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Estimation of elemental concentrations of Indian medicinal plants using Energy dispersive X-ray fluorescence (EDXRF)

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ABSTRACT : Energy Dispersive X-ray fluorescence technique (EDXRF) is used to analyze 13 elements (P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Se, Rb, and Sr) in 10 different medicinal plants often used in Indian Ayurvedic system. Elemental analysis was carried out in medicinal plant samples using EX-3600, EDXRF spectrometer. Most of the medicinal plants were found to be rich in one or more of the elements under study. The elemental concentration in different part of medicinal plants and their biological effects on human physiology are discussed.

Keywords: Trace elements; EDXRF; Medicinal plants.

INTRODUCTION

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this revitalization of interest, the research on plants of medicinal importance is growing phenomenally at the international level. Most of the developing countries have adopted traditional medical practice as an integral part of their culture.

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Historically, all medicinal preparations are derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. [Krishnaraju et al (2005)]. Medicinal plants contain both organic and inorganic constituents. Inorganic elements also play significant roles in human physiology. They are intimately involved in the physiological functions and are important co-factors in the production of enzymes. They are also necessary for the maintenance and regulation of cell, genes and membrane function etc.

The elemental deficiencies results in the reduced activity of the concerned enzymes. However, since each element is related to so many enzymes, deficiency of a single element is often not associated with any specific clinical manifestations, but rather manifests as a combination of various symptoms [O.Wada (2004)]. Relatively less attention was received in research work on inorganic constituents of the medicinal plants than estimation of organic elements.

Considering the importance of trace elements in various human metabolic processes and also considering their curative properties, in the present investigation we have applied one of the sensitive analytical techniques such as EDXRF to study the essential elemental content in different parts of Indian medicinal plants. The overall impact of these essential trace elements on human health is also discussed. Even though the direct link between the essential elemental content and their curative capacity is not yet established, the experimental data of the present work will be of immense importance in the synthesis of new Ayurvedic formulations. Also, it will help in deciding the proportion of various active constituents and managing dose of a particular formulation.

EXPERIMENTAL DETAILS

SAMPLING

Fifteen different medicinal plants (Table 1) were collected from in and around Regional forest centre, Rajahmundry, Andhra Pradesh, India. These samples were washed in tap water and rinsed thoroughly with double distilled water in order to remove surface contamination. Each plant sample was then dried at 60-650. Dried samples are grounded in an agate mortar to form homogenized powder.

A quantity of 0.2 gm of each powder sample was weighed and compressed using a 150 ton hydraulic press and made into pellets of 13 mm diameter and about 2 mm thickness. Triplicates of each sample were done. These pellets were then used as targets for the EDXRF experiment. Biological reference material NIST 1515 (Apple leaf) was used as a reference multi- elemental standard.

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Sample code	Botanical Name	Vernacular name (Telugu Name)	Part used
MP-1	Boerhavia diffusa	Attika mamidi	Leaves
MP-2	Leucas aspera	Tella tummi	Ariel
MP-3	Indigofera tinctoria	Neelimandhu	Leaves
MP-4	Cymbopogon citratus	Nimmagaddi	Leaves
MP-5	Psidium guajava	Jama	Leaves
MP-6	Butea monasperma	Modhuga	Flower
MP-7	Olanum nigrum L.	Kamanchi	Leaves
MP-8	Momordica charantia L.	Avisa	Leaves
MP-9	Piper longum	Pippala	Leaves
MP-10	Convolvulus pluricaulis	Shankupuspi	Ariel

Table-1: List of medicinal plants selected for present study with sample codes and parts used

The list of medicinal plants selected for present study, their botanical names, code used for representation and the corresponding parts of the plants used for analysis is given in Table 1.

ENERGY DISPERSIVE X-RAY FLUORESCENCE (EDXRF) ANALYSES

Several techniques, namely, AAS, PIXE, XRF, ICP-MS, ICP-AES, EDXRF, NAA etc. are generally used for the analysis of elements present in minor quantity down to the level of parts per million or parts per billion. Among these techniques, the energy-dispersive X-ray fluorescence (EDXRF) technique is being widely used for trace element detection in various fields of science. The present study was done using Energy dispersive X-ray fluorescence (EDXRF).

Present study was carried out at trace element laboratory, UGC-DAE CSR Kolkata center, Kolkata. The set-up consists of a Xenemetrix (previously Jordan Valley) EX-3600 EDXRF spectrometer. This consists of an X-ray tube with a Rh anode as the source of X-rays with a 50 V, 1 mA power supply, Si(Li) detector with a resolution of 143 eV at 5.9 keV and 10-sample turret enables mounting and analyzing 10 samples at a time.

The targets were positioned at an angle of 45^0 to the beam direction. The X-ray beam was collimated to a diameter of 4 mm and was made to fall on the target. The detector was kept at angle of 45^0 to the target position and at an angle of 90^0 to the X-ray beam direction. The characteristic X-rays emitted from each sample were recorded with a high resolution Si(Li) detector which has a sensitive area of 30 sq mm and provided with a thin beryllium window of 8 mm thickness. The spectra were collected for a sufficiently long time so that good statistical accuracies can be achieved. The quantitative analysis is carried out by using the software nEXT.

RESULTS AND DISCUSSION

The results of EDXRF measurements for the determination of the concentration of major, minor and trace elements in medicinal plants are presented in Table 2 & 3. Quantitative analysis of thirteen different elements namely, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr was done. Many of these elements are of cardinal importance in human metabolism. They are considered essential for the growth of living organisms.

Medicinal plants are rich in many trace elements and suggested that this is important in curative effect of plants [Ray DK et al (2004), singh V and Gargh AN (1997)]. Trace elements play a very important role in formation of active chemical composition present in medicinal plants and therefore responsible for medicinal as well as toxic properties [Rajukar and Damame (1998)]. Essential, trace elements and minerals in Indian medicinal plants have been investigated by researchers to strengthen the importance of elemental analysis with respect to human health [Nomita Devi et al (2008), Bhargava. et al (2008), Srividhya et al (2011)]. The chemical constituents present in the plants that are responsible for their medicinal properties include bases comprising alkaloids, amines and glycosides etc. Trace elements play an important role in the formation of these compounds. They are intimately involved in the physiological functions and are important co-factors in the production of enzymes. They are also necessary for the maintenance and regulation of cell, genes and membrane function etc. A number of minerals essential to human nutrition are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment.

Knowledge of the elemental content in medicinal plants is very important since many trace elements play significant roles in the formation of active constituents responsible for the curative properties. Moreover, some of these elements are vitally important for various metabolic processes in

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the human body. They are closely linked to human growth and general health [Cobanoglu et al (2010)]. The current study revealed that all the elements are accumulated to greater or lesser extents in all ten investigated plant species. Minerals serve a variety of functions. Some have only one function, but others serve multiple functions ranging from acting as cofactors in enzyme reactions to stabilizing and contributing to the hardness of bone. Mineral ions occur almost always bound to particular proteins where they often play a crucial part in maintaining the protein's three dimensional structure. If the protein happens to be an enzyme then the mineral may be needed for catalytic activity. Several of the minerals influence the expression of genes encoding one or more proteins by regulating the transcription and translation of the gene.

Zinc (Zn) is the component of more than 270 enzymes and its deficiency in the organism is accompanied by multisystem dysfunction [Zinpro Corporation (2000)]. Calcium (Ca) is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortality. Potassium (P) is the principal positively charged ion inside body cells. It plays a major role in maintaining fluid and electrolyte balance and cell integrity. It is also critical to maintaining the heartbeat [Frances Sizer and Elcanor Whitney (1999)]. Sulphur (S) has a long history of use for a variety of dermatological disorders, as an ingredient of ache ointments [N Tarimci et al (1997) and Lin (1998)]. Sulphur aids in healing of wounds via keratin and has a history of folklore usage as a remedy of skin rashes [Lin (1998)]. The role of Iron (Fe) in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells [Sigel (1978)]. Iron deficiency is the most prevalent nutritional deficiency in humans [Reddy et al., (1987)].

Manganese (Mn) is important for several enzymatic processes; it helps in eliminating fatigue and reduces nervous irritability (Hamilton et al., 1994; O'Dell and Sunde, 1997; Prasad, 1993).The leaves containing a fairly good amount of Bromine (Br) may be used for the preparation of the drugs in curing natural diuretic, phlegm eliminating and stomach invigorating diseases [Chen et al., 1993]. Copper (Cu) is an essential constituent of several enzymes; it is involved in many metabolic reactions. It is required for maintaining a healthy heart and blood vessels, for the synthesis of hemoglobin, melanin and phospholipids, and for the development of bones and nervous system. It strengthens connective tissue and is involved in energy production, in the protection of cells from free radical damage and in brain neurotransmitters. [Paul L Fox (2003)].

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Sample code	Name of sample	P	S	Cl	K	Ca	Mn
MP-1	Boerhavia diffusa	1702±44	1811.7±21	25832±801	29059±493	21543±337	70.2±3.8
MP-2	Leucas aspera	2569±80	1355±82	15545±500	11940.7±175	13928.6±90	205±5
MP-3	Indigofera tinctoria	1719.7±146	2767.8±249	579	12434±534	33037±992	274.6±5.7
MP-4	Cymbopogon citratus	934.5±54	1405±69	17234±382	17325±149	5871±184	102.3±5.5
MP-5	Psidium guajava	2007±97	2950±436	4385.7±162	16399±563	5382±132	32.8±1
MP-6	Butea monasperma	1495.5±15	1580±66.9	4648.6±193	18313±287	3536.5±106	36.9±1.7
MP-7	Olanum nigrum	2961.9±101	2617.6±41	23046±842	16572±303	12672±3131	58.175±2.2
MP-8	Momordica charantia	1443±77	2421±86	10130±187	11711±170	19035±272	75.0±0.36
MP-9	Piper longum	2433.7±159	1748.6±90	5945.3±188	19170±480	15903±165	175.6±8.7
MP-10	Convolvulus pluricaulis	2514±177	1284±69	14444±2071	26107±2062	17911±730	49.9±1.7
Standard	NIST(1515)	1590	1800	579	16100	15260	54

Table-2: Average elemental concentration with (±) standard deviation in ppm of P, S, Cl, K, Ca, Mn in medicinal plant samples

Sample code	Name of sample	Fe	Cu	Zn	Se	Br	Rb	Sr
MP-1	Boerhavia diffusa	307.04±15	6.2±0.85	9.8±0.4	0.53±0.71	123.9±0.91	190.9±2	75.32±1.4
MP-2	Leucas aspera	609±9	11.2±1.7	29.6±0.6	1.21±0.8	320.5±2.5	76.7±1	59.3±1
MP-3	Indigofera tinctoria	95±2	5.6±0.2	29.7±0.2	1.3±0.6	8.29±0.3	32.14±3	93.1±4
MP-4	Cymbopogon citratus	301.61±22	9.86±1.3	38.6±0.6	1.31±0.8	89.4±0.7	56.4±2.5	28.5±3
MP-5	Psidium guajava	87.9±1	17.5±0.3	18.6±0.8	0.15	2.7±0.2	12.7±1.2	46.9±2
MP-6	Butea monasperma	205.5±7.3	22.62±0.8	33.3±0.6	0.56±0.4	168.2±1.5	62.1±0.9	34.7±1.7
MP-7	Olanum nigrum	118.5±3	12.4±1	33.8±1	0.56±0.1	71.6±1	21.3±1.3	192.4±3.6
MP-8	Momordica charantia	118.8±1.9	5±0.9	10.49±1.9	0.10	114.6±0.3	23.1±0.4	257±2.6
MP-9	Piper longum	239.1±2	15.05±0.4	20.04±0.5	0.34±0.4	28.1±0.4	45.1±0.3	67.4±4
MP-10	Convolvulus pluricaulis	168.7±13.7	8.7±1.2	32.3±1.5	0.59±0.4	93.2±7	28.6±3	163.8±10.6
Standard	NIST(1515)	83.00	5.64	12.50	0.05	1.80	10.20	25.00

Table-3: Average elemental concentration with (±) standard deviation in ppm of Fe, Cu, Zn, Se, Br, Rb and Sr in medicinal plant samples

S.No	Name of the plant	Elements present (Concentration in Ppm)

	sample	With higher concentration	Concentration range	With lower concentration	Concentration range
1	Boerhavia diffusa	Cl - 25832±801 K - 29059±493 Rb - 190.9±2	25832 –579 29059 –11711 190.9 –12.7	Zn - 9.8 ±0.4	38.6 - 9.8
2	Leucas aspera	Fe - 609 ± 87. 9 Br - 320.5±2.5	609 – 87.9 320.5--9.8	K - 11940 ±175 Ca - 13928±90	29059 –11711 3303 – 5382
3	Indigofera tinctoria	Ca - 33037±992 Mn - 274.4±5.7	3303 – 5382 274.6 – 32.8	Cl - 579 K - 12434 ± 534	25832 – 579 29059 –11711
4	Cymbopogon citratus	Zn - 38.6±0.6 Se - 1.31±0.8	38.6 – 9.8 1.31 – 0.15	P - 934.5 ± 54 Sr - 28.5±3	2961.9 – 934.5 257 – 28.5
5	Psidium guajava	S -2950±536 Cu - 17.5±0.3	2950 – 1284 22.62 -- 5	Ca - 5382±132 Mn - 3208±1 Fe - 87.9±1	3303 – 5382 274.6 – 32.8 609 – 87.9
6	Butea monasperma	Cu- 22.62±0.8 Zn-33.3±0.6	22.62 -- 5 38.6 – 9.8	P - 1495.5±15 S - 1580±66.9 Ca - 3536.5±106 Mn - 36.9 ± 1.7	2961.9 – 934.5 2950 – 1284 3303 – 5382 274.6 – 32.8
7	Olanum nigrum L.	P - 2961.9±101 Zn - 33.8±1	2961.9 – 934.5 38.6 – 9.8	K - 16572±363 Ca –2672±3131	29059 –11711 3303 – 5382
8	Momordica charantia L.	Sr - 257±2.6 Cl -10130±187	257 – 28.5 25832 – 579	P - 1443± 77 K - 11711 ±170 Cu - 5± 0.9 Zn - 10.49 ± 1.9	2961.9 – 934.5 29059 –11711 22.62 -- 5 38.6 – 9.8
9	Piper longum	P -2433.7±189 Mn - 175.6±8.7 Cu - 15.05±0.4	2961.9 – 934.5 274.6 – 32.8 22.62 – 5	S - 1748.6± 90	2950 – 1284
10	Convolvulus pluricaulis	P - 2514±177 K - 26107±2067 Zn -32.3±1.5	2961.9 – 934.5 29059 – 11711 38.6 – 9.8	S - 1284±69 Mn - 49.9 ±1.7	2950 – 1284 274.6 – 32.8

Table 4: Higher and lower concentration of elements and range of concentrations in studied samples.

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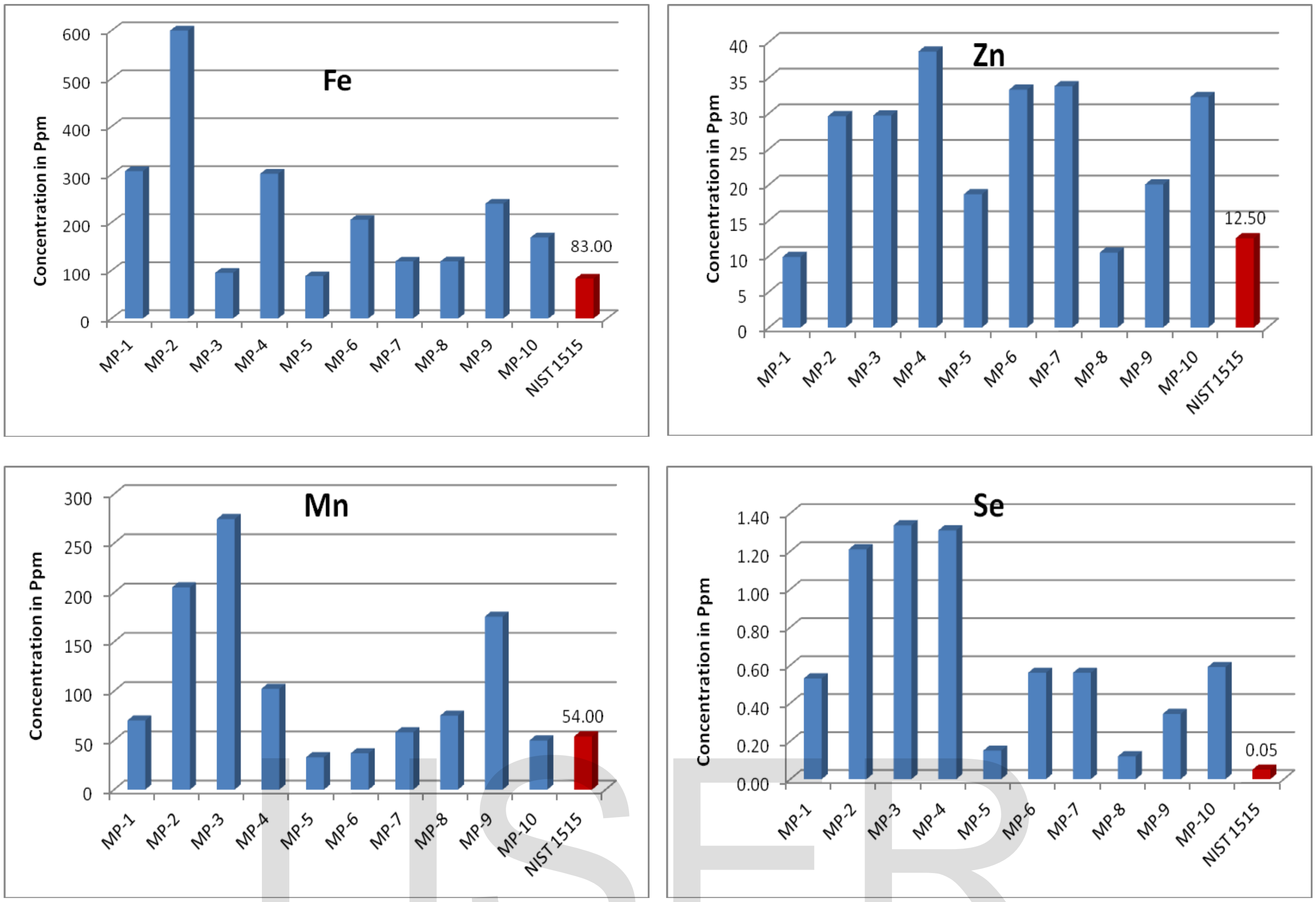

Figure 1: Plots of concentrations (in Ppm) of Fe, Zn, Mn, Se in studied plant samples

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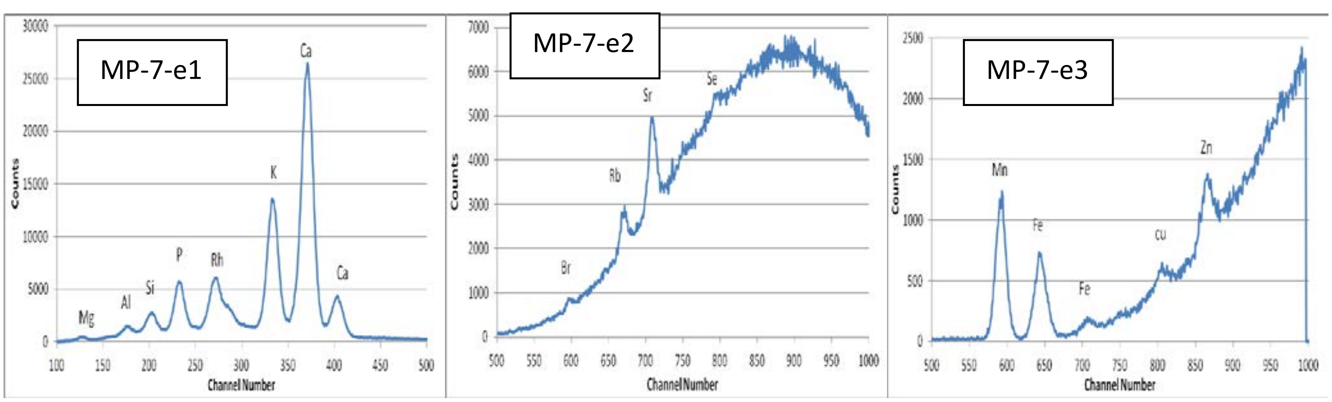

Figure 2: EDXRF Spectrums of Indigofera tinctoria (MP-7) sample

Chlorine (Cl) is a constituent of sodium chloride. Hence the metabolism of chlorine and sodium are intimately related. Chloride is involved in the regulation of acid-base equilibrium, fluid balance and osmotic pressure. These functions are carried out by the interaction of chloride with Na+ and K+. It is necessary for the formation of Hcl in the gastric juice. It activates the enzyme salivary amylase. [Kathryn E et al (2005)].

Plots of concentrations (in Ppm) of Fe, Zn, Mn, Se in studied plant samples

In the present study, the concentration of elements with higher and lower concentrations along with the range of concentrations in studied plant samples are given in table 4. Results of present study shows, all the samples under study are rich in many of the elements particularly Boerhavia diffusa (MP-1) have significantly higher concentrations of Cl, K, Rb, Indigofera tinctoria (MP-3) have significant concentrations in, Ca, Mn, Zn and Se. Concentration of Fe ,Zn, is found high in Cymbopogon citrates(MP-4). Piper longum (MP-9) have significant concentrations of P, Mn, and Cu. Plots of concentrations (in Ppm) of Fe, Zn, Mn, Se in studied plant samples is given in figure 1 and EDXRF Spectrums of Indigofera tinctoria (MP-7) sample is shown in figure 2.

CONCLUSIONS

Attempts have been made in the present study to list out various trace elements like P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Se, Rb, and Sr and their concentrations in ten different medicinal plants mentioned in different traditional systems and documented in literature. The elemental data of the present work will be of immense importance in the synthesis of new Ayurvedic formulations. Also, it will be useful to set new standards for prescribing the dosage and duration of administration of these herbal medicine to the diseased patients. Adequate and necessary care should be taken while supplementing the elements through these medicinal plants in order to avoid other complications like metal toxicity.

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