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ScienceDirect

Food Chemistry 104 (2007) 1454–1463

Food Chemistry

www.elsevier.com/locate/foodchem

Variation in essential, trace and toxic elemental contents in *Murraya koenigii* – A spice and medicinal herb from different Indian states

R. Paul Choudhury, A.N. Garg *

Department of Chemistry, Indian Institute of Technology, Roorkee 247 667, India

Received 18 July 2006; received in revised form 12 December 2006; accepted 12 February 2007

Abstract

Curry leaves (*Murraya koenigii*), collected from 19 different Indian states, were analyzed for six minor (Ca, Cl, K, Mg, Na and P) and 20 trace (As, Ba, Br, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mn, Rb, Sb, Sc, Se, Sr, Th, V and Zn) elements by instrumental neutron activation analysis (INAA). In addition, Ni, Cd and Pb were determined by atomic absorption spectrophotometry (AAS). Most elements vary over a wide range, depending on geo-environmental factors and local soil characteristics. Fe, Mn, Na, K, Rb, Se and P vary by a factor of 3–5 whereas Br, Cs, Sc, Th and Zn vary by an order of magnitude. Leaves collected from the southern zone were enriched in K, Mg, Mn, Cl and P but depleted in Se. However, leaves from the northern zone were particularly enriched in Ca whereas those from the western zone were enriched in Zn. Concentrations of most elements from the eastern zone were on par with the mean values. Cr, Fe, Cu, V and Zn are known to play an important role in the maintenance of normoglycemia by activating β-cells of pancreas. Percent contributions of diabetically important elements from curry leaves were 1–2% of daily dietary intake (DDI) but are likely to be in bioavailable form thus making them effective for treatment of diabetes. Rb and Cs are linearly correlated ($r = 0.93$) as their salts enhance the absorption of insulin in the lower respiratory tract by breakdown of glucose. Inorganic elements may remain complexed with organic ligands.

Keywords: *Murraya koenigii*; Instrumental neutron activation analysis; Atomic absorption spectrophotometry; Daily dietary intake; Rb and Cs correlation

1. Introduction

Murraya koenigii, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other south Asian countries. It is found almost everywhere in the Indian subcontinent, excluding the higher levels of the Himalayas (Rastogi & Mehrotra, 1998). Curry leaves are grown throughout India and they adorn every house yard, especially in southern parts, where most cuisines cannot do without the subtle flavouring of this highly aromatic leafy spice. The botanical name *M. koenigii* refers to two 18th century botanists: a Swede, Johann Andreas Murray (1740–1791) and a German, Johann Gerhard König (1728–1785) (Seidemann, 2005). The leaves are used to flavour a range of dishes and typically these are fried in oil until crisp to impart flavour to all types of curry preparations. Fresh leaves release strong aroma while cooking. However, in cities, its dry powder is also used. The plant has also been used in traditional Indian medicine systems for a variety of ailments (Chivallier, 1996; Sivarajan & Balachandran, 1996).

According to a news report in The Guardian, a research team led by Prof. Peter Houghton from the Pharmacy Department of King's College, London, claimed that curry leaves slow down the starch to glucose breakdown in diabetic patients (Houghton, 2004) though such claims have

* Corresponding author. Tel.: +91 1332 285218; fax: +91 1332 273560.
E-mail addresses: agargfcy@iitr.ernet.in, agargfcy@yahoo.com (A.N. Garg).

0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2007.02.013

already been made by some Indian workers (Santhakumari, Pillai, Pillai, & Nair, 1985). The oil derived from the leaves is also used in the perfume and soap industry. Leaves are rich in minerals, vitamins A and B, and are a rich source of carbohydrates, proteins, amino acids and alkaloids (Kong et al., 1986; Tee & Lim, 1991). Fresh juice of curry leaves, mixed with lime juice and sugar, cures morning sickness, nausea and vomiting due to indigestion. A glass of buttermilk with a pinch of salt and a spoonful of ground curry-leaf paste, taken on an empty stomach, relieves stomachache. Chewing the tender leaves helps control loose motions whereas fully-grown curry leaves are beneficial in controlling diabetes and weight loss. Leaves cooked in milk and ground to a paste, when applied to poisonous insect bites and other wounds and cuts, relieve pain and swelling. Leaves ground with turmeric, and taken daily, are an effective remedy for allergic reactions. Also their paste, applied on the foot, prevents cracking. Curry leaves and black pepper beaten with sour curd are beneficial for indigestion (Brahmananda, 2000). Leaves boiled in coconut oil are often used as a hair tonic for stimulating hair growth and retaining natural pigmentation. Several workers have extensively evaluated the aqueous extract of *M. koenigii* leaves for their hypoglycemic activity without any side effects or toxicity (Kesari, Gupta, & Watal, 2005; Math & Balasubramaniam, 2005; Santhakumari et al., 1985; Vinuthan, Girish, Ravindra, & Jayaprakash, 2004). The leaves have been shown to considerably reduce blood sugar levels, where Cr, V, Mn, Zn, Cu and Se are known to play an important role in biochemical processes and especially in diabetics.

Most studies on curry leaves reported in the literature pertain to the organic constituents, namely essential oils, coumarins, terpenoids and carbazole alkaloids, known for their antioxidant properties. Wang, He, Shen, Hong, and Hao (2003) isolated two carbazole alkaloids, murrayanine and 8,8″-biskoenigine, by oxidative coupling, using a solid-state reaction. Sukari et al. (2001) extracted murrayazoline and murrayacine from the $CHCl_3$ extract and elucidated their structure using high-resolution NMR (^{1}H NMR, ^{13}C NMR, HMQC and HMBC), IR and mass spectrometry. Chowdhury, Jha, Bhattacharyya, and Mukherjee (2001) isolated two alkaloids (1-formyl-3-methoxy-6-methylcarbazole and 6,7-dimethoxy-1-hydroxy-3-methylcarbazole). Adebajo and Reisch (2000) reported eight furocoumarins (xanthotoxin, isobyakangelicol, phellopterin, gosferol, neobyakangelicol, byakangelicol, byakangelicin and isogosferol) and β-sitosterol from *M. koenigii* seeds. Ramsewak, Nair, Strasburg, DeWitt, and Nitiss (1999) isolated three bioactive carbazole alkaloids, mahanimbine, murrayanol and mahanine, having antimicrobial and mosquitocidal activities. Palaniswamy, Caporuscio, and Stuart (2003) determined the levels of the antioxidant vitamins, α-tocopherol, β-carotene and lutein, in fresh curry leaves by using reversed phase gradient HPLC. Dasgupta, Rao, and Yadava (2003) studied the anticarcinogenic potential of the curry-leaf extract and suggested its use for the prevention of stomach and skin cancers. Tachibana, Kikuzaki, Lajis, and Nakatani (2003) evaluated antioxidative properties of 12 carbazole alkaloids. Earlier, we have reported three novel organic compounds, 3-methylthiopropanenitrile, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and 1-pentene-3-ol, in the ethanolic extract of curry leaves (Choudhury, Jain, & Garg, 2006).

Only scanty reports are available on the minor and trace element composition which plays a significant role in biochemical and enzymatic processes (Herber & Stoeppler, 1994; Kariyanna, 2003). These suggested dark and light green-coloured leaves as an indicator for high Mn and Fe contents, respectively. Gopalan et al. (1999) compiled eight essential elements, along with organic compounds, in curry leaves, which play a vital role in human metabolism. Narendhirakannan, Subramanium, and Kandaswamy (2005) analyzed the elemental composition in the leaves of *M. koenigii*, widely used in the treatment of diabetes-related metabolic disorders, by using atomic absorption spectrophotometry (AAS). Ray, Nayak, Rautray, Vijayan, and Jena (2004) determined K, Ca, Fe, Cr, Mn, Cu, Zn, Rb, Sr and Pb using an energy dispersive X-ray fluorescence technique (EDXRF). Rajurkar and Pardeshi (1997) reported essential and trace elements in curry leaves by NAA and AAS. NAA has been widely employed for determining 10–20 elements in curry leaves from Tirupati in Andhra Pradesh (Balaji et al., 2000), Nagpur in central India (Singh & Garg, 2006) and other parts of the country (Choudhury et al., 2006).

Earlier we reported the determination of up to 20 elements in 14 curry-leaf samples by short irradiation NAA and three new organic compounds (Choudhury et al., 2006). In the present study, we have carried out a more exhaustive study by analyzing 28 samples of fresh curry leaves (*M. koenigii*) from east, west, north and south zones from over 19 Indian states for six minor (Ca, Cl, K, Mg, Na and P) and 23 trace (As, Ba, Br, Cd, Ce, Co, Cr, Cs, Cu, Fe, Hg, La, Mn, Ni, Pb, Rb, Sb, Sc, Se, Sr, Th, V and Zn) elements by using thermal neutron activation analysis (TNAA) and AAS. An attempt has been made to explain variation in elemental contents from different geographical zones, to calculate the contribution of essential nutrient elements to daily dietary intake (DDI) and correlate trace element contents with medicinal properties.

2. Materials and methods

2.1. Sample collection

Tender leaves were collected from 28 cities/towns spread over 19 Indian states, as shown in Fig. 1. In each case, leaves were plucked from all parts of the plant, thus making the sample representative of that plant and of its immediate surroundings but not of the city. For the sake of convenience the whole country has been divided into four zones: East (Churachandpur, Silchar, Kolkata, Cuttack,

Fig. 1. Sample collection sites in Indian map.

Bokaro, Chapra, Jamshedpur); West (Bhilwara, Jaipur, Baroda, Mumbai, Pune, Nagpur, Indore); North (Kangra, Hissar, Pathankot, Roorkee, Bijnaur, Lucknow); South (Hyderabad, Visakhapatnam, Vijaywada, Pallakad, Pondicherry, Madurai). Samples from Mumbai and Hyderabad, the two megacities, were collected from two different places separated by >10 km. On average, ~100 g of leaves was collected from the very bottom to the top of the shrub, mostly from the residential areas, and kept in polyethylene bags. These were first wiped with tissue paper to remove any dirt and then thoroughly washed with distilled water to avoid any surface contamination. The leaves were first air-dried and then dried under an infrared lamp. These were then crushed to a homogeneous fine powder (100 mesh) in an agate mortar and stored in pre-cleaned polyethylene vials/bags. The samples were handled with extreme care in a glove box to avoid any contamination. A synthetic multielemental comparator standard was prepared by depositing 2–5 μg amounts of As, Co, Fe, Se, Hg and Zn salt (AR/HP grade) solutions, in chloride/nitrate form, on a Whatman filter paper, No. 42, strip. A single strip was packed for each irradiation. Two reference materials (RMs), Peach Leaves (SRM-1547) (1993) and Mixed Polished Herbs (MPH-2) (Dybczynski et al., 2004), were procured from the National Institute of Standards and Technology NIST, USA and The Institute of Nuclear Chemistry and Technology, Poland, respectively. All the samples and RMs were dried in an oven at 80 °C overnight before packing. Moisture content was 68.4 ± 1.3%, as reported earlier (Choudhury et al., 2006).

2.2. Irradiation and counting

About 50 mg each of powdered samples and RMs were accurately weighed and packed in alkathene/aluminium foil (Superwrap) for short (2 min)/long (1 d) irradiation in a CIRUS/Dhruva reactor, respectively, at the Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, India, at a thermal flux of $\sim 10^{13}$ n cm^{-2} s^{-1}. Short-lived activities were measured using an 80 cm^3 coaxial HPGe detector (EG&G ORTEC) and 4k MCA at the reactor site and later at the Radiochemistry Laboratories of BARC, Mumbai. Long irradiated samples were brought to our laboratories in Roorkee and γ-activity was measured using a HPGe detector with 2.1 keV resolution at 1332 keV of ^{60}Co and 8k MCA with GENIE-2000 software (Canberra,

Table 1
Irradiation and counting schedule, including nuclides identified and energies

Irradiation (reactor)	Delay	Counting	Nuclides identified (E_γ, keV)
2 min (CIRUS)	1 min	50, 100 s	^{27}Mg (1014), ^{49}Ca (3084), ^{52}V (1434)
	5 min	5 min	^{38}Cl (1643), ^{56}Mn (846)
	1 h	10 min	^{56}Mn, ^{76}As (559)
	1 d	1, 2 h	^{24}Na (1369), ^{42}K (1524), ^{82}Br (554, 776) ^{51}Cr (320), ^{64}Cu (511), ^{140}La (1596)
1 d (Dhruva)	10 d	2, 4 h	^{51}Cr (320), ^{85}Sr (514), ^{131}Ba(373), ^{233}Th (312)
	15 d	4 h	^{51}Cr, ^{233}Th, ^{59}Fe (1099, 1291), ^{141}Ce(145), ^{203}Hg (279), ^{75}Se (264), ^{134}Cs (605, 796)
	22 d	6 h	^{60}Co (1173, 1332), ^{124}Sb (603, 1691), ^{46}Sc (889, 1120), ^{86}Rb (1077)
	25 d	1 h	^{32}P (β_{max} = 1710)
	30 d	6 h	^{65}Zn (1115), ^{141}Ce, ^{203}Hg, ^{75}Se, ^{134}Cs
	60 d	12 h	^{60}Co (1173, 1332), ^{124}Sb

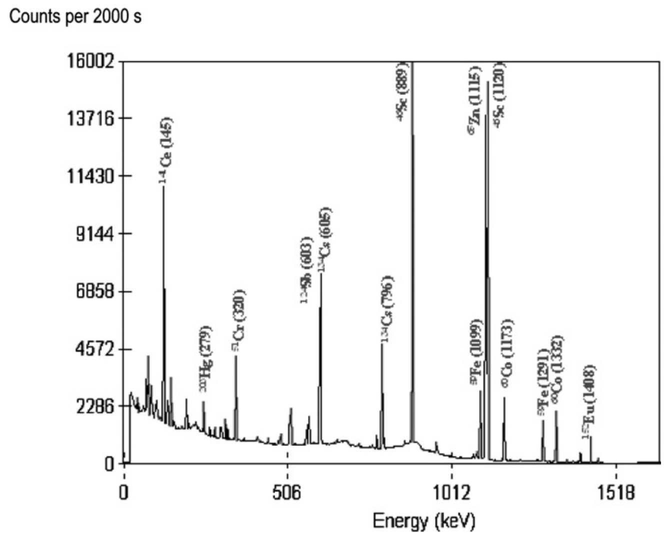

Fig. 2. Typical γ-ray spectra of long-lived radionuclides in curry leaves.

USA). Counting was followed for 1 h, 2 h, 6 h and 12 h at different intervals for up to 3 months. Irradiation and counting schedule, including nuclides identified in each case, are listed in Table 1. A typical γ-ray spectrum of long-lived radionuclides in curry leaves is shown in Fig. 2. Efforts were made to obtain maximum elemental information and the reproducibility of data was checked from more than one counting. Elemental contents were calculated by a comparator method, using RMs and synthetic standards as comparators. Phosphorus was determined by measuring β^- activity due to ^{32}P on an end-window G.M. counter, using a 27 mg cm^{-2} aluminium filter after a delay period of ~3 weeks (Garg, Kumar, & Choudhury, 2007; Weginwar, Samudralwar, & Garg, 1989).

2.3. AAS measurements

For the analysis of Ni, Cd and Pb, an atomic absorption spectrophotometer (GBC Avanta, Australia) with oxy-acetylene flame was used. About 2 g each of the powdered samples was accurately weighed and dissolved in a (5:1v/v) mixture of nitric and perchloric acids (Zhang, 2000) with repeated heating. After a clear solution was obtained, a few drops of HCl were added and the solution was filtered through Whatman filter paper No. 42 and finally made up to 25 ml. Prior to the analysis, the instrument was calibrated using ppm level standard solutions of Ni, Cd and Pb salts (AR/HP grade).

3. Results and discussion

3.1. General

Elemental concentrations were calculated using a synthetic multielemental standard and, in some cases, two RMs as comparators. Means ± SD data for SRM-1547, along with certified/information values, RSD and Z-score, are listed in Table 2. It is observed that our data match well within ±5–10% of the certified values, with the exception of As. Also, RSDs were <10% in all cases, suggesting a high order of precision. Further, Z-score values are all below 3, except Co, K, Sc, as shown in Fig. 3, suggesting that the data should be within a 95% confidence limit. Therefore, it is assumed that elemental concentration data for samples analyzed in this study should be reliable within

Table 2
Elemental concentrations in peach leaves (SRM-1547) used for data validation

Element	Peach leaves (SRM-1547)		% Error	% RSD	Z-score
	This work	Certified			
As (ng/g)	51.6 ± 0.7	(60 ± 18)	−14	1.4	−0.47
Ba (μg/g)	136 ± 7	(124 ± 4)	9.7	5.1	3.0
Br (μg/g)	10.3 ± 0.6	[11]	−6.4	5.8	–
Ca (mg/g)	16.3 ± 0.5	(15.6 ± 0.2)	4.5	3.1	3.5
Ce (μg/g)	10.1 ± 1.1	[10]	1.0	11.0	–
Cl (mg/g)	0.39 ± 0.03	(0.36 ± 0.02)	8.3	7.7	1.5
Co (ng/g)	64.6 ± 0.4	[70]	−7.7	0.6	–
Cr (μg/g)	1.10 ± 0.10	[1]	10	9.0	–
Cs (ng/g)	85.1 ± 0.4	{–}	–	0.5	–
Fe (μg/g)	221 ± 18	(218 ± 14)	1.3	8.1	0.21
Hg (ng/g)	28.0 ± 0.6	(31.0 ± 7.0)	−9.7	2.1	−0.73
K (mg/g)	24.7 ± 2.0	(24.3 ± 1.0)	1.2	8.1	0.40
La (μg/g)	7.98 ± 0.05	[9]	−11.3	0.6	–
Mg (mg/g)	4.72 ± 0.28	(4.32 ± 0.08)	9.3	5.9	5.0
Mn (μg/g)	101 ± 5	(98.0 ± 3.0)	3.0	5.0	1.0
Na (μg/g)	22.2 ± 1.0	(24.0 ± 2.0)	−7.5	4.5	−0.90
P (mg/g)	1.26 ± 0.10	(1.37±0.07)	−8.0	7.9	−1.57
Rb (μg/g)	21.6 ± 2.1	19.7 ± 1.2	9.6	9.7	1.58
Sb (ng/g)	17.9 ± 1.1	[20]	−10.5	6.1	–
Sc (ng/g)	42.1 ± 0.3	[40]	5.3	0.7	–
Se (ng/g)	101 ± 2	(120 ± 9)	−15.8	1.9	−2.11
Sr (μg/g)	49.2 ± 1.1	(53 ± 4)	−9.1	2.2	−0.95
Th (ng/g)	53.5 ± 2.0	[50]	7.0	3.7	–
Zn (μg/g)	18.9 ± 0.1	(17.9 ± 0.4)	5.6	0.5	2.5

In parentheses ( ) are certified values, in [ ] are information values, and in {–} no data available.

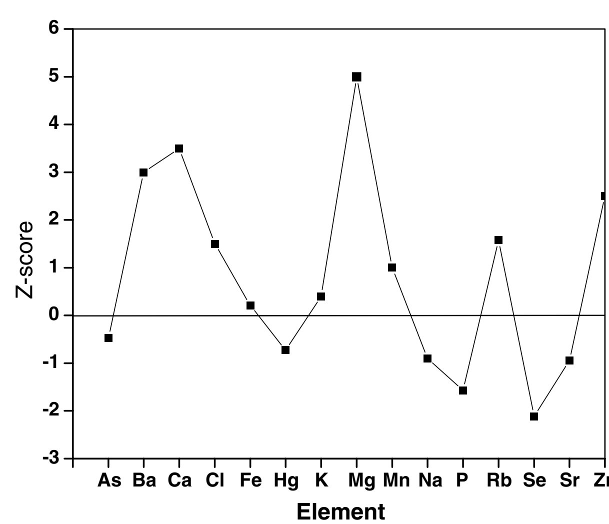

Fig. 3. Z-score plot for elements in peach leaves (SRM-1547).

Table 3
Comparison of ranges and mean elemental contents from east, west, north and south zones

Elements	Eastern zone ($n = 7$)		Western zone ($n = 8$)		Northern zone ($n = 6$)		Southern zone ($n = 7$)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
As (ng/g)	31.3–81.4	55.1 ± 18.5	40.7–105	77.5 ± 25.3	48.3–69.8	58.1 ± 10.9	39.6–271	111 ± 77
Ba (μg/g)	19.7–57.7	37.6 ± 11.5	22.7–80.4	53.3 ± 18.3	19.3–56.6	30.3 ± 14.1	26.0–67.8	44.6 ± 13.5
Br (μg/g)	4.18–10.4	6.63 ± 1.93	1.69–15.3	8.61 ± 5.44	2.24–7.49	4.21 ± 1.97	3.55–13.3	11.0 ± 4.8
Ca (mg/g)	12.3–18.4	15.0 ± 2.1	9.44–19.7	15.6 ± 3.64	21.6–33.2	25.7 ± 4.4	14.9–23.5	18.6 ± 3.3
Cd (μg/g)	0.98–5.17	2.38 ± 1.51	1.89–5.07	2.95 ± 0.99	1.39–3.74	2.55 ± 0.85	1.92–4.02	2.83 ± 0.80
Ce (μg/g)	1.81–5.79	3.39 ± 1.20	0.90–88.8	13.6 ± 30.5	1.16–4.26	2.16 ± 1.31	1.80–4.82	2.98 ± 0.94
Cl (mg/g)	0.76–1.86	1.39 ± 0.43	1.21–2.43	1.70 ± 0.40	1.67–3.64	2.50 ± 0.81	1.84–4.02	2.66 ± 0.82
Co (ng/g)	35.1–168	92.6 ± 47.4	48.6–148	103 ± 42	34.9–102	72.4 ± 25.3	20.4–78.6	49.3 ± 22.0
Cr (μg/g)	0.53–0.86	0.70 ± 0.12	0.52–2.05	1.02 ± 0.46	0.23–1.05	0.68 ± 0.32	0.67–1.02	0.82 ± 0.13
Cs (ng/g)	17.3–48.3	26.5 ± 14.2	12.2–55.4	33.4 ± 12.2	16.4–98.7	61.0 ± 35.0	36.2–50.2	42.3 ± 4.4
Cu (μg/g)	11.2–41.1	30.1 ± 21.3	7.52–21.7	12.1 ± 4.7	1.39–10.9	6.87 ± 3.61	3.66–63.3	16.1 ± 21.0
Fe (μg/g)	116–283	167 ± 57	72.5–228	171 ± 47.3	112–215	153 ± 34.9	101–211	140 ± 40
Hg (ng/g)	12.7–45.0	38.2 ± 12.2	1.23–76.5	41.6 ± 28.8	18.1–42.9	26.4 ± 9.1	15.7–49.1	32.0 ± 11.5
K (mg/g)	10.3–27.9	16.9 ± 7.0	10.9–30.3	19.5 ± 7.75	13.6–23.9	17.9 ± 3.7	15.6–22.0	18.5 ± 2.30
La (μg/g)	3.82–6.49	5.02 ± 0.97	2.06–4.98	3.20 ± 1.07	2.57–10.7	6.17 ± 3.55	1.13–5.58	3.33 ± 1.41
Mg (mg/g)	2.61–5.16	3.77 ± 0.95	1.14–6.90	4.19 ± 1.58	2.14–4.51	2.87 ± 0.83	4.03–7.19	5.51 ± 1.22
Mn (μg/g)	24.8–61.6	35.4 ± 12.3	27.8–56.6	40.7 ± 7.81	43.1–56.3	48.6 ± 5.28	41.7–63.0	55.0 ± 8.2
Na (μg/g)	107–346	203 ± 96	120–455	248 ± 106	70.9–214	139 ± 61	153–277	192 ± 42
Ni (μg/g)	1.24–4.17	2.78 ± 1.03	1.68–6.65	3.73 ± 1.79	1.57–4.52	3.21 ± 1.19	1.93–6.73	3.94 ± 1.69
P (mg/g)	0.80–1.48	1.07 ± 0.25	0.43–1.62	0.96 ± 0.44	0.58–2.16	1.45 ± 0.53	0.72–1.59	0.97 ± 0.29
Pb (μg/g)	3.42–37.3	18.1 ± 14.8	5.17–79.1	31.4 ± 27.9	7.12–38.4	16.9 ± 13.2	5.31–37.4	17.7 ± 11.7
Rb (μg/g)	5.15–15.5	10.5 ± 5.4	7.45–20.9	12.8 ± 4.22	6.99–27.2	20.3 ± 9.7	7.34–24.3	15.4 ± 6.2
Sb (ng/g)	21.1–83.9	49.3 ± 26.5	45.6–80.7	59.5 ± 11.5	14.1–57.4	34.2 ± 16.7	2.61–51.5	28.1 ± 18.1
Sc (ng/g)	13.6–49.1	32.8 ± 14.3	4.98–57.7	33.7 ± 18.7	21.4–60.6	35.6 ± 18.6	22.4–75.7	50.6 ± 20.8
Se (ng/g)	56.3–108	84.6 ± 21.2	50.9–131	85.7 ± 40.2	40.1–116	61.6 ± 27.3	24.1–45.8	34.5 ± 8.8
Sr (μg/g)	24.5–87.2	53.4 ± 19.9	22.4–50.2	36.3 ± 9.9	20.6–52.4	31.9 ± 11.1	25.5–57.1	39.0 ± 10.6
Th (ng/g)	9.33–80.1	41.1 ± 24.0	6.60–97.5	56.8 ± 37.7	20.0–115	54.8 ± 35.1	16.9–86.5	45.5 ± 22.8
V (μg/g)	0.69–2.31	1.54 ± 0.63	0.67–1.21	1.04 ± 0.27	1.02–2.73	1.94 ± 0.68	0.83–2.89	1.75 ± 0.79
Zn (μg/g)	17.0–30.2	22.4 ± 4.2	10.0–70.5	34.9 ± 21.0	7.90–30.1	19.6 ± 9.6	10.9–30.2	20.5 ± 6.4

Note. Cd, Ni and Pb were determined by AAS and ±SD were calculated on the basis of number of samples analyzed for each zone.

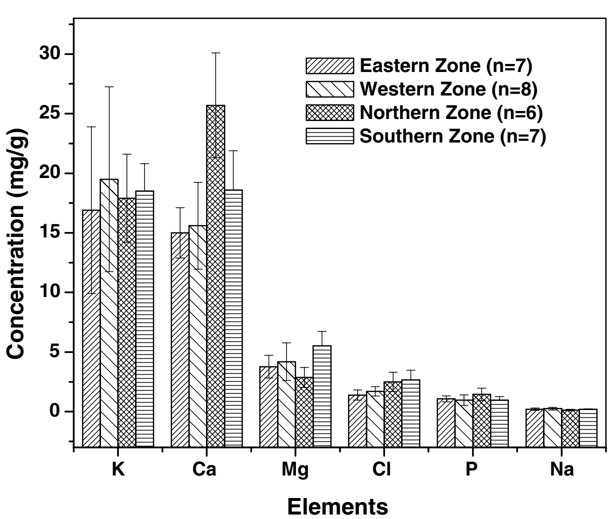

Fig. 4. Concentrations of minor elements in curry leaves from different zones in India.

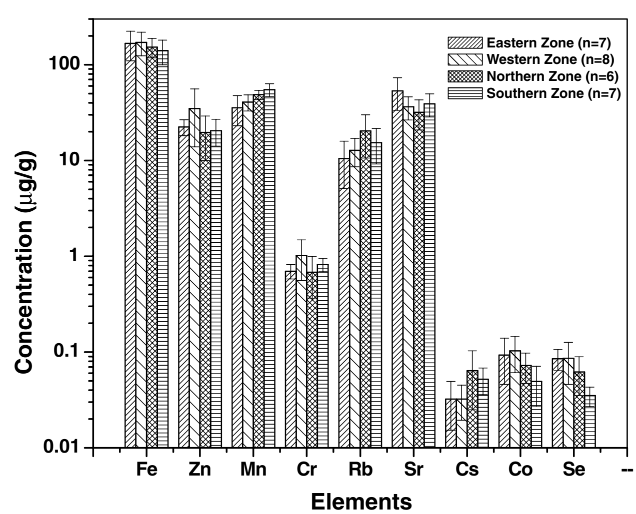

Fig. 5. Concentrations of essential trace elements in curry leaves from different zones in India.

±10%. Ranges and mean elemental concentrations in four different zones are listed in Table 3. Bar plots, comparing minor, trace and toxic element concentrations in four different zones, are shown in Figs. 4–6, respectively. Further ranges, means ± SD and medians ± SD, for all the elements in curry leaves, along with a comparison with the literature reports (Balaji et al., 2000; Gopalan et al., 1999; Singh & Garg, 2006), are listed in Table 4.

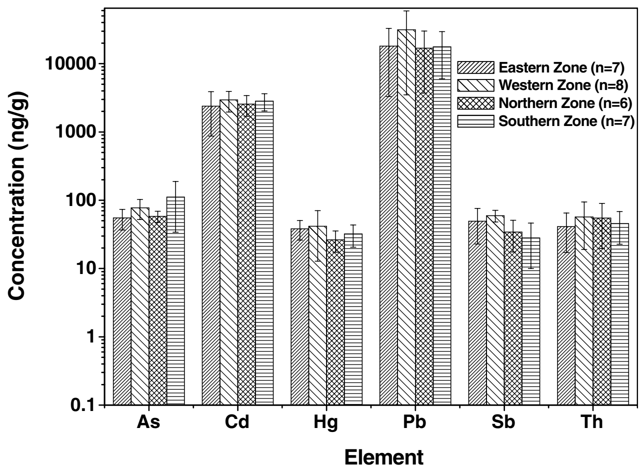

Fig. 6. Concentrations of toxic elements in curry leaves from different zones of India.

3.2. Elemental contents in four zones

A perusal of elemental contents in Table 3 shows that most elements in the eastern zone vary in a narrow range by a factor of 3 except for Cd, Co, Cu, Sb and Sc. Th and Pb contents vary by an order of magnitude. It is observed that leaves from Kolkata, a mega city, show the highest Ca (18.4 ± 0.5 mg/g), K (27.9 ± 1.8 mg/g) and P (1.48 ± 0.11 mg/g) contents. Leaves from Bokaro have the highest Na (313 ± 23 μg/g) and Cl (1.90 ± 0.21 mg/g) contents. Fe, Co, Cs and Rb contents are higher in leaves from Jamshedpur. Among the toxic elements, Hg, Sb, Cd and Ni contents are greater in the Jamshedpur sample while the Bokaro sample had the highest As (81.4 ± 3.9 ng/g) and Pb (35.5 μg/g) contents. Both of these cities (Bokaro and Jamshedpur) are known for steel plants in the state of Jharkhand and high concentrations of toxic elements in leaves from these cities indicate the effects of industrial emissions and other anthropogenic activities. Incidentally, leaves from Churachandpur, a sleepy and non-industrial town from the state of Manipur, show the lowest concentrations of As, Hg, Sb and Cd. However, leaves from Cuttack have higher concentrations of Cr (0.86 ± 0.03 μg/g) and Se (108 ± 4 ng/g), In general, elemental concentrations vary over a small range. Br content, however, from this seashore town, is very high.

It is observed that elemental contents in leaves from the west zone vary over a much wider range. Br, Ce, Hg, Pb, Sc and Th vary by an order of magnitude or more. Leaves from Baroda have higher concentrations of Cl, Mg and Zn while those from Indore have the highest Ca and K contents. Samples from Mumbai-2 (BARC, Mumbai) have higher Co, Cr, Fe, P and Se contents, whereas those from Pune are rich in Na. Among toxic elements, surprisingly,

Table 4
Ranges, mean and median elemental concentrations in curry leaves ($n = 28$) and comparison with the literature data

Elements	Curry leaves ($n = 28$)			Gopalan et al. (1999)	Balaji et al. (2000)	Singh and Garg (2006)
	Range	Mean ± SD	Median ± SD			
As (ng/g)	31.3–271	78.2 ± 47.7	69.8 ± 44.4	ND	ND	ND
Ba (μg/g)	19.3–80.4	42.2 ± 16.4	38.6 ± 10.7	ND	ND	ND
Br (μg/g)	1.69–15.3	7.74 ± 4.44	6.70 ± 2.38	ND	2.96 ± 0.35	64.1
Ca (mg/g)	9.44–33.2	18.4 ± 5.24	17.2 ± 4.16	8.30	22.9 ± 1.05	ND
Cd (μg/g)	0.98–5.17	2.69 ± 1.05	2.47 ± 0.55	ND	ND	ND
Ce (μg/g)	0.90–88.8	5.93 ± 16.3	2.92 ± 15.4	1.98	5.5 ± 0.2	5.43
Cl (mg/g)	0.76–4.02	2.04 ± 0.80	1.84 ± 0.57	ND	ND	740
Co (ng/g)	20.4–168	80.4 ± 40.3	72.2 ± 25.8	0.6	ND	2.27
Cr (μg/g)	0.52–2.05	0.82 ± 0.32	0.79 ± 0.27	ND	ND	ND
Cs (ng/g)	12.2–98.7	39.8 ± 21.8	36.2 ± 15.1	ND	ND	ND
Cu (μg/g)	1.39–63.3	16.5 ± 16.8	10.7 ± 10.8	ND	ND	ND
Fe (μg/g)	72.5–283	158 ± 45	154 ± 36.9	93	ND	1920
Hg (ng/g)	1.23–76.5	32.9 ± 17.6	27.7 ± 13.2	ND	ND	133
K (mg/g)	10.3–30.3	18.2 ± 5.5	17.5 ± 3.50	ND	17.8 ± 0.6	22.6
La (μg/g)	1.13–10.7	4.33 ± 2.19	4.01 ± 1.68	ND	ND	ND
Mg (mg/g)	1.14–7.19	4.13 ± 1.48	4.09 ± 1.06	4.4	8.3 ± 0.4	ND
Mn (μg/g)	24.8–63.0	44.6 ± 11.3	44.1 ± 6.69	15	67.15 ± 3.31	86.9
Na (μg/g)	70.9–455	199 ± 87	187 ± 67	ND	1710 ± 70	1020
Ni (μg/g)	1.24–6.73	3.44 ± 1.48	3.26 ± 0.96	ND	ND	ND
P (mg/g)	0.43–2.16	1.09 ± 0.42	1.02 ± 0.30	0.57	ND	8.1
Pb (μg/g)	3.42–79.1	21.5 ± 18.8	11.8 ± 13.2	ND	ND	ND
Rb (μg/g)	5.15–27.2	14.5 ± 7.0	14.3 ± 3.86	ND	ND	15.6
Sb (ng/g)	2.61–83.9	43.8 ± 21.8	45.6 ± 14.1	ND	ND	ND
Sc (ng/g)	4.98–75.7	38.1 ± 18.7	40.1 ± 12.4	ND	ND	730
Se (ng/g)	24.1–131	67.5 ± 33.7	58.5 ± 18.7	ND	ND	670
Sr (μg/g)	20.6–87.2	40.3 ± 15.0	37.8 ± 11.7	ND	ND	ND
Th (ng/g)	6.60–115	49.6 ± 29.8	41.9 ± 19.0	ND	ND	710
V (μg/g)	0.67–2.89	1.54 ± 0.65	1.02 ± 0.23	ND	ND	ND
Zn (μg/g)	7.90–70.5	24.9 ± 13.7	22.8 ± 11.0	20	ND	89.4

Bhilwara, the industrial city, has higher Hg and Sb contents. Higher Cd and Pb contents are observed in leaves from Mumbai-1 and Nagpur, mega cities of this zone. Yet again, samples collected from near the sea shore (Mumbai-2) showed higher Br contents.

Most of the samples collected from the north zone were from small suburban towns, except Lucknow. Hence, elemental contents did not vary much. Not surprisingly, leaves from Lucknow had the highest As, Br, Hg and Cd contents. However, the samples from this city were enriched in Co, Cs, Fe, K, Mn and Zn. Leaves from Bijnaur had the highest Ca, Cl, Cu and Rb contents while the Roorkee sample was rich in Na. Higher Sb and Pb contents were observed in leaves from Pathankot, an industrial town.

Elemental contents in leaves from south zone vary over a relatively wider range than the north zone. Cu and Sb contents vary by 20-fold. Our data show that leaves from Pallakad, the least polluted city, are enriched in Ca, Cl, Cu, Mg, Na, P and Se. However, leaves from Vizag (near Bay of Bengal), known for Navy and other heavy industries, are rich in Co, Cs, Fe, Rb and Zn. Pb content is highest in leaves from Hyderabad, a mega city. Similar to our observations from other zones, the Vizag sample has highest Br content.

3.3. Zonal variations

Bar plots, showing variation in minor, essential and toxic trace elemental contents in curry leaves from four zones, are shown in Figs. 4–6, respectively. A comparison of elemental contents of curry leaves from four different zones (Table 3) shows that leaves from the west zone are most enriched in minor (Na, K and Fe) and trace (Cr, Zn, Co and Se) elements. Leaves from the north zone are especially enriched in Na while those from the south zone show the highest Mg and Cl contents. Western zone leaves have higher contents of As, Hg, Pb, Sb and Th. This is understandable, as leaves were collected from the most populous cities. Br and Ni contents are higher in leaves from the south zone. Most elemental contents in leaves from the east zone are on par with the total mean contents, as listed in Table 4, except Cs, which is lowest. Ca content in the north zone is highest (25.7 ± 4.4 mg/g), whereas Se is lowest in the south zone (34.5 ± 8.8 ng/g). A look at Table 4 shows that, in all cases, medians are lower than the means by ~5%, though, in the cases of Ba, Cd, Cl, Cs and Zn, medians are still lower by 8–9%. It may be concluded that most variations in elemental contents are uniform all over India, irrespective of wide differences in soil characteristics, and seem to be real rather than artificial. Elemental uptake by a plant is its characteristic property and may depend on the use of fertilizers, irrigation water and different climatological/geo-environmental factors. It is observed that *Murraya koenegii* leaves are enriched in K, Mg, Ca and P as minor constituents (>1 mg/g) with trace amounts of Fe, Mn, Cu, Rb and Zn (>20 μg/g). However, V and Cr, whose compounds are considered insulin-like, are found at ~1 μg/g only. It seems that bioavailability rather than the total amount of an element is important.

Minor amounts of alkali and alkaline earth metals (sodium, potassium, magnesium and calcium), together with chloride ions, must be in balance in extracellular fluid, which is responsible for muscular irritability. Magnesium has been particularly shown to play a significant role as a regulatory cation in direct and indirect traumatic brain injury (Cerenak & Vink, 1999). Recently, it has been reported that trace amounts of rubidium and caesium help in the breakdown of starch to glucose (Backstrom, Dahlback, Edman, & Johannson, 2005). Rb and Cs in the east, west, north and south zones vary linearly with $r = 0.96$, 0.85, 0.82 and 0.66, respectively. However, the regression coefficient, for all 28 samples, is found to be 0.82, as shown in Fig. 7. Thus correlation is excellent in leaves from the east zone but poor in the south zone. The K/P ratio is considered to be an indicator of diagnostic importance (Batra, Bewley, Edwards, & Jones, 1976). It is found to vary over a large range of 6.39–65.9 in four zones with a mean value of 18.6 ± 11.2. However, the Ca/P ratio lies in a smaller range of 8.09–48.8 with a mean value of 19.2 ± 9.9. Thus, both the ratios are comparable, supporting the view that K, Ca and P all have comparable concentration ranges, irrespective of the zone from where curry leaves are derived.

A comparison of our data with those reported in the literature (Table 4) shows that the Zn content is comparable with those reported by Gopalan et al. (1999), while Ca, Ce, K and Mn contents are similar to those reported by Balaji et al. (2000). The Rb value is in perfect agreement with our earlier report (Singh & Garg, 1997a).

Haratake, Fukunaga, Ono, and Nakayama (2005) showed that when a V (IV, V) hydroxamic acid complex is injected into streptozotocin-induced diabetic mice, glucose levels are effectively lowered. Thus, vanadium complexes showed insulin-like activity. Chromium, required for maintenance of normal glucose metabolism, is directly

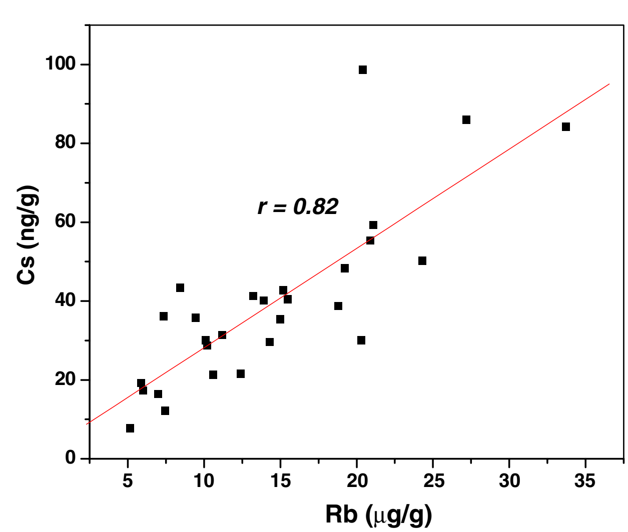

Fig. 7. Correlations of Rb vs Cs in curry leaves.

related to the function of insulin by way of the glucose tolerance factor (GTF). It contains glutamic acid, cysteine and niacin. Yang, Li, Dong, Ren, and Sreejayan (2006) reported that Cr(III) complexes play a key role in carbohydrate and lipid metabolism. The Cr-(phenylalanine)$_3$ complex has been shown to be insulin-sensitive.

Earlier, we observed that zinc level in the blood of diabetic patients is lowered (Garg, Kumar, Maheshwari, & Sharma, 2005). About 85% of zinc combines with protein for transport after its absorption and its turnover is rapid in the pancreas. Haase and Maret (2005) showed that insulin/zinc interactions, insulinomimetic effects of zinc and controlling of the cellular redox state, indicate the specific role of zinc in the pathobiochemistry of diabetes. Deficiency of zinc causes diabetic hyposmia, hypogeusia or coma. In a Russian patent, Akbarov and Aripkhodzhaeva (2000) have shown that a coordination compound of Mn with glutamic acid and vitamin C intensively lowers the blood glucose level in diabetes and shows diuretic action. Mueller, Bosse, and Pallauf (2006) have shown that glutathione peroxidase and thioredoxin reductases and selenium contribute to the maintenance of cellular antioxidative balance when taken up at the recommended dietary allowance (RDA) of 50–100 μg/d (Food and Nutrition Board, 1989). Thus, high doses of selenate (selenium VI) have been shown to normalize hyperglycemia. Also Cu deficiency has been correlated with type II diabetes (Frank, Sell, Danielsson, Fogarty, & Monnier, 2000). Thus all these metals, as coordination compounds, show strong correlation with diabetes and their supplementation in the form of natural herbs may be responsible for the antidiabetic action of *M. koenigii* leaves.

3.4. Toxic elements

Toxicity of medicinal herbs is of much greater concern today than ever before (Chan, 2003). In recent years, much emphasis is being laid on toxic element contents, as several western countries have banned *Ayurvedic* drugs. A cursory look at Table 4 shows that mean As (78.2 ± 47.7 ng/g), Cd (2.69 ± 1.05 mg/kg), Pb (21.5 ± 18.8 mg/kg) and Hg (23.2 ± 17.6 ng/g) levels are present in significant amounts. Though As and Hg contents are well below the permissible limits of 10 and 1 mg/kg, respectively, stated by USFDA, the Cd and Pb contents are somewhat higher, than the permissible limits of 0.3 and 10 mg/kg, respectively. High Pb contents could be due to vehicular and industrial emissions in cities. Similarly, Th, a radioactive element, is also present in significant amounts (49.6 ± 29.8 ng/g).

3.5. Dietary intake

Curry leaves are used principally as a spice and flavouring agent and their total consumption may be just about 5 g/d, which may of course vary, depending on individuals and availability. Based on this assumption, percent contribution of some essential nutrient elements from curry leaves to DDI was calculated, as shown in Table 5. Also included in Table 5 are RDA values (Food and Nutrition Board, 1989) for comparison. It is observed that curry leaves contribute only 1–2% of total DDI (Singh & Garg, 1997b). It seems that bioavailability of an element, rather than its total content, is important and curry leaves supply some of these elements, along with other components. Therefore, dietary intake may be insignificant as a cause of concern. It is supposed that inorganic elements may be coordinated with organic compounds present in the herb. In this regard, 3-methylthiopropanenitrile, reported by us (Choudhury et al., 2006), may be a potential ligand, besides carbazoles (Chowdhury et al., 2001) and alcoholic compounds (Chowdhury, 2000), which together enhance its medicinal importance, especially in diabetes and cancer.

Table 5
Contribution of essential mineral elements from curry leaves to DDI and comparison with RDA

Element	Daily dietary intake (DDI)[a]	DDI (total), Singh and Garg (1997b)	Percent contribution of DDI	Recommended dietary allowance (RDA), Food and Nutrition Board (1989)
K	55 mg	3.5 g	1.6	1.9–5.6 g
Ca	55 mg	0.5 g	11	400–500 mg
Mg	12 mg	–	–	300–350 mg
P	3.5 mg	1.2 g	2.9	1 g
Cr	2.5 μg/g	0.25 mg	1.0	50–100 μg
Mn	135 μg/g	8 mg	1.7	2–5 mg
Fe	480 μg/g	28 mg	1.7	10–18 mg
Se	0.21 μg/g	45 mg	0.5	50–100 μg
Zn	75 μg/g	12 mg	0.6	15 mg

[a] Based on the consumption of 5 g green or 3 g dried leaves per day.

4. Conclusion

Twenty eight leaves samples of *M. koenigii* from 19 states of India were analyzed for 29 elements by instrumental neutron activation analysis (INAA) and atomic absorption spectrophotometry (AAS). Mean elemental contents vary over a wide range, attributed to varying geo-environmental conditions and local soil characteristics from one zone to another. Curry leaves are a rich source of minor constituents, such as Ca, K, Mg, P, along with Fe, Mn, Se and Zn, in trace amounts. Mean contents of Cr, Fe, Cu, V and Zn, known to play a role in the maintenance of normoglycemia, were found to be ~1, 150, 15, 1, and 20 μg/g, respectively. Toxic element (As, Cd, Hg and Pb) contents were found to be below USFDA limits. Though percent contributions of diabetically important elements were 1–2% of DDI, their bioavailability seems to be more important. Inorganic elements remain complexed with organic ligands and make them bioavailable to the body system.

Acknowledgements

Thanks are due to Drs. A.V.R. Reddy and A.G.C. Nair, Radiochemistry Division of BARC, Mumbai, for providing necessary facilities during short irradiation work.

Financial assistance from the BRNS (DAE) and MHRD fellowship to R.P.C. is gratefully acknowledged.

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