AVAILABILITY OF ESSENTIAL TRACE ELEMENTS IN AYURVEDIC INDIAN MEDICINAL HERBS USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION SPECTROSCOPY

Pravin Singare*

Bhavan's College, Andheri, India

Ram Lokhande†

University of Mumbai, Vidyanagari, Santacruz, India

Mahadeo Andhale‡

University of Mumbai, Vidyanagari, Santacruz, India

Raghunath Acharya**

Bhabha Atomic Research Centre, India

Abstract: Elemental analysis of these medicinal plants was performed by employing Instrumental Neutron Activation Analysis (INAA) and Atomic Absorption Spectroscopy (AAS) techniques. The samples were irradiated with thermal neutrons in a nuclear reactor and the induced radio activity was counted by gamma ray spectrometry using an efficiency calibrated high resolution High Purity Germanium (HPGe) detector. Most of the medicinal plants were found to be rich in one or more of the elements under study.

*Corresponding author: Department of Chemistry, Bhavan's College, Andheri, Mumbai 400058, India; e-mail: pravinsingare@vsnl.net

†Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz, Mumbai 400 098, India; rama.lokhande@yahoo.com

‡Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz, Mumbai 400 098, India; mahadeoa@gmail.com

**Raghunath Acharya Radiochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India; raghunath@rediffmail.com

The variation in elemental concentration in same medicinal plant samples collected from different regions was studied and the biological effects of these elements on human beings are discussed. The study was also extended further to estimate the level of toxic elements like Cr, Cd, Ni, Pb and Hg in medicinal plants which arises due to environmental pollution. The results were discussed with careful reference to established role of essential and rare elements to the physiology and pathology of plant and human life.

Keywords: Instrumental neutron activation analysis; INAA; atomic absorption spectroscopy; AAS; medicinal plants; trace elemental analysis; inter-elemental correlations.

INTRODUCTION

There are many references to the curative properties of several herbs in the ancient Indian literature, *Rigveda*, though a more detailed account is found in the *Atharvaveda* from were *Ayurveda*, the Indian traditional health care system (*ayus* = life, *veda* = knowledge, meaning science of life) originated. Fairly comprehensive information about herbs has been recorded in two treatises *Charak Samhita* and *Shusruta Samhita* – a base for Ayurvedic system of medicine (Parchure, 1983; Sharma, 1993). These herbs are now being increasingly used in cosmetics, food as well as alternative medicine (Bakhru, 1998). Some of the ingredients of allopathic and most of the Ayurvedic and Homeopathy medicines are derived from plants. 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 body (lyengar, 1989). Some western scholars have pursued the analysis of various Indian plants and herbs for their medicinal properties (Ambasta, 1986). Most studies on such medicinal plants pertain to their organic contents, viz. essential oils, glycosides, vitamins, alkaloids and other active components and their pharmacological/therapeutic effects. Besides several organic compounds, it is now well established that many trace elements play a vital role in general well-being as well as in the cure of diseases (Prasad, 1993; Underwood, 1977). Several studies have reported elemental contents in plant extracts, which are consumed by us either as a herbal health drink or medicine (Abou Arab and Donia, 2000; Kumar et al., 2005; Powel et al., 1998). These elements are present at varying concentrations in different parts of the plants, especially in roots, seeds and leaves which are used as a dietary item as well as ingredient in the Ayurvedic medicinal preparation. The leaves of the plants are still used in some countries, as for instance, in Malaysia (Majid et al., 1995), Greece (Kanias et al., 1993) and India (Patel, 1986) particularly for their therapeutic effects. Since these trace elements constitute a minute fraction in different parts of the medicinal plants, a sensitive and reliable analytical technique is a prerequisite for obtaining precise and accurate data.

Considering the importance of trace elements in various human metabolic processes and also considering their curative properties, in the present investigation we have applied one of the sensitive analytical techniques such as NAA to study the essential elemental content in different parts of Indian medicinal plants and herbs. The overall impact of these essential trace elements on human health is also discussed. Due to increasing industrialisation and environmental pollution, the study was also extended to estimate the level of toxic elements present in these medicinal plant samples. Even though the direct link between the essential elemental content and their

curative capacity is not yet established, the experimental data of the present work will be of immense importance in the synthesis of new Ayurvedic formulations. Also, it will help in deciding the proportion of various active constituents and managing dose of a particular formulation.

EXPERIMENTAL

Sampling

In the present investigation medicinal plants most commonly used in Indian Peninsula were studied for quantification of their essential trace elemental content. The medicinal plants selected for the study are widely used in treatment of variety of diseases, in cosmetics, as food and in alternative medicine. The different medicinal plants were collected from Bhayander, Pravaranagar and Aurangabad regions in Maharashtra, India to study the soil effect on the mineral content of the plants. Botanical names of the plants analysed along with their parts used are listed in Table 1. Surface contaminants of the plant samples were removed by washing with deionized water twice and then with deionized double distilled water. The leaves were air dried in a clean drying chamber and then dried at 80°C for overnight in an oven. The samples were powdered in agate mortar and passed through 100-mesh sieve. Sampling is done from this powder. Two biological reference materials namely IAEA CRM V-10 and CTA VTL-2 (IJCT Poland) were used as a control and reference multielemental standard, respectively. The concentrations of all the elements investigated in this study are well certified in the reference material.

Irradiation and counting

About 50–80 mg of each sample was sealed in a polyethylene cover. Samples, reference standard and control sample were packed together and irradiated in the E8 position of the Apsara reactor, BARC. Irradiation time was varied between 30 min and 7 hr depending on the half lives of the activation products. The subcadmium neutron flux in this position is in the order of 1×10^{12} $cm^{-2}s^{-1}$. The samples were also irradiated at Dhruva reactor for 1 d in order to determine the elemental concentration of the long-lived radionuclides, such as Fe. The short irradiation and counting were conducted at

Table 1 Medicinal plant samples selected for the study

Sample number	Local name	Botanical name	Parts of plants: medicinal use
1	Boar	*Zizyphus jujuba*	Leaves: Diabetes, purify blood quality
2	Jambhul	*Eugenia jambolana*	Fruit: Diabetes
3	Todali	*Coccinia indica*	Leaf: Embryo growth
4	Limbu	*Citrus acida*	Fruit: Diarrhoea
5	Tulas	*Ocimum sanctum*	Whole plant: bronchitis, malarial fever, asthama, urinogenital disorder, vomiting, indigestion, antibacterial, antituberculosis, expectorant, anticatarrhal, antispasmodic, anthelminthic
6	Methi	*Trigonella foenumgraecum*	Seeds: diabetes insipidus, reduces fat, snake bite, cough

the reactor site followed by spectra unfolding at the Radiochemistry Division of BARC, Mumbai. The radionuclides used for the analysis and their γenergies are given in Table 2. All the samples and SRMs were counted at a calibrated sample-detector distance from a High Purity Germanium (HPGe) detector (Ortec) with 25% relative efficiency and 2.1 keV resolution at 1332.5 keV of ^{60}Co line, which was connected, to an IBM PC XT computer system.

Most of the short lived isotopes contributing to the dead time belong to the elements present in major Ca and minor Al levels. The presence of different elements analysed in various medicinal plants was confirmed by measuring their characteristic γ-ray energy as well as half lives which are in good agreement with the literature values. Radioactivity measuring times were chosen not to exceed 0.2 times the half lives of the radionuclide of interest. Long irradiated samples were brought to Radiochemistry Laboratory at Mumbai University and gamma activity was measured. Counting was followed for 1, 2, 6 and 12 hr at different intervals up to three months. Care was taken to obtain maximum elemental information from more than one counting and the reproducibility of data was checked. Elemental concentrations of various Ayurvedic medicinal plants were calculated by comparator method using control and reference multielemental standard as comparators.

Table 2 Radionuclides used for the analysis and their γ – energies

Nuclide	γ-ray energy in keV
^{42}K	1524
^{56}Mn	847
^{24}Na	1368
^{59}Fe	1099
^{65}Zn	1115
^{64}Cu	1040
^{60}Co	1332
^{82}Br	776
^{153}Sm	103
^{38}Cl	1642
^{140}La	1596
^{28}Al	1779

Note: Thermal neutron flux: 10^{12}–10^{13} n cm^{-2} s^{-1}.

Atomic absorption spectrometer measurement

The samples in the powdered form were accurately weighed and digested in (5:1) mixture of nitric acid and perchloric acid (Herber and Stoeppler, 1994). After digestion few drops of concentrated HCl was added. The solution was heated gently and then filtered. The residue was again subjected to digestion and filtrate was collected. The entire filtrate was diluted suitably with distilled deionized water. The dilute filtrate solution was used for analysis of elements of interest by Atomic Absorption Spectroscopy (AAS) (Perkin Elmer 3100 model) using suitable hollow cathode lamps. The concentration of various elements was determined by comparator method using A.R. grade solutions of elements of interest. The standard conditions for atomic absorption measurement are represented in Table 3.

RESULTS AND DISCUSSION

The SRMs of biological origin were analysed for quality control and method validation. It was observed that most elemental contents are within ±10% of the certified values. Standard deviations were also relatively small. The values listed in the Table 4 (which are averages of three independent measurements) are having the precision of ~ ±2 to 10%.

Table 3 Standard conditions for atomic absorption measurement

Element	Wavelength nm	Slit width nm	Lamp current mA
Cr	357.9	0.2	7
Ca	422.9	0.5	10
Cd	228.8	0.5	4
Ni	232.0	0.5	4
Pb	283.6	0.5	5
Hg	253.7	0.5	4

An examination of the data from Table 4 shows that different medicinal plants contain elements like K, Mn, Na, Fe, Zn, Cu, Co, Br, Sm, Cl, La, Al, Cr, Ca, Cd, Ni, Pb and Hg in various proportions. The presence of these elements in different plants was confirmed by measuring the half life of the corresponding radioisotope formed as well as their gamma energies. The elements Cr, Ca, Cd, Ni, Pb and Hg were analysed by AAS technique by measuring the absorbance of the species at its resonance wavelength. From the experimental data, it appears that the concentrations of some elements may vary by one order of magnitude or more within the leaves of the same plant. The variation in elemental concentration is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants are cultivated. Other factors responsible for a variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions (Rajurkar and Pardeshi, 1997). The active constituents of the medicinal plants are the metabolic products of the plant cells. A number of trace elements play an important role in the metabolism. These elements are called essential. An element is considered essential for a plant if the plant fails to grow normally and complete its life cycle in a medium adequately removed from the element were as in the presence of the suitable chosen concentration of that element it grows and reproduces normally.

An examination of the data from Table 4 shows that, for medicinal plants like Boar, Tulas and Jambhul, higher potassium content is observed in the samples collected from Aurangabad region. For Todali samples collected from Bhayander region, potassium and sodium content was observed to be maximum of 125.2 and 1.20 mg/g, respectively, while in Limbu the K content was high (43.5 mg/g), in samples collected from Pravranagar region. Potassium is accumulated within human cells by the action of the Na^+, K^+- ATPase (sodium pump). The regulation of such metal ion flows, especially of K and Na, is crucial to life and is most clearly exemplified by the ionic movements that occur in nerve cells during excitation and transmission of the action potential. The regulation of potassium is intimately involved with that of sodium and the two are largely interdependent. Potassium is an activator of some enzymes; in particular coenzyme for normal growth and muscle function (Birch and Padgham, 1994, p.531). The calcium content in various medicinal plant samples varies from 0.11 mg/g in Methi samples collected from Bhayander region to 7.65 mg/g in Jambhul samples collected from Aurangabad region. Calcium is essential for healthy bones, teeth and blood (Charles, 1992; Hughes, 1972). The health of the muscles and nerves

Table 4 Elemental concentration in various medicinal plants by NAA and AAS techniques

Medicinal plants	Regions	Elements																	
		K (mg/g)	Mn (μg/g)	Na (mg/g)	Fe (μg/g)	Zn (μg/g)	Cu (μg/g)	Co (μg/g)	Br (μg/g)	Sm (μg/g)	Cl (mg/g)	La (μg/g)	Al (mg/g)	Cr† (μg/g)	Ca† (mg/g)	Cd† (μg/g)	Ni† (μg/g)	Pb† (μg/g)	Hg† (μg/g)
Boar (*Zizyphus jujuba*)	1	21.40 (±0.15)	102 (±12)	0.20 (±0.04)	407 (±120)	28.8 (±6.1)	4.30 (±0.19)	0.80 (±0.09)	0.42 (±0.06)	0.44 (±0.05)	0.20 (±0.05)	0.25 (±0.03)	0.48 (±0.09)	104 (±23)	0.80 (±0.11)	0.21 (±0.04)	4.49 (±0.23)	2.24 (±0.18)	0.53 (±0.05)
	2	52.0 (±0.18)	77 (±10)	0.61 (±0.09)	355 (±83)	60.0 (±9.0)	2.65 (±0.08)	0.44 (±0.06)	1.20 (±0.08)	0.47 (±0.06)	0.38 (±0.06)	0.41 (±0.05)	1.65 (±0.07)	57.5 (±14.2)	0.45 (±0.06)	0.75 (±0.05)	2.67 (±0.18)	4.00 (±0.17)	0.66 (±0.05)
	3	65.3 (±0.15)	545 (±23)	0.26 (±0.05)	370 (±95)	75.5 (±13.3)	2.37 (±0.10)	0.76 (±0.08)	0.69 (±0.08)	0.85 (±0.05)	0.74 (±0.06)	0.64 (±0.08)	2.11 (±0.12)	66.4 (±8.1)	0.34 (±0.07)	0.44 (±0.06)	3.35 (±0.23)	ND	0.75 (±0.07)
Jambhul (*Eugenia-jambolana*)	1	4.32 (±0.09)	40.5 (±8.3)	0.47 (±0.09)	541 (±136)	88.4 (±13.8)	0.40 (±0.19)	0.31 (±0.04)	1.82 (±0.10)	0.10 (±0.02)	0.64 (±0.07)	0.66 (±0.07)	1.22 (±0.05)	40.7 (±6.3)	0.61 (±0.05)	0.84 (±0.07)	2.70 (±0.16)	3.34 (±0.12)	2.95 (±0.12)
	2	11.50 (±0.12)	166 (±15)	0.11 (±0.03)	740 (±150)	85.2 (±10.9)	0.80 (±0.15)	0.67 (±0.04)	1.64 (±0.11)	0.24 (±0.05)	0.55 (±0.05)	1.11 (±0.10)	2.68 (±0.08)	54.5 (±5.2)	2.45 (±0.20)	0.21 (±0.02)	7.48 (±0.30)	ND	0.40 (±0.06)
	3	54.0 (±0.14)	426 (±32)	0.54 (±0.08)	212 (±68)	40.6 (±12.0)	1.65 (±0.32)	1.33 (±0.06)	0.82 (±0.07)	0.43 (±0.06)	0.99 (±0.08)	0.82 (±0.09)	0.31 (±0.05)	74.2 (±8.0)	7.65 (±0.35)	0.50 (±0.05)	5.44 (±0.45)	1.00 (±0.10)	ND
Todali (*Coccinia indica*)	1	125.2 (±0.20)	45.8 (±12)	1.20 (±0.11)	425 (±117)	78.5 (±18.0)	6.25 (±0.49)	0.45 (±0.09)	5.51 (±0.20)	0.14 (±0.01)	0.74 (±0.10)	0.47 (±0.08)	3.81 (±0.07)	44.7 (±8.5)	0.66 (±0.09)	0.25 (±0.04)	1.75 (±0.14)	0.75 (±0.09)	2.32 (±0.14)
	2	112.3 (±0.18)	60.3 (±18.5)	0.84 (±0.10)	265 (±102)	91.0 (±14.6)	10.7 (±0.6)	0.76 (±0.07)	4.05 (±0.18)	1.10 (±0.13)	0.77 (±0.11)	0.71 (±0.06)	3.04 (±0.14)	40.4 (±8.6)	1.65 (±0.13)	4.82 (±0.14)	4.65 (±0.33)	0.48 (±0.08)	0.88 (±0.04)
	3	21.4 (±0.13)	72.1 (±17.3)	0.56 (±0.08)	384 (±97)	55.5 (±8.1)	5.81 (±0.41)	0.32 (±0.08)	1.00 (±0.12)	0.45 (±0.09)	0.44 (±0.08)	0.30 (±0.05)	1.19 (±0.10)	0.52 (±0.18)	2.11 (±0.18)	2.27 (±0.10)	0.50 (±0.09)	0.22 (±0.07)	0.47 (±0.04)

Limbu *(Citrus acida)*	1	6.02 (±0.05)	22.3 (±1.4)	0.64 (±0.09)	265 (±84)	97.2 (±8.5)	12.6 (±0.2)	0.71 (±0.06)	2.42 (±0.10)	0.31 (±0.07)	0.54 (±0.09)	0.66 (±0.08)	0.21 (±0.04)	26.6 (±5.9)	0.41 (±0.04)	6.44 (±0.22)	2.74 (±0.12)	1.88 (±0.13)	0.95 (±0.05)
	2	43.5 (±0.18)	17.5 (±2.0)	0.37 (±0.04)	123 (±63)	45.3 (±8.7)	35.5 (±0.8)	1.40 (±0.07)	2.95 (±0.11)	0.56 (±0.08)	0.84 (±0.11)	0.71 (±0.05)	0.40 (±0.07)	8.41 (±3.08)	0.74 (±0.07)	2.45 (±0.15)	3.77 (±0.17)	ND	1.23 (±0.07)
	3	20.6 (±0.12)	9.45 (±2.18)	0.84 (±0.09)	75.2 (±18.7)	20.4 (±3.0)	23.7 (±0.9)	0.52 (±0.07)	7.15 (±0.20)	0.74 (±0.07)	0.17 (±0.05)	0.14 (±0.02)	0.78 (±0.06)	5.12 (±8.15)	0.82 (±0.07)	1.72 (±0.11)	0.80 (±0.10)	2.00 (±0.15)	ND
Tulas *(Ocimum-sanctum)*	1	5.22 (±0.07)	48.5 (±8.3)	0.44 (±0.06)	345 (±123)	125 (±18)	13.4 (±0.3)	0.61 (±0.08)	4.30 (±0.14)	0.09 (±0.01)	0.54 (±0.07)	0.46 (±0.08)	0.31 (±0.07)	22.5 (±4.4)	0.29 (±0.03)	6.41 (±0.26)	8.72 (±0.25)	4.90 (±0.18)	3.45 (±0.18)
	2	2.43 (±0.04)	64.4 (±4.6)	0.70 (±0.08)	465 (±137)	211 (±21)	6.68 (±0.32)	0.71 (±0.09)	7.44 (±0.13)	0.20 (±0.06)	0.60 (±0.08)	0.11 (±0.03)	0.85 (±0.08)	7.50 (±0.98)	0.60 (±0.05)	4.33 (±0.20)	4.68 (±0.28)	6.84 (±0.20)	1.22 (±0.09)
	3	7.66 (±0.09)	55.0 (±3.8)	0.54 (±0.05)	183 (±43)	95 (±21)	1.05 (±0.13)	0.22 (±0.05)	6.11 (±0.17)	0.29 (±0.07)	0.84 (±0.09)	0.35 (±0.04)	0.71 (±0.07)	30.11 (±2.16)	0.21 (±0.02)	9.01 (±0.37)	2.80 (±0.20)	3.00 (±0.13)	7.45 (±0.14)
Methi *(Trigonel-lafoenum-graecum)*	1	1.20 (±0.07)	92.1 (±10.3)	0.20 (±0.03)	247 (±76)	77 (±15)	4.60 (±0.33)	0.30 (±0.07)	8.88 (±0.21)	0.31 (±0.08)	0.37 (±0.04)	0.21 (±0.01)	1.84 (±0.12)	2.50 (±0.89)	0.11 (±0.02)	2.42 (±0.19)	1.50 (±0.11)	ND	2.55 (±0.08)
	2	8.11 (±0.10)	30.5 (±4.1)	0.84 (±0.09)	760 (±173)	212 (±83)	7.35 (±0.58)	0.84 (±0.06)	1.75 (±0.13)	0.49 (±0.09)	0.78 (±0.05)	0.29 (±0.03)	3.55 (±0.26)	4.25 (±0.95)	1.45 (±0.09)	2.60 (±0.21)	5.20 (±0.19)	2.11 (±0.10)	ND
	3	4.65 (±0.06)	44.6 (±5.2)	0.65 (±0.07)	315 (±94)	620 (±47)	1.09 (±0.14)	0.41 (±0.07)	0.85 (±0.14)	0.60 (±0.07)	1.20 (±0.08)	0.61 (±0.07)	3.02 (±0.20)	25.0 (±3.2)	0.85 (±0.07)	7.85 (±0.41)	9.15 (±0.27)	4.36 (±0.11)	3.10 (±0.17)
CTA VTL-2 (Virginia Tobacco Leaves)		[10.3] {10.0} (±0.09)	[79.7] {80.2} (±7.5)	[0.312] {0.308} (±0.02)	[1083] {1100} (±104)	[43.3] {44.1} (±2.7)	[18.2] {18.9} (±0.9)	[0.429] {0.445} (±0.07)	[14.3] {15.2} (±0.09)	[0.157] {0.162} (±0.01)	[7.43] {7.25} (±0.31)	[1.01] {1.07} (±0.08)	[1.682] {1.704} (±0.07)	[1.87] {1.79} (±0.09)	[36.0] {35.4} (±1.9)	[1.52] {1.53} (±0.06)	[1.98] {2.01} (±0.10)	[22.1] {22.9} (±1.1)	[0.048] {0.050} ±0.002)

†Elements analysed by AAS technique; ND = Not Detected.

Note: 1. Bhayander, 2. Pravaranagar, 3. Aurangabad. (±) Standard Deviations [ ] Certified Values { } Measured Values.

depends on calcium. It is required for the absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine, for the activation of enzymes such as the pancreatic lipase. The recommended daily dietary allowance for Ca is for children between 500 and 1000 mg and 800 mg for adults. The elements like Zn, Fe and Cr are essential trace elements (micro nutrients) for living organisms. Zinc is relatively non-toxic (Prasad, 1982, p.3). From the results it appears that the concentration of Zn is high in the medicinal plant samples collected from Aurangabad region. The concentration of zinc varies from minimum in Limbu (20.4 μg/g) to maximum in Methi (620 μg/g). Zinc deficiency is characterised 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 zinc-deficient human subjects (Prasad, 1982, p.3). Zinc is necessary for the growth and multiplication of cells (enzymes responsible for DNA and RNA synthesis), for skin integrity, bone metabolism and functioning of taste and eyesight (Thunus and Lejeune, 1994, p.333, 667). Tulsi, known for numerous medicinal properties in Ayurvedic literature, has been used as an antifertility agent (Khanna et al., 1986). Due to their low zinc content (<211 μg/g), the leaves of Tulsi may be recommended as antifertility agent. The role of zinc in spermatogenesis (Prasad, 1993) may be correlated with low zinc content and its antifertility effect. Iron occupies a unique role in the metabolic process. The role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells (Sigel, 1978, p.417). Iron deficiency is the most prevalent nutritional deficiency in humans (Reddy et al., 1987, p.429) and is commonly caused by insufficient dietary intake, excessive menstrual flow or multiple births. In this case, it results especially an anemia. Fe is important because it eliminates phlegm and strengthens the function of stomach. Hence the daily intake of iron is necessary. The requirement of Fe for an adult is 20 mg/day and for a child is 10 mg/day. The Fe concentration in the medicinal plant samples analysed ranges from 75.2 μg/g in Limbu samples collected from Aurangabad region to 760 μg/g in Methi samples collected from Pravranagar region. The concentration of Fe in Boar, Jambhul Todali, Limbu, Tulas and Methi were observed to be higher. Hence the use of these medicinal plants may be advised to compensate for an iron deficiency. Chromium plays an important role in diabetes treatment. It is an important element required for the maintenance of normal glucose metabolism. The function of chromium is directly related to the function of insulin, which plays a very important role in diabetes. Chromium is found in the pancreas, which produces insulin. One usable form of chromium is the Glucose Tolerance Factor (GTF) (Zetic et al., 2001), an inorganic compound containing glutamic acid, cysteine and niacin. The absorption of the trivalent chromium in GTF is about 10 to 25%. Only 1% of inorganic chromium is absorbed. GTF is essential for the efficient use of insulin. It enhances the removal of glucose from the blood. Chromium also acts as an activator of several enzymes. Deficiency of chromium decreases the efficiency of insulin and increases sugar and cholesterol in the blood. Deficiency of chromium decreases the efficiency of insulin and increases sugar and cholesterol in the blood. Cr is important in the potentiating of insulin as a constituent of GTF (Anderson, 1989). Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be a risk factor in artherosclerotic disease (Mertz, 1982, p.315). From the result obtained, it is observed that the chromium content is high

in the medicinal plant samples like Boar, Todali and Limbu collected from Bhayander region. The chromium content is fairly high in Boar and Jambhul samples (40.7 to 104.0 μg/g). Hence the use of this medicinal plants may be advised for the treatment and control of diabetics .The leaves containing a fairly good amount of Br may be used for the preparation of the drugs in curing natural diuretic, phlegm eliminating and stomach invigorating diseases (Chen et al., 1993). In experiments conducted by Anke et al. (1988) with growing, gravid and lactating goats, a poor Br nutrition (<1 mg/g dry matter) led to a significantly reduced growth, a worse conception rate, reduced milk fat production and decreased hemoglobin content. Since Br accumulates well in plants, application of agricultural chemicals such as methyl bromide as fumigant leads to Br enrichment in agricultural products. From the results obtained, it is observed that the concentration of Br (8.88 μg/g) is high in Methi samples collected from Bhayander region. However, further investigations regarding possible essentiality of Br is necessary. Mn is also an essential element required for various biochemical processes (Gunther and Konieczynski, 2003). In most of the constituents, it is found to be <200 g/g. From the results presented in Table 4, it is observed that the concentration of Mn (545 μg/g) is high in Boar samples collected from Aurangabad region. Mn is important for several enzymatic processes; it helps in eliminating fatigue and reduces nervous irritability (Hamilton et al., 1994; O'Dell and Sunde, 1997; Prasad, 1993). Cobalt is an essential element for the plants having the capability to fix nitrogen in the root tubercles. Animals are able to synthesise vitamin B12, which is the main source of Co in animal foods. Nevertheless, only a part of Co in food derived from animals is present in the form of cobalamines. The recommended daily intake of vitamin B12 for adults is 3 mg (0.13 mg Co), taking into account that only 50% is absorbed in the intestine (Thunus and Lejeune, 1994, p.333, 667). In humans, deficiency of vitamin B12 leads to a megaloblastic anemia. Cobalt also plays also an important role in thyroid metabolism in humans. The elements like Hg, Pd, Cd and Ni are supposed to be toxic in nature and their presence in trace amount in various medicinal plant sample analysed is due to the pollution arising from automobile and industrial activities.

Inter-elemental correlations

Several literature reports suggest interrelationship of various elements (Herber and Stoeppler, 1994; Kumar et al., 2005; Lokhande et al., 2009a,b; Underwood, 1977). From the experimental data, it is observed that K/Na ratio in Boar is 85.2 in Pravaranagar region, while it increases to 251.2 in Aurangabad region. This indicates that the K content in Boar is 85.2 times of Na content in Pravranagar region while in Aurangabad region it increases to 251.2. Also the plant samples collected from Pravranagar region, the K content is 104.5 times of Na content in Jambhul, while in Limbu it is 117.6 and it increases to 133.7 in Todali. The regional variation of K/Na ratio for different plant samples is graphically represented in Figure 1. There exists a strong linear relationship between Cr and Zn, in medicinal plants like Methi and Limbu having correlation coefficient (r) 0.985 and 0.984, respectively as shown in Figures 2 and 3. The two transition elements Cr and Zn are well known for their role in biochemical processes. This is interesting because the availability of Zn in the range of 14.8–28.4 μg/g may be beneficial for diabetic patients as its deficiency has been correlated with acute and chronic mal absorption states (Garg et al., 2005; O'Dell and Sunde, 1997). Cr (III) may be bound with glycine, cysteine

Figure 1 Regional variation of K/Na ratio in different medicinal plant samples

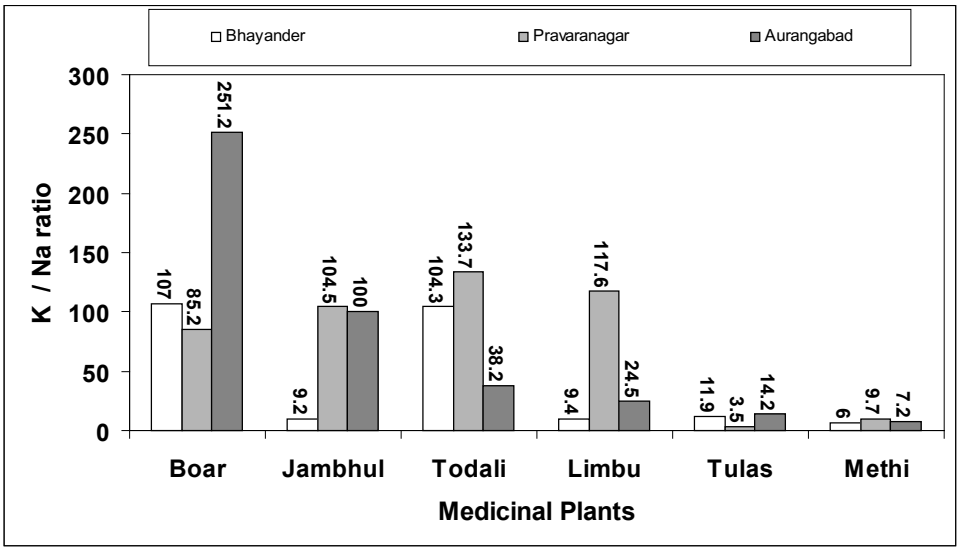
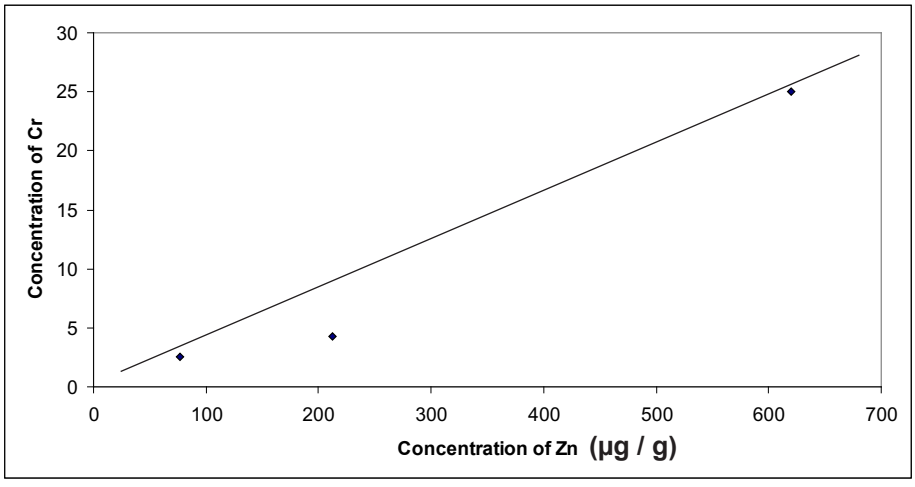
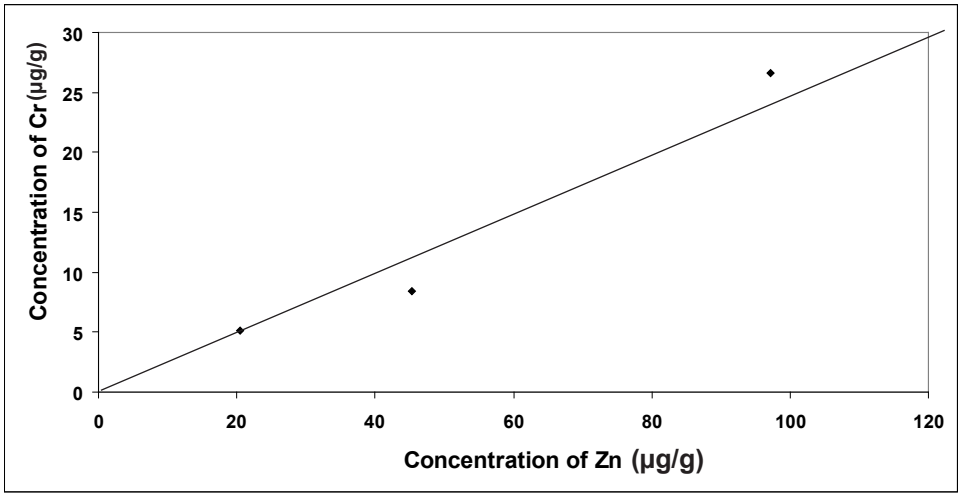


Figure 2 Correlation between Zn and Cr in Methi *(Trigonella foenum-graecum)*



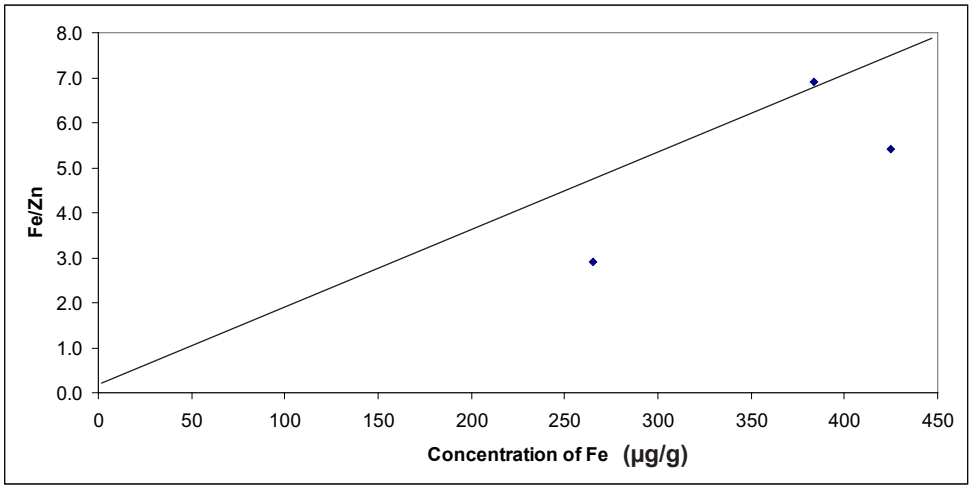
Note: Correlation coefficient (*r*) = 0.985.

Figure 3 Correlation between Zn and Cr in Limbu *(Citrus acida)*



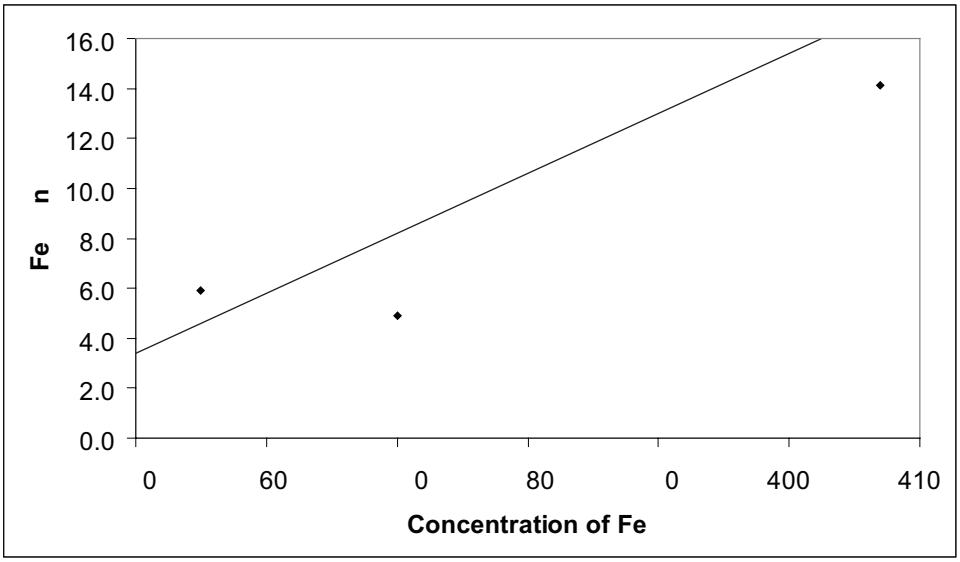
Note: Correlation coefficient (*r*) = 0.984.

Figure 4 Variation in Fe/Zn ratio vs. Fe concentration in Todali *(Coccinia indica)*



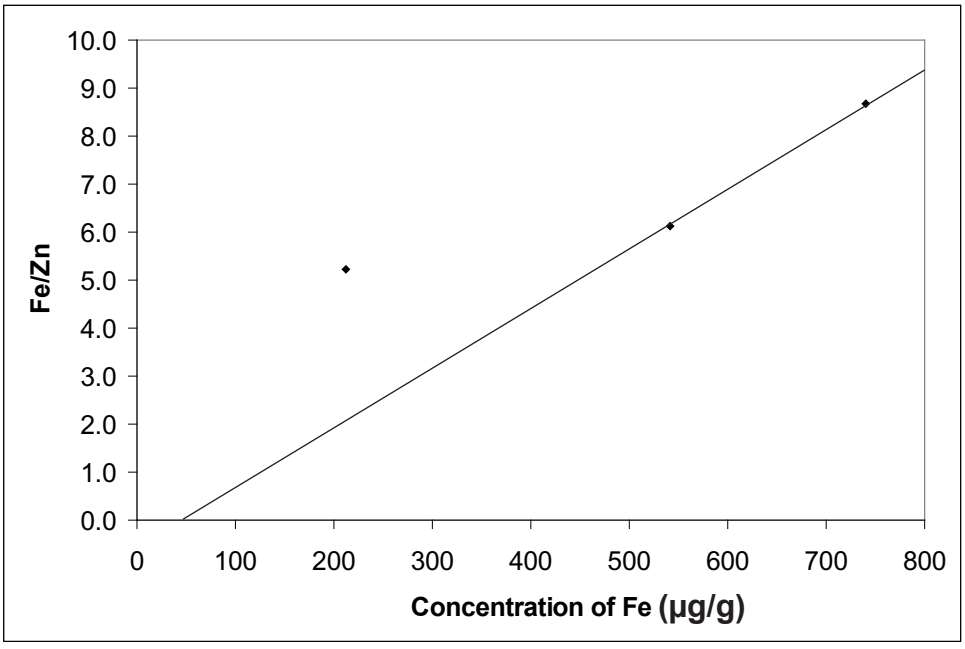
Note: Correlation coefficient (*r*) = 0.808.

Figure 5 Variation in Fe/Zn ratio versus Fe concentration in Boar *(Zizyphus jujuba)*



Note: Correlation coefficient (*r*) = 0.927.

Figure 6 Variation in Fe/Zn ratio versus Fe concentration in Jambhul *(Eugenia jambolana)*



Note: Correlation coefficient (*r*) = 0.915.

and glutamic acids to form a complex molecule called GTF (Zetic et al., 2001). Fe and Zn are essential elements for biochemical processes (Kumar et al., 2005). For the medicinal plants like Todali, a plot of Fe versus Fe/Zn ratio shows linear relationship (Figure 4) with $r = 0.808$, which represents somewhat poor relationship. However for medicinal plants like Boar and Jambhul, a plot of Fe versus Fe/Zn ratio shows an excellent linear relationship with $r = 0.927$ and 0.915, respectively (Figures 5 and 6). In general, it may be mentioned that interrelationship of several elements in medicinal herbs suggest synergistic or antagonistic effects, thus providing various elements to the body in bioavailable form in a balanced manner with almost no harmful effects except some environmental contaminants. These, however, should be avoided by collecting herbs grown in a clean and well controlled environment (Kumar et al., 2005).

CONCLUSIONS

One of the main problems of ethnomedicine, Ayurvedic system is lack of solid scientific evidence regarding safety, efficacy, quality of practices and their precise molecular mechanisms. However, many Ayurvedic preparations appear to demonstrate significant success in treatment of many complex diseases. Presumably Ayurvedic medicines contain trace elements in a bioavailable form and their impact on the overall pharmacological action cannot be ruled out. Although the direct link between elemental content and curative capability is yet to be established, such studies are vital to understanding the pharmacological action of herbs. The data obtained in the present work will be helpful in the synthesis of new Ayurvedic drugs which can be used for the control of various diseases. In order to develop a stronger basis for appreciating the curative effects of medicinal plants, there is a need to investigate their elemental composition, which is very often overlooked in biochemical assays. It has been demonstrated that Instrumental Neutron Activation Analysis (INAA), with multielemental characterisation over a wide range of concentration, its blank free-nature and minimum sample preparation, is ideal for such studies. The data obtained on elemental concentration of the medicinal plants studied will be useful in deciding the dosage of the Ayurvedic drugs prepared from these plant materials. The results of the present research work will be helpful to Ayurvedic clinicians and scientists who would like to pursue further research in the areas of Ayurvedic and alternative medicines.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. A.G.C. Nair and Dr. A.V.R. Reddy, Radiochemistry Division, B.A.R.C., Mumbai, for providing the irradiation, counting facilities and also for the valuable suggestions provided during the experimental work.

BIOGRAPHY

Ram Lokhande completed his Masters degree in Physical Chemistry from Mumbai University in 1976, PhD (Chemistry) from Advanced Study Centre in Nuclear Chemistry, University of Pune in 1981. He joined University of Mumbai in 1982 as a Lecturer of Chemistry. He is presently working as a Professor of Chemistry and has about 200 research papers presented, 140 research papers published in various National and International Journals. He is also the research guide for MSc and PhD in Mumbai University and has guided 40 PhD and 20 MSc (By Research) students

in Chemistry. His research areas of interest are Radioanalytical Nuclear Chemistry, Environmental Chemistry, Solvent extraction, Ion exchange techniques. He is the Member of Indian Society of Analytical Scientists, National Association for Application of Radio Isotopes and Radiation in Industry (NAARI), Indian Nuclear Society, Bhabha Atomic Research Centre, (B.A.R.C.), Anushaktinagar, Mumbai and Indian Council of Chemists, Agra, India. He is on the Editorial Board of Asian *Journal of Chemistry*, *International Journal of Chemical Sciences*, Jaipur, India.

Pravin Singare completed his Masters degree in Inorganic Chemistry in 1997, PhD (Chemistry) from Mumbai University in 1999. He is expert in concentration of multimetal sulphide ore by froath flotation technique and subsequent analysis by various instrumental methods. He has worked in Sikkim Minning Corporation, Sikkim, India on the project related to the concentration of Cu/Pb/Zn sulphide ores. He is presently working as an Assistant Professor in Chemistry at Bhavan's College, Andheri, Mumbai. He has guided 15 students for their MPhil in Chemistry. He has presented 17 research papers and published 80 research papers in various National and International Journals. His research areas of interest are Radioanalytical Nuclear Chemistry, Ion exchange techniques, Environmental analysis, Trace elemental analysis in medicinal plants by INAA and AAS techniques. He is the Member of Indian Society of Analytical Scientists, National Association for Application of Radio Isotopes and Radiation in Industry (NAARI), Indian Nuclear Society, Bhabha Atomic Research Centre, (B.A.R.C.), Anushaktinagar, Mumbai, and Indian Council of Chemists, Agra, India.

Mahadeo Andhale completed his Masters and MPhil from University of Pune. He was awarded with PhD from University of Mumbai in 2007. He has presented several research papers on application of INAA technique in various National conferences and has international research papers to his credit. Presently, he is working as an Assistant Professor in Chemistry at Vartak College, Mumbai.

Raghunath Acharya received his Masters degree from Utkal University, India and then joined 37th batch of BARC Training School in 1993. After graduation from Training School he joined Radiochemistry Division. He received his PhD from University of Mumbai in year 2000. He did his postdoctoral studies during 2000–2002 at Dalhousie University, Canada. He is instrumental in optimising a relatively new method of NAA called k_o-NAA method, which as capability to obtain multielemental profiles using a single comparator instead of multielement standard. He is also actively engaged in speciation analysis of trace elements like arsenic and iodine by chemical separation and NAA. He has significant contribution in Nuclear chemistry as evident from his research publications in National and International journals.

REFERENCES

Abou Arab, A.A.K. and Donia, M.A.A. (2000) 'Heavy metals in Egyptian spices and medicinal plants and the effect of processing on their levels', *Journal of Agricultural and Food Chemistry*, Vol. 48, No. 6, pp.2300–2304.

Ambasta, S.P. (1986) *The Useful Plants of lndia*, CSIR, New Delhi, India.

Anderson, R.A. (1989) 'Essentiality of chromium to humans', *Science of the Total Environment*, Vol. 86, Nos. 1–2, pp.75–81.

Anke, M., Groppel, B., Arnhold, W. and Larger, M. (1988) 'Essentiality of the trace element Bromine', in P. Briitter and P. Schramel (Eds). *Trace Element Analytical Chemistry in*

Medicine and Biology, Vol. 5, pp.619–629, Berlin, New York: Walter de Gruyter.

Bakhru, H.K. (1998) *Herbs that Heal Natural Remedies for Good Health*, New Delhi, India: Orient Paperbacks, Division of Vision Book Pvt. Ltd.

Birch, N.J. and Padgham, C. (1994) 'Potassium', in H.G. Seiler, A. Sigel and H. Sigel (Eds). *Handbook on Metals in Clinical and Analytical Chemistry*, pp.531–535, New York: Marcel Dekker.

Charles, P. (1992) 'Calcium absorption and calcium bioavailability', *Journal of Internal Medicine*, Vol. 231, No. 2, pp.161–165.

Chen, K.S., Tseng, C.L. and Lin, T.H. (1993) 'Trace elements in natural drugs determined by INAA', *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 170, No. 1, pp.265–280.

Garg, A.N., Kumar, A., Maheshwari, G. and Sharma, S. (2005) 'Isotope dilution analysis for the determination of zinc in blood samples of diabetic patients', *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 263, No. 1, pp.39–43.

Gunther, W. and Konieczynski, P. (2003) 'Speciation of Mg, Mn, and Zn in extracts of medicinal plants', *Analytical and Bioanalytical Chemistry*, Vol. 375**,** No. 8, pp.1067–1073.

Hamilton, E.M.N., Whitney, E.N. and Sizer, F.S. (1994) *Nutrition: Concepts and Controversies*, 4th edition, St. Paul, MN, USA: West Publishing Co.

Herber, R.F.M. and Stoeppler, M. (Eds.) (1994) *Trace Element Analysis in Biological Specimens*, New York: Elsevier.

Hughes, M.N. (1972) *The Inorganic Chemistry of Biological Processes*, London: Wiley.

lyengar, G.V. (1989) *Elemental Analysis of Biological Systems-Biomedical Environmental, Compositional and Methodological aspects of Trace Elements*, Florida, Boca Raton: CRC Press.

Kanias, G.D., Kilikoglou, V., Tsitsa, E. and Loukis, A. (1993) 'Determination and statistical analysis of trace element and active constituent concentrations in the medicinal plant Eucalyptus Camaldulensis Dehnh (E. Rostratus schlecht)', *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 169, No. 2, pp.483–491.

Khanna, S., Gupta, S.R. and Grover, J.K. (1986) 'Effects of long term feeding of tulsi Ocimum sanctum on reproductive performance of adult albino rats', *Indian Journal of Experimental Biology*, Vol. 24, No. 5, pp.302–304.

Kumar, A., Nair, A.G.C., Reddy, A.V.R. and Garg, A.N. (2005) 'Analysis of essential elements in Pragya-peya- a herbal drink and its constituents by neutron activation', *Journal of Pharmaceutical and Biomedical Analysis*, Vol. 37, No. 4, pp.631–638.

Lokhande, R.S., Singare, P.U., Andhale, M.L., Acharya, R., Nair, A.G.C. and Reddy, A.V.R. (2009b) 'Study of some ayurvedic Indian medicinal plants for the essential trace elemental contents by instrumental neutron activation analysis and atomic absorption spectroscopy techniques', *Radiochimica Acta*, Vol. 97, No. 6, pp.325–331.

Lokhande, R.S., Singare, P.U., Andhale, M.L., Acharya, R., Nair, A.G.C. and Reddy, A.V.R. (2009a) 'Analysis of mineral content of some medicinal plants by NAA and AAS techniques', *Radiochemistry*, Vol. 51, No. 3, pp.321–325.

Majid, A.A.B., Sarmani, S., Yusoe, N.I., Wie, Y.K. and Hamzah, F. (1995) 'Trace elements in Malaysian medicinal plants', *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 195, No. 1, pp.173–183.

Mertz, W. (1982) 'Clinical and public health significance of chromium', in A.S. Prasad (Ed). *Clinical, Biochemical and Nutritional Aspects of Trace Elements*, pp.315–323, New York: Alan R. Liss, Inc.

O'Dell, B.L. and Sunde, R.A. (Eds.) (1997) *Handbook of Nutritionally Essential Mineral Elements*, New York: Marcell Dekker Inc.

Parchure, S.N. (1983) *Charak Samhita*, Pune, India: Sagar Publications.

Patel, N.G. (1986) 'India's traditional medicine: Ayurveda', in R.P. Steiner (Ed). *Folk Medicine: The Art and the Science*, pp.41–45, Washington, DC: American Chemical Society.

Powel, J.J., Burden, T.J. and Thompson, R.P.H. (1998) 'In vitro mineral availability from digested tea: a rich dietary source of manganese', *Analyst*, Vol. 123, No. 8, pp.1721–1724.

Prasad, A.S. (1982) 'Clinical and biochemical spectrum of zinc deficiency in human subjects', in A.S. Prasad (Ed). *Clinical, Biochemical and Nutritional Aspects of Trace Elements*, pp.3–6, New York: Alan R. Liss, Inc.

Prasad, A.S. (1993) *Essential and Toxic Elements in Human Health and Disease: an Update*, New York: Wiley-Liss.

Rajurkar, N.S. and Pardeshi, B.M. (1997) 'Analysis of some herbal plants from India used in the control of diabetes mellitus by NAA and AAS techniques', *Applied Radiation and Isotopes*, Vol. 48, No. 8, pp.1059–1062.

Reddy, M.B., Chidambaram, M.V. and Bates, G.W. (1987) '**Iron** Bio-availability', in G. Winkelmann, D. van der Helm and J.B. Neilands (Eds). *Iron Transport in Microbes, Plants and Animals*, pp.429–443, New York: VCH.

Sharma, P.V. (1993) *Dravya Guna Vigyan*, Varanasi, India: Chaukhamba Bharati Academy.

Sigel, H. (Ed.) (1978) 'Iron in model and natural compounds', *Metals in Biological Systems*, Vol. 7, pp.417–425, New York: Marcel Dekker.

Thunus, L. and Lejeune, R. (1994) 'Cobalt, Zinc', in H.G. Seiler, A. Sigel and H. Sigel (Eds). *Handbook on Metals in Clinical and Analytical Chemistry*, pp.333–667, New York: Marcel Dekker.

Underwood, E.J. (1977) *Trace Elements in Human and Animal Nutrition*, New York: Academic Press.

Zetic, V.G., Tomas, V.S., Grba, S., Lutilsky, L. and Kozlek, D. (2001) 'Chromium uptake by Saccharomyces cerevisiae and isolation of glucose tolerance factor from yeast biomass', *Journal of Biosciences*, Vol. 26, No. 2, pp.217–223.