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Quantitative Determination of Essential and Trace Elements in Indian Ayurvedic Medicinal Herbs by WD-XRF spectrometry

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Abstract

Medicinal plants contain essential and trace elements, which are very important for the human body. India had a rich experience of health benefits through utilising many varieties of Ayurvedic medicinal herbs. Our emphasis in the current paper is on the characterization of five Medicinal plants (Oroxylum indicum (Linn.) Vent., Leucas indica (L.) R. Br. Ex Vatke, Premna tomentosa (Willd.), Piper chaba (Hunter) and Hedychium spicatum (Buch.-Ham.) for their macro and microelement contents using wavelength dispersive X-ray fluorescence (WD-XRF) spectrometry. The applicability of the method particularly for the simultaneous determination of sixteen elements (Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Ba and Pb) in biological matrices has been evaluated in terms of the detection limit, precision and accuracy. The method was validated by analyzing five Chinese certified reference materials (NCS ZC73012 (cabbage), NCS ZC73013 (spinach), NCS ZC73017 (apple), NCS ZC85006 (tomato) and NCS DC73348 (bush, branches and leaves)) of vegetable standards. In general, good agreement was achieved between certified values and measured ones with recoveries ranging from 92 to 108%. The WD-XRF method proposed proved to be an effective tool for the investigation and quality control processes of vegetation samples.

Keywords

Trace elements, X-ray fluorescence, Quantification, Reference materials

INTRODUCTION

Medicinal plants are an important part of the biological heritage of the earth therefore; they have constantly been valued as a source for the treatment of a variety of diseases and having a vital role in providing the modern medical science with novel substances for developing life saving drugs and chemical entities. It is a fact, that the development of number of modern scientific medicines is attributed to active principles isolated from medicinal plants. Countries like China, India, Egypt and Greece had a rich experience of the health benefits and the utility of many types of plants.

Though synthetic drugs and chemotherapy made headway in the twenty-first century, according to WHO, more than 70% of the world's population still depends on traditional medicines, while about half of the population in industrialized countries use this as an alternative medicine for their primary health care needs [1]. One reason for the use of herbal products may be because of their observed and proven efficacy and being free from some toxic effects associated with synthetic drugs. Nowadays,

plant materials are widely used by many countries world over as home remedies and nutritional supplements [2] or as raw materials by the pharmaceutical industry, representing a substantial proportion of the global market.

Medicinal plants contain essential and trace elements, which can be available to the human body from any kind of consumption of herbs. Nearly four decades ago, many attempts have been made, particularly in the field of medical science, to establish relationships between the concentration of elements and specific biological dysfunctions or disease [3, 4]. These studies have successfully been established the impact of the concentration of the elements (particularly toxic) on the human health. Every element has some specific importance in the life system, though it may be present in the human organism in very minute quantities. Their excess or deficiency is responsible for upsetting the equilibrium and normal functioning of the human system. In addition, the geographical origin of plants belonging to the same species can result in different concentrations of elements and their bioavailability, depending on the soil features and environmental pollution. Quantitative characterization of these elements may provide useful information about the origin of the elements and helps to understand their pharmacological action. Further, these results are helpful to estimate the dosage of the herbal medicine prepared from these plant materials.

The concentration of heavy metals plays a key role in making raw plants admissible in the production of synthetic drugs. The permissible limit of heavy metals in the dietary contents as per the WHO is, lead (10 ppm), cadmium (0.3 ppm), and arsenic (3 ppm) [5]. In India, the department of AYUSH (*Ayurveda*, *Yoga* & *Naturopathy*, *Unani*, *Siddha* and *Homeopathy*) under the ministry of Health and Family Welfare, Govt. of India, issued an order in October 2005 (F.No. K-11020/5/97-DCC (AYUSH)), for new permissible limits of heavy metals in *Ayurvedic* herbal products following the WHO guidelines. Keeping in view the importance of the medicinal herbs, we present and discuss here, the elemental composition and concentration of five important medicinal plants investigated by wavelength dispersive X-ray fluorescence (WD-XRF) technique.

EXPERIMENTAL

Instrumental details

Samples were characterized using WD-XRF spectrometer (Bruker S4 Pioneer), equipped with a 4 kW, Rh anode X-ray tube with six

analyzer crystals (LiF(200), PET, OVO-55, OVO-N, OVO-C and OVO-B). It has a sealed proportional counter for lighter elements and a scintillation counter for heavy element detection. X-ray exposure time and power conditions were adjusted for each element by a pre-calibrated program. The recorded spectra were evaluated by fundamental parameters method using the software linked to the equipment. The validation of the results was based on the analysis of five Chinese certified reference materials of vegetable standards (NCS ZC73012 (cabbage), NCS ZC73013 (spinach), NCS ZC73017 (apple), NCS ZC85006 (tomato) and NCS DC73348 (bush, branches and leaves)).

Materials and Methods

The samples of the *Ayurvedic* medicinal herbs analyzed were obtained from the natural product division of our laboratory. Originally, all these herbs were collected by natural product division of our laboratory from Tirumala forest, Tirupati, Andhra Pradesh, India. Authentication was done by Dr K Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. A voucher specimen was deposited in the herbarium of the Botany department, Sri Venkateswara University, Tirupati. The name and other details of the herbs analyzed are listed in Table 1.

Table 1 Nomenclature and medicinal use of the plants for experimental study

Code	Family	Common name	Part	Use
S1	Bignoniaceae	Dundilum	Stem-bark	Anti-tumor, anti-inflammatory and anti-bacterial
S2	Lamiaceae	Tummi	Whole plant	
S3	Verbanaceae	Krishna palai	Stem-bark	Anti-microbial and urinary track problems
S4	Piperaceae	Pepper	Roots	Liver disorders and hepatotoxicity
S5	Zingiberaceae	Kapura kachri	Rhizomes	Analgesic, anti-inflammatory and diuretic
				Liver disorders, bronchial asthma and nausea

Sampling and pre-treatment for the WD-XRF analysis

Five species of medicinal herbs traditionally used in India were studied (Table 1): *Oroxylum indicum* (S1), *Leucas indica* (S2), *Premna tomentosa* (S3), *Piper chaba* (S4) and *Hedychium spicatum* (S5). All the samples were oven dried at 70°C for 24 h and then crushed into fine powder using an agate mortar. Later, the powder was passed through an 80µ mesh to get the particle size uniform. Five grams of each original sample

was taken in an aluminium cup and pressed into a pellet using a hydraulic press (HERZOG, type: TP40/2D) at 15 tons to obtain pellet of moderate thickness. Care has been taken to reduce the time between pelletization and analysis as short as possible in order to avoid deformation of the flat surfaces of the pellets [6, 7].

RESULTS AND DISCUSSION

Table 2 lists the concentrations of sixteen minor and trace elements determined in five medicinal herbs by WD-XRF.

Table 2 Chemical composition of medicinal plants used in the study (percent (%)

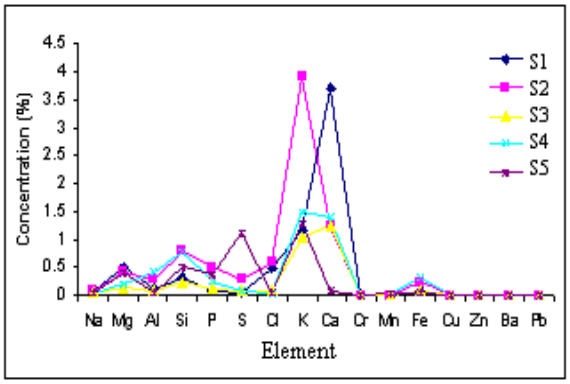
Element	S1	S2	S3	S4	S5
Na	0.0771	0.0834	0.0598	0.0527	0.0661
Mg	0.518	0.425	0.140	0.212	0.400
Al	0.1088	0.2729	0.0747	0.4412	0.0640
Si	0.318	0.808	0.230	0.776	0.522
P	0.088	0.513	0.109	0.262	0.362
S	0.057	0.279	0.084	0.097	1.118
Cl	0.490	0.601	0.086	0.026	0.051
K	1.159	3.915	1.031	1.490	1.292
Ca	3.694	1.226	1.245	1.392	1.116
Cr	0.0002	0.0003	0.0002	0.0002	0.0003
Mn	0.0014	0.0107	0.0049	0.0155	0.0185
Fe	0.0787	0.2351	0.0856	0.2995	0.0519
Cu	0.0011	0.0004	0.0005	0.0006	0.0005
Zn	0.0019	0.0030	0.0067	0.0024	0.0046
Ba	0.0024	0.0013	0.0022	0.0039	0.0019
Pb	ND	ND	0.0025	0.0016	0.0031

ND: not detected; values are expressed as mean of two replicates

The accuracy of the method was validated by analyzing five Chinese certified reference materials: NCS ZC73012 (cabbage), NSC ZC73013 (spinach), NCS ZC73017 (apple), NCS ZC85006 (tomato) and NCS DC73348 (bush branches and leaves) and the values are given in Table 3. The measured values are in good agreement (with recoveries ranging from 92 to 108%) with the certified values. Table 4 gives calibration data for sixteen elements generated with five standards (those were used for method validation). Out of sixteen elements listed, which are considered to be essential to the life system [8, 9] nine are macro nutrients (Na, Mg, Al, Si, P, S, K, Ca and Fe) and five are defined as micro-nutrients (Cr, Mn, Cu, Zn and Ni). The main constituent elements of the plants are Mg, K, Ca, Si and Cl. Out of five medicinal herbs, three of them (S3, S4 and S5) are having all the 16 elements and the remaining two (S1 and S2) are having 15 elements except Pb.

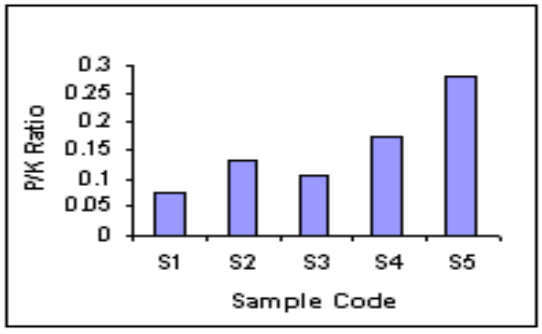
Calcium & Potassium are the most important elements for the human body. It is necessary for the membrane function, muscle contraction and nerve impulse transmission. In all these medicinal plants Ca and K are present in relatively high concentration (Ca, 3.694 - 1.116%; K, 3.915 - 1.013%). Another essential element Mg, which is useful for brain and liver function, was found to be between 0.518% (S1) and 0.140% (S3). Concentration of Al, Si and P was also high in all the herbs with ranges as follows; Al (0.4412 – 0.064%), Si (0.808-0.23%) and P (0.513-0.088%). Another important element Sulphur which is found in many amino acids and is responsible in regulating the heart and brain function was detected in all the herbs (1.118%, high concentration) in S5 and a low value in (0.057%) in S1. The variation in the elemental concentrations of Mg, Si, S, K, and Ca in the individual herbs was much larger than that of the rest of the elements as demonstrated in Fig. 1.

Figure 1 Variation of elemental concentrations of Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Cu, Zn, Ba and Pb in herbs of S1, S2, S3, S4 and S5.



It is well known that, nutrition in the form of food and fluids, mainly supplies trace elements to the human body. The essential trace elements, Fe, Cu, Mn, Zn, Ni, and Cr were found at 0.01 to 0.001% in these medicinal herbs. Lead is known to be toxic and non-essential to the human body. Three of the medicinal herbs S3 (0.0025%), S4 (0.0016%) and S5 (0.0031%) had lead in excess quantities as against the WHO standards[5]. None of the herbs were having other toxic elements like, As and Cd at detectable levels.

Figure 2 Variation in P/K ratio in herbs of S1, S2, S3, S4 and S5.



Literature survey reveals that, some plants are having all or some of the toxic elements [10, 11]. It could probably be in part due to the natural variation of these trace elements in the soils on which these medicinal plants grow or plant species, stage of maturity,

yield, climate, fertilizers and mineral contents and other factors on which trace element concentrations in plants are dependent. The absence of the toxic heavy metals (except Pb in S3-S5) in all the medicinal herbs indicates that these herbs were unpolluted in their natural habitats.

In the human body, the elemental uptake systems are never entirely specific for a single element. These systems often show competition between similar chemical species of different elements, resulting in inhibition of uptake of essential elements and uptake of competing potentially toxic elements. Because of these competitive interactions, ion ratios often control the cellular uptake of toxic and nutrient elements. Several researchers [12, 13], have already been suggested about the study of the interrelationship of various elements. In this context, we have plotted P/K ratio against the medicinal herb (code, S1-S5) as shown in Fig. 2. It can be seen that S1, S3 (P/K= 0.076 & 0.106) and S2, S4 (0.131 & 0.176) exhibit close values. A plot of Fe/Ca ratio versus Fe showed a linear relationship with correlation factor R^2=0.9446 (Fig. 3), which represents somewhat strong relationship. On the contrary, Cu/Mn versus Cu showed a poor relationship with R^2=0.512 as demonstrated in Fig. 4. Further, interrelationship of some elements in these medicinal herbs may suggest some antagonistic or synergistic effects, thereby supplying the elements to the body in a balanced manner with nearly no harmful effects [14].

Figure 3 Variation in Fe/Ca ratio against Fe concentration in herbs of S1, S2, S3, S4 and S5.

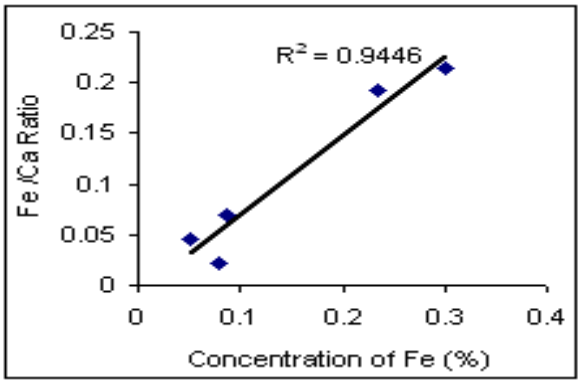


Figure 4 Variation in Cu/Mn ratio against Cu concentration in herbs of S1, S2, S3, S4 and S5

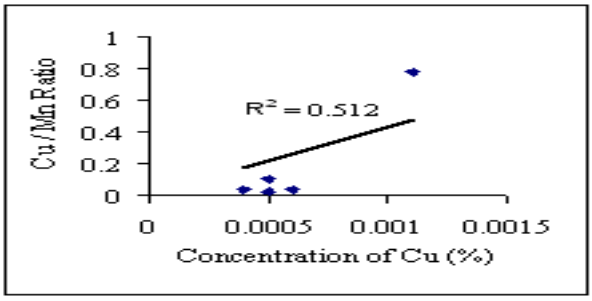


Table 3 Results of the determination of the chemical composition in the reference materials (vegetable) by WD-XRF (percent (%))

Element	NCS ZC73012 Cabbage		NCS ZC73013 Spinach		NCS ZC73017 Apple		NCS ZC85006 Tomato		NCS DC73348 Bush, branches and leaves	
	Certified	Reference	Certified	Reference	Certified	Reference	Certified	Reference	Certified	Reference
Na	1.090±0.060	1.060±0.05	1.500±0.06	1.540±0.04	0.116±0.009	0.118±0.01	0.130±0.025	0.126±0.015	1.10±0.01	1.140±0.030
Mg	0.241±0.015	0.247±0.016	0.552±0.015	0.529±0.020	0.039±0.006	0.036±0.004	0.736±0.057	0.746±0.044	0.287±0.018	0.282±0.014
Al	0.017±0.002	0.019±0.003	0.061±0.006	0.062±0.005	0.007±0.001	0.006±0.001	0.295±0.043	0.284±0.044	0.214±0.022	0.210±0.016
Si	0.024±0.005	0.023±0.004	0.212±0.024	0.216±0.022	0.005±0.001	0.005±0.001	NA	0.905±0.049	0.580±0.04	0.543±0.041
P	0.460±0.03	0.471±0.025	0.360±0.020	0.351±0.018	0.066±0.004	0.068±0.005	0.530±0.035	0.509±0.031	0.083±0.004	0.082±0.005
S	0.720±0.05	0.701±0.05	0.450±0.040	0.426±0.032	0.063±0.004	0.066±0.005	NA	0.922±0.036	0.320±0.03	0.333±0.025
Cl	0.640±0.07	0.653±0.05	1.080±0.07	1.073±0.068	0.008±0.001	0.009±0.001	NA	0.186±0.030	1.130±0.041	1.119±0.03
K	1.550±0.06	1.575±0.05	2.490±0.11	2.465±0.099	0.770±0.040	0.784±0.042	0.579±0.052	0.570±0.060	0.850±0.05	0.841±0.045
Ca	0.700±0.02	0.686±0.03	0.660±0.03	0.671±0.038	0.049±0.001	0.043±0.002	5.31±0.190	5.20±0.200	2.22±0.13	2.226±0.145
Cr*	1.80±0.3	2.0±0.5	1.4±0.2	<2.0±0.5	0.3±0.06	<1.0±0.2	NA	2.5±0.4	2.3±0.3	3.0±0.5
Mn*	18.7±0.8	20.0±1	41.0±3	38.0±2	2.7±0.2	3.0±0.3	87.1±5.6	89.0±4.0	58.0±6	62.0±7
Fe*	98.0±10	94.0±7	540.0±20	528.0±15	16.0±2	17.0±2	1380±150	1366±125	1020±67	1026±81
Cu*	2.7±0.2	3.0±0.4	8.9±0.4	10.0±0.5	2.5±0.2	3.0±0.4	21.1±2.5	21.0±2	5.2±0.5	6.0±0.5
Zn*	26.0±2	24.0±2	35.3±1.5	37.0±2	2.1±0.4	2.4±0.3	36.2±3.1	38.0±2	20.6±2.2	20.8±3.0
Ba*	12.0±2	11.0±2	9.0±0.8	9.0±0.5	2.5±0.3	2.7±0.3	55.2±5.2	52.0±4	19.0±3	17.0±3.0
Pb*	0.19±0.03	<2.0±0.5	11.1±0.9	12.0±0.5	0.084±0.032	ND	4.97±0.54	5.0±1	7.1±1.1	7.5±1.0

Experimental values are presented as mean of three replicates with corresponding standard deviation in ppm

NA: not available, ND: not detected, *: concentrations are in ppm

CONCLUSION

In the present study WD-XRF technique was used for the quantitative determination of some major elements (Na, Al, Mg, Si, P, S, Cl, K, Ca), trace elements (Cr, Mn, Fe, Cu, Zn) and non-essential elements (Ba, Pb) in five different vegetation samples. This technique is very versatile and non-destructive, doesn't require any chemical sample preparation, though it has a limitation (ppm levels) over the detection limit. Good agreements were achieved between certified values and data obtained by WD-XRF with recoveries ranging from 92 to 108%. To summarize, it could be concluded that this method provides sufficient accuracy and precession for the estimation of elemental concentration in Indian *Ayurvedic* medicinal herbs by WD-XRF spectrometry. We believe that the results obtained will be useful in understanding the pharmacological activities of the plant concerned and also helpful to estimate the dosage of the herbal medicine prepared from these plant materials.

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Table 4 Calibration data for vegetation matrices [#]

Element	Calibration range (%)	Correlation coefficient (R^2)
Na	0.118 – 1.540	0.9982
Mg	0.036 – 0.746	0.9978
Al	0.006 – 0.284	0.9328
Si	0.005 – 0.905	0.9975
P	0.068 – 0.509	0.9972
S	0.066 – 0.922	0.9975
Cl	0.009 – 1.119	0.9997
K	0.570 – 2.465	0.9994
Ca	0.048 – 5.280	0.9999
Cr*	1.0 – 3.0	0.9271
Mn*	3.0 – 89.0	0.9949
Fe*	17.0 – 1366.0	0.9998
Cu*	3.0 – 21.0	0.9972
Zn*	2.4 – 38.0	0.9901
Ba*	2.7 – 52.0	0.9992
Pb*	2.0 – 12.0	0.9987

[#] number of standards used for each element = 5; *: concentrations are in ppm

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