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Availability of essential trace elements in medicinal herbs used for diabetes mellitus and their possible correlations

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Four plant parts (leaves, roots, fruits and seeds) of twenty samples of sixteen antidiabetic herbs including three commercially marketed capsules have been analyzed for 6 minor (Na, K, Ca, Cl, Mg, and P) and 21 trace (As, Ba, Br, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hg, La, Mn, Rb, Sb, Sc, Se, Sm, Th, V and Zn) elements by instrumental neutron activation analysis (INAA). Further, Ni, Cd and Pb contents were determined by AAS. Elemental data were validated by simultaneously analyzing reference material (RM), MPH-2 Mixed Polish Herbs. Several elements such as Cr and V (1–2 μg/g), Rb (10–40 μg/g), Cs (80–300 ng/g), Se (~100 ng/g) and Zn (25–60 μg/g) play an important role in diabetes mellitus. Interelemental linear correlations have been observed for Cu vs. Zn ($r = 0.89$) and Rb vs. Cs ($r = 0.87$). K/P ratio varies in a narrow range with a mean value of 6.2±1.4. Toxic elements As and Hg were found in <1 μg/g whereas Cd and Pb were in ~5 μg/g and <10 μg/g, respectively.

Introduction

Various plant parts have been a major source of drugs for the treatment of chronic ailments in Ayurveda, the ancient Indian medicine system. Despite tremendous advances in synthetic medicinal chemistry, several diseases are not cured successfully due to their adverse side effects or diminution in response after prolonged use. In recent years a global trend has been noticed for revival of interest in traditional medicines. Diabetes mellitus is one such common endocrine disorder that has affected more than hundred million people worldwide and is on continuous rise. In India, the prevalence rate of diabetes is estimated to be ~8%, especially in urban population.[1] According to the traditional Chinese medicine (TCM) system, diabetes mellitus is referred to as thirst disease which occurs due to excessive heat or fire chiefly due to insulin deficiency.[2] Further complications arise from cardiovascular, cerebro-vascular and peripheral vascular diseases leading to myocardial infarction, strokes and amputations causing morbidity and mortality.[3] Providing modern medical healthcare in developing countries such as India is still a far-reaching goal due to economic constraints. Therefore, it is prudent to look for options in herbal medicine for diabetes.

Continuous clinical research suggests that the body's balance of mineral trace elements is disrupted by diabetes. Conversely, it has also been suggested that early imbalances of essential elements may play an important role in insulin metabolism.[4]. Many plants are considered as a rich source of essential and trace elements and are prescribed because of their good bioavailability and least side effects, in addition to being of low cost.[5] Hence, a search for antidiabetic herbs has been initiated. Ethnobotanical information reports about 800 plants that may possess antidiabetic potential.[6] However, most literature reports pertain to hypoglycemic activities of their extracts[7–11] and isolation of organic constituents.[12,13] Only scanty reports are available on the role of micronutrients,[14,15] which play an important role in the formation of active constituents responsible for their curative properties.[16,17]

A number of techniques such as atomic absorption spectrometry (AAS), voltammetry, inductively coupled plasma atomic emission spectrometry (ICP-AES) and instrumental neutron activation analysis (INAA) is routinely used to determine trace elements in medicinal herbs. INAA is a versatile, non-destructive analytical tool widely used for the determination of minor and trace elements in complex biological samples. Present work was undertaken to determine the minor and trace element concentrations in 20 samples of 16 antidiabetic herbs (commercially marketed as powder or capsule). Two reference materials (RM) of biological origin were used as comparator standards and for evaluating the accuracy of the method with respect to each other.

Experimental

Sample collection and preparation

Four plant parts; leaves of *Vijaysar* (*P. marsupium*), *Neem* (*A. indica*), *Tejpatta* (*C. tamala*) and *Tejpan* (*L. nobilis*); root of *Kutki* (*P. kurrooa*); seeds of *Kali Jeera* (*C. cyminun*) and *Fenugreek* (*T. foenum*) and fruits of *Bitter Gourd* (*M. charantia*), *Amalaki* (*E. officinalis*) and *Jaamun* (*S. cuminii*) in powder form were procured from Vyas Pharmacy, Indore. Similarly, leaves of *Gurmaar* (*G. sylvestre*) and *Palas* (*B. monosperma*); roots of *Giloy* (*T. cordifolia*), and *Naagarmotha*

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(*C. rotundeus*) and *Morarphali* (*H. isora*), a fruit were procured from Yogi Pharmacy, Haridwar. *Jaamun* and *Fenugreek* seeds were procured locally. Capsules of *Neem*, *Garlic* (*A. sativum*) and *Bitter Gourd* were procured from Himalaya Drugs and Pharmaceuticals, Bangalore. All the samples chosen for this study are consumed by middle-income group. The raw material in the form of leaves, roots, fruits and seeds were wiped to clean for any surface contamination, dried under an IR lamp at ~80 °C and crushed to powder in an agate mortar to a uniform particle size (100 mesh). Capsules were cut opened and the powder was used as such. The samples were stored in precleaned polyethylene capped bottles (with dil HNO_3 and rinsed with doubly distilled water and dried) and handled in a glove box. Peach leaves[18] (SRM-1547) and Mixed Polish Herbs[19] (MPH-2) were from NIST (USA) and INCT (Poland), respectively.

Irradiation and counting

About 40 mg each of the samples and RMs in dried form were packed in polythene/aluminum foil and irradiated in batches of 5/10 at a thermal flux of $\sim 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$ for 2 min/3 days, respectively, in the CIRUS reactor at the Bhabha Atomic Research Centre (BARC), Mumbai. For short pneumatic irradiation (2 min), counting was followed by a 40 cm^3 coaxial HPGe detector (EG & G Ortec) and 4k MCA with DOS based pcaiii software at the reactor site. Long irradiated samples (3 d) were air lifted to our laboratory in Roorkee and counting was done for up to 2 months on 80 cm^3 coaxial HPGe detector with 8k MCA (Canberra, USA) and GENIE-2k software for durations of 2, 4, 6 and 12 hours. The detector system had a resolution of 1.8 keV at 1332 keV of ^{60}Co. Elemental contents were calculated by the comparator method using Peach Leaves (SRM-1547) as multielemental comparator. Mixed Polish Herbs (MPH-2) was used for data validation. Phosphorus was determined by measuring the β-activity[20] of ^{32}P on an end window GM counter (Nucleonix, Hyderabad) using Al-absorber of 27 $mg \cdot cm^{-2}$. Statistically significant counts ($>10^4$) were collected so that counting error was <±1%.

Results and discussion

Mean elemental concentrations in MPH-2 Mixed Polish Herbs calculated on the basis of replicate analyses, different irradiations and γ-ray energies are listed in Table 1. A comparison of our data with certified or information values show agreement within ±10% except for Ce, Cl, Hf, Sm and Th. Further, *Z*-score values for all elements in Table 1 and Fig. 1 are below ±2.58 except for Cl, suggesting that all our elemental data must be true within 99% confidence limit. Therefore, it is presumed that all the elemental concentrations in the samples should be accurate and precise within ±10%. Mean concentrations of minor (Na, K, Mg, Ca, P, Cl), essential trace (Ba, Ce, Co, Cr, Cs, Cu, Fe, La, Mn, Ni, Rb, Sc, Se, V and Zn) and toxic (As, Br, Cd, Pb, Sb, Hg, Th) elements in 20 samples of 16 anti-diabetic herbs as obtained from two irradiations/using more than one photo peaks and both RMs as comparators are listed in Tables 2 and 3; ±SD values were calculated on the basis of replicate values. In a few cases where only one value was available, ±SD was calculated on the basis of counting statistics. Four samples of *Bitter Gourd*, *Jamun*, *Neem* and *Fenugreek* were from more than one source. Further, Eu, Hf and Sm were also determined but not included in the tables. All the elements vary in a large concentration range. Also included in these tables are median ±SD. A perusal of mean and median values in Tables 2 and 3 shows that K, Mg, P, Cr, Fe, Cu and Zn are comparable whereas those for As, Br, Ca, Cl, Na, Ba, Mn, Sc, Se, Th and V differ widely. This may be attributed to the difference in geo-environmental factors or soil characteristics where these plants are grown.[21] Implications of elemental data vis-à-vis their role in treatment of diabetes mellitus is discussed below.

Minor constituents

A perusal of elemental data in Tables 2 and 3 shows that K (5.30–60.9 mg/g), Ca (4.98–47.8 mg/g), Mg (0.43–1.92 mg/g), Cl (0.21–11.9 mg/g) and P (0.59–6.11 mg/g) form minor constituents as these are found in 0.02–6% amounts. Also Na (0.03–5.67 mg/g) and Fe (0.11–0.27 mg/g) are found in <0.5% amounts. These electrolytic or structural elements play an important role in the fluid balance.[22] It is observed that Na content shows largest variation by two orders of magnitude whereas Mg varies by a factor of 4 only. Incidentally Na content in *Bitter Gourd* (Karela), a household medicine for diabetes from Vyas pharmaceuticals is highest and so also Cl content in this particular sample. Probably common salt must have been added to suppress its bitter taste. Similarly its capsule powder (Himalaya Drugs) contains elevated amounts of K (60.9±1.8 mg/g) and P (6.11±0.11 mg/g), which could possibly be due to deliberate addition of some preservatives. It is observed that *Vijaysar (P. marsupium)*, the most potent flora against diabetes[23,24] is deficient in Na (0.03±0.01 mg/g), Ca (4.98±0.12 mg/g) and Cl (0.21 ±0.01 mg/g) but enriched in Mg and P. It is observed that our data for K and Cl in *Neem, Fenugreek, Bitter Gourd* and *Naagarmotha* are in excellent agreement with those reported by RAJURKAR and PARDESHI[15] even though these are from widely different places.

Table 1. Elemental concentrations in INCT-MPH-2 Mixed Polish Herbs used for data validation

Element	This work	Certified[18]	Error, %	RSD, %	*Z*-score
As (μg/g)	207 ± 7	191 ± 23	+8.38	3.38	0.70
Ba (μg/g)	34.3 ± 1.8	32.5 ± 2.5	+5.54	5.24	0.72
Br (μg/g)	7.60 ± 0.5	7.71 ± 0.61	–1.43	6.58	–0.18
Ca (mg/g)	10.1 ± 0.9	10.8 ± 0.7	–6.48	8.91	–1.0
Ce (μg/g)	1.36 ± 0.2	1.12 ± 0.10	+21.4	14.7	2.40
Cl (μg/g)	2.16 ± 0.11	2.84 ± 0.20	–23.9	5.09	–3.40
Co (ng/g)	186 ± 15	210 ± 25	–11.4	8.06	–1.36
Cr (μg/g)	1.73 ± 0.02	1.69 ± 0.13	+2.37	1.16	0.31
Cs (ng/g)	82 ± 5	76 ± 7	+7.89	6.10	0.85
Cu (μg/g)	6.78 ± 0.51	7.77 ± 0.53	–12.7	7.52	–1.87
Eu (ng/g)	17.3 ± 0.7	15.7 ± 1.8	+10.2	4.05	0.89
Fe (μg/g)	481 ± 22	460	+4.56	4.57	–
Hf (ng/g)	274 ± 17	236 ± 20	+13.8	6.20	1.90
Hg (ng/g)	19.8 ± 1.6	17.6 ± 1.6	+11.1	8.08	1.38
K (mg/g)	17.9 ± 1.4	19.1 ± 1.2	–6.28	7.82	–1.0
La (μg/g)	595 ± 34	571 ± 46	+4.20	5.71	0.52
Mg (mg/g)	6.27 ± 0.36	–	–	5.74	–
Mn (μg/g)	178 ± 14	191 ± 12	–6.81	7.87	–1.08
Na (μg/g)	365 ± 18	350	+4.29	4.93	–
P (mg/g)	2.38 ± 0.08	2.5	–4.80	3.36	–
Rb (μg/g)	11.2 ± 0.4	10.7 ± 0.7	+4.67	3.57	0.77
Sb (ng/g)	61.6 ± 4.2	65.5 ± 9.1	–5.95	6.81	0.43
Sc (ng/g)	115 ± 4	123 ± 9	–6.50	3.48	–0.89
Se (ng/g)	136 ± 11	–	–	8.09	–
Sm (ng/g)	111 ± 7	94.4 ± 8.2	+17.2	6.31	2.02
Th (ng/g)	173 ± 4	154 ± 13	+12.3	2.31	1.50
V (μg/g)	1.01 ± 0.02	0.95 ± 0.16	+6.32	1.98	0.38
Zn (μg/g)	37.1 ± 1.9	33.5 ± 2.1	+10.7	5.12	1.71

–: No result available.

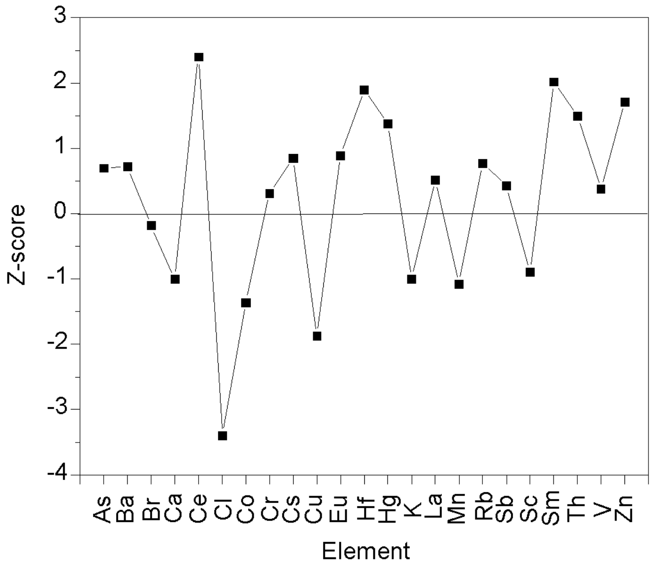

Fig. 1. Z-score plot for elements in MPH-2

Table 2. Concentrations of minor and trace elements in herbs and herbal formulations used as anti-diabetic drugs ($n = 20$)

Sample	Na, mg/g	K, mg/g	Ca, mg/g	Mg, mg/g	Cl, mg/g	P, mg/g	V, μg/g	Cr, μg/g	Mn, μg/g	Fe, μg/g	Co, ng/g	Ni, μg/g	Cu, μg/g	Zn, μg/g
Gurmaar (Y)	0.36 ± 0.01	24.8 ± 0.7	17.3 ± 1.2	0.49 ± 0.02	1.25 ± 0.07	2.32 ± 0.01	2.36 ± 0.16	1.94 ± 0.10	53.2 ± 1.4	261 ± 4	94 ± 8	0.59	13.4 ± 0.8	44.8 ± 1.9
Giloy (Y)	0.08 ± 0.01	19.1 ± 0.6	8.30 ± 0.42	0.83 ± 0.04	2.33 ± 0.16	2.34 ± 0.01	1.69 ± 0.11	1.04 ± 0.04	73.8 ± 2.6	175 ± 3	55 ± 5	1.03	2.46 ± 0.21	32.7 ± 1.3
Jaamun (L)	0.04 ± 0.01	8.90 ± 0.3	35.3 ± 2.80	1.01 ± 0.08	1.49 ± 0.08	1.20 ± 0.03	2.97 ± 0.18	1.96 ± 0.07	42.7 ± 3.1	186 ± 3	58 ± 5	0.90	7.31 ± 0.44	35.2 ± 1.1
Jaamun (V)	0.10 ± 0.01	13.1 ± 0.3	31.6 ± 1.2	1.92 ± 0.08	0.98 ± 0.03	0.99 ± 0.03	2.01 ± 0.11	2.00 ± 0.02	22.8 ± 0.4	147 ± 4	42 ± 3	1.86	4.25 ± 0.39	28.3 ± 1.5
Palas (Y)	0.10 ± 0.01	24.0 ± 0.7	12.6 ± 1.2	0.72 ± 0.04	3.25 ± 0.22	2.75 ± 0.08	0.96 ± 0.05	1.54 ± 0.06	58.6 ± 4.9	231 ± 4	73 ± 6	2.29	15.9 ± 1.1	45.4 ± 2.1
Marorphali (Y)	1.36 ± 0.13	7.10 ± 0.2	15.8 ± 1.3	1.24 ± 0.07	1.68 ± 0.09	0.93 ± 0.02	0.79 ± 0.04	1.79 ± 0.07	22.2 ± 1.4	276 ± 5	115 ± 10	0.79	39.9 ± 2.7	58.9 ± 2.2
Naagarmotha (Y)	1.40 ± 0.04	8.70 ± 0.3	19.2 ± 1.6	0.88 ± 0.05	2.53 ± 0.17	1.28 ± 0.03	1.02 ± 0.06	1.42 ± 0.10	34.3 ± 2.6	214 ± 3	92 ± 8	0.67	8.69 ± 0.53	38.3 ± 1.5
Fenugreek (L)	1.66 ± 0.04	12.2 ± 0.4	18.6 ± 2.3	1.41 ± 0.11	8.63 ± 0.12	1.87 ± 0.05	2.15 ± 0.14	1.47 ± 0.20	24.3 ± 0.8	266 ± 5	91 ± 8	1.38	31.5 ± 2.9	50.6 ± 2.6
Fenugreek (V)	0.08 ± 0.01	16.1 ± 0.2	19.9 ± 0.4	1.22 ± 0.07	6.36 ± 0.31	2.13 ± 0.10	2.47 ± 0.13	1.16 ± 0.14	37.8 ± 2.6	257 ± 24	58 ± 7	0.42	7.17 ± 0.57	30.2 ± 1.8
Neem (H)	0.68 ± 0.02	20.2 ± 0.6	28.3 ± 1.6	0.58 ± 0.04	9.21 ± 0.33	2.89 ± 0.03	1.77 ± 0.12	1.84 ± 0.07	29.2 ± 1.2	173 ± 3	59 ± 5	1.70	7.32 ± 0.33	35.1 ± 1.4
Neem (V)	0.21 ± 0.01	15.7 ± 0.2	24.8 ± 1.9	0.49 ± 0.03	11.9 ± 0.9	1.86 ± 0.06	1.82 ± 0.07	1.58 ± 0.19	32.9 ± 2.1	131 ± 12	46 ± 6	1.05	4.24 ± 0.34	28.6 ± 2.2
Garlic (H)	0.29 ± 0.01	5.30 ± 0.2	7.56 ± 0.27	0.43 ± 0.02	7.03 ± 0.62	0.59 ± 0.01	1.29 ± 0.07	1.01 ± 0.04	28.3 ± 1.5	159 ± 3	55 ± 5	1.04	3.72 ± 0.26	31.5 ± 1.3
B. Gourd (H)	1.55 ± 0.04	60.9 ± 1.8	11.3 ± 1.0	0.49 ± 0.03	10.6 ± 0.4	6.11 ± 0.11	2.26 ± 0.18	1.28 ± 0.05	55.6 ± 3.7	180 ± 3	72 ± 7	1.57	10.5 ± 0.6	35.2 ± 1.3
B. Gourd (V)	5.67 ± 0.17	43.7 ± 1.5	8.63 ± 0.42	1.45 ± 0.13	8.27 ± 0.43	4.36 ± 0.15	2.51 ± 0.14	1.58 ± 0.02	58.8 ± 2.1	242 ± 6	65 ± 4	0.54	3.57 ± 0.22	32.9 ± 2.1
Vijaysaar (V)	0.03 ± 0.01	13.2 ± 0.6	4.98 ± 0.12	0.52 ± 0.03	0.21 ± 0.01	2.31 ± 0.06	1.93 ± 0.13	0.99 ± 0.01	13.7 ± 0.4	216 ± 10	84 ± 5	0.30	5.63 ± 0.47	30.6 ± 1.6
Tejpata (V)	0.22 ± 0.01	9.76 ± 0.2	47.8 ± 3.5	1.36 ± 0.12	6.00 ± 0.01	1.36 ± 0.04	0.88 ± 0.05	0.59 ± 0.01	54.0 ± 3.3	247 ± 8	41 ± 3	1.41	5.13 ± 0.44	49.7 ± 1.8
Kaalijeera (V)	1.04 ± 0.04	14.9 ± 0.9	17.5 ± 0.1	0.97 ± 0.04	6.11 ± 0.16	1.87 ± 0.05	0.91 ± 0.06	1.16 ± 0.01	356 ± 10	260 ± 6	62 ± 4	1.78	6.24 ± 0.28	33.2 ± 1.3
Tejpan (V)	0.19 ± 0.04	14.3 ± 0.8	21.6 ± 0.6	0.69 ± 0.04	3.63 ± 0.20	1.55 ± 0.04	0.64 ± 0.03	0.88 ± 0.01	68.9 ± 4.8	111 ± 3	52 ± 3	2.06	3.72 ± 0.31	23.4 ± 1.2
Amalaki (V)	0.27 ± 0.05	9.03 ± 0.30	13.9 ± 0.8	0.88 ± 0.05	8.32 ± 0.21	1.12 ± 0.07	1.36 ± 0.09	0.27 ± 0.02	29.6 ± 1.9	112 ± 3	37 ± 3	0.89	3.14 ± 0.28	26.5 ± 1.4
Kutki (V)	0.13 ± 0.03	17.2 ± 0.1	24.6 ± 1.6	1.18 ± 0.09	1.96 ± 0.07	2.06 ± 0.08	1.01 ± 0.05	2.15 ± 0.02	64.8 ± 4.2	268 ± 12	54 ± 3	1.28	8.04 ± 0.65	36.1 ± 2.0
Mean±SD:	0.77 ± 1.28	17.9 ± 13	19.5 ± 10.5	0.94 ± 0.41	5.09 ± 3.58	2.09 ± 1.27	1.64 ± 0.69	1.38 ± 0.50	58.1 ± 72.3	206 ± 55	65 ± 21	1.18 ± 0.5	9.59 ± 9.67	36.4 ± 9.1
Median:	0.52 ± 0.29	15.7 ± 3.5	16.6 ± 0.8	0.78 ± 0.06	2.89 ± 0.36	2.10 ± 0.23	1.25 ± 0.24	1.45 ± 0.06	38.5 ± 4.2	200 ± 13	73 ± 5	1.04 ± 0.4	8.91 ± 7.83	36.8 ± 4.2

Sm and Eu were also determined but not included here.
Y = Yogi Pharmacy, Haridwar, H = Himalaya Drugs and Pharmaceuticals, Bangalore, India; V = Vyas Pharmacy, Indore and L = Local.

Table 3. Concentrations of essential trace and toxic elements in herbs and herbal formulations used as anti-diabetic drugs ($n = 20$)

Sample	Ba, μg/g	Rb, μg/g	Se, ng/g	Cd, μg/g	Cs, ng/g	As, ng/g	Br, μg/g	Ce, μg/g	Hg, ng/g	Sb, ng/g	La, μg/g	Pb, μg/g	Sc, ng/g	Th, ng/g
Gurmaar (Y)	40.3 ± 2.3	25.3 ± 1.6	126 ± 7	6.73	152 ± 11	635 ± 40	25.1 ± 1.5	4.69 ± 0.74	67 ± 3	22.1 ± 1.5	3.07 ± 0.14	11.4	103 ± 5	56 ± 4
Giloy (Y)	46.5 ± 2.7	11.1 ± 0.5	121 ± 8	4.75	84 ± 4	279 ± 18	1.29 ± 0.1	1.81 ± 0.22	69 ± 4	15.7 ± 1.3	1.03 ± 0.05	14.2	33 ± 2	21 ± 2
Jaamun (L)	81.6 ± 4.3	13.8 ± 0.8	73 ± 5	4.95	107 ± 9	489 ± 36	1.30 ± 0.1	1.60 ± 0.20	51 ± 5	42.7 ± 0.6	0.68 ± 0.03	5.33	29 ± 1	17 ± 1
Jaamun (V)	71.8 ± 1.9	17.2 ± 0.4	98 ± 6	6.03	124 ± 6	277 ± 8	1.50 ± 0.1	1.99 ± 0.20	43 ± 3	38.9 ± 4.1	0.90 ± 0.06	10.2	182 ± 6	146 ± 7
Palas (Y)	33.7 ± 2.5	28.8 ± 1.7	126 ± 6	3.15	143 ± 11	796 ± 49	103 ± 6.4	1.94 ± 0.24	88 ± 6	27.0 ± 1.7	1.07 ± 0.05	8.95	36 ± 2	24 ± 7
Marorphali (Y)	67.9 ± 3.9	22.5 ± 1.3	169 ± 12	5.63	148 ± 17	827 ± 53	17.5 ± 1.1	11.0 ± 1.3	92 ± 7	27.4 ± 9.7	8.35 ± 0.38	9.15	93 ± 5	474 ± 36
Naagarmotha (Y)	58.2 ± 3.4	22.0 ± 1.3	1044 ± 70	4.88	113 ± 15	930 ± 60	16.5 ± 1.0	15.8 ± 1.9	47 ± 3	18.0 ± 1.1	10.3 ± 0.5	14.8	80 ± 5	291 ± 17
Fenugreek (L)	99.3 ± 5.7	11.0 ± 0.6	291 ± 19	4.95	106 ± 8	569 ± 41	89.9 ± 5.5	2.54 ± 0.31	143 ± 9	24.6 ± 1.4	1.83 ± 0.08	2.70	55 ± 3	36 ± 3
Fenugreek (V)	81.9 ± 5.6	23.8 ± 2.0	72 ± 4	3.20	159 ± 7	156 ± 12	6.86 ± 0.3	3.58 ± 0.59	32 ± 2	25.0 ± 1.6	3.59 ± 0.26	7.48	229 ± 16	365 ± 27
Neem (H)	204 ± 12	14.6 ± 0.9	100 ± 7	2.78	88 ± 7	358 ± 29	398 ± 48	3.31 ± 0.41	67 ± 4	20.2 ± 0.9	1.12 ± 0.05	4.80	32 ± 2	173 ± 11
Neem (V)	201 ± 14	27.5 ± 2.3	80 ± 5	4.08	165 ± 8	211 ± 15	36.8 ± 1.6	3.18 ± 0.53	35 ± 2	15.6 ± 1.0	3.65 ± 0.26	4.20	291 ± 24	314 ± 21
Garlic (H)	23.6 ± 1.4	4.15 ± 0.2	267 ± 13	2.95	57 ± 3	868 ± 55	72.4 ± 4.4	1.42 ± 0.18	63 ± 4	43.3 ± 3.6	0.73 ± 0.03	5.15	29 ± 2	162 ± 9
B. Gourd (H)	33.8 ± 2.0	39.1 ± 2.8	91 ± 6	3.70	158 ± 13	1013 ± 65	433 ± 27	1.56 ± 0.19	33 ± 2	53.4 ± 3.7	0.99 ± 0.05	11.5	29 ± 1	162 ± 9
B. Gourd (V)	37.3 ± 1.0	44.7 ± 1.1	135 ± 9	4.25	185 ± 12	1439 ± 123	203 ± 6	2.05 ± 0.20	54 ± 3	55.1 ± 8.1	2.18 ± 0.10	6.65	138 ± 5	119 ± 6
Vijaysaar (V)	51.2 ± 1.4	19.4 ± 0.5	116 ± 7	3.33	128 ± 7	277 ± 7	18.5 ± 1.3	2.11 ± 0.21	30 ± 2	34.7 ± 3.5	6.59 ± 0.10	9.23	62 ± 2	397 ± 23
Tejpata (V)	31.2 ± 0.8	15.9 ± 0.4	93 ± 5	4.90	81 ± 4	803 ± 22	74.1 ± 2.1	2.59 ± 0.25	27 ± 2	21.9 ± 1.6	3.51 ± 0.10	4.75	27 ± 1	129 ± 10
Kaalijeera (V)	68.7 ± 1.8	51.5 ± 1.2	250 ± 18	2.90	279 ± 18	441 ± 21	18.7 ± 0.6	5.90 ± 0.54	68 ± 4	34.6 ± 4.4	1.04 ± 0.08	6.80	14 ± 1	83 ± 6
Tejpan (V)	71.9 ± 1.9	27.8 ± 0.6	112 ± 5	4.28	114 ± 12	125 ± 5	70.8 ± 0.5	3.47 ± 0.34	38 ± 2	23.6 ± 3.0	0.99 ± 0.08	9.55	56 ± 2	87 ± 5
Amalaki (V)	54.0 ± 1.5	28.6 ± 0.7	153 ± 9	5.88	97 ± 8	129 ± 4	4.07 ± 0.1	2.08 ± 0.20	42 ± 3	21.8 ± 2.8	0.33 ± 0.06	2.93	63 ± 2	168 ± 8
Kutki (V)	42.4 ± 1.1	42.9 ± 1.1	227 ± 13	4.88	278 ± 19	99 ± 4	2.62 ± 0.2	8.39 ± 0.82	23 ± 2	26.1 ± 1.6	0.51 ± 0.01	6.30	115 ± 4	236 ± 18
Mean±SD:	70.0 ± 49.5	24.6 ± 12.3	187 ± 212	4.41 ± 1.12	138 ± 58	536 ± 367	79.7 ± 125	4.05 ± 3.70	55.6 ± 28.5	29.6 ± 11.7	2.62 ± 2.78	7.80 ± 3.39	85 ± 74	173 ± 134
Median:	52.4 ± 36.1	17.9 ± 4.1	244 ± 103	4.28 ± 1.06	110 ± 3	868 ± 10	48.8 ± 94.7	2.24 ± 2.96	65 ± 19	24.8 ± 1.6	1.09 ± 2.11	8.95 ± 2.97	35 ± 12	232 ± 98

Y = Yogi Pharmacy, Haridwar, H = Himalaya Drugs and Pharmaceuticals, Bangalore, India; V = Vyas Pharmacy, Indore and L = Local.

Essential trace elements

It is observed that most elemental concentrations vary by an order of magnitude except Co (65±21 ng/g), Cr (1.38±0.50 μg/g), Fe (206±55 μg/g), V (1.64±0.69 μg/g) and Zn (36.4±9.1 μg/g), where variations are in a narrow range. This variation in elemental concentrations might be due to the differential uptake by the plants from the soil or due to inherent nature of the plant species[21] grown in that region. Bar plots of V, Cr, Mn, Fe, Co, Cu, Zn, Rb, Cs and Se all essential trace elements (Fig. 2) exhibit wide variations with Co being in least amount (65±21 ng/g) and Mn the highest (58.1±72.3 μg/g). It is observed that mean concentration of Cu, Mn, Rb and Zn are in >10 μg/g whereas Cr and V are in >1 μg/g amounts.

Elements of importance in diabetes

It is well known that elements such as Cr, V, Zn, and Se play an important role in curing diabetes mellitus.[17,24,25] In general, 20 herbal samples analyzed in this study can be broadly classified into three groups; plant parts such as root, leaves, fruits and seeds, commercially available herbal powders and some powders marketed as capsules (from Himalaya Drugs). Some of these are vegetable (*Bitter Gourd*) and others are spices (*Fenugreek seeds* and *Tejpatta*). In general, no single plant part is enriched in all the elements. *Tejpatta*, leaves used as a spice in Indian curry preparations, is rich in Ca (47.8±3.5 mg/g) while roots like *Kutki* and *Naagarmotha* are enriched in Cr (2.15±0.02 μg/g) and Se (1.04±0.07 μg/g), respectively. Clinical based trials have shown evidences that Cr based supplements act as complementary therapies in Type 2 diabetes by increasing tissue sensitivity to insulin.[26] Selenium is a dietary antioxidant and its supplement can be a useful therapeutic measure to delay the onset of diabetic nephropathy.[27] *Marorphali* (a fruit) contains elevated concentrations of Fe, Cu and Zn, three essential elements of importance in diabetes. HEIKKILA and CABBAT[28] observed that an iron chelate of diethylenediaminepentaacetic acid (DETPAC) given to Swiss-webstar mice protects them from diabetogenic action of alloxan. The problem of diabetes may be mediated through oxidative stress and though indirectly, Zn plays a key role in cellular antioxidative defence.[29] Earlier we have observed ~14% lowering of Zn concentration in blood of diabetic patients.[28] Seeds of *Jaamun* and *Kaalijeera* are considerably enriched in V (2.97±0.18 μg/g) and Mn (356±10 μg/g), respectively. SEKAR et al.[31] and FUGUNO and SAKURAI[32] have shown that V salts play a major role in the therapeutic efficacy in treating diabetes mellitus. MARZBAN and MCNEILL[33] observed that treatment of STZ-diabetic Wistar rats with bis (maltolato) oxovanadium(IV) in a dose of 0.75–1 mg/ml in drinking water for 3 weeks corrected hyperglycemia. Mn is considered to play an important role in controlling diabetes[34] though it varies in a wide range (13.7–356 μg/g) in the samples analyzed.

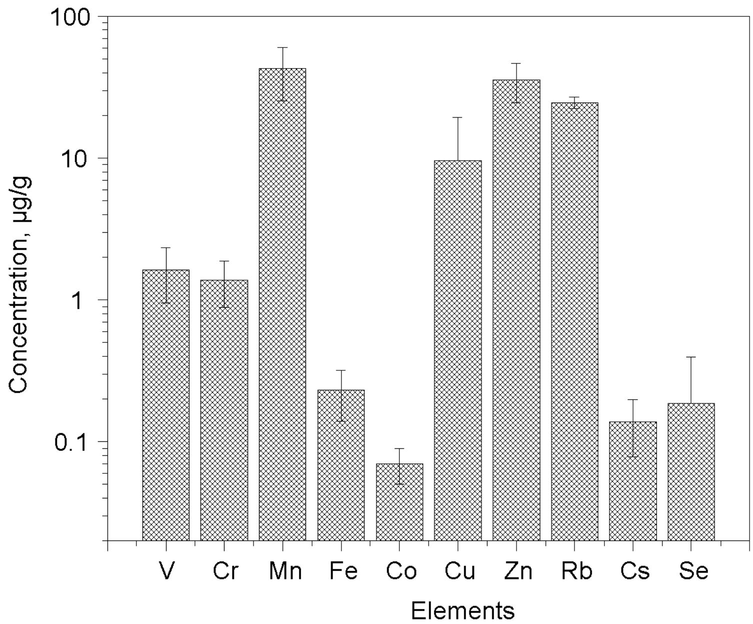

Fig. 2. Variation in mean concentration of essential trace elements in 20 medicinal herbs used in diabetes mellitus

The differing Mn levels can be attributed to the selective uptake by the plants from the soil[35] and not to any other parameter connected with essentiality. Out of two samples of *Jaamun* seed powder, the one from Vyas Pharmacy contained higher amounts of Na, K, Mg, Rb and Cs while the local variety was enriched in P, V, Mn, Fe, Cu and Zn. Similarly *Bitter Gourd* powder from Vyas Pharmacy is enriched in Na, Mg and Se whereas the capsules from Himalaya contained significantly higher amounts of K, Ca, Cl, P but comparable amounts of Cu. Cr, Mn, Fe, Zn, Rb, Cs and Se. These observations suggest possible addition of a preservative to enhance shelf life of capsules. Also *Neem* powder in capsule is enriched in Na whereas all other elements are comparable with the sample from Vyas Pharmacy.

Also, contents of four rare earth elements (REE) La (0.33–10.3 μg/g), Ce (1.42–15.8 μg/g), Sm (83–903 ng/g) and Eu (5.3–159 ng/g) were determined which vary by an order of magnitude. It is reported that the REE uptake may be governed by the plant species, growing season and environmental factors including soil characteristics.[21] However, there are no precise evaluations for the long-term biological effects of REE on the human health though some acute toxicity and sub chronic toxicity tests have shown that low doses of REE had no significant teratogeny and mutagenicity.[36,37]

Toxic elements

Contents of toxic elements in *Ayurvedic* drugs have been a matter of concern because of ban imposed by several western countries. Health Canada issued a warning to consumers[38]and made a public list of “unapproved Indian *Ayurvedic* products” including *Bitter Gourd* (Himalaya) on its website in July 2005. Its both forms (from Himalaya as well as Vyas Pharmacy) were found to contain As >1 μg/g compared to its permissible limit of 0.5 μg/g specified by the US FDA.[39] It may probably be due to spray of pesticides where good agricultural practice may not have been followed[40] or due to inherent nature of the *Bitter Gourd* plant itself. Also Br content is >200 μg/g. Hg content varies in a wide range of 23–143 ng/g but well below the permissible limit of 1 μg/g. Some of these elements such as As and Hg form an integral part of Ayurvedic medicines because of their therapeutic properties. Also mean contents of Cd (2.78–6.73 μg/g) and Pb (2.70–14.8 μg/g) determined by AAS were found to be 4.41±1.12 μg/g and 7.80±3.39 μg/g, respectively. Mean content of radioactive Th is found to be 173±134 ng/g. In general, toxic elements were found below the permissible level.

Interelemental correlations

Literature suggest interrelationship of various elements in plant species.[41,42] K/P ratio often used to assess the diagnostic value[43] lies in a close range of 6.45–10.7 with a mean value of 8.2±1.4. TEKELI and ATES[44] observed a K/P = 6.31 in White Clover grass (*Trifolium Repens L.*), which matches well with our value. Balance of mineral elements is essential to maintain good health.[40] Cu and Zn, essential elements for biochemical processes,[15] are well correlated with $r = 0.89$ in our study as shown in Fig. 3. Another linear relationship exists between Rb and Cs with $r = 0.87$ (Fig. 4). An US patent claimed that Rb and Cs help in the breakdown of starch to glucose ratio and thus play an important role in diabetes mellitus.[45]

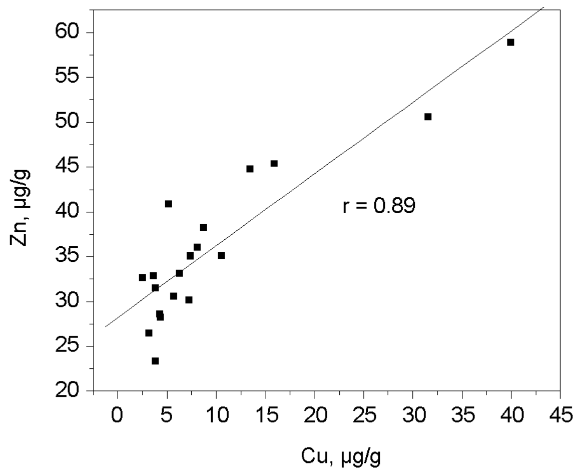

Fig. 3. Correlation of Zn vs. Cu in anti-diabetic herbs

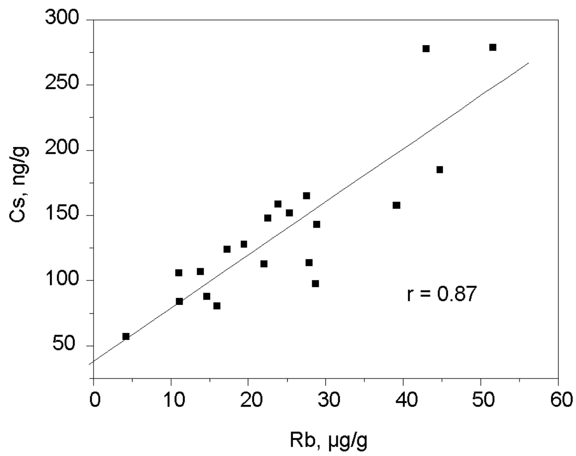

Fig. 4. Correlation of Rb vs. Cs in anti-diabetic herbs

Diabetes is now a global problem. WHO has pointed out that prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be an attainable target.[46] INAA data obtained for the minor, essential trace and toxic element concentrations in medicinal herbs can be used to evaluate their potentiality. This study aims to open new ways for the improvement of medicinal uses of indigenous antidiabetic plants on a firm scientific footing and add value to the natural resources for the well being of the population in developing countries.

Conclusions

Leaves, roots, fruits and seeds of twenty anti-diabetic herbs including three commercial capsules have been analyzed for 6 minor (Na, K, Ca, Cl, Mg, and P) and 21 trace (As, Ba, Br, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hg, La, Mn, Rb, Sb, Sc, Se, Sm, Th, V and Zn) elements by instrumental neutron activation analysis (INAA). Further, Ni, Cd and Pb were determined by AAS. Elemental contents vary in a wide range, in some cases even by an order of magnitude. K, Ca, Mg, Cl and P are present in mg/g amounts while Na, Fe, Zn, Cu, Mn and V are found in μg/g amounts. Toxic elements as As, Cd, Hg, Pb and Sb, are present below the permissible limits. K/P ratio in all 20 samples lies in a very close range (6.45–10.7). Also, Cu and Zn ($r = 0.89$) and Rb and Cs ($r = 0.87$) exhibit strong correlationship.

*

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