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RESEARCH ARTICLE

CONCENTRATION OF TRACE ELEMENTS IN SELECTED INDIAN ANTIDIABETIC MEDICINAL PLANTS BY USING EDXRF TECHNIQUE

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ABSTRACT

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Diabetes mellitus has experimentally been shown to be managed by medicinal plant extracts. Among the factors attributable to the hypoglycemic potential of the medicinal plants, are the trace elements present in them. This study was designed to determine the content of trace elements in nineteen Indian antidiabetic medicinal plants traditionally used to manage diabetes mellitus , using Energy Dispersive X-ray Fluorescence (EDXRF) technique. P, S, Cl , K , Ca, Mn , Fe, Cu, Zn, Br , Se, Rb and Sr were identified and their contents estimated. The results of the present study provide justification for the usage of these medicinal plants in the treatment of diabetes mellitus since they are found to contain the elements, Cr, Zn, Cu, Ni, Mn and Se which play vital roles in blood glucose reduction, thereby aiding in management of diabetes mellitus. Our results show that the analyzed medicinal plants can be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus. Moreover, these results can be used to set new standards for prescribing the dosage of the herbal drugs prepared from these plant materials.

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INTRODUCTION

Diabetes mellitus has been shown to be associated with abnormalities in the metabolism of some trace elements such as Zinc, Chromium, Copper, Magnesium and Manganese^{1,2}.

Macro and micronutrients have been investigated as potential preventive and therapeutic agents for diabetes mellitus and common diabetic complications of diabetes ^{3,4}. Literature regarding the active components of such types of plant extracts, which may be responsible for lowering blood glucose levels, is still unavailable. Believably, some trace elements, such as Zinc, Copper, Manganese, and Selenium, play a major role in protecting the insulin secreting pancreatic -cells, which are sensitive to free radical damage.

It is against this background that this study was designed in order to determine the presence, identity and levels of antidiabetic trace elements in nineteen medicinal plants traditionally used in management of diabetes mellitus in India. The nineteen plants have previously been bioscreened for their antidiabetic potential and found to lower blood glucose levels appreciably in alloxan-induced diabetic mice 5.6. It is justifiably postulated that their hypoglycemic potential is due to, among others, antidiabetic trace elements contained in them.

MATERIALS AND METHOD

Experimental details

Sampling

Nineteen different anti-diabetic 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

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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. The anti-diabetic activity of these plants is clearly documented in literature

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° 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° to the target position and at an angle of 90° 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.

RESULTS

Upon analysis of trace elemental composition of the nineteen antidiabetic medicinal plants, it was found that iron, zinc, selenium, manganese, calcium and copper were present in detectable quantities in all the nineteen plants at varying levels; Zinc was present in detectable quantities in two plants Abelmoschus moschatus Medik, Bnincasa hispida. The concentration of Calcium varies from (51655 $ppm \pm 2594$ ppm). Calcium is found to be high in Aegle marmelos (51655 \pm 2594 Ppm) (MP15), Annona Squamosa ррт (38524±632ppm), (*MP10*) Bahuniaaccuminata and (36550±298.09ppm (MP19). The concentration of Mn varied from 2329.24 ppm to 19.05 ppm In our study leaves of Strychnosnux Vomica L (MP-13) also have Manganese 2329.24 ppm and followed by Lawsonia alba (MP-3) 249.50 ppm .The values varied from 2329.24 ppm to 19.05 ppm. In the present study Selinium is found to be present in most of the plant samples and their concentrations are significant varies from 1.79 ppm to 0.15ppm. To found to be high in Carica papaya (Mp-14). Capparis Zevalancica L (Mp-06). Copper was detected in appreciable quantities in Sesbaniajrandiflora (Mp-7) and Bnincasahispida (MP 15).

DISCUSSION

The hypoglycemic activity of the nineteen medicinal plants can justifiably be attributed to, among others, the trace elements in them. These metal ions play vital roles in hypoglycemic antidiabetic activity. Literature is rich with information regarding the mode of antidiabetic activity of these elements.

It was established in this study that zinc was present in all the studied plants in significant quantities (Table 1 and 2). Abnormalities in the metabolism of chromium and zinc have been associated with diabetes. Impairment of Manganese and zinc status has been reported as aggravating factors in the progression of diabetes.

S.no	Sample No	Botanical name	Local name	Used part
1	MP1	Andrographics paniculatta	Nelavemu	leaves
2	MP2	Aegle marmelos	Bilva	pulp
3	MP3	Lawsonia alba LAM	Gorintha	leaves
4	MP4	Azadiraktha indica	Vepa	leaves
5	MP5	Aegle marmelos	Maredu	bark
6	MP6	Capparis Zevlancia L	Pudeena	leaves
7	MP7	Sesbaniagrandiflora	Avisa	seeds
8	MP8	Melochia corchorifolia L	Pindikura	leaves
9	MP9	Coriandrum sativum	Kottimeera	leaves
10	MP10	Annona Squamosa	Seetaphalam	leaves
11	MP11	Saraca asoca	Seeta asoka	bark
12	MP12	Panicum frumentaceum	Korralu	seeds
13	MP13	Strychnos nux-vomica	Musini	leaves
14	MP14	Carica papaya	Boppai	seeds
15	MP15	Bnincasahispida	Budita gummadi	seeds
16	MP16	Lady finger	Benda	fruit
17	MP17	Luffa acutangula	Beera	fruit
18	MP18	Abelmoschus moschatus Medik	Karpoora benda	seeds
19	MP19	Bahunia accuminata	Devakanchana	leaves

 Table 1 List of medicinal plants and their useful parts with sample code

Zinc (**Zn**) and insulin concentrations in the pancreas change in the same direction in a variety of situations in humans³. Zinc plays vital roles in insulin biosynthesis, storage, and secretion¹⁸. It no wonder zinc deficiency is associated with a number of metabolic disturbances including impaired glucose tolerance, insulin degradation, and reduced pancreatic insulin content⁹. It is also postulated to improve glycaemia, and a restored Zinc status in type 2 diabetics may counteract the deleterious effects of oxidative stress, thereby helping to prevent complications associated with diabetes mellitus²⁰. In othe present study the concentration of zinc is 96.64 ppm very high in Bnincasahispida(MP 15).

Copper (Cu) was detected in appreciable quantities in Sesbaniajrandiflora (Mp-7) and Bnincasahispida (MP-15) in (table 1). Owing to its redox chemistry, copper is both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical. Although virtually all cells must acquire copper to drive important biochemical reactions, the potential toxicity of copper demands an exquisite level of vectorial transport and homeostatic control²¹. Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications²².

Manganese(Mn) was found in detectable quantities in the leaves of Strychnosnux Vomica L (MP-13) also have Manganese 2329.24 ppm and followed by Lawsonia alba (MP-3) 249.50 ppm. The values varied from 2329.24 ppm to 19.05 ppm. It acts as a cofactor for the antioxidant enzyme, Manganese Superoxide Dismutase (MnSOD). The levels of this enzyme are reported to be lower in the white blood cells of diabetics than in those of non diabetic controls. By still obscure mechanisms, Manganese is essential for glucose metabolism and deficiency may result in glucose intolerance similar to diabetes mellitus in some animal species. However, studies examining the Manganese status of diabetic humans have generated contradictory results, where diabetics showed lower level of Manganese than that of normal subjects but the difference did not reach to the level of statistically significant difference

Selenium (Se), an essential trace element, is a critical component of numerous seleno proteins involved in antioxidant defence systems, such as glutathione peroxidase, which actively protect against damage from free radicals and reactive oxygen species.

Table 2 Average elemental concentration with (\pm) standard deviations in ppm

Sample no	Р	S	Cl	K	Ca	Mn
MP-1	953.46±44.17	2110.80±84.24	4698.70±117.4	16927.5±243.25	17884.31±364.68	143.39±1.27
MP-2	937.41±66.55	577.27±25.59	579±0	12259.15±389.17	2943.36±34.29	22.35±1.68
MP-3	607.12±22.52	999.75±18.29	1596.03±26.8	7941.63±68.82	10119.81±34.58	249.10±4.65
MP-4	2343.93±52.83	1995.34±25.31	14374.53±184	19162.16±303.76	19178.50±493.57	64.74±1.38
MP-5	272.90±33.4	812.45±8.55	579±0	1340.31±41.64	51655.28±2594.18	19.84 ± 1.64
MP-6	3661.43±94.47	3181.25±65.33	9298.68±563.86	16278.33±375.74	10172.9±235.4	70.79±1.63
MP-7	6749.53±639.38	3355.55±296.25	579.0±0	8740.24±635.79	2517.51±210.07	83.85 ± 5.98
MP-8	2538.01±351.38	2557.16±187.8	18249.82±1901	27790.83±2233.	13481.0±481.66	35.01±1.68
MP-9	4339.03±151.75	2542.89±79.33	24413.15±757	31646.24±579.9	9383.37±147.46	77.69±1.85
MP-10	734.48±14.35	1548.95±28.67	1915.22±61.12	5663.78±166.12	38524.18±632.51	43.33±2.71
MP-11	972.69±52.75	830.07±11.46	579.0±0	3727.72±52.01	24424.52±142.06	52.48±2.14
MP-12	10821.41±367.44	1603.42±15.42	579.0±0	7197.21±160.3	969.36±20.08	36.36±1.8
MP-13	1025.1±28.34	4678.38±114.45	3291.52±71.57	10701.57±121.5	20163.27±195.28	2329.24±18.3
MP-14	8671.54±202.4	10747.63 ± 420.88	579.0±0	9540.12±58.85	9255.50±306.27	49.78±0.9
MP-15	15944.70±523.17	3105.81±2333.73	4389.52±231.03	16512.63±528.56	4349.5±115.87	106.46±4.36
MP-16	6320.64±198.69	2200.07±54.42	10781.19±515	21212.67±642.35	6349.75±293.47	37.43 ± 2.40
MP-17	8029.98±116.08	1696.53±51.46	10942.60±173	28341.96±218.92	4040.11±27.76	19.05±1.22
MP-18	10130.30±477.88	3084.58±88.02	2064.32±114	9661.10±896	8093.63±330.25	76.74±2.73
MP-19	899.64±34.53	1658.39±37.43	1125.30±44.34	5067.41±127.79	36550.85±298.09	92.89 ± 2.46

Table 3 Average elemental concentration with (±) standard deviations in ppm

		U				11	
Sample No	Fe	Cu	Zn	Se	Br	Rb	Sr
MP-1	2955.18±77.58	5.84±0.29	44.47±1.96	0.26±0.23	23.17±1.10	19.19±0.72	128.20±2.36
MP-2	2707.64±58.69	7.14 ± 0.98	8.13±0.45	0.39±0.47	2.63±0.26	19.46 ± 1.09	16.18±0.86
MP-3	3704.9±78.22	11.39±0.99	11.86 ± 0.41	0.19 ± 0.06	27.01±0.8	35.64±2.44	128.90±3.99
MP-4	1075.53±7.28	26.66±0.48	0.25 ± 1.94	0.25±0.21	51.21±1.51	23.02±0.33	114.02±6.31
MP-5	475.80±44.53	5.26 ± 41.64	1.26 ± 0.85	0.33±0.11	1.24 ± 0.28	1.15 ± 1.08	476.17±20.81
MP-6	691.3±22.38	13.73±0.76	35.19±1.35	0.15±0.04	18.09±0.66	57.01±1.48	53.76±1.09
MP-7	2312.07±82	24.47±0.27	79.36±6.29	1.12±0.45	1.10 ± 1.42	22.28±1.76	10.54±1.93
MP-8	547.86±36.97	5.46 ± 0.66	19.30±1.5	$0.73 \pm .54$	28.0±1.19	21.76±1.26	86.55±4.38
MP-9	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-10	1209.78±131	7.14 ± 0.82	35.36±0.61	0.51±0.62	18.46±0.25	4.81±1.95	192.32±6.06
MP-11	492.14±10.87	10.84 ± 0.7	37.74±1.46	0.97±0.61	6.41±1.77	10.19 ± 2.38	102.91±2.33
MP-12	181.82±13.63	16.63±0.63	80.28±5.03	0.43±0.54	2.31±0.75	25.34±1.21	6.03±2.6
MP-13	303.96±2.57	3.61±1.69	4.26 ± 1.12	0.58 ± 0.4	137.79±1.95	41.26±3.12	69.06±1.95
MP-14	217.13±5.19	11.74 ± 1.82	77.70±6.38	1.79 ± 2.37	2.17±0.74	14.06 ± 1.96	28.85±3.19
MP-15	322.44±7.67	22.81±1.71	96.64±6.8	0.48 ± 0.44	5.51±1.02	8.48 ± 1.65	11.31±2.67
MP-16	106.30±5.27	11.44±0.6	42.79±1.10	0.73±1	22.66±0.75	21.71±1.7	52.44±2.49
MP-17	315.65±10.05	18.53±1.1	50.07±1.88	0.15±0.06	9.14±1.47	9.94±1.49	19.10±1.04
MP-18	202.37±13.08	16.5±1.95	81.96±6.12	0.28 ± 0.28	3.69±0.22	4.38±1.09	24.95±1.95
MP-19	187.28±4.69	6.37±0.58	23.04±1.1	0.27±0.17	75.37±1.75	15.59 ± 2.37	222.55±13.27

Increased free radical levels impair glucose-stimulated insulin secretion, decrease gene expression of key -cell genes, and induce cell death. Selenium modulates the cellular response and protects against oxidative stress and the production of reactive oxygen In the present study Selinium is found to be present in most of the plant samples and their concentrations are significant varies from 1.79 ppm to0.15ppm. to found to be high in Carica papaya (Mp-14) and Capparis Zevalancica L (Mp-06).

Calcium (Ca) has been suspected as modifiers of diabetes risk. Calcium and Vitamin D insufficiency has long been suspected as a risk factor for type 1 diabetes based on animal and human observational studies. More recently there is accumulating evidence to suggest that altered calcium homeostasis may also play a role in the development of type 2 diabetes {5}. Although the evidence to date suggests that calcium deficiency influences glycemia post response prandial and insulin while supplementation may be beneficial in regulating these processes, the exact mechanisms by which calcium may promote cell function or ameliorate insulin resistance is incomplete. The concentration of varies from 51655 ppm to 2594 ppm Calcium is found to be high in Aegle marmelos $(51655 ppm \pm 2594 Ppm)$ (MP15), Annona Squamosa (38524 \pm 632 ppm), (MP10) and Bahuniaaccuminata (36550±298.09ppm (MP19).

K and Cl act as electrolytes in the human body. Cl acts as an anion of the extracellular, lymph, connective tissue cartilage and bone (Etuk and Mohammed, 2009). Potassium is important, mainly in the intercellular fluid as the primary ion. Potassium together with sodium helps to regulate the water balance within the body. It regulates the transfer of nutrients to the cell, transmits electrochemical impulses and is necessary for normal growth and enzymatic reactions (Shendkar et al., 2011). Potassium is a well proven insulin secretagogue in the intact organism and the isolated pancreas. Insulin is a key defender against exogenous, K load by using intracellular buffering to minimize hyperkalemia before renal excretion (Nguyen et al., 2011; Dluhy et al., 1972). The distributions of the concentration of Cl and K in the studied plants are shown in table. The concentration of Cl ranges from 24413.15±757 ppm to 579 ppm. Pottassium concentration varies between 31646.24±579.9 ppm to 3727.72±52.61 ppm.





Figure1 EDXRF Spectrums of Strychnosnux-vomica L(MP13) "After 2nd image





Sample No

Figure2 Plots of Concentration of elements in ppm in studied samples" After 5th image

CONCLUSION

Interest in the biochemical and clinical consequence of trace element 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. This study has established that the 19 antidiabetic plants analyzed have appreciable quantities of some of the trace elements associated with glucose lowering effects.

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