L* were found to be richest in Calcium. *Morus alba L* exhibited the highest concentration of calcium at >3325.13 ppm, followed by *Morus nigra L* at >2817.37 ppm and *Morus indica L.* at >2796.61. (Figure 1)

Calcium is an integral part of bones, teeth and blood. Required for absorption of dietary Vitamin B and for the activation of enzyme pancreatic lipase [15]. Deficiency of calcium leads to bone mass reduction and osteoporosis. High calcium levels cause poor bone health, pancreatitis, kidney stones and abnormal heart and brain function.

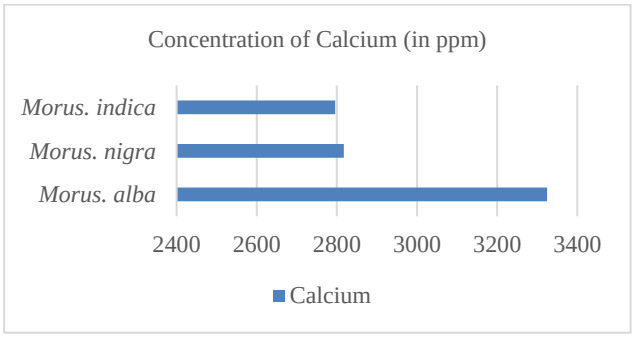

Figure 1: Concentration of Calcium in the leaf extracts of three different plant species of Mulberry, namely *Morus alba L, Morus nigra L. and Morus indica L.*

The second richest element in all the three species was found to be Sodium, with *Morus alba L.,* showing the highest concentration at 57.315 ppm, followed by *Morus nigra L.* at 43.222 ppm and *Morus indica L.* at 39.806 ppm. (Figure 2). Sodium an essential element is one of the major electrolytes in the blood, keeps the body hydrated, only needed in small amounts. High intakes of this element are associated with increased blood pressure and risk of cardiovascular disease. High level of sodium in the blood causes electrolyte imbalance also called Hypernatremia. (Severe sweating or fever; vomiting and diarrhea). [16]

Iron was the third richest element with *Morus nigra L.* having the highest concentration amongst all the three species at 37.402 ppm, followed by *Morus alba L.* at 34.539 ppm and*, Morus indica L.* at 30.905 ppm. (Figure 2).

Iron is a crucial mineral for sustaining homeostasis in the human body. It is required for several cellular processes, like, oxygen transfer through hemoglobin, oxidation-reduction reactions, immune responses, cell division and growth, DNA synthesis and thyroid hormone regulation. It is also an essential component of various enzymes involved in metabolic processes. [17]. High doses of iron can cause hepatotoxicity. Iron deficiency leads to anemia, adverse pregnancy outcomes and impaired, physical work performance.

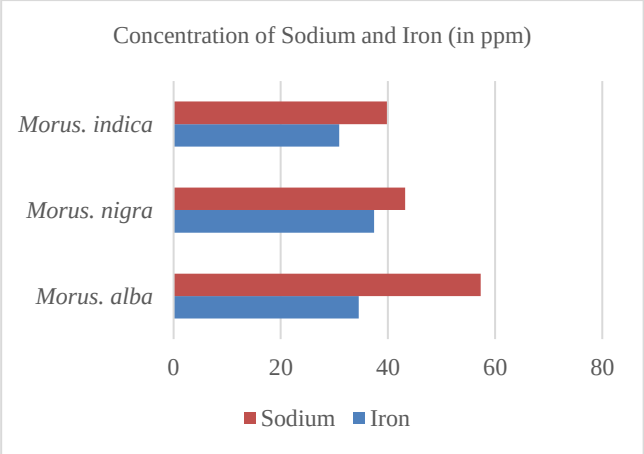

Figure 2: Concentration of Sodium and Iron in the leaf extracts of three different plant species of Mulberry, namely *Morus alba L., Morus nigra L. and Morus indica L.*

Highest concentration of Chromium, Zinc and Nickel among the three species was exhibited by *Morus nigra* at 2.179 ppm, 7.6 ppm and 0.14 ppm respectively. Nickel concentration in *Morus alba L.* (0.052 ppm) was found to be lower than that of *Morus indica L.* (0.073 ppm).

Morus alba L. and *Morus indica L.* exhibited 2.16 ppm and 1.55 ppm of chromium concentration respectively. (Figure 3).

Chromium is an essential trace element and plays an important role in glucose metabolism by serving as a cofactor for insulin action, it is one of the key minerals in controlling blood sugar and lipid levels. Chronic exposure to Cr may result in liver, kidney and lung damage and deficiency of chromium increases sugar and cholesterol level in the blood. [18].

Copper is an essential trace element and transition metal found in both humans and animals. It acts as a cofactor of many redox enzymes. It is involved in several biological processes such as antioxidant defense, neuropeptide synthesis and immune function. Cu deficiency may result in impaired development of the cardiovascular system, bone malformation and neurological and immunological abnormalities in infants. In adults Cu deficiency has been associated with alterations in cholesterol metabolism. Cu is toxic at high levels, causing oxidative cell damage and cell death. [19].

Morus alba showed the highest concentration of Copper 1.653 ppm, followed by *Morus nigra L.* at 1.032 ppm *and Morus indica L.* at 0.851 ppm. (Figure 3).

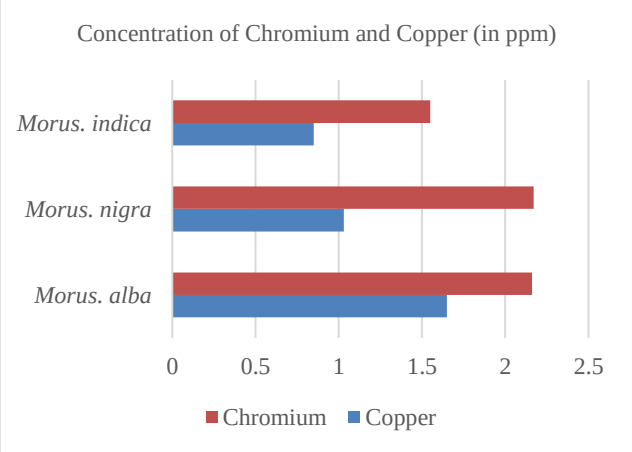

Figure 3: Concentration of Chromium and Copper in the leaf extracts of three different plant species of Mulberry, namely *Morus alba L., Morus nigra L. and Morus indica L.*.

Morus alba L. and *Morus indica L.* contain 5.92 ppm and 3.47 ppm of Zinc respectively. (Figure 4). Zinc (Zn) is a trace element essential for cell proliferation and differentiation. It is a structural constituent of many enzymes and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins. Zinc is not stored in the body and excess intakes result in reduced absorption and increased excretion. [20].

Zn deficiency can inhibit the growth of various organs like heart, brain, lungs, kidneys. Toxicity symptoms (nausea, vomiting, epigastric pain, lethargy, and fatigue) occur with extremely high zinc intakes. [21].

Morus indica L. showed the highest concentration of Manganese at 6.67 ppm, followed by *Morus nigra L.* at 6.478 and *Morus alba L.* at 6.171. (Figure 4).

Manganese is essential for several catalytic enzymes involved in bone formation. In addition, manganese reduces inflammatory pain, gastrointestinal dysfunction associated with alcohol, and can treat Helicobacter pylori diseases. Manganese deficiency has been reported in animals but rarely in humans. [22]· Deficiency of Mn causes human myocardial infarction and other cardiovascular diseases. [23]

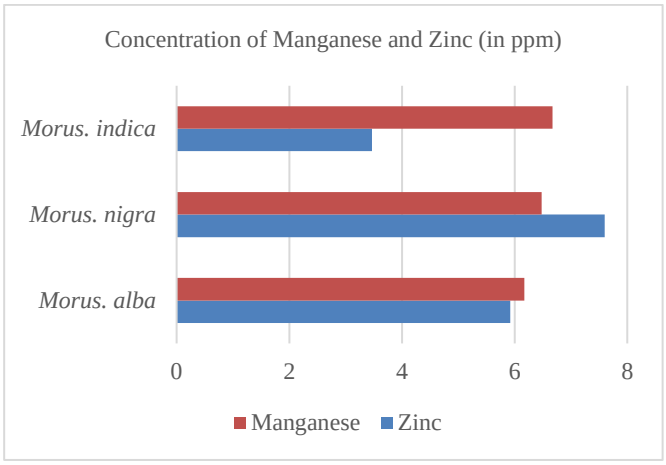

Figure 4: Concentration of Manganese and Zinc in the leaf extracts of three different plant species of Mulberry, namely *Morus alba L., Morus nigra L. and Morus indica L.*

Morus alba L. had the highest concentration of Lead at 0.154 ppm, followed by *Morus nigra L.* at 0.13 ppm and *Morus indica L.* at 0.103 ppm. (Figure 5).

Lead is a naturally occurring toxic metal found in the Earth's crust. Lead in the body is distributed to the brain, liver, kidney and bones. It is stored in the teeth and bones and strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content. [24].

Nickel is a micro nutrient essential for proper functioning of the human body, as it increases hormonal activity and is involved in lipid metabolism. Large doses of nickel or prolonged contact with it could cause a variety of side effects. Harmful effects of Nickel are genotoxicity haematotoxicity, teratogenicity, immunotoxicity and carcinogenicity. [25]

Cadmium, was not detected in all the leaf extracts- *Morus alba L., Morus nigra L. and Morus indica L.*, which means that its concentration was less than 0.01 ppm.

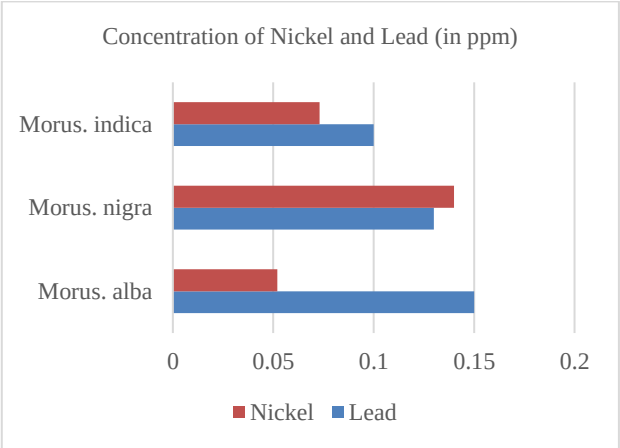

Figure 5: Concentration of Nickel and Lead in the leaf extracts of three different plant species of Mulberry, namely *Morus alba L., Morus nigra L. and Morus indica L.*

Accumulation of Cd in kidney leads to high blood pressure and renal diseases. Its accumulation also leads in damaging the nerve cells, inhibition of release of acetylcholine and activation of choline esterase enzyme. Cadmium (Cd) is a heavy metal that is of great concern in the environment, because of its toxicity to animals and humans. [26]

Moreover, amongst all the three species *Morus alba L.* was found to be richest in almost all the ten elements detected, followed by *Morus nigra L. and* lastly *Morus indica L.*. These variations in elemental composition among different species may be caused due to their genotype, climatic conditions, region of

growth, methods of cultivation, and soil nutrient content.

IV. CONCLUSION

Basic objective of this original study was to analyze ten major, micro and trace elements, namely, copper, chromium, iron, manganese, sodium, zinc, calcium, nickel, lead and cadmium in the leaf extracts of three different species of Mulberry, namely *Morus alba L, Morus nigra L. and Morus indica L.,* by using Inductively Coupled Plasma -Atomic Emission Spectroscopy (ICP-AES), and to estimate their elemental concentrations at ppm level. The variation in elemental concentrations may be due to the differences in chemical structure, mineral content of the soil, the area of growth, the climate, age of the plant, water quality and environmental conditions.

Our results show Calcium as the most abundant element in all the three different species of Mulberry, namely *Morus alba L, Morus nigra L. and Morus indica L.,*, which are similar to the results given by D. Yigit, F. Akar in their research paper. [27]. Eva María Sánchez-Salcedo and Asunción Amorós have also confirmed in their research that the main mineral element in both *Morus alba L. and Morus nigra L.* leaves were found to be Calcium. [28]

Literature survey shows that very few papers have been published on the elemental analysis of *Morus* species. This study is very unique as it gives a great comparison between the elemental composition of leaf extracts of three different species of Mulberry, which has not been attempted before. Also, no research paper on elemental analysis of *Morus indica L.* has been published till now. To the best of our knowledge, there is a lack of information regarding mineral concentrations in these plant species, Therefore, it is a first study on elemental composition of *Morus indica L.* Furthermore, this original study concurs that the quantities of toxic elements determined are well below the WHO permissible limits set for medicinal plants and major and minor trace elements are not harmful to human health.

It is thus concluded that medicinal plants like Mulberry are an important source of essential elements, namely, Calcium, Iron, Sodium, Zinc and Manganese and it is essential that the concentrations of these elements must be investigated thoroughly, before the plant material can be utilized in the form of nutraceuticals or herbal drugs.

The data generated by our study will be helpful in determining the type of extract, exact dosage, therapeutic potential and the efficacy of Mulberry leaf samples in the treatment of different diseases. It will also be beneficial for pharmacologists, chemists, ayurvedic professionals to carry out future research in the field of herbal and alternative medicines.

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