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Original article**Trace element profile of some selected medicinal plants of Manipur, India****C.h.B. Devi^{a,*}, N.K.S. Singh^b, S.S. Ram^c, M. Sudarshan^c, A. Chakraborty^c, N. Rajmuhon^a**^a*Department of Chemistry, Manipur University, Canchipur, Imphal-795 003, India.*^b*Department of Physics, Manipur University, Canchipur, Imphal-795 003, India.*^c*UGC-DAE Consortium for Scientific Research, Kolkata Centre, Kol;kata-700 098, India.*^{*}Corresponding author; Department of Chemistry, Manipur University, Canchipur, Imphal-795 003, India.

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Herbal remedies are considered the oldest forms of health care known to mankind on this earth. The different elemental concentration at trace level of medicinal plant also plays an important role in the treatment of diseases. Different parts of the plants such as roots, leaves, stem, bark, fruits, seeds etc depending on the plant species are generally used for the preparation of traditional medicines. In this present work, some selected medicinal plants were collected from different parts of Manipur, India and analysed by using EDXRF (Energy dispersive X-ray fluorescence), a fast multi-elemental analytical technique. Fourteen elements namely, Potassium, Calcium, Titanium, Vanadium, Chromium, Manganese, Iron, Copper, Zinc, Arsenic, Selenium, Bromine, Rubidium and Strontium were detected. Also the elemental content of each plant part has been correlated with its potential application as herbal medicine.

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1. Introduction

Man has been dependent upon nature for survival since time immemorial. Plants and plant derived products are part of health care since human civilization. Prior to the development of modern medicine, the traditional systems of medicine that have evolved over the centuries within various communities, are still maintained as a

great traditional knowledge base in herbal medicines¹. It evolves a unique system of knowledge about plant wealth by trial and error methods². Traditionally, this treasure of knowledge passed orally from generation to generation without any written document^{3, 4}, and is still retained by various indigenous people of the world. The World health Organization estimates that about 80% of the world's population relies mainly on herbal medicine for primary health care^{5, 6}. India is the largest producer of medicinal herbs and is called as botanical garden of the world⁷. A number of medicinal plants, traditionally used for over 1000 years named rasayan are present in herbal preparations of Indian traditional health care systems⁸. Traditional Indian medical herbs used for strengthening the body immune system are known to have many essential and nutritional elements. Their excess or deficiency may disturb normal biochemical functions of the body⁹. The World health Organization in 1992¹⁰ and the Drug Council Authority Malaysia¹¹ have emphasized the quality of medicinal plant as raw materials and their products, including the analytical control methods for heavy metals in order to establish their quality, safety, and efficacy. Physiological concentration of trace elements must always be maintained for proper maintenance of cellular functions of animals. However, the normal concentration of trace elements in different cells mainly depends on the dietary concentration, absorption, and homeostatic control mechanisms of the body¹². The concentration of some minerals in the living cells and the human requirement are to some extent lower than those of bulk elements and they were not easily quantified by early analytical methods, hence the name trace elements. There are at least 50 elements that are vital for the well being of humans¹³. It is believed that the great majority of them act as key components of essential enzyme systems or other proteins, e.g., the haemoprotein haemoglobin which performs vital biochemical functions^{13- 15}.

Manipur is north east state of India bordering Burma and lies in the Indo-Burma hotspot. About 90% of the total area of state is hill and remaining 10% is valley which is surrounded by the hills. The state has a rich species of medicinal plants as north-east is genetic treasure house of bio-resources. The evolution of Ayurveda and plant based remedies through day to day life experience is a part of cultural heritage of India and so is for the north east as well^{16, 17}. In this report we have applied energy-dispersive X-ray fluorescence (EDXRF), a reliable multi-elemental analytical technique for the determination of the essential and toxic minerals in eight medicinal plants commonly used in Manipur. The results would be an addition to such relevant informations available on these plants and represents the first report on the concentration of the major, minor and trace elements of these plants.

2. Materials and methods

2.1. Sample preparation

The eight medicinal plants were collected from their natural habitats in and around Eco-park, Kakching and the traditional medicinal values of them are given in table 1. Each of the plant samples was washed extensively in distilled water in order to remove superficial dust. The samples were oven dried at 60°C for 48 hours. The dried samples were ground into fine powder and homogenized using an mortar pestle. Three sub-samples of each plant sample were then prepared and pressed into thick pellets of 13mm diameter using a table-top K-Br press (Pressure: 100-110 kg/cm² for 5min).

2.2. EDXRF analysis

The elemental analysis of medicinal plants were carried out using a Jordan Valley Ex-3600 Energy dispersive X-ray fluorescence EDXRF spectrometer, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV, current 1 mA). The measurements were carried out in vacuum using different filters (between the sources and sample) for optimum detection of elements. For example, for Na, Si and Al, no filter was used, and a voltage of 8 kV and current of 85 mA were used. A 0.05-mm-thick Ti filter was used in front of the source for K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu and Zn, with an applied voltage of 20 kV and a current 400 mA. For higher Z elements such as Pb, Bi, Ag and As, an Fe filter of 0.05 mm thickness was used at a voltage of 35 kV and 500 mA current. All measurements were carried out for 1200 s. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm² Si (Li) semiconductor detector (resolution 150 eV at 5.9 KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software ExWIN integrated with the system¹⁸. Standard reference materials (SRM) from National Institute of Standards and Technology (NIST) – Apple leaf, SRM1515; bovine liver, SRM 1577b; coal fly ash, SRM 1633b; and estuarine sediment, SRM 1646a – were used for quantification of the elements and checking the reliability of the data obtained by the system.

3. Results and discussion

Some of the trace elements known to be essential to our body are Cr, As, Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, Sn, V, Zn, and the other essential major elements are C, H, O, N, S, Ca, P, K, Na, Cl and Mg totaling twenty six essential elements. So the different trace elements in the different medicinal plants will have their definite role for smooth functioning of our body. Almost, fourteen elements viz. K, Ca, Fe, Mn, Zn, Cu, Se, Ti, Cr, Rb, Sr, Br, V, As have been detected in all the plants. The concentrations of the elements in the medicinal plants are given in Table 2. The roles of the detected elements are given below.

Potassium is essential to all organisms as a major cation and is important in nerve action. Na and K ions play an important role in diseases related to renal disorder and diarrhea¹⁹. K is an activator of some enzymes, in particular co-enzyme for normal growth and muscle function. Potassium works with sodium to maintain the body's water balance, thus, helping to maintain the blood pressure²⁰⁻²¹. Thus, potassium has a protective effect against hypertension. From the results obtained, it is observed that the concentration of K ranges from 25,715 µg/g in *Stephania* root to 5,572 µg/g in *Syzygium* leaf. The *Lindernia* leaf and *Stephania* root are found to have appreciable quality of potassium; hence they could be use in the treatment of hypertension.

Calcium is essential for all organisms and used in cell walls, healthy bones, teeth and blood and some cells as structural component; important electrochemically and by coordination. The health of the muscles and nerves depends on Ca. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as pancreatic lipase. The recommended daily allowance for Ca is for children between 500 and 1000 mg and for adults 800 mg. To achieve a Ca level of nearly one percent of the total diet would be rather difficult²². From the data obtained the concentration of Ca ranging from 16,926 µg/g in *Syzygium* to 1,129 µg/g in *Smilax ovalifolia*.

Table 1

Botanical names and uses of various medicinal plants in the treatment of diseases.

Sl. No	Local name	Common/ Eng name	Botanical name of plant	Parts of plants: medicinal use
1	Heibung	Dampel	<i>Garcinia xanthochymus</i>	Fruit: dysentery, jaundice, gout
2	Heinouman	<i>Syzygium fruticosum</i>	Leaf: urinary problems, tonic
3	Koubruyai	<i>Stephania glabra</i> (Roxb)	Root: Diabetes, stomach tumors, leprosy, obesity, gout, paralysis, leucoderma, fever, colic, cough, asthma, rheumatism, amoebiasis, purified blood, eye complaint, backache.
4	Kwamanbi	<i>Smilax ovalifolia</i>	Root: skin diseases, muscular sprain, stomach pain and rheumatic complaint
5	Manahi/ Bahera	Yellow myrobalan	<i>Terminalia chebula</i>	Fruit: chronic ulcers, toothache, bleeding gum, diuretic.
6	Mukthroobi	Winged leaf prickly ash	<i>Zanthoxylum alatum</i> Roxb	Leaf: tonic, fever, dyspepsia, dental troubles, scabbies, insect repellent.
7	Shamba	<i>Ovoxylum indicum</i>	Fruit: Cancer patients, jaundice, dysentery, headache, rheumatism, body ache, worms cases, madness skin tuberculosis, etc.
8	Yenakhat		<i>Lindernia ciliata</i>	Leaf: Jaundice, urinary disturbances, bronchitis, headache, liver complaints, spleen diseases, worms cases, constipation, fever, lost of appetite, asthma, cough, skin diseases.

Table 2Measured concentrations in ($\mu\text{g/g}$) of trace elements in the selected medicinal plants.

Element	<i>Zanthoxylum alatum</i>	<i>Syzygium fruticosum</i>	<i>Terminalia chebula</i>	<i>Lindernia ciliata</i>	<i>Smilax ovalifolia</i>	<i>Stephania glabra</i>	<i>Ovoxylum indicum</i>	<i>Garcinia xanthochymus</i>
K	18,586 \pm 1783	5,572 \pm 388	6,022 \pm 604	25,236 \pm 917	7,392 \pm 818	25,715 \pm 595	19,339 \pm 1823	11,568 \pm 341
Ca	11,785 \pm 485	16,926 \pm 892	9,278 \pm 692	9,958 \pm 166	11,29 \pm 105	9,441 \pm 229	3,880 \pm 338	2,137 \pm 75
Fe	398 \pm 32	191 \pm 11	202 \pm 14	5039 \pm 339	2128 \pm 387	384 \pm 28	154 \pm 21	564 \pm 18
Mn	68 \pm 4	146 \pm 8	86 \pm 6	1,981 \pm 68	56 \pm 7	35 \pm 3	40 \pm 3	46 \pm 2
Zn	82 \pm 6	36 \pm 2	64 \pm 6	78 \pm 2	61 \pm 3	70 \pm 4	34 \pm 2	32 \pm 1
Cu	10.2 \pm 0.4	21 \pm 1	13 \pm 1	11.4 \pm 0.5	11 \pm 1	21 \pm 4	27 \pm 1	10 \pm 1
Se	2.8 \pm 0.2	2.7 \pm 0.3	2.2 \pm 0.3	3.04 \pm 0.6	8.9 \pm 0.6	10.4 \pm 0.5	3.1 \pm 0.6	12.8 \pm 0.4
Ti	29 \pm 9	20 \pm 7	26 \pm 15	826 \pm 66	219 \pm 49	21 \pm 6	8 \pm 1	6.3 \pm 0.8
Cr	10 \pm 6	35 \pm 6	14 \pm 8	25 \pm 4	63 \pm 10	23 \pm 8	19 \pm 1	29 \pm 3
Rb	15.3 \pm 0.9	10.1 \pm 0.4	16 \pm 2	54 \pm 3	62 \pm 9	102 \pm 49	56 \pm 5	20 \pm 3
Sr	120 \pm 3	16 \pm 2	152 \pm 5	137 \pm 6	30 \pm 3	126 \pm 5	33 \pm 2	25 \pm 2
Br	1.6 \pm 0.4	-----	16.1 \pm 0.6	3.4 \pm 0.2	5.7 \pm 0.8	1.1 \pm 0.1	5.1 \pm 0.4	1.2 \pm 0.2
V	-----	-----	-----	26 \pm 3	10 \pm 3	-----	-----	-----
As	-----	4 \pm 1	-----	6 \pm 2	-----	-----	-----	10 \pm 2

The probable reason for the traditional use of *Terminalia chebula* (9 mg/g) and *Zanthoxylum alatum* (11 mg/g) in dental problems could be presence of high concentration of calcium in these plants. *Syzygium* and *Zanthoxylum* (Ca values 16 and 11 mg/g, respectively) could be use as the supplement of calcium deficiency in human body. In the present study, calcium was found to be in appreciable amounts in all the plant samples.

Fe occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with haemoglobin, in the oxygenation of red blood cells. It is needed for a healthy immune system and for energy production. Iron deficiency is the most prevalent nutritional deficiency in humans and is commonly caused by insufficient dietary intake, excessive menstrual flow or multiple births. Severe iron deficiency results in anaemia and red blood cells that have a low haemoglobin concentration. Fe is important because it eliminates phlegm and strengthens the function of stomach²². In young children, iron deficiency can manifest in behavioral abnormalities (including reduced attention) reduced cognitive performance and slow growth. In adults, severe iron deficiency anemia impairs physical work capacity. The requirement of Fe for an adult is 20 mg/day and for a child is 10 mg/day. From the results obtained, it is observed that the concentration of Fe ranges from 5039 µg/g in *Lindernia* to 154 µg/g in *Ovoxylum*. The high concentration of iron in *Lindernia* (5039 µg/g) and *Smilax* (2128 µg/g) may be advised to compensate for the treatment of anemia. Hence, the use of *Syzygium fruticosum* (191µg/g) and *Zanthoxylum alatum* (398 µg/g) in general tonic preparation as well as *Stephania glabra* (384 µg/g) as blood purifier.

It is well known that elements such as Cr, V, Zn and Se play an important role in curing diabetes mellitus²³⁻²⁵. Clinical based trials have shown evidences that Cr based supplements act as complementary therapies in type 2 diabetes by increasing tissue sensitivity to insulin. Cr is found in the pancreas, which produces insulin. Chromium is essential functioning as a glucose tolerance factor (GTF)²⁶, an inorganic compound containing glutamic acid, cysteine and niacin. It is also used for insulin signaling for biological role and thus to sugar metabolism and diabetes. Cr also acts as an activator of several enzymes. The requirement of Chromium is 0.05-0.2 mg per day. Deficiency of chromium decreases the efficiency of insulin and increases sugar and cholesterol in the blood. Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be risk factor in arteriosclerotic disease²⁷. From the results obtained, it is observed that Cr content is high in roots of *Smilax ovalifolia* (63 µg/g) and *Syzygium* (35 µg/g), *Lindernia* (29 µg/g), and *Garcinia* (29 µg/g). Hence the use of this medicinal plant may be advised for the treatment and control and diabetics. The traditional used of *Stephania glabra* for the treatment of diabetes could also be correlated with the presence of high concentration of Chromium in the samples analysed.

Among the essential elements, zinc is found to be the most abundant. More than 300 enzymes are known to be related to zinc²⁸. Zn has been found to be related to the eye functions viz. to interact with vitamin A, to modify photoreceptor plasma membranes, to regulate the light-rhodopsin reaction, to modulate synaptic transmission, and to serve as an antioxidant²⁹. It increases the excitability of dopaminergic neurons³⁰. Zinc has a low order of toxicity compared with most of the other trace elements and it enhances the effectiveness of insulin secretion³¹. Zinc is essential to all organisms and it is an important trace element having a definite role in the metabolism, growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis), for skin integrity, bone metabolism and functioning of taste and eyesight³². Clinical materials prove that Zn can have good effect on eliminating ulcer and promoting healing wounds. Zn plays an important role in blood clotting ³³. Zn deficiency can cause hair loss, diarrhea, fatigue, decreased growth rate, slow mental development in infants, and reduced taste activity³⁴. Zn deficiency may contribute to age-related macular degeneration^{35, 36-38}. Zn deficiencies are characterized by recurrent infections, lack of immunity and poor growth. Growth retardation, male hypogonadism, skin changes, poor appetite and mental lethargy are some of the manifestations of chronically Zn deficient human subjects³⁹. The problem of diabetes may be mediated through oxidative stress and though indirectly, Zn plays a key role in cellular antioxidative defence⁴⁰. Zn deficiencies also cause stunning and impaired immune function, both of which are risk factors for diarrheal illness. Behavioral changes and altered morphology in brain were also associated with Zn deficiency⁴¹. The requirement of Zinc is 15 mg per day. From the results obtained, it is observed that the concentration of Zn ranges from 82 µg/g in *Zanthoxylum alatum* to 13 µg/g in *Syzygium fruticosum*. The use of *Garcinia xanthochymus* and *Ovoxylum indicum*, *alatum* in the treatment of dysentery could be correlated with the presence of zinc. The high concentration of zinc in *Zanthoxylum alatum*, *Lindernia ciliata* and *Smilax ovalifolia* suggests its possible use in tonic, treatment of worms and skin disease⁴². The appreciable high concentration of Zn in *Stephania glabra* may also be helpful in treatment of eye trouble. The traditional used of *Terminalia chebula* for the treatment of ulcer could also be correlated with the presence of high concentration

of zinc. *Zanthoxylum*, *alatum* *Lindernia ciliata* and *Stephania glabra* are found to have appreciable have quality of zinc; hence, they could possibly be use in the treatment of diabetes.

The function Vanadium will be probably part of protection system against injury of tissues. Vanadium salts also play a major role in the treapeutic efficacy in treating diabetes mellitus 43, 44. The presence of Vanadium (26 $\mu\text{g/g}$) and Zinc (78 $\mu\text{g/g}$) in *Lindernia* root could possibly be use in the treatment of diabetes.

Mn is an essential element required for various biochemical processes⁴⁵. Manganese is an antioxidant nutrient⁴⁶ and is important in the breakdown of amino acids and production of energy. It is essentially required for the metabolism of vitamins B1, C and E and for the activation of various enzymes which are important for the proper digestion, utilization of foods, and, hence, in regulating immune response of the body. The functional values of Manganese are as a Lewis acid and catalyst for oxidation. It is essential to all organisms, activates numerous enzymes. It helps in eliminating fatigue and reduces nervous irritability 47-49. Hence, the use of *Lindernia ciliate* (1981 $\mu\text{g/g}$) in medicinal preparations may help to supplement Mn for various body functions. The requirement of Manganese is 2.5-5.0 mg per day. Copper is an essential micronutrient for the body and is necessary for the growth, development, and maintenance of bone, connective tissue, brain, heart, and many other body organs. Copper stimulates the immune system to fight infections, repair injured tissues, and promote healing. *Ovoxyllum indicum* is traditionally used for treating mental disorder. The probable reason for this could be the presence of high concentration of Cu (27 $\mu\text{g/g}$) in *Ovoxyllum indicum* (fruit) with the neuro regulatory action.

Through an intervention study the protective effects of selenium were discovered. The research showed that supplementation with selenium had protective benefits, but could not reverse heart failure⁵⁰. Selenium is the constituent of glutathoioine peroxidase and other enzymes and has the antioxidant property. Selenium also plays an important role in the redox reactions of metallothionein and glutathione⁵¹⁻⁵². Selenium counteracts the oxidative effects of various heavy metals by sequestering them in non-toxic complex, as in brain⁵³. The deficiency of Se may lead to muscle and pancrease degeneration, haemolysis. Symptoms of Se deficiency include muscle pain, weakness, and loss of pigment in the hair and skin and the whitening of the nail beds⁵⁴. The requirement of Se is 55 $\mu\text{g/g}$ per day. The Se content of *Stephania* (10.4 $\mu\text{g/g}$) and *Garcinia* (12.8 $\mu\text{g/g}$) reflects its possible use for the treatment of liver complaints (jaundice) and skin diseases. The presence of high concentration of selenium in *Smilax ovalifolia* (8.9 $\mu\text{g/g}$) in the traditional use for the treatment of muscular pain, skin diseases, stomach pain and rheumatic complaint can be understood from this. Though Arsenic is considered as toxic element, at lower concentration it plays a role in the metabolism of methyl compounds and the deficiency of it would lead to impairment of growth, reproduction and heart function. The presences of arsenic in *Syzygium* bark (36 $\mu\text{g/g}$) *Lindernia* leaf (6 $\mu\text{g/g}$) and *Garcinia* fruit (10 $\mu\text{g/g}$) may be due to the elemental uptake by the plants from the arsenic contaminated soil of Kakching area. The World Health Organisation Maximum Tolerable Daily Intake (WHO-MTDI) value of As is 2 $\mu\text{g day}^{-1} \text{ Kg}^{-1}$ body weight⁵⁵. Hence suggests appropriate care must be taken if these plant parts are used as supplement of other nutrient except As. The function of Titanium is known yet. It is harmless to our body. Bromine may be essential to in mammals. It is non-toxic except in oxidizing forms. The function of Rubidium and strontium are not known to human body. The Karl Pearson's coefficient of correlation is shown in Table 3. As evident from the table, the highest coefficient of correlation 0.997 is found in between V and Fe; followed by V and Ti; V and Mn with 0.991, 0.924 coefficients. So, the coefficient of correlation among the four elements viz. Fe, Mn, Ti, V are almost high leading to the conclusion that if the concentration of one of the elements increases, the remaining three elements will also increase.

4. Conclusion

The variation of concentration of elements in different plants is observed even if they were collected from the same area. This will be due to the absorption of different elemental concentration from the soil by the different plants. So the medicinal plants are used for specific purpose. The medicinal plants analysed so far, contain Se which has strong antioxidant property leading to the potential use for cancer treatment.

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Table 3

Karl Pearson's co-efficient of Correlation of different elements.

Elements	K	Ca	Fe	Mn	Zn	Cu	Se	Ti	Cr	Rb	Sr	Br	V	As
K	1	0.025	0.367	0.467	0.449	0.188	0.050	0.408	-0.412	0.631	0.450	-0.379	0.351	0.041
Ca		1	-0.083	0.182	0.249	0.171	-0.589	0.030	-0.398	-0.312	0.324	-0.178	-0.058	-0.088
Fe			1	0.919	0.444	-0.409	-0.079	0.987	0.278	0.246	0.257	-0.060	0.997	0.307
Mn				1	0.406	-0.258	-0.285	0.966	-0.052	0.118	0.386	-0.068	0.924	0.375
Zn					1	-0.461	-0.221	0.457	-0.213	0.254	0.828	0.148	0.436	-0.387
Cu						1	-0.202	-0.341	-0.150	0.345	-0.278	-0.139	-0.363	-0.341
Se							1	-0.196	0.423	0.404	-0.310	-0.343	-0.139	0.372
Ti								1	0.156	0.211	0.329	-0.033	0.991	0.291
Cr									1	0.225	-0.586	-0.149	0.276	0.079
Rb										1	0.159	-0.165	0.244	-0.319
Sr											1	0.415	0.257	-0.295
Br												1	-0.025	-0.383
V													1	0.264
As														1

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