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

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Abstract
The elements present in medicinal plants play an important role in the treatment of diseases as described in Ayurveda. Energy Dispersive X-ray fluorescence spectroscopy (XRF) has been applied for quantitative analysis of multi elements present in medicinal plant. XRF technique, one of the most powerful and quick multi elemental analysis with high sensitivity has been used. The data shows presence of thirteen elements in varying concentration. Such information could be helpful in standardization of herbal products since the Indian medicinal plants play an important role in maintenance of human health.

Keywords: Trace elements, Indian medicinal plants, EDXRF

1. Introduction
Due to their minor side effects, the medicinal plants are widely used to treat many human diseases [1, 2]. The human body needs a number of minerals in order to maintain good health [3, 4]. Macro- and microelements influence biochemical processes in the human organism. Active constituents of medicinal plants i.e. metabolic products of plant cells and a number of mineral elements play an important role in the metabolism [5, 6]. Vartika *et al*. and co-workers concluded that the medicinal values of some plant species used in homoeopathic system may be due to the presence of Ca, Cr, Cu, Fe, Mg, K and Zn [7]. These elements also take part in neuro chemical transmission and serve as constituents of biological molecules and in a variety of different metabolic processes [8]. Determination of mineral elements in plants is very important since the quality of many foods and medicines depends upon the content and type of minerals [9]. Malnutrition is of major concern for many tropical developing countries. Deficiency or excess of elements may cause a number of disorders. For example, Iron deficiency anaemia affects one third of the world population [10, 11]. Low levels of Zn can induce the pathogenesis of lung cancer [12]. Breast cancer patients had low levels of Ca, Mg, Fe, Cu, Mn and Zn in their hair [13].
Therefore, it is of major interest to establish the levels of some metallic elements in common used plants because, at elevated levels, these metals could be dangerous and toxic [14, 15]. Determination of metals in medicinal plants is a part of quality control to establish their purity, safety and efficacy according to the World Health Organization (WHO) [16]. Although several attempts have been reported for determination of elemental concentration of medicinal and aromatic plants from all over the world [17], reports of plants growing in the Asian country like India.
Most of the medicinal plants were used after soaking in water and thus only this water extract is taken for the cure of disease. However, some are taken as a whole in the form of powder mixed with milk, honey and yoghurt or eaten as a fruit. So this study aimed to determine the concentration of some micro- and macro elements in selected medicinal plants extensively used in the preparation of herbal products in the India.

2. Materials and Method
2.1. Experimental details
2.1.1. Sampling
Ten different medicinal plants (Table 1) were collected from in and around Regional Forest Centre, Rajahmundry, Andhra Pradesh, India. Samples consist of different parts of plants including leaves, aerial parts, roots, fruits and rhizomes. 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, ground and homogenized in an agate mortar. 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 (Apple leaf-1515) was used as a reference multi- elemental standard. 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.

2.2 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 centre, 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 analysing 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 an 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.

3. Results

Thirteen elements P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Se, Rb and Sr were determined by using Energy Dispersive X-ray Fluorescence (EDXRF) in selected Indian medicinal plants namely, *Bougain villea, Lawsonia alba LAM, Adhatoda vasica,Aegle marmelos, Cuminumcyminum,Brassica nigra,Coriandrum sativum, Carica papaya, Bnincasahispida and Abelmoschus moschatus Medik. Brassica nigra* has the highest concentration of Potassium (93761.56±7884.45ppm), Sulphur (10747.63±420.88 ppm), Zn (96.65±6.8ppm) and Rb (63.11±0.68ppm). The highest concentration of Cu (26.66±1.37 ppm) was found in Adhatoda vasica.The highest concentration of Se (1.79 ±2.37 ppm) was found in Carica papaya. Bougain villea had the highest concentration of Mn (576.9±8.94 ppm), Br (96.21±0.95ppm) and Sr (114.02±2.21ppm) respectively. Such information could be helpful in standardization of herbal products since the Indian medicinal plants play an important role in maintenance of human health.

4. Discussion

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 the human body. They are closely linked to human growth and general health [18]. In this study, a total of thirteen elements i.e.,P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Br, Se, Rb and Sr were determined in the powdered medicinal plant samples by using EDXRF. The mean concentrations of various metals in the plant samples were shown in following tables

Table 1: Concentration of elements in various samples with standard deviations

SAMPLE CODE	P	S	Cl	K	Ca	Mn
MP 1	617.10±58.56	3208.91±70.61	6740.25±181.20	21686.43±474.34	19347.51±424.25	576.94±8.94
MP 2	607.12±22.52	999.96±18.29	1596.03±26.80	7941.63±68.82	10119.82±34.58	249.10±4.65
MP 3	2343.93±19.49	1995.35±16.61	14374.53±224.55	19162.13±231.31	19178.51±347.23	64.74±1.8
MP 4	272.91±33.4	812.45±18.55	579±0	1340.321±41.64	51655.28±2594.18	19.84±1.64
MP 5	1119.65±77.1	2999.66±58.84	79726.78±1064.7	21957.42±188.57	9181.27±123.58	41.05±1.92
MP 6	92033.56±7112.3	14896.±1851.02	579±0	7733.18±598.19	3851.71±287.37	36.74±0.71
MP 7	4339.03±151.75	2542.89±79.53	24413.89±757.94	31643.24±579.9	9383.38±147.46	77.69±1.85
MP 8	8671.54±202.4	10747.63±420.88	579±0	9540.12±58.85	9255.5±306.27	49.78±0.9
MP 9	15944.7±523.17	4339.13±205.81	4389.53±231.03	16512.64±528.56	4349.50±115.87	106.46±4.36
MP 10	6320.64±198.69	2200.08±54.42	10781.19±515.61	21212.67±642.35	6349.762±293.47	11.73±2.98

SAMPLE CODE	Fe	Cu	Zn	Se	Br	Rb	Sr
MP 1	500.52±14.59	6.03±0.88	13.33±1.17	0.95±0.32	96.21±0.95	78.40±0.97	6[illegible].65±0.63
MP 2	3704.9±78.22	11.4±0.99	11.87±0.41	0.19±0.06	27.01±0.8	35.64±2.4	128.9±3.99
MP 3	1075.53±17.99	26.66±1.37	0.26±1.71	0.26±0.07	51.22±1.78	23.03±1.27	114.02±2.21
MP 4	475.81±44.53	5.26±0.85	1.27±0.11	0.3±0.1	1.24±1.01	1.15±0.81	476.18±20.89
MP 5	219.25±14.14	8.17±0.82	14.71±0.75	0.47±0.2	48.18±2.98	22.03±2.12	82.62±14.4
MP 6	148.49±9.89	7.98±0.55	67.88±1.41	0.2±0.1	0.73±0.64	63.[illegible]1±0.68	35.36±1.33
MP 7	838.85±9.29	8.04±0.42	33.10±1.21	0.37±0.39	27.67±0.95	31.73±1.18	47.93±0.41
MP 8	217.13±5.19	11.74±1.82	77.71±6.38	1.79±2.37	2.18±0.74	14.06±1.96	28.85±3.19
MP 9	322.44±7.67	22.81±1.71	96.65±6.8	0.49±0.44	5.5[illegible]±1.02	8.48±1.65	1[illegible].31±2.67
MP 10	106.31±5.27	11.44±0.41	42.79±1.10	0.73±1	22.67±0.75	21.71±1.7	52.45±2.49

Table 2: List of medicinal plants with their botanicl names and used parts

S.NO	Sample Code	Local Name	Botanical Name	Used Part
1	MP1	Kagitham Poolu	Bougain villea	Flowers
2	MP 2	Gorintha	Lawsonia alba LAM	Leaves
3	MP 3	Addasaram	Adhatoda vasica	Leaves
4	MP 4	Maredu	Aegle marmelos	Bark
5	MP 5	Jeelakarra	Cuminumcyminum	Fruit
6	MP 6	Aavalu	Brassica nigra	Seeds
7	MP 7	Kottimera	Coriandrum sativum	Leaves
8	MP8	Boppai	Carica papaya	Seeds
9	MP 9	Booditha Gummadi	Benincasa hispida	Seeds
10	MP 10	Karpoora Benda	Abelmoschus moschatus Medik	Seeds

The current study revealed that all the metals were accumulated to greater or lesser extents by all ten investigated plant species.

Copper is an essential nutrient that plays an important role in the production of hemoglobin, myelin, collagen and melanin [12]. Cu concentrations varied from 5.26±0.85 ppm to 26.66±1.37 ppm, with showed that they contained large amounts of nutrients and were rich in K and Ca. The high concentration of Potassium in plants is needed for many essential processes including enzyme activation, photosynthesis, water use efficiency, starch formation and protein synthesis. Potassium participates actively in the maintenance of the cardia rhythm [19] and in constipation. Concentrations of potassium were in the range (31643.24±7884.45ppm to 7941.63±68.82ppm. *Coriandrum sativum* (31643.24 ±579.9 ppm) had the highest K concentration followed by *Cuminumcyminum* (21957.42±188.57 ppm) Calcium 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. Calcium concentrations were in the range 51655.28 ppm to 3851.5 ppm. Three samples have concentrations in the range 10119.82 ppm to 19347.51 ppm. *Aegle marmelos* had the highest Ca concentration (51655.28 ppm) whereas Brassica nigra had the lowest (3851.5 ppm).

Manganese concentration level ranged from 11.73±2.98 ppm to 576.94±8.94ppm. *Bougain villea* had the highest Mn concentration and *Abelmoschus moschatus Medik* had the lowest. Deficiency of Mn in human causes myocardial infarction and other cardiovascular diseases, also disorder of bony cartilaginous growth in infants and children and may lead to immunodeficiency disorder and rheumatic arthritis in adults [20].

Iron is an essential element for human beings and animals and is an essential component of hemoglobin. It facilitates the oxidation of carbohydrates, protein and fat to control body weight, which is very important factor in diabetes. The Fe concentrations varied from 29 to 1426 ppm. Four samples have concentrations ranging from 106.31±5.27 ppm to 3704.9±78.22 ppm, other three samples in the range 500.53–1075.53ppm. Lawsonia *Alba* Lam had the highest Fe concentration followed by Adhatoda vasica and *Coriandrum Sativum* respectively. *Brassica nigra* had the lowest Fe concentration. According to FAO/WHO, the concentration of Fe in *H. johannis* was found to exceed the maximum permissible limit [21]. However, previous study [22] showed that *H. johannis* dried roots and ethanol extract induced toxic effect in rats and they suggested that, the apparent lack of clinical signs of acute toxicities in human when administered the extract orally, may be a reflection of the low dose administration as well as short duration of exposure. Moreover, the root was rich in tannins [23]. Suggesting that iron can be found chelated with tannic acid and this subsequent chelation may be eliminated faster from the body as compared to non-chelated iron [24]. Nevertheless, the safety of this plant in the traditional medicine should be verified by much further testing, including *in vivo* experiments and clinical studies.

Chromium is important in the utilization of glucose. According to Perry [25], Cr, Mg and Zn have important roles in the metabolism of cholesterol as well as heart diseases. The presence of Cr and Mn in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases [25]. The toxic effects of Cr intake is skin rash, nose irritations, bleeds, upset stomach, kidney and liver damage, nasal itch and lungs cancer. Cr deficiency is characterized by disturbance in glucose lipids and protein metabolism [26]. The daily intake of Cr 0.05-0.20 mg has been recommended for adults by US National Academy of Sciences [27].

Zinc is the component of more than 270 enzymes [28]. and its deficiency in the organism is accompanied by multisystem dysfunction. Besides, Zn is responsible for sperm manufacture, fetus development and proper function of immune response [29]. The Zn concentrations varied from 01.27±0.21 to 96.6±6.8 ppm. *Bnincasahispida* had the highest Zn concentration and *Adhatoda vasica* had the lowest.

Bromine is considered as a non-essential element for living organisms [31]. The concentrations of this element varied from0.73±0.64ppm to 96.21±0.95 ppm. Most samples have concentrations between 22.67ppm to 51.22ppm and. *Bougain villea had* the highest Br concentration and *Brassica nigra had* the lowest. Rubidium is also considered as non-essential element for human organism [32]. Rubidium concentrations varied from 1.15 to 78.4 ppm. Most samples have concentrations in the range 21.7 to 63.7 ppm. *Bougain villea* had the highest Rb concentration followed by *Brassica nigra and Aegle marmelos* had the lowest

Finally, the Strontium concentrations were in the range 11.31±2.67ppm to 128.9±3.99 ppm. Four samples being in the 61.65 ppm to-114.02 ppm range. *Lawsonia alba Lam* had the highest Sr concentration and *L. Bnincasahispida* ha*d* the lowest.

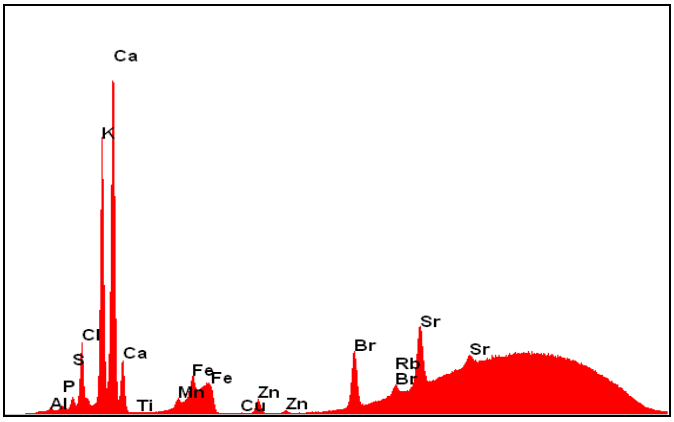

Fig 1: EDXRF sample spectrum

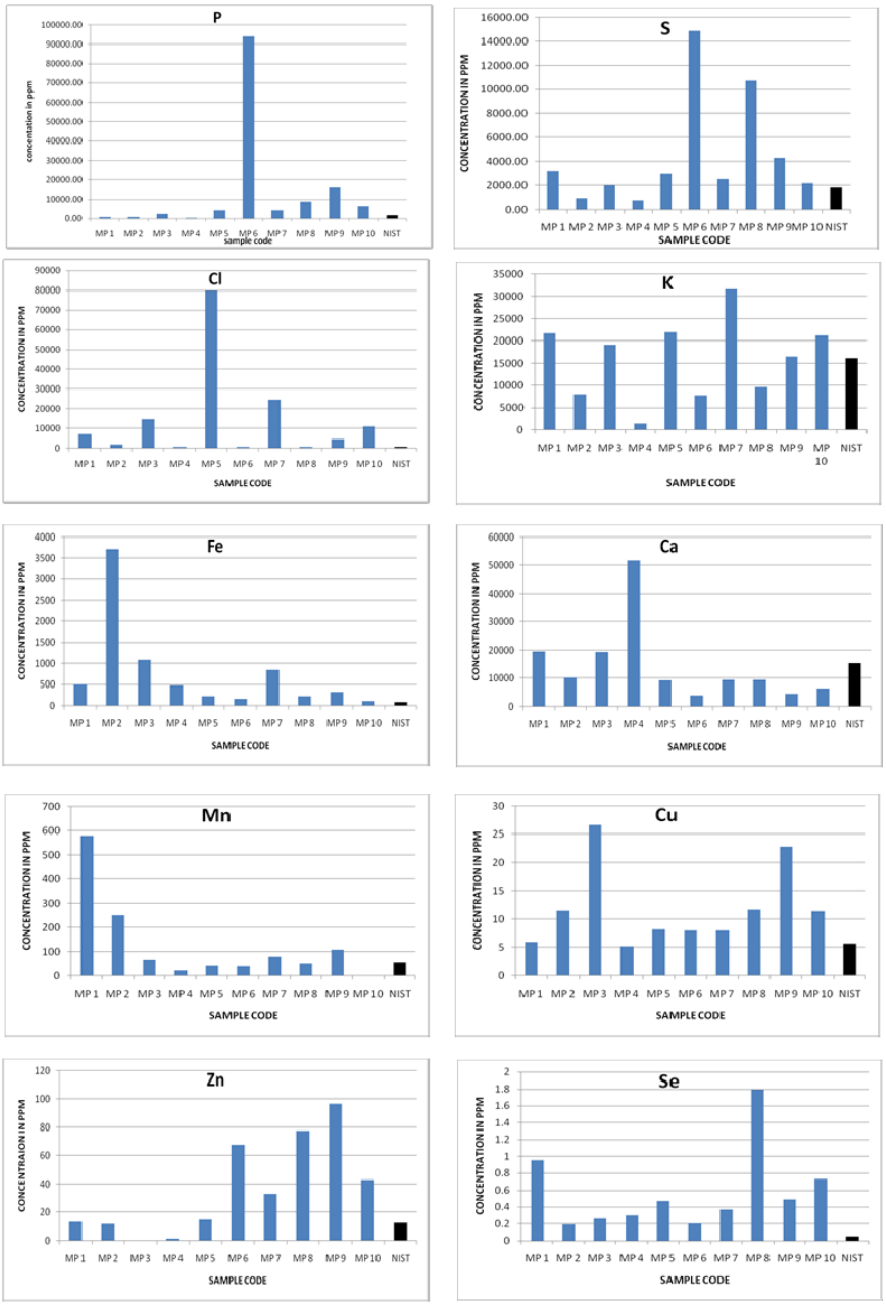

Fig 2: Graphs of elements concentration in studied plant samples

Table 3: Pharmacognostic Features of the studied Plants

S.no	Botanical name	Part used	Medicinal properties	References
1	Bougain villea	Flowers	antidiabetic,anticancer	33,34
2	Lawsonia alba lam	Leaves	hypoglycemic,hepatoprotective, immunostimulant, anti-inflammatory,antibacterial, antimicrobial, antifungal, antiviral, antidermatophytic, antioxidant, antifertility and anticancer	35
3	Adhatoda vasica	Leaves	asthma,chronic bronchitis,malarial fever,snake bites,antidiabetic	36,37,38,
4	Aegle marmelos	Bark	diabetes, cholesterol, peptic ulcer, inflammation, diarrhoea, and dysentery, anticancer, cardio protective, anti-bacterial, anti-fungal, radio protective, anti-pyretic, analgesic, antioxidant, hepatoprotective, wound healing	39,40
5	Cuminum cyminum	Fruit	Anti-microbial,Anti-diabetic,Anti-cancer,Anti-oxidant,,Anti-asthmatics,	41
6	Brassica nigra	Seeds	antibacterial, astringent, diaphoretic, hypoglycemic,	42
7	Coriandrum sativum	Leaves	Antioxidant Activity, Anti-diabetic Anti-convulsant activity, Sedative Hypnotic Activity, Anti-microbial Activity, Anti mutagenic, Anthelmintic activity	43, 44
8	Carica papaya	Seeds	Anticancer activity, treatment for dengue fever, antidiabetic activity, and antifertility effects.	45
9	Benincasa hispida	Seeds	anti-inflammatory, antiasthmatic, diuretic, nephroprotective, antidiabetic, hypolipidemic and antimicrobial effects	46
10	Abelmoschus moschatus Medik	seeds	antiseptic, stomachic, cooling, tonic	47

5. Conclusions

In view of the above facts, the medicinal plants studied are a source of biologically important elements, which may play part in the observed therapeutic properties of these plants. Metabolism has been steadily increasing. Trace elements have important physiological effects when present at concentrations other than those associated with classical toxicity or with extreme deficiency. Among the various elements detected in different medicinal plants of same genus used in the treatment of various diseases. The data obtained in present study will be helpful in the synthesis of new modern drugs with various combinations of plants which can be used in the cure of many diseases ethno medicinally. However, more detailed analysis of chemical composition of the following medicinal plants is required. The elemental results of wild edible fruits shows that many of these fruit contain elements of vital importance in man's metabolism and that are needed for growth, developments, prevention and treatment of many diseases. It is evident that they are important sources of essential mineral elements in reasonable concentrations which have required in treatment of many diseases.

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